



HAL
open science

Fat content of striped mice decreased during the breeding season but not 1 during the food-restricted dry season

Rebecca Rimbach, Stéphane Blanc, Alexandre Zahariev, Jean-Patrice Robin,
Carsten Schradin

► To cite this version:

Rebecca Rimbach, Stéphane Blanc, Alexandre Zahariev, Jean-Patrice Robin, Carsten Schradin. Fat content of striped mice decreased during the breeding season but not 1 during the food-restricted dry season. *Journal of Experimental Biology*, 2019, 10.1242/jeb.208504 . hal-02362355

HAL Id: hal-02362355

<https://hal.science/hal-02362355>

Submitted on 13 Nov 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Fat content of striped mice decreased during the breeding season but not**
2 **during the food-restricted dry season**

3

4 Rebecca Rimbach^{*a}, Stéphane Blanc^b, Alexandre Zahariev^b, Jean-Patrice Robin^b, Neville
5 Pillay^a, Carsten Schradin^{a,b}

6

7 ^a School of Animal, Plant & Environmental Sciences, University of the Witwatersrand,
8 Private Bag 3, WITS 2050, Johannesburg, SOUTH AFRICA

9 ^b Université de Strasbourg, CNRS, IPHC UMR 7178, F-67000 Strasbourg, FRANCE

10

11 *Corresponding author: rimbach@gmail.com

12 **Abstract**

13 Individuals that are capable of accumulating appropriate fat stores are assumed to have
14 selective advantages when food becomes scarce. Similar to species from temperate zones,
15 some species inhabiting arid areas accumulate fat stores prior to periods of food limitation.
16 Yet, we have little knowledge concerning seasonal variation in body composition and the
17 relationship between fat store size and disappearance risk in species from arid habitats. Using
18 the water dilution method, we examined the body composition of African striped mice
19 (*Rhabdomys pumilio*) living in a seasonal habitat with a long food-restricted dry season. We
20 tested for seasonal changes in body composition (N = 159 measurements of 113 individuals)
21 and whether dry season survival was related to fat mass (N = 66 individuals). Fat stores were
22 similar in size at the onset and the end of the dry season, but surprisingly smaller at the onset
23 of the moist breeding season. Fat stores showed a negative relationship with food availability.
24 Individual variation in fat stores was not associated with disappearance risk, but there was a
25 positive association of disappearance risk with body mass. Increased disappearance risk of
26 heavy individuals suggests elevated dispersal rates in competitive individuals. This study
27 suggests that non-breeding philopatric striped mice do not accumulate large fat stores prior to
28 the food-limited dry season but that they might mobilize fat stores at the onset of the breeding
29 season to satisfy the energetic demands of reproduction and/ or to decrease costs associated
30 with larger fat stores such as increased predation risk.

31

32 **Key words:** disappearance, energetics, fat reserves, lipid analysis, method validation,
33 survival

34

35 **Introduction**

36 Food shortages are pervasive and re-occurring impediments encountered by most animals,
37 especially species living in seasonal environments (Fretwell, 1972). To increase survival
38 probability during periods of limited food availability and to fuel demands for maintenance
39 and/ or reproduction, species can rely on internal or external nutrient stores (Giannoni et al.,
40 2001; Gutman et al., 2006; McNamara and Houston, 1990; Parker et al., 2009; Worden and
41 Pekins, 1995; Young, 1976). For example, many species from temperate areas undergo a
42 fattening period during summer or autumn to sustain them during a nutritionally demanding
43 winter (Baldwin and Kendeigh, 1938; Bednekoff and Houston, 1994; Fleharty et al., 1973).
44 Endogenous lipid stores are the main source of energy, catabolized during periods of food
45 limitation (John, 2005; McCue, 2010; McCue, 2012; Mellanby, 1942). Quantifying energy
46 stores and determining their influence for individual survival is of prime importance to
47 understand how animals cope with food constraints imposed by their natural environment.

48 Animals have to continuously adjust physiologically to changes in their environment
49 (called allostasis), and when energy expenditure exceeds energy acquisition, allostatic
50 overload occurs (McEwen, 2000; McEwen and Wingfield, 2003; Romero et al., 2009). This
51 leads to a decrease in body condition, pathologies (e.g. reduced immune function (Tannock
52 and Smith, 1972) and decline in health status (Schoepf et al., 2017)), and finally, if it persists,
53 starvation leading to death. Individuals that are capable of accumulating appropriate energy
54 stores are assumed to have a selective advantage during energetically highly demanding
55 periods or when energy supplies do not meet short-term needs (Lindstedt and Boyce, 1985;
56 Young, 1976). The majority of studies that examined the relationship between fat stores and
57 survival probability has been conducted on animals in temperate zones with a focus on their
58 over-winter survival (Boos et al., 2002; Boos et al., 2005; Buskirk and Harlow, 1989; Dugan
59 et al., 1981; Monteith et al., 2013; Murie and Boag, 1984; Piper and Wiley, 1990). For

60 example, both mule deer *Odocoileus hemionus* (Bender et al., 2007) and subordinate great tits
61 *Parus major* (Gosler, 1996) with larger fat stores have higher survival. Thus, it has been well
62 documented that fat stores can help animals to survive the food-restricted winter in temperate
63 zones (Bender et al., 2007; Boos et al., 2002; Murie and Boag, 1984; Waite, 1992).

64 Food shortages also occur in semi-deserts in the subtropics (Nagy, 1988), and some
65 species inhabiting these areas accumulate fat stores prior to periods of food limitation. For
66 example, several Malagasy lemurs, such as *Lemur catta*, *Eulemur sp.*, *Cheirogaleus medius*
67 and *Microcebus murinus*, as well as Australian fat-tailed dunnarts *Sminthopsis crassicaudata*
68 have an elevated body fat content when entering periods of reduced food availability (Fietz
69 and Dausmann, 2006; Morton, 1978; Schmid, 1999; Simmen et al., 2010). In addition to food
70 shortages, animals from semi-arid areas also face the problem of water scarcity (Degen,
71 1997; Nagy, 1994). Body water plays a central role in nutrient transport, maintenance of cell
72 volume, and thermal regulation (McKinley et al., 2008; Siri, 1956) and thus, water
73 conservation is especially important for animals from arid areas. Yet, we know little about
74 seasonal changes in fat stores and body hydration (i.e. water conservation) from animals
75 inhabiting semi-arid areas that simultaneously experience food scarcity and dryness.
76 Important would also be to understand the relationship between energy stores and survival of
77 species living in such areas.

78 The African striped mouse *Rhabdomys pumilio* (Sparrman 1784) is an ideal species in
79 which to examine such questions because they live in a habitat with pronounced seasonal
80 variation in food and water availability (Schradin and Pillay, 2006). Individuals are born in
81 spring and subsequently have to survive the food-restricted dry season during summer and
82 autumn before they can reproduce in the following winter (moist season) (Schradin et al.,
83 2012). Body mass of striped mice varies seasonally (Schradin and Pillay, 2005), which may
84 indicate seasonal changes in fat mass. Approximately 70% of striped mice disappear from our

85 study population before reaching the end of their first year (Schradin and Pillay, 2005), while
86 in some years this rate is much higher at 99% (Schradin et al., 2010). Striped mice experience
87 nutritional limitation during the dry season, which results in the mobilization of somatic
88 reserves (Rimbach et al., 2017; Schoepf et al., 2017). This precipitates a decline in their
89 health status, which progressively deteriorates during the dry season (Schoepf et al., 2017).
90 Especially blood parameters that are indicative of nutritional state (i.e. albumin, glucose and
91 total protein) are impaired in individuals that disappear during the dry season (Schoepf et al.,
92 2017). However, we do not know to what extent striped mice store fat during the moist
93 season to be able to cope with the coming food-restricted dry season and whether their
94 survival is influenced by the amount of fat they store.

95 **Our study addressed the main aims to:** (1) describe seasonal changes in body
96 composition (fat and water content); (2) assess in how far this relates to changes in food
97 availability (N = 159 measurements); and (3) to investigate whether the size of fat stores at
98 the onset of the dry season is associated with disappearance probability until the subsequent
99 breeding season (N = 66 individuals). We hypothesized that (i) throughout the year, variation
100 in fat mass (FM) will show a positive relationship with variation in food availability; and (ii)
101 that striped mice with larger fat stores are more likely to survive the dry season. **To determine**
102 **FM in free-living striped mice we validated the water dilution method by comparing**
103 **estimates acquired via this indirect method with those obtained via the ‘golden standard’**
104 **method (i.e. biochemical analysis) of body composition measurement.** We aimed to
105 determine the hydration coefficient of striped mice, a species living in a semi-arid habitat,
106 because to date, the hydration coefficient of animals adapted to arid habitats has not been determined,
107 which may cause errors in studies of animals that inhabit arid environments. This validation was
108 important to ensure that estimates of body composition acquired via the water dilution
109 method were accurate, especially in lean animals.

110

111 **Materials and methods**

112 *A) Data collection on free-living striped mice*

113 *Study site and species*

114 We collected data in the Succulent Karoo semi-desert of South Africa. Here, striped mice
115 typically live in social groups, consisting of one breeding male, two to four breeding females
116 and their adult philopatric offspring of both sexes, and groups can contain up to 30 adult
117 individuals of both sexes during the dry season (Schradin and Pillay, 2004). Summers
118 (December – February), and also most part of autumns (March-May) are hot and dry with
119 low food availability (Schradin and Pillay, 2004; Schradin and Pillay, 2006). Striped mice
120 typically breed in the austral winter / spring (August – November / December) when food is
121 abundant. Striped mice can reach sexual maturity at around 6 weeks of age, but typically do
122 not start reproducing in the breeding season in which they were born (Schradin and Pillay,
123 2004). Females show a higher level of natal philopatry than males, which are more likely to
124 disperse than females (Solmsen et al., 2011). We studied individuals that were born in either
125 August – December 2014 (N = 70; data used to estimate daily energy expenditure in another
126 study (Rimbach et al., 2018a)) or in September – December 2016 (N = 89; Table 1).

