



HAL
open science

Prey consumption does not restore hydration state but mitigates the energetic costs of water deprivation in an insectivorous lizard

Chloé Chabaud, George Bruschi, Anouk Pellerin, Olivier Lourdaï, Jean-François Le Galliard

► To cite this version:

Chloé Chabaud, George Bruschi, Anouk Pellerin, Olivier Lourdaï, Jean-François Le Galliard. Prey consumption does not restore hydration state but mitigates the energetic costs of water deprivation in an insectivorous lizard. *Journal of Experimental Biology*, 2023, 226 (17), 10.1242/jeb.246129 . hal-04189871

HAL Id: hal-04189871

<https://cnrs.hal.science/hal-04189871v1>

Submitted on 14 Nov 2023

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 **Prey consumption does not restore hydration state but mitigates the energetic costs of**
2 **water deprivation in an insectivorous lizard**

3

4 Chloé Chabaud^{1,2}, George A. Bruschi^{1,4}, Anouk Pellerin², Olivier Lourdais¹, Jean-François Le
5 Galliard^{2,3}

6 1. Centre d'Etudes Biologiques de Chizé, Université La Rochelle, CNRS, UMR 7372, 405
7 Route de Prissé la Charrière, 79360 Villiers-en-Bois, France

8 2. Sorbonne Université, UPEC, UPCité, CNRS, INRAE, IRD, Institut d'Ecologie et des
9 Sciences de l'Environnement de Paris (iEES Paris - UMR 7618), 75005 Paris, France

10 3. École normale supérieure, PSL Research University, Département de biologie, CNRS,
11 UMS 3194, Centre de recherche en écologie expérimentale et prédictive (CEREPEP-Ecotron
12 IleDeFrance), 78 rue du château, 77140 Saint-Pierre-lès-Nemours, France.

13 4. Biological Sciences, California State University San Marcos, San Marcos, CA, 92096,
14 USA

15

16 Corresponding author: Chloé Chabaud chloe.chabaud@normalesup.org

17 Running title: Absence of dietary water intake in a lizard

18

19

20

21 **Abstract**

22 To cope with **limited availability of drinking water** in their environment, terrestrial animals
23 have developed numerous behavioral and physiological strategies including maintaining an
24 optimal hydration state through dietary water intake. Recent studies performed in snakes,
25 which are carnivorous reptiles with a specialized diet, suggest that benefits of dietary water
26 intake are negated by hydric costs of digestion. Most lizards are generalist insectivores that
27 can shift their prey types, but firm experimental demonstration of dietary water intake is
28 currently missing in these organisms. Here, we performed an experimental study in the
29 common lizard *Zootoca vivipara*, a keystone mesopredator from temperate climates
30 exhibiting a great diversity of prey in its mesic habitats, in order to investigate the effects of
31 food consumption and prey type on physiological responses to water deprivation. Our results
32 indicate that common lizards cannot improve their hydration state through prey consumption,
33 irrespective of prey type, suggesting that they are primarily dependent upon drinking water.
34 Yet, high-quality prey consumption reduced the energetic costs of water deprivation,
35 potentially helping lizards to conserve a better body condition during periods of limited water
36 availability. These findings have important implications for understanding the physiological
37 responses of ectotherms to water stress, and highlight the complex interactions between
38 hydration status, energy metabolism, and feeding behavior in insectivorous lizards.

39
40 Keywords: physiology - lizards – water balance – dietary water – metabolites - reptiles

41

42 **1. Introduction**

43 As climatic conditions shift towards longer and more frequent warm and dry spells in many
44 regions (IPCC 2019), it is critical to understand and quantify the contributions of different
45 water sources in wild animals. Optimal hydration state can be maintained by drinking free-
46 standing water (Karasov & del Rio, 2007), by food consumption (dietary water intake, Nagy
47 & Knight, 1994) **or cutaneous drinking (e.g., Jørgensen, 1994)**, or by internal metabolic
48 production (metabolic water, Bailey, 1923; Jindra & Sehnal, 1990). Free-standing water is the
49 most widespread, primary source of water intake in animals, but it is not continuously
50 available in many terrestrial ecosystems and most organisms have developed strategies to
51 cope with limited water availability. For example, water requirements can be met through
52 dietary shifts (Leinbach, McCluney, & Sabo, 2019), utilizing internal water reservoirs (e.g.,
53 urinary bladder, Davis & DeNardo, 2007), or even harvesting water from fog (Seely, 1979).
54 Conversely, water loss can be minimized through behavioral changes in activity patterns
55 (Bissonette, 1978) or microhabitat use (Dupoué *et al.*, 2015; Dezetter, Le Galliard, &
56 Lourdais, 2022), but also through internal osmoregulatory strategies (e.g., nitrogen excretion
57 via increased uric acid production, Braun, 1999). Finally, some species can tolerate
58 hyperosmolality and deviate from homeostatic set-points to cope with water constraints
59 (Peterson, 1996; Bradshaw, 1997). This diversity of strategies has important implications for
60 the capacity to survive in arid environments (Cloudsley-Thompson, 1975), but has been little
61 investigated in non-arid adapted species.