127

128 *Trapping and disappearance probability*

129 We trapped the study population continuously throughout the study and continued after this
130 study ended. Striped mice were trapped using Sherman-style live-traps (26 x 9 x 9 cm) baited
131 with wheat bran flakes, raisins, sea salt and sunflower oil. We placed traps close to a group's
132 nest in the morning and the late afternoon and checked them 30–45 min later. We weighed
133 captured individuals using an electronic scale (± 0.1 g) and recorded their reproductive state
134 (males: whether they were scrotal; females: whether nipples were visible and/ or their vagina

135 was perforate). For individual recognition, all mice were marked with ear tags (National
136 Band and Tag Co., Newport, KY, USA). The use of experimental animals complied with
137 South African animal welfare laws. Animals were captured, handled and euthanized (N = 24
138 free-living mice were euthanized; 113 individuals were handled and subsequently released)
139 following protocols approved by the Animal Ethics Screening Committee of the University of
140 the Witwatersrand (AESC 2012/37/2A extended until December 2019, AESC 2014/40/B,
141 AESC 2017/01/02/B, Department of Environment and Nature Conservation Permit No.
142 0474/2017).

143 We regarded an individual as having disappeared after it had not been trapped or
144 observed for at least one year, which is more than the average life span of striped mice at our
145 study site. We set the date it was last trapped as its day of disappearance. At the study site,
146 the breeding season typically starts in July/August (Schradin and Pillay, 2004). Individuals
147 can immigrate and disperse throughout the year, although a peak of dispersal occurs around
148 June (Vuarin et al 2019). Immigration is much lower than disappearance, and consequently,
149 most mice that disappeared can be assumed to have died (Vuarin et al 2019). Individuals that
150 were still present on June 1st were regarded as having survived the dry season.

151

152 *Food availability*

153 Using the Braun-Blanquet method (Werger, 1974), we conducted plant surveys on the 1st and
154 15th of each month. The number of food plants species, for which the palatability was known
155 from behavioural observations (Schradin and Pillay, 2006), was recorded in eight monitoring
156 plots (4 m² each) within the field site. As a measure of food availability, we used the number
157 of species averaged over all monitoring plots (Table 1). For analyses, we used food
158 availability averaged over 4 weeks prior to the measurement of body composition.

159

160 *Body condition index*

161 We calculated an index for body condition as body mass divided by body length. Thus, the
162 larger this index, the heavier an individual for its given body length. We measured body
163 length as the distance from the tip of the nose to the anus to the nearest 1 mm. Body length
164 measurements were available for 89.3% of the body composition measurements (142 out of
165 159; Table 1), and they were obtained within 6.4 ± 9.9 days of injection with doubly labelled
166 water (DLW) (i.e. when body mass was measured).

167

168 *Estimation of body composition from body water*

169 Using the water dilution method (Speakman, 1997), we estimated FM and fat free mass
170 (FFM) of 113 free-living striped mice (159 measurements; Table 1). We trapped individuals
171 in the morning at their nest, brought them to the research station, weighed them (± 0.1 g),
172 anesthetized them with di-ethyl ether and took a first blood sample (~ 100 μ l) from the sub-
173 lingual vein into glass capillaries, which we then flame-sealed. After disinfecting a part of the
174 abdomen for an injection site, we injected highly-enriched DLW intraperitoneally at a dose of
175 3.38 g/kg body mass (1.0 g of 97 % ^{18}O and 0.35 g of 99.9 % ^2H). We weighed syringes
176 immediately before and after administration (± 0.0001 g, Mettler-Toledo balance). In small
177 vertebrates, an isotopic equilibration in body water is typically reached within 1 hour (Degen
178 et al., 1981; Poppitt et al., 1993). Thus, after 1 hour, we weighed individuals (± 0.1 g),
179 anesthetized them with di-ethyl ether, took a second blood sample (~ 100 μ l) to determine the
180 maximum isotope enrichment. Between blood samplings, animals were awake and kept in
181 Sherman-style live-traps without food and water to reduce stress, as striped mice are used to
182 these traps and are shielded from visual stressors while in the dark traps. Subsequently,
183 individuals were released at their nest. We kept sealed capillaries in a fridge at 4°C until
184 transport to the IPHC-DEPE laboratory in Strasbourg, France for isotope analysis.

185 Isotope analysis was conducted as previously described in detail (Chery et al., 2015;
186 Rimbach et al., 2018a). Blood samples were vacuum distilled for 5 min and 0.1 µl distillate
187 was injected into an elemental analyser with thermal conversion (TC/EA, Thermo, Bremen,
188 Germany) which was connected to a continuous flow isotope ratio mass spectrometer (IRMS-
189 DELTA V PLUS, Thermo, Bremen, Germany). Distillates were pyrolyzed at 1400°C into H₂
190 and CO gas in a glass carbon tube under pure He flow at 90 ml min⁻¹. H₂ and CO were further
191 separated at 110°C on a molecular sieve GC column before sequential analysis in the isotope
192 ratio mass spectrometer. Results were first drift corrected and optionally a memory effect
193 correction was applied. Results were normalized versus the VSMOW2/SLAP2 international
194 scale. All analyses were performed in quadruplicate. TBW was calculated from the ¹⁸O
195 dilution space divided by 1.007 to correct for *in vivo* isotopic exchange (Racette et al., 1994).
196 The average isotope dilution space ratio (R) was 1.029 ± 0.018 (mean ± s.d.; N =159). FFM
197 was derived from TBW assuming a hydration coefficient of 73.2% (Blanc et al., 2005). FM
198 was calculated from the difference between body mass and FFM.

199

200 *B) Validation of the water dilution method by chemical analysis*

201 It is challenging to accurately measure fat stores, especially in small animals and particularly
202 in free-living individuals. To ensure our estimates obtained by the water dilution method
203 were reliable, we compared estimates acquired via this indirect method with those obtained
204 via a direct method of body composition measurement. This validation was important
205 because estimates of FM were surprisingly low and we wanted to ensure that these estimates
206 were accurate. Carcass lyophilisation is the ‘golden standard’, and a direct and accurate
207 method for determining body reserves (Robbins, 1993), but it requires killing of the study
208 animals in order to chemically analyse their body composition. This procedure is time
209 consuming and the killing of animals makes it ethically undesirable, especially when working

210 with free-living animals. In contrast, the water dilution method is indirect, less invasive and
211 can be used repeatedly on the same individuals both in the laboratory and under free-living
212 conditions.

213 For the validation study, we used a total of 54 mice (24 free-living and 30 captive
214 individuals). We trapped 4 males and 5 females in May 2017 and 5 males and 4 females in
215 October 2017 at Klein Goegap (a farm included into the Goegap Nature Reserve recently)
216 that we injected with DLW (see details in the previous section ‘Estimation of body
217 composition’). In February 2018, we also injected 8 females and 6 males from the captive
218 colony (Garnier and Schradin, 2019) at the IPHC-DEPE in Strasbourg with DLW with the
219 same procedure. After the 2nd blood sample for the water dilution method was taken, animals
220 were euthanized via cervical dislocation. In addition, we determined body composition via
221 biochemical analysis of an extra 22 striped mice (free-living: 4 in May and 2 in October;
222 captive: 6 females and 10 males in February 2018) for which DLW samples could not be
223 analysed. We weighed animals and immediately placed them in a plastic bag to avoid water
224 loss resulting from bleeding or dehydration. Carcasses were stored at -20°C until
225 lyophilisation at the CNRS, Strasbourg, France.

226 For the carcass analysis, we used standard procedures applied at our laboratory (Boos et
227 al., 2005; Mata et al., 2006). To ensure an adequate elimination of all body water, we cut the
228 carcasses into small pieces ($< 1 \text{ cm}^3$) and broke all bones. Subsequently, we freeze-dried
229 carcasses in a freeze dryer (Christ Martin ALPHA 1-4) to constant mass for 2 weeks. We
230 calculated TBW as the difference between fresh and dry body mass. After drying, we ground
231 the carcasses and homogenized each one into a fine powder, which we stored at -20°C in
232 sealed boxes until chemical analyses. Samples were lyophilized for 48 h just before analysis
233 to eliminate any traces of water. We determined body lipid content using chloroform/
234 methanol (2/1, vol/vol) solution as extraction solvent on 1 g of powdered tissue (Folch et al.,

235 1957). Using 100 – 150 mg sample we determined the nitrogen content of the carcasses using
 236 the Kjeldahl method, and we calculated protein content as nitrogen content * 6.25 (Robbins,
 237 1993). We measured ash content on 1 – 2 g of sample after total combustion at 500°C for 24
 238 h in a muffle furnace. The sum of the ash, lipid, protein, and water masses represented $98.4 \pm$
 239 1.0% of the fresh body mass. The average $R = 1.035 \pm 0.016$ ($N = 32$).

240

241 **Table 1.** Overview of variation in food availability, and sample sizes for measurements of
 242 body composition (via DLW) and body condition in different months for two birth cohorts
 243 (individuals born in the same breeding season). Light red indicates dry season onset, dark red
 244 indicates end of the dry season and blue indicates the onset of the moist season.

Birth cohort	Year	Month	Food availability (mean no. of plant species/4m ² ± s.d.)	Body composition (estimated)	Body condition
2014	2014	Oct	4.31 ± 1.47	3	3
	2014	Nov	2.81 ± 0.86	6	6
	2014	Dec	2.33 ± 0.88	5	5
	2015	Jan	1.85 ± 0.90	3	3
	2015	Feb	1.50 ± 0.84	16	16
	2015	Mar	1.13 ± 0.73	19	19
	2015	April	1.25 ± 0.57	0	0
	2015	May	1.80 ± 0.89	2	2
	2015	June	2.94 ± 1.04	2	2
	2015	July	5.88 ± 1.72	8	8
	2015	Aug	6.60 ± 1.78	6	3
SUBTOTAL				70	67
2016	2017	Jan	2.49 ± 0.66	23	20
	2017	Feb	1.31 ± 0.88	0	0
	2017	Mar	1.09 ± 0.74	0	0
	2017	April	1.19 ± 0.77	0	0
	2017	May	1.50 ± 0.78	28	25
	2017	June	1.50 ± 0.54	0	0
	2017	July	2.30 ± 0.83	0	0
	2017	Aug	7.10 ± 1.04	23	18
	2017	Sep	6.40 ± 1.58	0	0
2017	Oct	3.38 ± 1.72	15	12	
SUBTOTAL				89	75

TOTAL	159	142
--------------	------------	------------

245

246 *Statistical analyses*

247 We analysed all data using R 3.5.1 (R Core Team, 2018). Data are presented as means \pm s.d.

248

249 Body composition by direct chemical analysis

250 We used ANCOVAs, with body mass as a co-variate, to examine differences in FM, TBW,
251 protein and ash between free-living and captive striped mice. We included the interaction
252 term between condition (free-living vs captive) and body mass, and removed non-significant
253 interaction terms.

254

255 Validation of the water dilution method

256 To assess whether differences in body water determinations we obtained from O¹⁸, deuterium
257 or after lyophilisation are related to body mass, FM or FFM, we regressed delta water values
258 (O¹⁸ and deuterium; O¹⁸ or deuterium and water measured after lyophilisation) with body
259 mass, FM and FFM.

260 We assessed the agreement between the estimates of FM and TBW obtained via water
261 dilution method and lyophilisation using the 95% limits of agreement (LoA) method (Altman
262 and Bland, 1983; Bland and Altman, 2003). We used Shapiro-Wilk tests to assess whether
263 data was normal distributed. TBW was normal distributed and we log-transformed FM to
264 reach normal distribution which is required for the LoA (Altman and Bland, 1983). We
265 calculated the mean difference (i.e. bias) and the LoA (bias \pm 2 s.d.) between both methods
266 using the function 'bland.altman.stats' from the package 'BlandAltmanLeh' (Lehnert, 2015).
267 We set a priori limits of agreement at 5 - 8 % for FM and 1 - 2 % for TBW (Schoeller, 2005).
268 We plotted Bland-Altman plots (mean-difference plots) using the package 'ggplot2'
269 (Wickham, 2016).

270

271 Seasonal variation in body composition and TBW

272 To assess whether FM and % TBW changed between the onset of the dry season (December
273 2014 – March 2015, January 2017; N = 66), the end of the dry season (May 2015 and May
274 2017; N = 30) and the moist season (August 2015 and August 2017; N = 29), we used two
275 linear mixed models (LMM) with Gaussian error structure and identity link using the package
276 ‘lme4’ (Bates and Maechler, 2010). We used the square root of FM to reduce
277 heteroscedasticity when fitting the model. We used study period (dry onset, dry end and
278 moist) and sex as explanatory variables, and individual ID as a random factor because several
279 individuals were sampled in more than one period. In addition, we included body mass as a
280 covariate in the FM model. In the FM model, we included the interaction terms between sex
281 and body mass and between body mass and study period. Moreover, we included the
282 interaction between sex and study period in both models and removed non-significant
283 interaction terms.

284 We used an additional LMM to assess the relationship between FM, food availability,
285 body condition and sex using data collected throughout the year (N = 142). Plotting the raw
286 data suggested curvilinear relationships but including quadratic terms of food availability and
287 body condition as additional fixed effects was not possible because raw data and their
288 quadratic terms were highly correlated. A model including only the raw data and a model
289 including only their quadratic terms produced similar AIC values and results, and thus we
290 present the results of the model including the quadratic terms. We standardized (z-
291 transformed) all numeric predictors for more accurate model fitting and to facilitate
292 comparisons of model estimates (Schielzeth, 2010). We tested interactions between all
293 explanatory variables and removed non-significant interaction terms.

294 We verified models by inspecting Q–Q plots and by plotting model residuals against
295 fitted values. Prior to running each model, we checked for multi-collinearity by calculating
296 variance inflation factors (Zuur et al., 2010) for the predictor variables using the ‘vif’
297 function in the car package (Fox and Weisberg, 2011). Vifs did not indicate collinearity (all
298 vifs < 2). To assess model stability, we ran diagnostics (dfbetas) that did not suggest the
299 existence of influential cases.

300

301 Body composition and disappearance probability

302 We examined whether FM stored at the onset of the dry season influences disappearance
303 probability until the subsequent breeding season using a Cox Proportional Hazards model
304 (function ‘coxph’ of the ‘survival’ package). For this analysis, we used measurements of FM
305 collected from 66 non-breeding philopatric individuals at the onset of the dry season
306 (December 2014 – March 2015, January 2017). We used survival time (days since the
307 measurement of body composition) as the dependent variable. The censoring date was set to
308 the 1st of June. Survival time was then calculated as the number of days from the
309 measurement of body composition until the last trapped date or the censoring date if the
310 individual was still present in the population and trapped later. In addition to FM, we also
311 included body mass and sex into the model. The interactions of FM with body mass and sex
312 were also included. We checked whether our model satisfied the proportional hazard
313 assumption using scaled Schoenfeld residuals and assessed the significance of effects using
314 loglikelihood ratio statistics (‘anova’ function). We assessed nonlinearity through visual
315 inspection of Martingale residuals, which exhibited no obvious trends. We visualized
316 significant effects of variables on hazard ratios using parametric simulations in the package
317 ‘simPH’ (Gandrud, 2013). We used ribbons to indicate the areas containing the central 95%
318 and 50% of 1000 simulations, reflecting an empirical confidence interval.

319

320 **Results**

321 *Body composition by direct chemical analysis*

322 Carcasses of free-living striped mice (males and females combined) consisted of 3.3 g FM,
323 26.8 g TBW, 1.8 g ash and 7.3 g protein and carcasses of captive individuals consisted of 6.3
324 g FM, 32.8 g TBW, 2.0 g ash, 9.2 g protein on average (Fig. 1; see Table S1 for values as %
325 of body mass). After controlling for body mass, captive and free-living individuals did not
326 differ regarding FM (ANCOVA: $F = 1.885$, $P = 0.176$; Fig. 1A), TBW ($F = 1.411$, $P = 0.240$;
327 Fig. 1B) or protein ($F = 1.368$, $P = 0.248$; Fig. 1D). Free-living individuals had a higher ash
328 content ($F = 5.541$, $P = 0.022$; Fig. 1C). The median hydration coefficient (ratio of body
329 water and FFM) determined via chemical analysis was $73.19 \pm 1.4\%$.

330

331 *Validation of the water dilution method*

332 Delta water values (O^{18} and deuterium; O^{18} or deuterium and water measured after
333 lyophilisation) were not significantly related to body mass, adiposity or FFM (all $r < 0.32$).
334 When comparing measurements from chemical analysis and O^{18} or deuterium, respectively,
335 the mean differences for FM were 0.23 g and 0.27 g (Table 2; Fig. S1), which were
336 equivalent to average discrepancies of 4.9 % and 5.8 % FM. For TBW, the mean differences
337 were 0.65 g and 0.70 g (Table 2; Fig. S1), which were equivalent to average discrepancies of
338 2.1 % and 2.3 % TBW. Both FM and TBW were slightly underestimated by the water
339 dilution method, but this underestimation was within the precision limits of the water dilution
340 method.

341

342 **Table 2.** Overview of the bias, critical difference and 95% confidence intervals of the bias
 343 between measurements of FM (log-transformed; in g) and TBW (in g) by chemical analysis
 344 and stable isotope dilution (water dilution method).

Comparison ^a	Bias ^b	Critical difference ^c	95% CI of the bias
FM (L vs O)	0.2736	1.0674	0.0703 – 0.4770
FM (L vs H)	0.2306	0.8342	0.0717 – 0.3895
TBW (L vs O)	0.6528	1.76501	0.3281 – 0.9774
TBW (L vs H)	0.7028	1.80661	0.3704 – 1.0351

345 ^a L = lyophilisation; O = determined via stable isotope ¹⁸O; H = determined via stable isotope ²H; ^b
 346 Mean difference; ^c Two times standard deviation of differences, equals half the difference of lower CI
 347 limit and upper CI limit

348

349 *Seasonal variation in body composition*

350 During the study period, average FM of striped mice was 3.1 g (0.2 – 10.6 g), which equalled
 351 8.0 % of striped mice body mass (range: 0.6 – 26.3 %). FM was similar at the onset (3.2 ± 1.9
 352 g) and the end of the dry season (3.4 ± 1.2 g; LMM: Estimate: 0.041 ± 0.106, t = 0.390, P =
 353 0.69; Fig. 2). FM was lower at the beginning of the moist season (2.8 ± 1.6 g) than at the
 354 onset and at the end of the dry season (onset vs moist: Estimate: -0.360 ± 0.126, t = -2.841, P
 355 = 0.005; end vs moist: Estimate: -0.401 ± 0.133, t = -3.004, P = 0.003; Fig. 2). There was no
 356 difference in FM between the sexes (Estimate: -0.045 ± 0.096, t = -0.471, P = 0.63). FM
 357 increased with increasing body mass (Estimate: 0.026 ± 0.007, t = 3.377, P = 0.001; Fig. 2B).
 358 Percent TBW was similar the onset of the dry season (66.7 ± 3.5 %) and the end of the dry
 359 season (67.1 ± 2.3 %; LMM: Estimate: 0.323 ± 0.684, t = 0.473, P = 0.63). Percent TBW was
 360 higher at the beginning of the moist season (69.0 ± 2.4 %) than at the onset and at the end of
 361 the dry season (onset vs moist: Estimate: 2.278 ± 0.695, t = 3.275, P = 0.001; end vs moist:
 362 Estimate: 1.954 ± 0.810, t = 2.411, P = 0.017). There was no difference in % TBW between
 363 the sexes (Estimate: -0.076 ± 0.559, t = -0.136, P = 0.89).

364 FM showed a negative relationship with food availability (Estimate: -0.083 ± 0.042 , $t =$
 365 -1.976 , $P = 0.051$; Fig. 3A) and a quadratic relationship with body condition (Estimate: 0.121
 366 ± 0.044 , $t = -2.695$, $P = 0.008$; Fig. 3B), where individuals with a low and a high body
 367 condition had lower FM than individuals with a medium body condition. Sex (Estimate:
 368 0.054 ± 0.089 , $t = 0.608$, $P = 0.54$) did not influence FM.

369

370 *Body composition and disappearance probability*

371 37.9 % of the individuals disappeared during the dry season and 62.1 % remained in the
 372 population. FM and its interactions with body mass and sex were not associated with
 373 disappearance probability (Table 3). Body mass was positively associated with disappearance
 374 probability (Table 3, Fig. 4).

375

376 **Table 3.** Analysis of the deviance table for the effects of fat mass at the onset of the dry
 377 season, body mass and sex, and the interactions of fat mass with each of the other covariates,
 378 on striped mice disappearance before the breeding season (Cox proportional hazards model,
 379 $N = 66$).

Variable	Chisq	df	<i>p</i>
Fat mass	0.7728	1	0.3793
Body mass	6.4161	1	0.0113
Sex	0.0019	1	0.9656
Fat mass:Body mass	1.5053	1	0.2199
Fat mass:Sex	1.1478	1	0.284

380

381 **Discussion**

382 In our study, fat stores of striped mice did not change from the onset of the dry season until
 383 its end. Instead, fat stores subsequently decreased until the beginning of the moist season.