62 A strong dependence of water budgets on dietary water intake has been shown in some
63 animals living in semi-arid or arid environments characterized by low annual rainfall, high
64 ambient temperatures, and recurrent drought episodes (Nagy, 2004). For example, many
65 herbivorous desert vertebrates feed on plant tissues or seeds to sustain their standard activity
66 and water balance even when water is not available in the environment (in mammals, Nagy &

67 Gruchacz, 1994; in birds, Tieleman 2002; in turtles, Nagy & Medica, 1986; in lizards,
68 Minnich & Shoemaker, 1970). Such dietary water intake is thought to provide a considerable
69 advantage in these environments and some species display adaptive shifts in food preferences
70 toward resources with higher water content during the dry season (Karasov, 1983). However,
71 recent studies in carnivorous squamate reptiles (i.e., lizards, snakes, and amphisbaenians),
72 suggest that they do not rely on dietary water intake due to the lower water content of their
73 prey compared to plant material, and the important protein content that increases the hydric
74 cost of digestion and leads to more nitrogenous waste (Wright, Jackson, & DeNardo, 2013;
75 Lillywhite, 2017). Even in arid environments, these species do not receive net hydric benefits
76 from their diet (Murphy & DeNardo, 2019). Indeed, fluid secretion is required during
77 digestion to aid in transport and absorption of nutrients, and this hydric cost can be
78 particularly important when carnivorous reptiles are already dehydrated (Murphy & DeNardo,
79 2019). Feeding may also involve behavioral changes that increase water loss rates, such as
80 locomotor activity during foraging and basking during digestion, and trade-offs can occur
81 between food acquisition and water loss (Gregory, Crampton, & Skebo, 1999). In addition,
82 carnivorous reptiles often feed intermittently and it has been suggested that this may increase
83 the hydric cost of digestion and reduce opportunities for dietary shifts toward prey with higher
84 water content (Lillywhite, 2017).

85 Currently, there is limited knowledge about dietary water intake and hydric costs of
86 digestion in insectivorous reptiles, which include the vast majority of lizards (see Table 1;
87 Meiri, 2018). In addition, it remains unknown if inferences drawn from studies of arid adapted
88 species apply to species from moister tropical or temperate zones, where free-standing water
89 is more regularly available. Most insectivorous lizards are generalist mesopredators with a
90 non-intermittent diet dominated by invertebrates such as insects, spiders, or other arthropods
91 (Meiri, 2018). The invertebrate prey of these lizards are highly variable in size, digestibility,

92 and macro- and micro-nutrient content including water content (McCluney & Date, 2008).
93 Generalist insectivorous lizards can thus shift their diet to meet different demands depending
94 on prey availability and resource expenditures across the activity season or between different
95 life stages (Bestion *et al.*, 2019). Previous work has focused on the energetic quality of food
96 and its **adequacy** to optimal diet models based on energetic constraints (Stamps, Tanaka, &
97 Krishnan, 1981; Dearing & Schall, 1992), but very few studies have investigated dietary shifts
98 due to water constraints in reptiles (but see Mautz & Nagy, 1987). Yet, when prey have
99 different water and protein content or different digestibility, this may influence the dietary
100 water intake, leading to dietary shifts caused by water balance instead of energy balance. For
101 example, if free-standing water is limited in the environment, insectivorous lizards might shift
102 their diet towards high-water content prey to maintain water balance, which can impact the
103 population dynamics and trophic ecology of these prey species (Bestion *et al.*, 2015). Thus, an
104 understanding of the net dietary water intake and hydric costs of digestion of insectivorous
105 lizards should include the possibility of different prey types that are representative of their
106 standard diet. To our knowledge, no study has examined if different prey types are more or
107 less effective at maintaining water balance in these organisms.

108 Water deprivation induces multiple physiological responses to restore hydration state,
109 including lipolysis, reduced hepatic acid synthesis, or protein catabolism to release bound
110 water, as previously demonstrated in birds, lizards, and snakes (Gerson & Guglielmo, 2011;
111 Bruschi *et al.*, 2018, 2020). These physiological responses are known to induce energy and
112 structural costs with reduced body reserves and muscle condition (Dezetter *et al.*, 2021;
113 Bruschi *et al.*, 2022). Therefore, besides the direct effect of diet on the water budget, we need
114 to consider the whole-organism metabolic response according to different prey types, since
115 food might be important to recoup the energetic and structural costs of water deprivation.
116 Here, we designed a laboratory experiment to test if consumption of different prey types can

117 compensate for water deprivation. We investigated these responses in the common lizard
118 (*Zootoca vivipara*, Lichtenstein 1823), a generalist mesopredator with a widespread
119 distribution across Eurasia, which is dependent on cool and moist environments inside humid
120 grasslands and bogs. In these natural habitats, the common lizard is a keystone mesopredator
121 due to its great diversity of invertebrate prey and its abundance (densities as high as 1,000
122 lizards per ha, Avery, 1971; Bestion *et al.*, 2015). Previous field and laboratory studies have
123 suggested that the species is dependent on free-standing water to maintain water balance
124 (Grenot *et al.*, 1987; Lorenzon *et al.*, 1999; Rozen-Rechels *et al.*, 2020). Additionally, water
125 deprivation causes a range of systemic responses associated with a reduction of body mass,
126 proximal tail width loss (where lipids are stored, Chapple & Swain, 2002), and limb thickness
127 reduction (due to muscle wasting, Bruschi *et al.*, 2018). However, no previous study has
128 investigated the link between diet, water balance, and body condition.