384 Striped mice breed in the moist season, in which they show increases in behavioural activity

385 and in resting metabolic rate, explaining their overall higher daily energy expenditure when
386 compared to the dry season (Rimbach et al., 2016; Rimbach et al., 2018b; Rimbach et al.,
387 2018a). Our findings suggest that they mobilize their fat stores to satisfy these energetic
388 demands of increased maintenance costs and reproduction. Such reductions in fat stores
389 during times when food is plentiful and the risk of starvation is low can also decrease costs
390 associated with carrying extra fat mass, such as predation risk. Individual variation in fat
391 content at the onset of the dry season was not associated with disappearance risk of striped
392 mice. Together, these results suggest that striped mice do not rely on fat storage for dry
393 season survival.

394

395 *Validation*

396 It is challenging to accurately measure fat stores, especially in small animals and particularly
397 in free-living individuals. Our study found that striped mice living in a semi-arid habitat have
398 a hydration coefficient of FFM of 0.7319. This value is nearly identical to the theoretical
399 value of 0.732. Such validation is important to accurately estimate body condition using the
400 water dilution method in wild species living in semi-arid habitat, especially when the
401 obtained estimates are surprisingly low. Further, estimates of body water were not related to
402 body mass, adiposity or FFM. These findings strengthen the validity of the FFM calculations
403 and thereafter adiposity. Our validation study on striped mice showed that the mean
404 differences ('bias') between both methods (5.3 % for FM and 2.2 % for TBW) lie within the
405 a priori limits of agreement we expected at 5 – 8 % for FM and 1 – 2 % for TBW. This
406 indicates that estimates of body composition obtained with the indirect water dilution method
407 reflect estimates from the biochemical analysis.

408

409 *Seasonal variation in body composition*

410 Free-living striped mice showed large individual variation in adiposity, ranging from 0.6 –
411 23.1 % of total body mass (0.2 – 10.6 g) over the course of the entire study period. Average
412 adiposity was 9 % at the onset and at the end of the dry season, indicating that fat stores were
413 not reduced throughout the food-restricted dry season. Many small rodents from temperate
414 zones accumulate fat stores before winter, which often resembles a nutritionally challenging
415 period, and lose fat during winter (Table 4). Fat content of *R. dilectus*, a sister species of *R.*
416 *pumilio*, living in the Eastern Cape, South Africa varies between 21 – 31 % of dry body mass
417 with no clear seasonal changes, and fat content was similar to dry fat mass in our study (28.7
418 % of dry body mass in *R. dilectus*, 24.5 % in *R. pumilio*) (Perrin, 1981). The reason for the
419 difference in seasonal variation in fat content between the two sister species remains to be
420 determined.

421 Unexpectedly, we found that fat content dropped from 9 % in the dry season to 6 % in
422 the moist season, even though food availability increased. Striped mice have a 43 % higher
423 energy expenditure in the moist season compared to the dry season (Rimbach et al., 2018a),
424 and they have a higher metabolic rate and spent more time active (Rimbach et al., 2016;
425 Rimbach et al., 2018b). There are two possible explanations for a decrease in fat content in
426 the moist season. Firstly, striped mice might mobilize their fat stores in the moist season to
427 satisfy the energetic demands of increased maintenance costs and reproduction (Rimbach et
428 al., 2016; Rimbach et al., 2018a; Rimbach et al., 2018b), similar to other rodents such as
429 muskrats *Ondatra zibethicus* (Virgl and Messier, 1992), house mice *Mus domesticus* (Mutze,
430 1990), and old-field mice *Peromyscus polionotus* (Caldwell and Connell, 1968). Secondly,
431 they might reduce costs associated with carrying extra fat, such as predation risk (Houston et
432 al., 1993; Lima, 1986), in the season when food is abundant. These two explanations are not
433 mutually exclusive because reduced fat stores due to higher maintenance costs will also
434 decrease costs associated with larger fat stores. We found that striped mice with a low or a

435 high body condition index (i.e. that are light or heavy for their body length, respectively)
436 have smaller fat stores than individuals with an intermediate body condition. In sum, while
437 average fat content of striped mice is comparable to that of small rodents inhabiting
438 temperate areas, we found no indication that they store fat in spring to cope with the food
439 restricted summer dry season.

440 Knowledge about seasonal changes in body composition of free-living animals from the
441 subtropics is limited. Here, we showed that fat stores of striped mice remained unchanged
442 during the dry season, and decreased in the moist season when breeding started. These results
443 indicate that striped mice, which live in an arid habitat with large seasonal variation in food
444 abundance, are well adapted to this environment. Although some species from arid habitats
445 and many species in temperate zones increase fat stores prior to periods of food limitation
446 (Table 4), we did not find this pattern in striped mice. Philopatric striped mice reduce their
447 energy expenditure in the dry season by reducing their resting metabolic rate and physical
448 activity level (Rimbach et al., 2018a). Estimates of their physical activity level are lower than
449 predicted by allometric equations, suggesting that they display lower levels of physical
450 activity than small mammals living in other habitat types (Rimbach et al., 2018a). Low
451 energy expenditure may explain why philopatric striped mice did not rely on fat storage for
452 dry season survival, at least in the two study years. However, we did not track changes in
453 body composition and energy expenditure continuously throughout the dry season. Thus, it is
454 possible that compensatory effects of energy expenditure and behavioural activity influence
455 body composition, as has been reported in Svalbard rock ptarmigan *Lagopus mutus*
456 *hyperboreus* (Stokkan et al., 1986). Changes in the substrate type catabolized and associated
457 production of metabolic water (i.e. from fat to carbohydrates (Frank, 1987; Takei et al.,
458 2012)) in the course of the dry season might explain why we did not find changes in fat mass
459 and body water during the dry season. Together with previous studies (Rimbach et al., 2016;

460 Rimbach et al., 2019), our results suggest that philopatric striped mice do not invest in fat
 461 storage but rather into somatic growth. Reaching a large body mass increases the competitive
 462 ability of striped mice (especially males) and influences their reproductive tactic (Hill et al.,
 463 2015; Schradin et al., 2009) and ultimately their fitness (Schradin and Lindholm, 2011).

464 Animals from semi-arid areas are faced with the problem of water scarcity (Degen,
 465 1997; Nagy, 1994). If a lack of water hampers with body water homeostasis, important
 466 physiological processes, such as nutrient transport, maintenance of cell volume, and thermal
 467 regulation, can be disturbed (McKinley et al., 2008; Siri, 1956). Body water of striped mice
 468 remained unchanged during the dry season and increased in the moist season, when the rate
 469 of daily water turnover is also higher compared to the dry season (Rimbach et al., 2018a).
 470 The daily water turnover rate describes the replacement of body water in a day, and is used to
 471 assess body water homeostasis. However, we were unable to estimate this rate in the current
 472 study because its measurement requires the collection of an additional blood sampling 24 h
 473 after injection with DLW. Our study shows that striped mice have a higher percentage of
 474 body water in the moist season, when most annual rain falls and fresh water-rich vegetation
 475 grows.

476

477 **Table 4.** Overview of seasonal changes in fat content in small mammals from temperate and
 478 subtropical areas (^a % of dry body mass; ^b % of wet body mass; ^c % of ingesta-free body mass).

Study species	Changes in fat content	Zone	Reference
Yellow-necked field mice, <i>Apodemus flavicollis</i>	14% ^a in autumn, 21 % in winter, 12 % in spring and summer	temperate	(Sawicka-Kapusta, 1968)
Cotton rats, <i>Sigmodon hispidus</i>	10 % ^b in winter, 4 % at the end of spring	temperate	(Flehart et al., 1973)
Muskrats, <i>Ondam zibethicus</i>	Accumulate fat in summer and autumn, peak of 16 % ^c in winter, 2 % in summer	temperate	(Virgl and Messier, 1992)
Harvest mouse, <i>Reithrodontomys megalotis</i>	7 % ^b in winter, 6 % in summer	temperate	(Flehart et al., 1973)
Deer mouse,	6.5 % ^b in winter, 5 % in summer	temperate	(Flehart et al., 1973)

<i>Peromyscus maniculatus</i>			
Prairie voles, <i>Microtus ochrogaster</i>	No seasonal changes in fat content (4 % ^b)	temperate	(Flehart et al., 1973)
European rabbit <i>Oryctolagus cuniculus</i>	No seasonal changes in fat content (3.6 % ^b)	temperate	(Boos et al., 2005)
House mice, <i>Mus domesticus</i>	Accumulate fat between late autumn and early winter (8 – 11% ^b), 3 % in spring	subtropical	(Mutze, 1990)
Ring-tailed lemurs, <i>Lemur catta</i>	18.6 % ^b at the end of the rainy season, 5.6 % in dry season	subtropical	(Simmen et al., 2010)
Fat-tailed dunnarts, <i>Sminthopsis crassicaudata</i>	Little caudal fat in the breeding season, caudal fat is stored in autumn	subtropical	(Morton, 1978)
Striped mouse, <i>Rhabdomys dilectus</i>	Variable, no clear peak in winter (average 28.7 % ^a)	subtropical	(Perrin, 1981)
Striped mouse, <i>Rhabdomys pumilio</i>	9 % ^b at the onset and the end of the dry season, 6 % in the moist season (24.7 % ^a at the end of the dry season)	subtropical	present study

479

480 *Body composition and disappearance probability*

481 We did not find that individuals with smaller fat stores at the onset of the dry season were
482 more likely to disappear than individuals with larger fat stores. This indicates that in the two
483 study years, the survival of individuals did not depend on their fat stores. Thirty-eight percent
484 of the individuals disappeared up to the start of the next breeding season. The two dry
485 seasons included in this study were harsher than the average dry season at the study site. The
486 dry seasons of 2015 and 2017 were characterized by lower food availability and higher
487 maximum ambient temperatures than dry seasons in the previous or subsequent years (Table
488 S2). It is possible that under yet harsher conditions, variation in fat stores would be more
489 predictive of the disappearance risk of individuals. For example, the harshest dry season in
490 our long-term study occurred in 2003, when 99% of striped mice disappeared (Schradin et al.,
491 2006; Schradin et al., 2010), the survivors being the ancestors of our study population. It is
492 possible that under such extreme conditions, fat stores are more important. Thus, it is likely
493 that proximate and ultimate factors we did not consider here played a more important role for
494 the disappearance risk of striped mice than the size of fat stores during the two years included
495 in the current study.

496 Body mass influenced disappearance risk, where heavier individuals were at a higher
497 risk than lighter ones. Reproductive tactics of male and female striped mice are body mass
498 dependent (Hill et al., 2015; Schradin et al., 2009). The most competitive (i.e. heaviest) males
499 typically disperse into neighbouring territories where they become breeders (Schradin and
500 Lindholm, 2011), and individuals of intermediate size with lower competitive ability are
501 more likely to disperse over larger distances than heavy individuals, while small individuals
502 do not disperse (Solmsen et al., 2011). The majority (85 %) of individuals weighing < 30 g
503 remained in the population, whereas 42 % weighing 30 – 45 g and 66 % of individuals > 45 g
504 disappeared. Thus, light individuals remained in the population, and individuals of
505 intermediate and large size disappeared either via dispersal or predation. Storing large fat
506 stores increases an individual's body mass. Large body mass can incur a cost in terms of an
507 increased mortality risk due to higher vulnerability of predation (Houston et al., 1993; Lima,
508 1986), which some species avoid by decreasing their body mass in the presence of a predator
509 (Carlsen et al., 1999; Gosler et al., 1995; Zimmer et al., 2010; Zimmer et al., 2011). Taken
510 together, our study suggests that both dispersal patterns and predation risk might explain the
511 observed relationship between disappearance risk and body mass.

512

513 *Conclusions*

514 Our study contributes information about seasonal variation in body composition and its
515 dependence on environmental factors in species from arid habitats. In our two study years,
516 larger fat stores at the onset of the dry season did not generate a selective benefit in the form
517 of a reduced disappearance risk, nor did they decline during the dry season. Animals from
518 arid habitats may be restricted in the amount of fat they can store because large amounts of
519 subcutaneous fat could interfere with temperature regulation via the prevention of heat loss
520 (Young, 1976), explaining the evolution of fat-tailed species. It is possible that under

521 extremely harsh conditions, variation in fat stores would be more predictive of the
522 disappearance risk of striped mice. This study suggests that philopatric striped mice do not
523 invest into fat storage but rather into somatic growth, which can directly influence their
524 reproductive tactic and thus fitness. Accordingly, fat stores were reduced in the moist season,
525 likely to satisfy the high energetic demands of reproduction.

526

527 **List of symbols and abbreviations**

528 Bm = body mass

529 DLW = doubly labelled water

530 ^{18}O = heavy form of oxygen

531 ^2H = deuterium, heavy form of hydrogen

532 R = isotope dilution space ratio

533 FM = fat mass

534 FFM = fat free mass

535 TBW = total body water

536

537 **Acknowledgements**

538 This study was made possible with the support of the Succulent Karoo Research Station
539 (registered South African NPO 122-134), Goegap Nature Reserve and several field assistants.

540 We thank Gildas Lemonier for help with the vacuum distillations and two anonymous
541 reviewers for comments that improved the manuscript.

542

543 **Competing Interest**

544 No competing interests declared.

545

546 **Author contributions**

547 RR, CS, NP, SB, JPR contributed to conception and design; RR and CS collected the data;
548 AZ analysed the blood samples, JPR analysed mice carcasses, RR analysed the data; RR, CS
549 and NP led the writing of the manuscript. All authors contributed to the drafts and gave final
550 approval for publication.

551

552 **Funding**

553 This work was supported by the University of Strasbourg Institute for Advanced Study
554 (USIAS); the University of the Witwatersrand; the Claude Leon Foundation; the National
555 Research Foundation [grant number 87769]; and the CNRS.

556

557 **References**

- 558 **Altman, D. and Bland, J.** (1983). Measurement in medicine: the analysis of method
559 comparison studies. *Statistician* **32**, 307–217.
- 560 **Baldwin, S. P. and Kendeigh, S. C.** (1938). Variations in bird weights. *Auk* **55**, 416–467.
- 561 **Bates, D. and Maechler, M.** (2010). lme4: Linear mixed-effects models using S4 classes.
- 562 **Bednekoff, P. A. and Houston, A. I.** (1994). Fat reserves over the entire winter: a dynamic
563 model. *Oikos* **71**, 708–415.
- 564 **Bender, L. C., Lomas, L. A. and Browning, J.** (2007). Condition, survival, and cause-
565 specific mortality of adult female mule deer in north-central New Mexico. *J. Wildl.*
566 *Manage.* **71**, 1118–1124.
- 567 **Blanc, S., Colman, R., Kemnitz, J., Weindruch, R., Baum, S., Ramsey, J. and Schoeller,**
568 **D.** (2005). Assessment of nutritional status in rhesus monkeys: comparison of dual-
569 energy X-ray absorptiometry and stable isotope dilution. *J. Med. Primatol.* **21**, 130–138.
- 570 **Bland, J. and Altman, D.** (2003). Applying the right statistics: analyses of measurement

571 studies. *Ultrasound Obstet. Gynecol.* **22**, 85–93.

572 **Boos, M., Zorn, T., Le Maho, Y., Groscolas, R. and Patrice Robin, J.** (2002). Sex
573 differences in body composition of wintering Mallards (*Anas platyrhynchos*): possible
574 implications for survival and reproductive performance. *Bird Study* **49**, 212–218.

575 **Boos, M., Thouzeau, C., Delacour, G., Artois, M., Marchandeurs, S., Jean-Claude, P.**
576 **and Robin, J. P.** (2005). Body condition assessment and prediction of fasting endurance
577 in wild rabbits (*Oryctolagus cuniculus*). *Wildl. Res.* **32**, 75–83.

578 **Buskirk, S. W. and Harlow, H. J.** (1989). Body-fat dynamics of the American marten
579 (*Martes americana*) in winter. *J. Mammal.* **70**, 191–193.

580 **Caldwell, L. D. and Connell, C. E.** (1968). A precis on energetics of the old-field mouse.
581 *Ecology* **49**, 495–505.

582 **Carlsen, M., Lodal, J., Leirs, H. and Jensen, T. S.** (1999). The effect of predation risk on
583 body weight in the field vole, *Microtus agrestis*. *Oikos* **87**, 277–285.

584 **Chery, I., Zahariev, A., Simon, C. and Blanc, S.** (2015). Analytical aspects of measuring
585 $2\text{H}/1\text{H}$ and $18\text{O}/16\text{O}$ ratios in urine from doubly labelled water studies by high-
586 temperature conversion elemental analyser-isotope-ratio mass spectrometry. *Rapid*
587 *Commun. Mass Spectrom.* **29**, 562–572.

588 **Degen, A. A.** (1997). *Ecophysiology of small desert mammals*. Berlin, Germany: Springer.

589 **Degen, A. A., Pinshow, B., Alkon, P. U. and Arnon, H.** (1981). Tritiated water for
590 estimating total body water and water turnover rate in birds. *J. Appl. Physiol.* **51**, 1183–
591 1188.

592 **Dugan, P. J., Evans, P. R., Goodyer, L. R. and Davidson, N. C.** (1981). Winter fat reserves
593 in shorebirds: disturbance of regulated levels by severe weather conditions. *Ibis (Lond.*
594 *1859)*. **123**, 359–363.

595 **Fietz, J. and Dausmann, K.** (2006). Big is beautiful: fat storage and hibernation as a strategy

596 to cope with marked seasonality in the fat-tailed dwarf lemur (*Cheirogaleus medius*). In
597 *Lemurs: ecology and adaptation* (ed. Gould, L.) and Sauther, M.), pp. 97–110. New
598 York: Springer.

599 **Fleharty, E. D., Krause, M. E. and Stinnett, D. P.** (1973). Body composition, energy
600 content, and lipid cycles of four species of rodents. *J. Mammal.* **54**, 426–438.

601 **Folch, J., Lees, M. and Stanley, G. H. S.** (1957). A simple method for the isolation and
602 purification of total lipids from animal tissues. *J. Biol. Chem.* **226**, 497–509.

603 **Fox, J. and Weisberg, S.** (2011). *An R companion to applied regression*. 2nd ed. London:
604 Sage Publications.

605 **Frank, C. L.** (1987). The effects of moisture content and metabolic water production on
606 desert rodent seed preferences.

607 **Fretwell, S. D.** (1972). *Populations in a seasonal environment*. Princeton, N.J.: Princeton
608 University Press.

609 **Gandrud, C.** (2013). simPH: an R package for illustrating estimates from cox proportional
610 hazard models including for interactive and nonlinear effects. *J. Stat. Softw.* **65**, 1–20.

611 **Garnier, L. and Schradin, C.** (2019). Pair bonding in monogamously and polygynously
612 kept African striped mice, *Rhabdomys pumilio*. *Anim. Behav.* **150**, 69–76.

613 **Giannoni, S. M., Dacar, M., Taraborelli, P. and Borghi, C. E.** (2001). Seed hoarding by
614 rodents of the Monte Desert, Argentina. *Austral Ecol.* **26**, 259–263.

615 **Gosler, A. G.** (1996). Environmental and social determinants of winter fat storage in the
616 great tit *Parus major*. *J. Anim. Ecol.* **65**, 1–17.

617 **Gosler, A. G., Greenwood, J. J. D. and Perrins, C.** (1995). Predation risk and the cost of
618 being fat. *Nature* **377**, 621–623.

619 **Gutman, R., Choshniak, I. and Kronfeld-Schor, N.** (2006). Defending body mass during
620 food restriction in *Acomys russatus*: a desert rodent that does not store food. *Am. J.*

621 *Physiol. - Regul. Integr. Comp. Physiol.* **290**, R881-891.

622 **Hill, D. L., Pillay, N. and Schradin, C.** (2015). Alternative reproductive tactics in female
623 striped mice: heavier females are more likely to breed solitary than communally. *Anim.*
624 *Ecol.* **84**, 1497–1508.

625 **Houston, A. I., McNamara, J. M. and Hutchinson, J. M.** (1993). General results
626 concerning the trade-off between gaining energy and avoiding predation. *Philos. Trans.*
627 *R. Soc. B Biol. Sci.* **341**, 375–397.

628 **John, D.** (2005). Annual lipid cycles in hibernators: integration of physiology and behavior.
629 *Annu. Rev. Nutr.* **25**, 469–497.

630 **Lehnert, B.** (2015). BlandAltmanLeh: plots (slightly extended). *R Packag. version 0.3.1.*

631 **Lima, S. L.** (1986). Predation risk and unpredictable feeding conditions: determinants of
632 body mass in birds. *Ecology* **67**, 377–385.

633 **Lindstedt, S. L. and Boyce, M. S.** (1985). Seasonality, fasting endurance, and body size in
634 mammals. *Am. Nat.* **125**, 873–878.

635 **Mata, A. J., Caloin, M., Robin, J.-P. and Le Maho, Y.** (2006). Reliability in estimates of
636 body composition of birds: oxygen-18 versus deuterium dilution. *Physiol. Biochem.*
637 *Zool.* **79**, 202–209.

638 **McCue, M. D.** (2010). Starvation physiology: reviewing the different strategies animals use
639 to survive a common challenge. *Comp. Biochem. Physiol. Part A* **156**, 1–18.