129 We tested for the occurrence of dietary water intake by comparing changes in plasma
130 osmolality of mildly dehydrated lizards following the consumption of three distinct types of
131 invertebrate prey with contrasted nutrient and water contents (crickets, spiders, and woodlice).
132 These prey represent the trophic diversity of prey in natural communities (see Bestion *et al.*,
133 2015). These lizards were fed but had no access to free-standing water during the 6 days of
134 the experimental treatment in a temperature-controlled room. They were compared to two
135 groups of unfed lizards: one “hydrated control” group with no food but *ad libitum* access to
136 free-standing water and one “dehydrated control” group without food or water. Using this
137 experimental design, we tested the following three hypotheses:

138 1) If lizards are able to extract dietary water resulting in a net water gain, we predict that
139 lizards held with food will have responses closer to hydrated versus dehydrated control
140 lizards.

141 2) Alternatively, if eating imposes a net hydric cost, lizards held with food would exhibit
142 responses closer to those of dehydrated rather than hydrated control lizards.

143 3) Third, we hypothesized that prey characteristics should have an impact on the response.
144 Notably digestively “costly” prey (low energy content and hard exoskeleton) such as
145 woodlice should provide limited energy and water benefits, and could induce a shift from
146 hypothesis one to two.

147

148 **2. Material and methods**

149 2.1. Study species, sampling, and acclimation

150 The common lizard *Zootoca vivipara* is a small lacertid lizard (Reptilia: Lacertidae) with a
151 wide Eurosiberian distribution (Surget-Groba *et al.*, 2006). It inhabits cold and wet habitats,
152 such as humid grasslands and bogs, and is highly sensitive to water deprivation due to high
153 standard water loss rates (Lorenzon *et al.*, 1999; Massot *et al.*, 2002; Dupoué *et al.*, 2017).

154 This lizard is a mesopredator in its food web with a diet of various invertebrates (hexapods,
155 spiders, isopods, annelids) ranging in size from 1 to 9 mm (Avery, 1996), which includes
156 decomposers, herbivorous, and predatory species (Bestion *et al.*, 2019). Common lizards used
157 in this study were captured in semi-natural populations maintained in 25 outdoor enclosures in
158 CEREEP-Ecotron IleDeFrance in Saint Pierre lès Nemours, France (48°17'N, 2°41'E).

159 Experiments were carried out in accordance with institutional guidelines, under license from
160 the French Ministry of the Environment (permit APAFIS#25281-2020042915424095).

161 We captured 128 lizards by hand in June 2020 and brought them back to a laboratory
162 located nearby. We selected only sexually mature males based on external morphology, body
163 size, and known age **from previous population monitoring**. Upon capture, one person
164 measured snout-vent length (SVL, mean=59.3 ± 6.5 mm SD) and total length (TL) to the
165 nearest millimeter with a plastic ruler as well as body mass (BM) to the nearest milligram

166 **using** an electronic balance (mean=4.19 ± 1.62 g SD). Animals were then kept separated in
167 individual terraria (18×11×12 cm) with sterilized peat substrate, a basking rock, a shelter
168 buried in the substrate and a water cup with *ad libitum* access to water. During captivity, we
169 provided all lizards with the same thermal conditions in a temperature-controlled room (23°C
170 for 9h per day 09:00-18:00, and 16°C at night) in addition to heating lamp and UV above their
171 terrarium during daytime (33°C below heating lamp). Lizards were fed with live crickets
172 (*Acheta domesticus*) every two days and had water sprayed on the sides of their cages three
173 times a day during acclimation.

174 After one week of acclimation, we collected morphology data of hydrated and fed
175 lizards (referred to as T0 measurements). During the same day, they were weighed with an
176 electronic balance and photographed with a digital scanner (ventral view, CanoScan LiDE
177 700F, Canon) to measure tail width, a trait linked to fat and muscle storage of the tail. Tail
178 width was measured at the 7th and 10th subcaudal scales by processing the digital images with
179 the software ImageJ (Schneider, Rasband & Eliceiri, 2012). We also assessed the transverse
180 muscle thickness of the right hind leg at the mid region of the upper limb with digital calipers
181 (ID-C1012BS, Mitutoyo, Japan, ±0.01 mm). These three morphological variables (body mass,
182 tail width, and hindlimb width) were used as indicators of dehydration and well-being during
183 the whole study. We also took a blood sample from the post-orbital sinus using 1 to 2 micro-
184 capillary tubes (ca. 20-40 µl whole blood). In the laboratory, samples were centrifuged at
185 11,000 rpm for 5 min to separate plasma from red blood cells. Plasma samples (approx. 10 µl)
186 were immediately frozen at -28°C until osmolality assays (see section 2.3).

187 2.2. Experimental design

188 In order to investigate the effects of food consumption on water and energy balance in mildly
189 dehydrated lizards, we first exposed all lizards to a moderate water restriction for 10 days (Fig
190 1A). To do so, we removed the water cup from terraria and reduced water spray to once every

191 two days while feeding lizards every two days. Water spray only provided lizards a few
192 milliliters of water **which they can ingest by licking**, but that dried in less than 30 minutes
193 (no water at all during 6 days is fatal for adults in this species). At the end of the 10th day, we
194 weighed lizards and measured tail and muscle width as indicated above (referred to as T1
195 measurements). We collected blood samples using 2 to 3 micro-capillary tubes (ca. 30-40 μ l
196 whole blood) and stored plasma samples like indicated above until osmolality and metabolite
197 assays (see section 2.3).

198 We selected three types of prey known to be part of this species diet and with distinct
199 trophic roles. First, we provided some lizards with crickets *Acheta domesticus*, an herbivorous
200 insect that can be purchased at reptile breeding shops. Crickets were kept with dry food and
201 with or without water in a climatic chamber at 25°C based on earlier studies showing that
202 such water restriction conditions can trigger dehydration (McCluney & Date, 2008). We also
203 wanted to compare a same type of prey but with different water content, in order to test the
204 quality of prey in addition to prey type. To do so, we provided lizards either with “hydrated”
205 crickets (held with access to water) or with “dehydrated” crickets (held for at least a week
206 without access to water). Second, we provided other lizards with woodlice (several species
207 including *Armadillum vulgare*, *Porcellio scaber*, *Philloscia muscorum* and *Oniscus asellus*),.
208 Woodlice are detritivorous isopods and were collected in the same outdoor enclosures as the
209 lizards. They were kept in a dark, humid box with leaf and compost until being provided to
210 the lizards. Third, we provided some lizards with spiders (several species of Lycosidae or
211 wolf spiders), which are carnivorous invertebrates. All arachnids were collected by hand in
212 the nearby forest plot as well as in outdoor enclosures the day before feeding the lizard and
213 were kept alone with humid cotton and no food prior to being used. The three species of prey
214 are different in their macronutrient components (proteins, carbohydrates and lipids, see
215 Reeves *et al.*, 2021) and differences in those ratios (spiders having higher protein: lipid ratio

216 than crickets and woodlice) can inform us about the hydric cost of digestion depending on
217 prey type.

218 To measure prey water content, invertebrates were first weighed alive individually
219 with a high precision scale (± 0.01 mg). Then, they were put in the freezer at -20°C for a few
220 hours to be killed, before being dried in a **laboratory** oven at 60°C for 24 hours and weighed
221 again to determine dry mass. Water content was calculated as the difference between wet
222 mass and dry mass. Despite the absence of a statistically significant difference in cricket water
223 amounts (Table 2), we chose to maintain the two groups as distinct treatments. In addition,
224 energy content was determined with dry aliquots (0.5-1.1g) by using an isoperibol bomb
225 calorimeter (Parr 6200), with benzoic acid as a standard. Woodlice differed significantly in
226 water content and energetic content, being of poorer quality, while the other prey types were
227 similar (Table 2).

228 We separated lizards into six different treatments groups (G1 to G6) for six days (Fig
229 1A) including a “hydrated control” where lizards had access to *ad libitum* water but no food
230 (G1), four groups of lizards that did not have access to *ad libitum* water but were provided
231 200 mg per day of either hydrated crickets (G2), dehydrated crickets (G3), woodlice (G4), or
232 spiders (G5) and a “dehydrated control” group where lizards did not have access to *ad libitum*
233 water or food (G6). We provided the same quantity of each prey type which corresponded to
234 similar energy intake for crickets and spiders but not for woodlice (Table 2).

235

236 2.3. Parameters considered and plasma assays:

237 We assessed plasma osmolality before and after the period of food consumption to get a direct
238 indicator of the response: a decrease in plasma osmolality indicates that lizards can
239 compensate for a lack of drinking water by consuming food, whereas an increase in plasma
240 osmolality indicates that meal consumption (including secretion, digestion, absorption, and

241 associated behavioral changes) has a non-negligible hydric cost (Wright *et al.*, 2013). We also
242 tracked plasma circulating metabolites associated with catabolism and fasting in reptiles. We
243 considered glucose which is liberated in the liver during fasting from stored glycogen,
244 triglycerides stored in adipose tissue or neoglucogenesis, and also ketone bodies produced by
245 the breakdown of fatty acids in the liver during periods of food restriction (McCue,
246 Lillywhite, & Beaupre, 2012; Price *et al.*, 2013). In particular, the brain, unlike other organs,
247 can only use glucose and ketone bodies to function, which makes them particularly essential
248 during food restriction (Price, 2017). We also monitored morphology over time (tail and leg
249 width) as indicators of structural mobilization. We measured the body mass of lizards every
250 two days, and we weighed any remaining food in the terrarium to assess how much was eaten
251 by each individual (Table 2). On the 7th day, we measured body mass, leg and muscle width
252 (T2 measurement), and collected a blood sample to measure plasma osmolality and
253 metabolites. All lizards were then removed from the experiment, fed and provided water *ad*
254 *libitum* for 2-3 days, and released into the outdoor enclosures.