640 **McCue, M. D.** (2012). *Comparative physiology of fasting, starvation, and food limitation.*
641 Springer.

642 **McEwen, B.** (2000). Allostasis and allostatic load: implications for
643 neuropsychopharmacology. *Neuropsychopharmacology* **22**, 108–124.

644 **McEwen, B. S. and Wingfield, J. C.** (2003). The concept of allostasis in biology and
645 biomedicine. *Horm. Behav.* **43**, 2–15.

646 **McKinley, M. J., McAllen, R. M., Whyte, D. and Mathai, M. L.** (2008). Central
647 osmoregulatory influences on thermoregulation. *Clin. Exp. Pharmacol. Physiol.* **35**,
648 701–705.

649 **McNamara, J. M. and Houston, A. I.** (1990). The value of fat reserves and the tradeoff
650 between starvation and predation. *Acta Biotheor.* **38**, 37–61.

651 **Mellanby, K.** (1942). Metabolic water and desiccation. *Nature* **150**, 21.

652 **Monteith, K. L., Stephenson, T. R., Bleich, V. C., Conner, M. M., Pierce, B. M. and**
653 **Bowyer, R. T.** (2013). Risk-sensitive allocation in seasonal dynamics of fat and protein
654 reserves in a long-lived mammal. *J. Anim. Ecol.* **82**, 377–388.

655 **Morton, S. R.** (1978). An ecological study of *Sminthopsis crassicaudata* (Marsupialia:
656 Dasyuridae) III. reproduction and life history. *Aust. Wildl. Res.* 183–211.

657 **Murie, J. O. and Boag, D. A.** (1984). The relationship of body weight to overwinter survival
658 in Columbian ground squirrels. *J. Mammal.* **65**, 688–690.

659 **Mutze, G. J.** (1990). Fat cycles, breeding and population changes in house mice. *Aust. J.*
660 *Zool.* **38**, 347–362.

661 **Nagy, K. A.** (1988). Seasonal patterns of water use and energy balance in desert vertebrates.
662 *J. Arid Environ.* **14**, 201–210.

663 **Nagy, K. A.** (1994). Seasonal water, energy and food use by free-living, arid-habitat
664 mammals. *Aust. J. Zool.* **42**, 55–63.

665 **Parker, K. L., Barboza, P. S. and Gillingham, M. P.** (2009). Nutrition integrates
666 environmental responses of ungulates. *Funct. Ecol.* **23**, 57–69.

667 **Perrin, M. R.** (1981). Seasonal changes in the body fat content of two coexisting rodents,
668 *Rhabdomys pumilio* and *Otomys irroratus*. *South African J. Wildl. Res.* **11**, 21–27.

669 **Piper, W. H. and Wiley, R. H.** (1990). The relationship between social dominance,
670 subcutaneous fat, and annual survival in wintering white-throated sparrows (*Zonotrichia*

671 *albicollis*). *Behav. Ecol. Sociobiol.* **26**, 201–208.

672 **Poppitt, S., Speakman, J. R. and Racey, P. A.** (1993). The energetics of reproduction in the
673 common shrew (*Sorex araneus*): a comparison of indirect calorimetry and the doubly
674 labeled water method. *Physiol. Biochem. Zool.* **66**, 964–982.

675 **R Core Team** (2018). R: a language and environment for statistical computing. R
676 Foundation for Statistical Computing, Vienna, Austria. www.R-project.org/.

677 **Racette, S. B., Schoeller, D. A., Luke, A. H., Shay, K., Hnilicka, J. and Kushner, R. F.**
678 (1994). Relative dilution spaces of 2H- and 18O-labeled water in humans. *Am. J.*
679 *Physiol.* **30**, E585–E590.

680 **Rimbach, R., Willigenburg, R., Schoepf, I., Yuen, C. H., Pillay, N. and Schradin, C.**
681 (2016). Young but not old adult African striped mice reduce their activity in the dry
682 season when food availability is low. *Ethology* **122**, 828–840.

683 **Rimbach, R., Pillay, N. and Schradin, C.** (2017). Both thyroid hormone levels and resting
684 metabolic rate decrease in African striped mice when food availability decreases. *J. Exp.*
685 *Biol.* **220**, 837–843.

686 **Rimbach, R., Blanc, S., Zahariev, A., Gatta, M., Pillay, N. and Schradin, C.** (2018a).
687 Seasonal variation in energy expenditure in a rodent inhabiting a winter-rainfall desert.
688 *J. Comp. Physiol. B* **188**, 877–888.

689 **Rimbach, R., Jäger, J., Pillay, N. and Schradin, C.** (2018b). Food availability is the main
690 driver of seasonal changes in resting metabolic rate in African striped mice (*Rhodomys*
691 *pumilio*). *Physiol. Biochem. Zool.* **91**, 826–833.

692 **Rimbach, R., Blanc, S., Zahariev, A., Pillay, N. and Schradin, C.** (2019). Daily energy
693 expenditure of males following alternative reproductive tactics: solitary roamers spend
694 more energy than group-living males. *Physiol. Behav.* **199**, 359–365.

695 **Robbins, C. T.** (1993). *Wildlife feeding and nutrition*. San Diego, CA: Academic Press.

696 **Romero, L. M., Dickens, M. J. and Cyr, N. E.** (2009). The reactive scope model - a new
697 model integrating homeostasis, allostasis, and stress. *Horm. Behav.* **55**, 375–389.

698 **Sawicka-Kapusta, K.** (1968). Annual fat cycle of field mice, *Apodemus flavicollis*
699 (Melchior, 1834). *Acta Theriol. (Warsz.)*. **13**, 329–339.

700 **Schielzeth, H.** (2010). Simple means to improve the interpretability of regression
701 coefficients. *Methods Ecol. Evol.* **1**, 103–113.

702 **Schmid, J.** (1999). Sex-specific differences in activity patterns and fattening in the gray
703 mouse lemur (*Microcebus murinus*) in Madagascar. *J. Mammal.* **80**, 749–757.

704 **Schoeller, D. A.** (2005). Hydrometry. In *Human body composition* (ed. Heymsfield, S.),
705 Lohman, T. G.), Wang, Z.), and Going, S.), p. Human Kinetics.

706 **Schoepf, I., Pillay, N. and Schradin, C.** (2017). The pathophysiology of survival in harsh
707 environments. *J. Comp. Physiol. B* **187**, 183–201.

708 **Schradin, C. and Lindholm, A. K.** (2011). Relative fitness of alternative male reproductive
709 tactics in a mammal varies between years. *J. Anim. Ecol.* **80**, 908–917.

710 **Schradin, C. and Pillay, N.** (2004). The striped mouse (*Rhabdomys pumilio*) from the
711 Succulent Karoo, South Africa: a territorial group-living solitary forager with communal
712 breeding and helpers at the nest. *J. Comp. Psychol.* **118**, 37–47.

713 **Schradin, C. and Pillay, N.** (2005). Demography of the striped mouse (*Rhabdomys pumilio*)
714 in the succulent karoo. *Mamm. Biol. - Zeitschrift für Säugetierkd.* **70**, 84–92.

715 **Schradin, C. and Pillay, N.** (2006). Female striped mice (*Rhabdomys pumilio*) change their
716 home ranges in response to seasonal variation in food availability. *Behav. Ecol.* **17**, 452–
717 458.

718 **Schradin, C., Schubert, M. and Pillay, N.** (2006). Winter huddling groups in the striped
719 mouse. *Can. J. Zool.* **84**, 693–698.

720 **Schradin, C., Scantlebury, M., Pillay, N. and König, B.** (2009). Testosterone levels in

721 dominant sociable males are lower than in solitary roamers: physiological differences
722 between three male reproductive tactics in a sociably flexible mammal. *Am. Nat.* **173**,
723 376–388.

724 **Schradin, C., König, B. and Pillay, N.** (2010). Reproductive competition favours solitary
725 living while ecological constraints impose group-living in African striped mice. *J. Anim.*
726 *Ecol.* **79**, 515–521.

727 **Schradin, C., Lindholm, A. K., Johannesen, J., Schoepf, I., Yuen, C.-H., König, B. and**
728 **Pillay, N.** (2012). Social flexibility and social evolution in mammals: a case study of the
729 African striped mouse (*Rhabdomys pumilio*). *Mol. Ecol.* **21**, 541–553.

730 **Simmen, B., Bayart, F., Rasamimanana, H., Zahariev, A., Blanc, S. and Pasquet, P.**
731 (2010). Total energy expenditure and body composition in two free-living sympatric
732 lemurs. *PLoS One* **5**, e9860.

733 **Siri, W. E.** (1956). The gross composition of the body. *Adv. Biol. Med. Phys.* **4**, 239–280.

734 **Solmsen, N., Johannesen, J. and Schradin, C.** (2011). Highly asymmetric fine-scale
735 genetic structure between sexes of African striped mice and indication for condition
736 dependent alternative male dispersal tactics. *Mol. Ecol.* **20**, 1624–1634.

737 **Speakman, J. R.** (1997). *Doubly-labelled water: theory and practice*. London: Chapman and
738 Hall.

739 **Stokkan, K. A., Mortensen, A. and Blix, A. S.** (1986). Food intake, feeding rhythm, and
740 body mass regulation in Svalbard rock ptarmigan. *Am. J. Physiol.* **25**, R264–R267.

741 **Takei, Y., Bartolo, R. C., Fujihara, H., Ueta, Y. and Donald, J. A.** (2012). Water
742 deprivation induces appetite and alters metabolic strategy in *Notomys alexis*: unique
743 mechanisms for water production in the desert. *Proc. Nutr. Soc.*

744 **Tannock, G. W. and Smith, J. M. B.** (1972). The effect of food and water deprivation
745 (stress) on salmonella-carrier mice. *J. Med. Microbiol.* **5**, 283–289.

746 **Virgl, J. A. and Messier, F.** (1992). Seasonal variation in body composition and morphology
747 of adult muskrats in central Saskatchewan, Canada. *J. Zool.* **228**, 461–477.

748 **Waite, T. A.** (1992). Winter fattening in gray jays: seasonal, diurnal and climatic correlates.
749 *Ornis Scand.* **23**, 499–503.

750 **Werger, M. J. A.** (1974). On concepts and techniques applied in the Zürich-Montpellier
751 method of vegetation survey. *Bothalia* **11**, 309–323.

752 **Wickham, H.** (2016). *ggplot2: elegant graphics for data analysis*. New York: Springer-
753 Verlag.

754 **Worden, K. A. and Pekins, P. J.** (1995). Seasonal change in feed intake, body composition,
755 and metabolic rate of white-tailed deer. *Can. J. Zool.* **73**, 452–457.

756 **Young, R. A.** (1976). Fat, energy and mammalian survival. *Am. Zool.* **16**, 699–710.

757 **Zimmer, C., Boos, M., Petit, O. and Robin, J.-P.** (2010). Body mass variations in disturbed
758 mallards *Anas platyrhynchos* fit to the mass-dependent starvation-predation risk trade-
759 off. *J. Avian Biol.* **41**, 637–644.

760 **Zimmer, C., Boos, M., Poulin, N., Gosler, A., Petit, O. and Robin, J.-P.** (2011). Evidence
761 of the trade-off between starvation and predation risks in ducks. *PLoS One* **6**, e22352.

762 **Zuur, A. F., Ieno, E. N. and Elphick, C. S.** (2010). A protocol for data exploration to avoid
763 common statistical problems. *Methods Ecol. Evol.* **1**, 3–14.

764

765

766 **Figure legends**

767

768 **Figure 1.** Body composition of free-living (N = 24, black circles) and captive (N = 30, grey
769 triangles) striped mice. Comparison of (A) fat mass, (B) total body water (TBW), (C) ash and
770 (D) protein of free-living and captive striped mice.

771

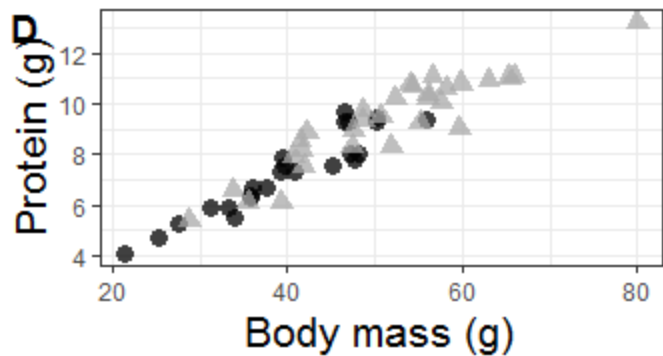
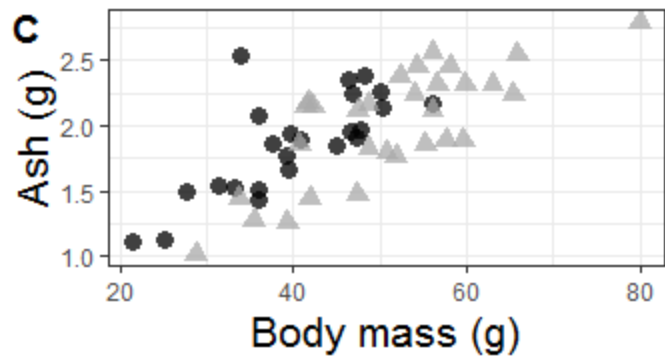
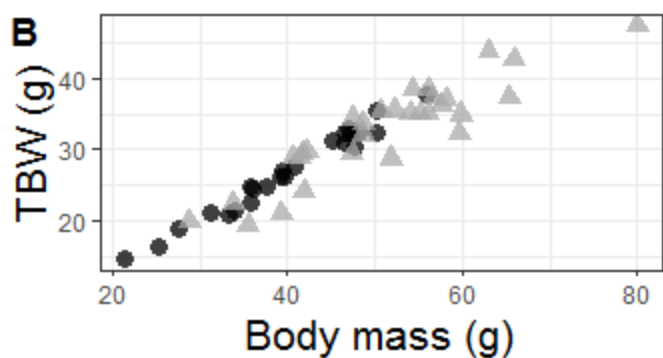
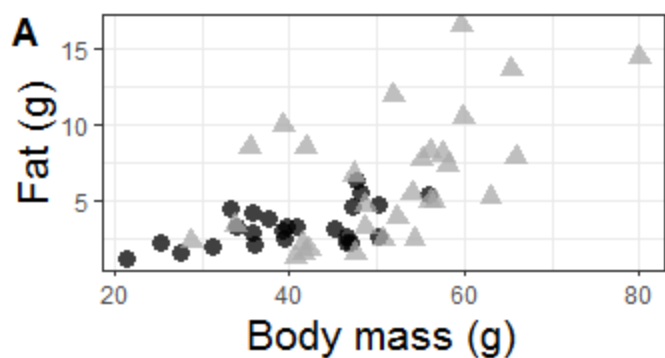
772 **Figure 2.** (A) Violin plots of fat mass of striped mice at the onset (N = 66) and end (N = 30)
773 of the dry season and the beginning of the moist season (N = 29); the shaded area represents
774 the kernel density plot, white boxplots show median values as well as 1st and 3rd quartiles and
775 filled dots show outliers. (B) The relationship between body mass and fat mass (N = 125),
776 filled circles indicate data from the onset of the dry season, grey squares data from the end of
777 the moist season and open triangles data from the moist season. Linear regression line is
778 shown ($y = 0.96727 + 0.05825x + 1.71$; $R^2 = 0.05$, $P = 0.008$).

779

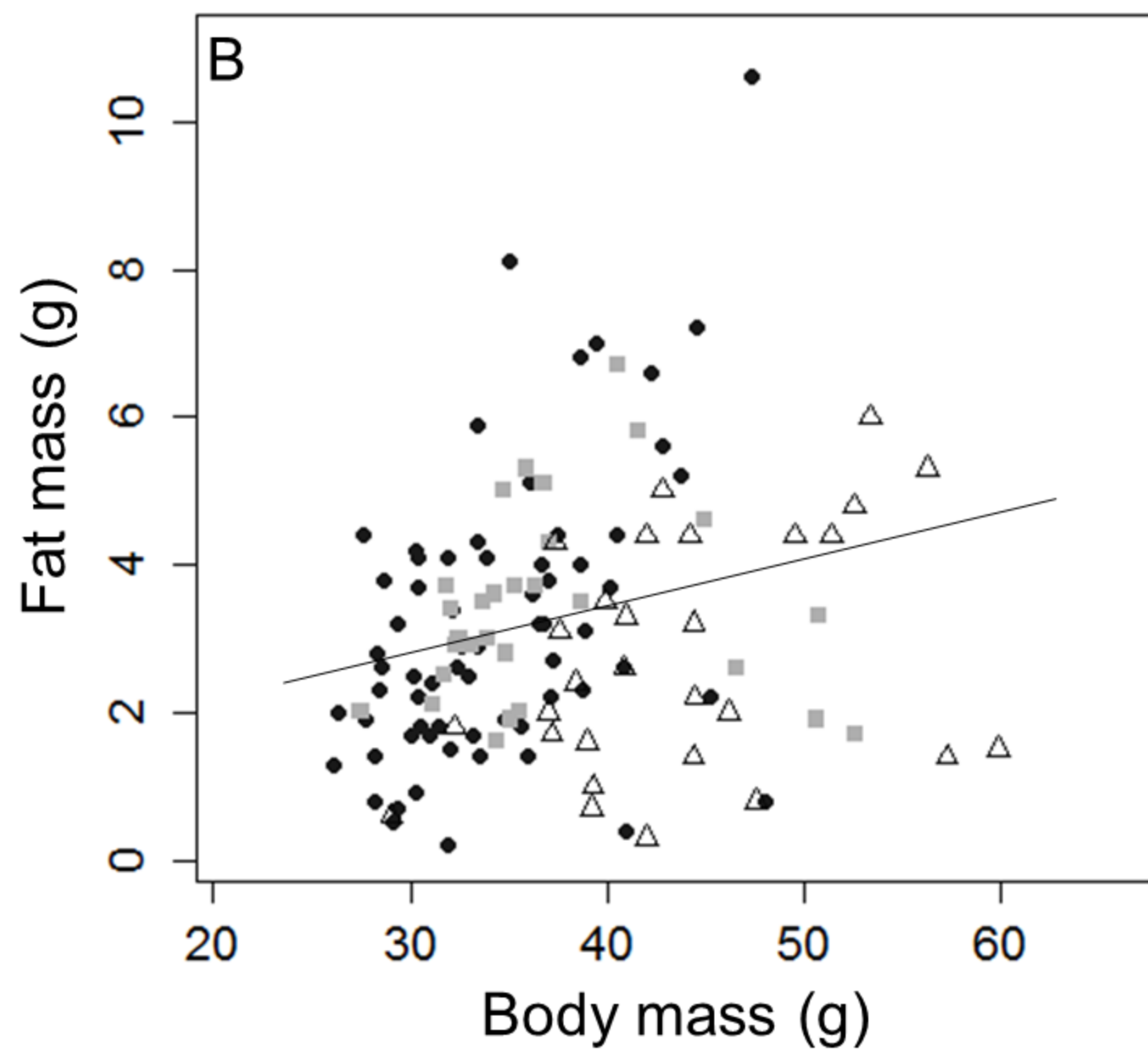
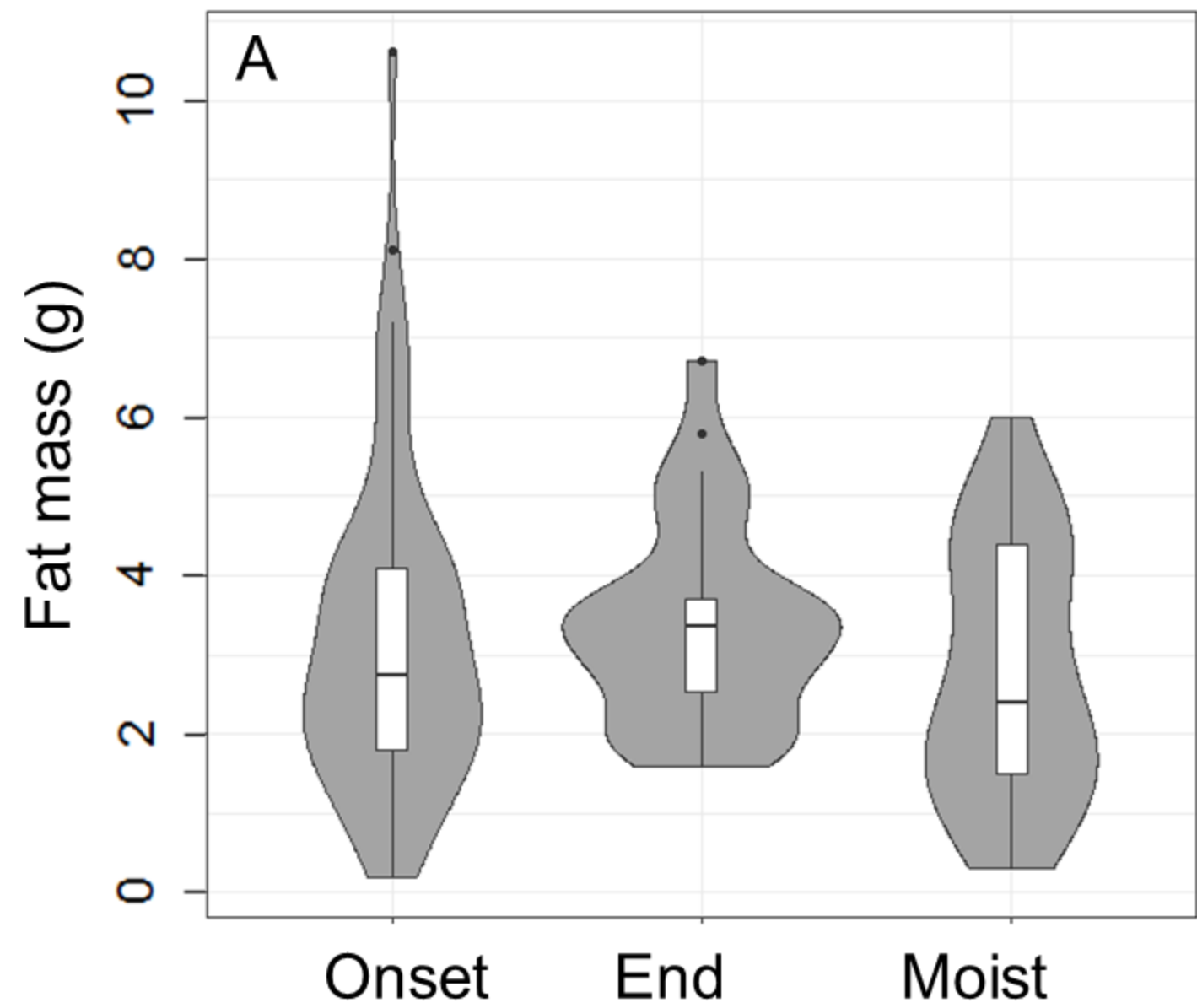
780 **Figure 3.** (A) Relationship between fat mass and food availability (mean number of food
781 plant species per 4m² averaged over 4 weeks prior to the measurement of fat mass). (B)
782 Relationship between fat mass and body condition index. Blue lines indicate the quadratic
783 regression lines and the shaded areas show the 95% confidence intervals.