255 First, plasma osmolality was determined using a vapor pressure osmometer (Vapro
256 5600, ELITechGroup) with the protocol described in Wright *et al.* (2013) adjusted to small
257 plasma volumes (Dupoué *et al.*, 2017). Before analyses, plasma was diluted (1:3) in standard
258 saline solution (280 mOsm kg⁻¹) to obtain duplicates for each sample (CV<0.4%). Normosmic
259 plasma osmolality values (280-320 mOsm kg⁻¹) indicate that lizards are hydrated, whereas
260 hyperosmotic values (> 350 mOsm kg⁻¹) indicate that lizards are moderately to sub-clinically
261 dehydrated (Dupoué *et al.*, 2018). Second, we measured plasma concentrations of ketone
262 bodies β -Hydroxybutyrate (β -HB) with a colorimetric method (β -Hydroxybutyrate Ketone
263 Body Colorimetric Assay Kit, ref n°700190, Cayman Chemical, USA). β -HB are
264 carbohydrates that are produced in the liver as a byproduct of the catabolism of fatty acid β -
265 oxidation during fasting periods in a process known as **ketogenesis**. β -HB can be used as an

266 energy source for the brain during periods of starvation and thus provide information on the
267 nutritional status (McCue, 2008; McCue *et al.*, 2012). Here, plasma samples were diluted to
268 1:8 (using 8 μ l of plasma) in the dilution buffer of the assay kit. Absorbance was measured at
269 450 nm and then transformed to concentration values using a dilution range provided by the
270 kit. We performed duplicates on a few samples to measure a coefficient of variation within
271 plates ($CV_{\text{within}}=7\%$) and between plates ($CV_{\text{between}}=11\%$) Third, we measured levels of
272 circulating triglycerides in plasma by a colorimetric assay (Triglyceride Colorimetric Assay
273 kit, ref n°10010303, Cayman Chemical, USA) using 2.5 μ l of plasma diluted at 1:4 in
274 provided dilution buffer. This method quantifies the total triglycerides using a chain of three
275 enzymatic reactions ending up in hydrogen peroxide production, which is converted into
276 quinoneimine dye quantified by absorbance at 540 nm ($CV_{\text{within}}=12\%$; $CV_{\text{between}}=13\%$).
277 Finally, we measured glucose concentration in the plasma with a colorimetric assay (Glucose
278 Colorimetric Assay Kit, ref n°10009582, Cayman Chemical, USA). We used 1.5 μ l of plasma
279 diluted at 1:13 in the provided dilution buffer as the glucose concentrations were very high
280 compared to kit sensibility. After three chemical reactions, the product of glucose is pink and
281 quantified by absorbance at 500 nm ($CV_{\text{within}}=4\%$; $CV_{\text{between}} > 30\%$ so we only compared
282 samples measured on the same kit).

283 2.4. Statistical analyses

284 All statistical analyses were performed using R statistical software (version 3.6.3; R Core
285 Team 2020). First, we checked plasma osmolality values during the first phase of our
286 manipulation to verify that lizards were mildly dehydrated and dehydration was similar
287 among treatment groups. This test was performed with an analysis of variance of the intra-
288 individual change in plasma osmolality from T0 to T1. There was no difference among groups
289 ($F_{5,120}=0.89$, $p=0.49$) since all lizards had similar increases in plasma osmolality (mean \pm SE:
290 41.08 ± 3.86 mOsm kg^{-1}). Then, to assess the effects of treatments on morphology, plasma

291 osmolality, and metabolites over the course of the experiment, we performed independent
292 linear models for each response variable. We calculated intra-individual changes as the
293 difference between T1 and T2 (referred to as Δ variable) and included the fixed effects of
294 treatment groups and initial value of the response variable in a linear regression model fitted
295 with the *lm* procedure. Prior to statistical tests, we used diagnostic plots of residuals to verify
296 the model assumptions with regard to the normality and homogeneous variance of residuals
297 (Heumann, Schomaker, & Shalabh, 2016). *Post-hoc* tests of differences among groups were
298 performed using the *multcomp* package (Hothorn *et al.*, 2016). All results are reported as
299 means \pm SE unless otherwise stated. As we noticed a difference in the amount of food
300 consumed among treatment groups with access to food ($F_{3,83}=10.22$, $p<0.0001$), we conducted
301 a second set of analyses with fed lizards using the total amount of food consumed during the
302 manipulation period as a covariate.

303 **3. Results**

304 3.1. Dehydration and water balance

305 Treatment groups had a significant effect on Δ osmolality ($F_{5,120}=11.27$, $p<0.0001$; Fig 1B).
306 Lizards in the hydrated control group restored their water balance to some extent, whereas
307 plasma osmolality increased in all other groups including lizards provided with food,
308 irrespective of their diet. Additionally, among fed lizards, plasma osmolality increased more
309 when lizards consumed more food across all treatment groups, as shown by the mild positive
310 correlation between increased food consumption and change in plasma osmolality during the
311 manipulation ($F_{1,79}=3.92$, $p=0.05$; Fig 1C).