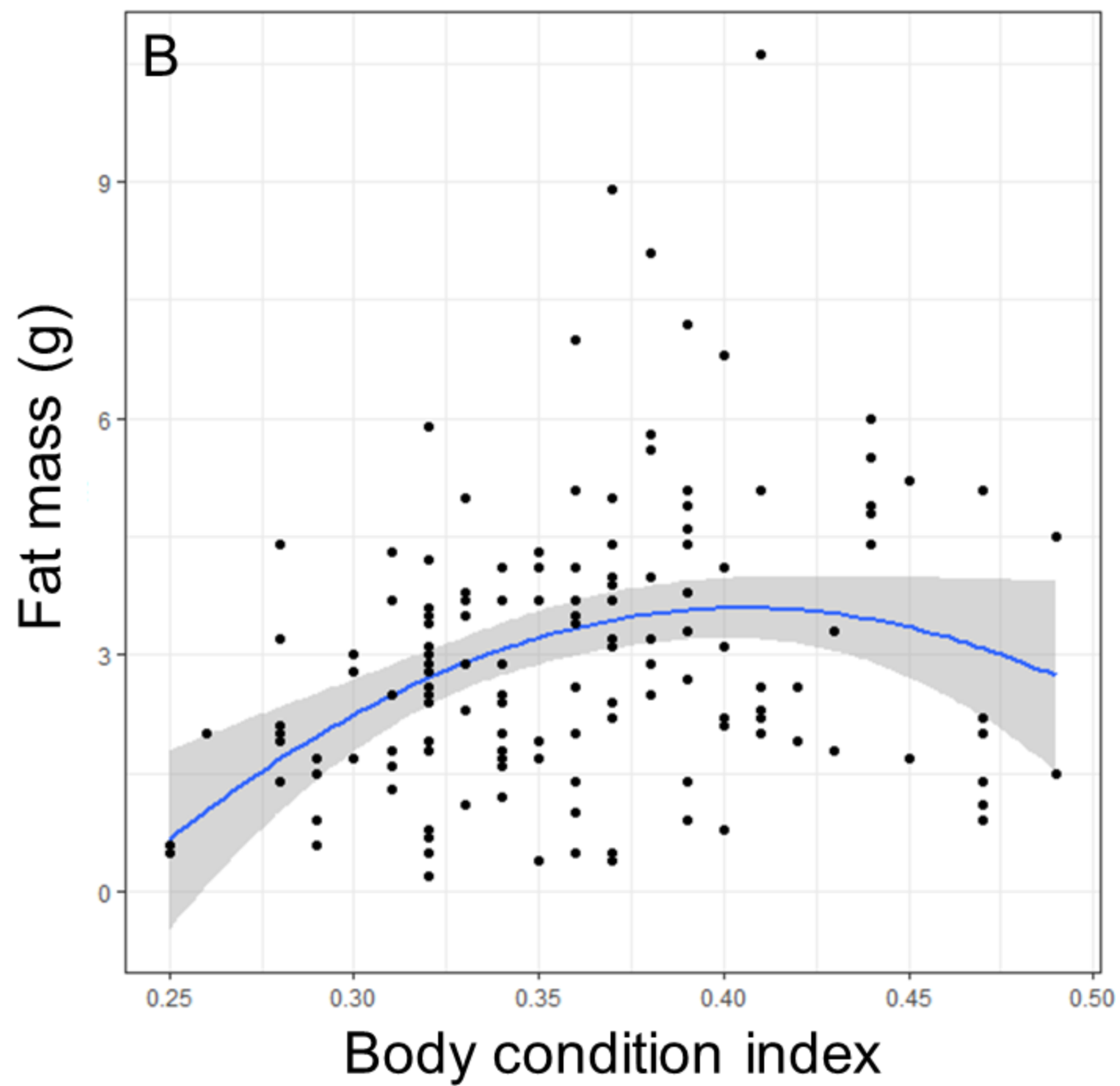
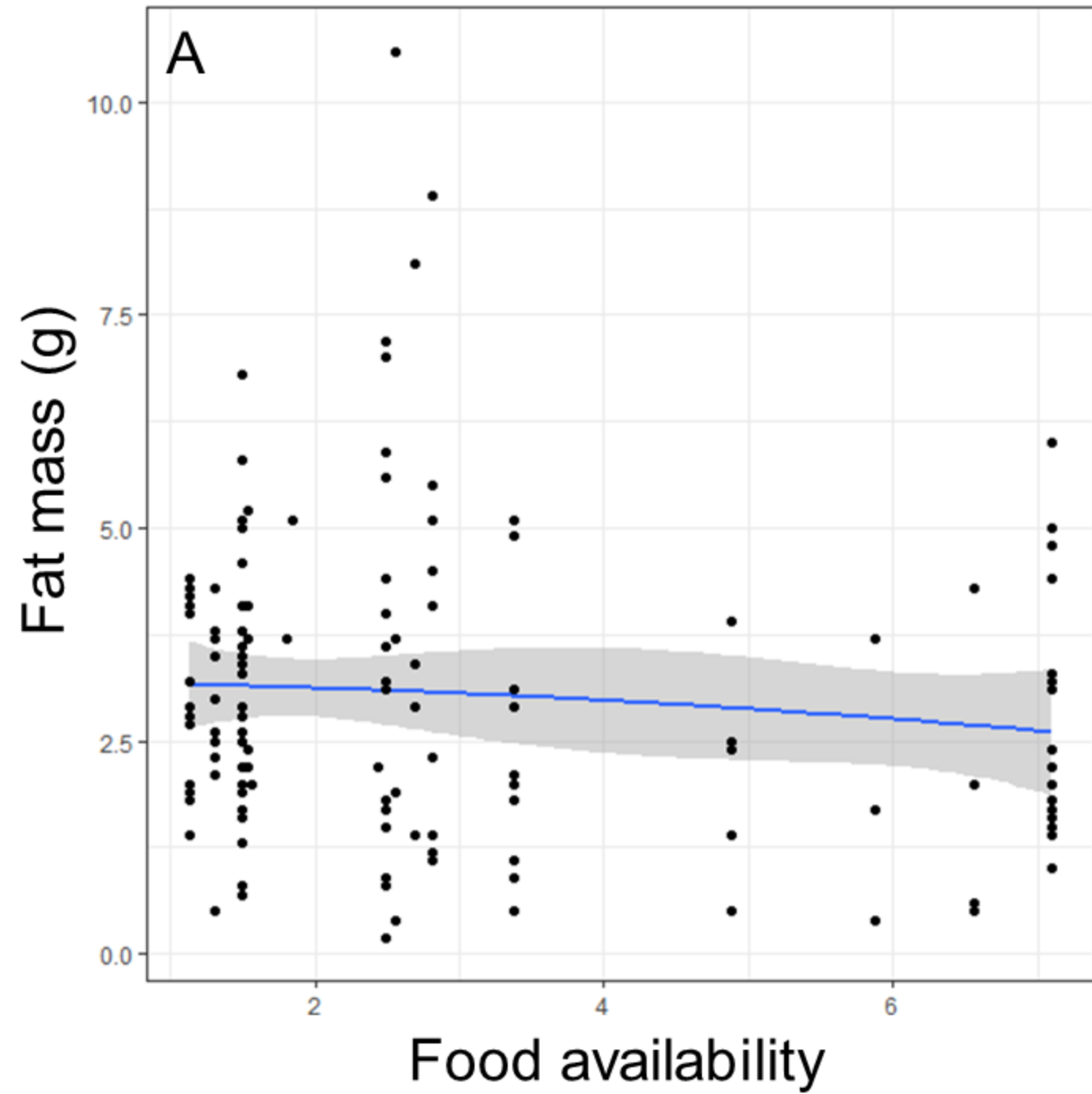
784

785 **Figure 4.** Relationship between dry season disappearance risk and body mass (Cox
786 proportional hazards model, N = 66). The simulated hazard ratio (line), 50% (dark blue
787 shaded area) and 95% (light blue shaded are) confidence intervals are shown.



condition ● Field ▲ Lab





Hazard Ratio

2.0
1.5
1.0
0.5

25 30 35 40 45

Body mass (g)