312 3.2. Morphological changes

313 Mean initial body mass was 3.81 ± 0.05 g with no difference among treatment groups
314 ($F_{5,123}=0.64$, $p=0.67$), but body mass change between T1 and T2 (Δ BM) differed strongly
315 among treatment groups ($F_{5,128}=34.23$, $p<0.0001$; Fig 2A). Lizards in hydrated control group

316 lost less body mass than other treatment groups (Tukey *post-hoc* tests, all $p < 0.005$). Lizards
317 provided with spiders or crickets lost less mass than those provided with woodlice or
318 dehydrated control ($p < 0.0001$). Of the fed lizards, those provided with woodlice lost the most
319 body mass and their body mass loss was not statistically different than in lizards deprived of
320 free-standing water and food ($p = 0.29$; Fig 2A). With regard to tail width, it did not change on
321 average in hydrated control lizards or those provided with spiders or crickets (contrast: $p > 0.3$;
322 Fig 2B), whereas a significant and similar reduction in tail width was observed in the
323 dehydrated control and lizards fed with woodlice (respectively, contrasts: $\beta = -0.24$, $p = 0.01$ and
324 $\beta = -0.26$, $p = 0.02$; Fig 2B). At the same time, hindlimb width decreased on average in all
325 lizards (mean Δ hindlimb = -0.15 ± 0.009 mm, Fig 2C). Hindlimb width loss was similar and
326 low in hydrated control lizards and those provided with spiders or hydrated cricket (Tukey
327 *post-hoc* tests : all $p > 0.2$, Fig 2C). Meanwhile, Δ hindlimb width was **greatest** in lizards from
328 dehydrated control and in lizards provided with woodlice and dehydrated crickets (Tukey
329 *post-hoc* tests : $p < 0.05$). The amount of food consumed by each lizard did not correlate with
330 the morphological changes (Δ BM $F_{1,82} = 0.66$, $p = 0.42$; Δ tail $F_{1,76} = 1.65$, $p = 0.21$; Δ hindlimb
331 $F_{1,80} = 0.0004$, $p = 0.98$).

332 3.3. Plasma metabolites

333 There was a strong effect of food treatment on triglyceride concentration changes in plasma
334 ($F_{5,88} = 37.25$, $p < 0.0001$; Fig 3A), as well as an effect of initial concentration ($F_{1,88} = 111.20$,
335 $p < 0.0001$). *Post-hoc* analyses indicated three different treatment group categories. First,
336 triglyceride concentrations on average decreased slightly in control lizards with no food
337 irrespective of water availability. Second, triglyceride concentrations did not change on
338 average in lizards provided with woodlice. Third, lizards fed with hydrated or dehydrated
339 crickets and spiders had higher concentrations of plasma triglycerides (respectively, contrasts:
340 $\beta = 420.8$, $\beta = 413.4$ and $\beta = 286.5$, all $p < 0.0001$; Fig 3A) compared to the other groups. Across

341 all treatment groups with access to food, the amount of food consumed was positively
342 correlated with the change in triglyceride concentrations ($F_{1,53}=7.83$, $p=0.007$). At the same
343 time, plasma β -HB concentrations slightly increased with a low variability among lizards and
344 there was no significant difference among treatment groups ($F_{5,118}=1.44$, $p=0.22$; initial
345 concentration: $F_{1,118}=2.69$, $p<0.0001$). Instead, intra-individual change of glucose
346 concentration was highly variable and differed among treatment groups ($F_{5,73}=4.04$,
347 $p=0.0027$; $F_{1,73}=5.99$, $p=0.017$; Fig 3B). In the dehydrated control treatment, lizard glucose
348 concentrations increased (estimate= 67.83 ± 18.94 , $p=0.0006$) above the levels observed in the
349 hydrated control treatment, whereas lizards provided with food had intermediate values. We
350 found no effect of food consumption on glucose concentration changes ($F_{1,45}=0.01$, $p=0.92$).

351

352 **4. Discussion**

353 In this study, we tested if prey consumption by a generalist mesopredator lizard could
354 compensate for water deprivation via dietary water intake. Notably, our results revealed that
355 eating does not restore water balance, irrespective of prey type. On the contrary, osmolality
356 increased similarly in all water-restricted lizards, with or without access to prey, whereas
357 access to free-standing water was associated with restoration of an optimal hydration status as
358 expected. This result is remarkable given that non-fed animals lost about 0.8 g of body mass
359 during the experiment, which corresponds to average water loss through excretion in addition
360 to respiratory and cutaneous evaporative water losses (Le Galliard *et al.*, 2021). At the same
361 time, we provided 1.2 g of food with a mean water content of 72%, which is the equivalent of
362 0.85 g potential dietary water intake (Table 2). Thus, if lizards could extract significant
363 amount of water from their food, they would have been able to compensate fully or partly for
364 the loss of drinking water, especially when fed with high quality prey types, but this was
365 obviously not the case. In addition, our results suggest that the more the lizards eat, the more

366 dehydrated they get, which would imply that food intake compromises water balance although
367 this result must be interpreted with caution given that it is based on a correlation (Fig 1C).
368 Altogether, these results imply that food consumption and digestion have a significant water
369 cost that counterbalance dietary water intake whatever the prey type and that common lizards
370 are therefore dependent on free standing water intake (i.e., drinking) or air moisture to
371 maintain their water balance (Gettinger, 1984).

372 Only a few previous studies have investigated dietary water intake in squamate
373 reptiles using experimental approaches. Direct tests on herbivorous and insectivorous species
374 have found similar results, with food consumption reducing body mass loss due to water
375 deprivation (Table 1, Munsey, 1972). However, osmolality was not measured, so previous
376 knowledge of the effect of food consumption on animal physiological hydration state is
377 lacking. Studies focusing on carnivorous species explain the water costs of digestion through
378 intermittent feeding and consumption of protein-rich prey, two traits associated with a
379 stronger water use during digestion, metabolism, and excretion (Wright *et al.*, 2013;
380 Lillywhite, 2017). Thus, food consumption and metabolism, as well as the elimination of
381 metabolic wastes, can increase the requirement for water, potentially exacerbating
382 dehydration during periods of drought in carnivorous reptiles, such as snakes (Lillywhite,
383 2017). In our study, lizards are insectivores that fed continuously, but increased dehydration
384 due to feeding could be the result of an increased activity and body temperature due to the
385 thermal and energetic requirements of foraging and digestion (Reilly, McBrayer, & Miles,
386 2007). These behavioral aspects may be of great importance in explaining the differences
387 between non-fed and fed individuals, particularly in widely foraging and active lizards of
388 small body size. Our results further suggest that lizards provided with low-quality diet such as
389 woodlice favored their energetic balance rather than their water balance, but we cannot
390 determine if it was due to a digestion constraint or the result of a behavioral choice from

391 woodlice-fed lizards to thermoregulate more, as we had no data on locomotor activity and
392 thermoregulation. If locomotion for foraging and thermoregulation to reach an optimal
393 temperature for digestion are mostly responsible for increased water losses and negate the
394 possible dietary water gains, we expect these costs to be even more significant in the field.
395 Indeed, hunting for prey should have a much higher energetic and hydric cost in natural
396 environments than in captivity (Anderson & Karasov, 1981). Therefore, complementary
397 studies are needed to quantify the hydric costs of digestion and the behavioral trade-offs
398 between feeding and hydration.

399 Despite no evidence for the hypothesis that water can be extracted from food from our
400 data on plasma osmolality, a relevant metric of whole-organism hydration status (Capehart *et*
401 *al.*, 2016), we found that food consumption can mitigate the structural and energetic costs
402 caused by chronic water deprivation, which included a general loss of body condition, muscle
403 loss from the limb due to protein catabolism to release bound water (as shown in reptiles
404 Bruschi *et al.*, 2018) and lipid catabolism from the reserves stored in the tail (Price, 2017). We
405 chose three prey types corresponding to the natural diet of *Zootoca vivipara* (Vacheva &
406 Naumov, 2019) and representing different trophic roles ranging from decomposers to
407 insectivores (Bestion *et al.*, 2019) with distinct mobility strategies, morphology, as well as
408 energy and nutrient content (Reeves *et al.*, 2021). In natural populations, common lizards
409 forage actively on a wide diversity of invertebrate species including larvae and adult spiders,
410 insects, isopods or gastropods (Vacheva & Naumov, 2019). For example, Avery (1966) found
411 mostly spiders and homopters in stomach content of common lizards at two localities but also
412 rarer prey such as ants and woodlice. Here, lizards provided with woodlice ate less readily
413 during the experiment (on average 0.8 g instead of 1 g for the other prey types), possibly
414 because these prey are less water and energy-rich (Table 2) and are less easy to handle and
415 digest, making them distasteful for lizards (see Pekár, Líznavá, & Řezáč, 2016). The results

416 on metabolites indicate that the concentration of plasma triglycerides was aligned with the
417 amount of food consumed per treatment, suggesting that food intake was associated with
418 nutrient uptake and transport. Triglycerides are the transport form of lipids and can be
419 hydrolyzed to produce fatty acids that enter the ATP production pathways. The low levels of
420 plasma triglycerides in non-fed lizards and those fed with woodlice indicate little food intake
421 and little lipid transport and storage, which is consistent with previous results seen in other
422 reptiles (Price *et al.*, 2013). As a consequence, the general body condition of woodlice-fed
423 and water deprived lizards was much more impacted than in lizards fed with higher quality
424 food such as crickets and spiders: these lizards lost more body mass, more muscle and more
425 fat reserves from their tail, and were virtually indistinguishable from those held without food
426 nor water. Thus, despite providing no significant net intake of water, high quality food and
427 strong food consumption were important to recoup some of the energy costs of water
428 deprivation and reduce the body condition loss induced by water stress. These results
429 highlight that feeding is important to maintain body condition during water restriction,
430 including muscular mass and reserves that are essential for future survival. Thus, while food
431 may be important to cope with limited rainfall and water availability, it does not primarily
432 provide common lizards with water. Rather, food intake enables them to survive longer by
433 maintaining a higher body condition, and improves their future prospects for reproduction by
434 preserving muscles and reserves. Of course, this hypothesis will require confirmation through
435 more accurate assessments of the lizards' condition and improved tests with prey that are both
436 hydrated and less hydrated since our manipulation of crickets did not yield reliable results in
437 this regard (Table 2). In this context, we predict that shifting diet toward higher water content
438 prey would not allow this generalist lizard to avoid dehydration, but it can reduce the short-
439 term costs of drought and long-term fitness consequences (Dezetter *et al.*, 2022).

440 Unexpectedly, we found a significant difference between hydrated and dehydrated
441 control groups in their plasma glucose concentration levels but no effect of food provisioning
442 and water deprivation on plasma concentrations of ketone bodies (β -Hydroxybutyrate). It is
443 predicted that ketone bodies' concentration should elevate during the second stage of fasting
444 when carbohydrate stores are depleted and animals mobilize lipid stores instead (McCue *et*
445 *al.*, 2012). However, previous studies have shown that plasma ketone concentrations do not
446 necessarily increase during fasting in reptiles, especially when fasting is too short, and may
447 even decrease when starvation is extremely long (Pontes *et al.*, 1988). Conversely, the
448 glucose levels in water-deprived lizards were maintained at relatively high levels, regardless
449 of their feeding status. In addition, lizards with neither water nor food had much higher
450 glucose concentration at the end of the manipulation than those from the hydrated control
451 group. In vertebrates exposed to periods of fasting or intense exercise, maintenance of high
452 glucose concentrations in the blood is ensured by gluconeogenesis in the liver (a metabolic
453 pathway that generates glucose from non-carbohydrate carbon substrates; Zain-ul-Abidin &
454 Katorski, 1967) and glycogenolysis (the degradation of glycogen stored in adipose tissues or
455 muscles; Storey, 1996). Gluconeogenesis involves glucose production in the liver from
456 transported blood glycerol that is released by triglyceride hydrolysis in the tail or other
457 abdominal reserve tissues. Glycerol levels and liver gluconeogenesis activity can increase
458 during periods of fasting in snakes (Price, 2017). Thus, high glucose concentrations in the
459 blood of food and water-deprived lizards may represent a higher capacity for gluconeogenesis
460 due to lipid catabolism induced by fasting and dehydration. Similarly, water deprivation in the
461 absence of food led to stronger muscle wasting, which may end up in muscle glycogen
462 degradation and increased glucose production. This conversion of muscle stored glycogen
463 into plasma circulating glucose was previously demonstrated in turtles (Emerson, 1967) and
464 in amphibians and lizards after vigorous exercise, which is close to simulated conditions of

465 fasting (Gleeson, 1982, 1991). These observations are interesting because our understanding
466 of how stored macronutrients are utilized during fasting in reptiles still remains limited
467 compared to our knowledge of the physiology of fasting in mammals and birds (Price, 2017).
468 Our findings suggest that muscle breakdown (catabolism) in reptiles may commence earlier
469 than expected in the starvation process, particularly in cases of water scarcity (Brusch *et al.*,
470 2018). Further physiological investigations would aid in elucidating these pathways and how
471 they may be influenced by hydration status.

472 The effect of environmental temperatures on ectotherm metabolic rates is now well-
473 studied (Schulte, 2015) but we know little about the other abiotic factors involved in the
474 regulation of metabolism and energetic functions of the organisms. If digestion is strongly
475 dependent on body temperature regulation in reptiles (e.g. Harlow, Hillman, & Hoffman,
476 1976; Naulleau, 1983), how it is related to water balance and how water availability impacts
477 nutrient assimilation is poorly studied. When body temperature increases, evaporative water
478 losses also increase, and these losses are further accentuated by higher metabolism and
479 respiration rates (Reilly *et al.*, 2007), which are closely linked to food intake. It is therefore
480 crucial to account for potential effects of food consumption and digestion on temperature and
481 water balance regulation in reptiles. Changes in diet have been observed in other ectothermic
482 species, where omnivores increase their herbivory in response to higher temperatures
483 (Carreira *et al.*, 2016, 2017; Zhang *et al.*, 2020). While these studies primarily focus on
484 aquatic ectotherms, the impact of environmental factors on the diet of terrestrial ectotherms
485 remains poorly known (Zimmerman & Tracy, 1989). Our findings indicate that insectivorous
486 species face trade-offs between water and energy balance, and their survival in water-deprived
487 regions may depend on food intake. This has potential implications for ecosystems, such as
488 increased top-down regulation of intermediate levels on primary producers with global change
489 (Zhang *et al.*, 2020). Further research is needed to gain a deeper understanding of how

490 digestive physiology affects various aspects of an individual's life history traits (Karasov &
491 Diamond, 1988), as trade-offs between linked physiological traits are crucial in understanding
492 organism responses to environmental constraints (Urban *et al.*, 2016; Riddell *et al.*, 2019).

493 **5. Acknowledgements**

494 The authors want to thank Jean-Patrice Robin and the IPHC UMR 7178 for their help with the
495 analysis of the invertebrate calorimetric contents. We also thank the intern students who help
496 us with the experiment and animal care, especially Anna Marcout and Jules Domalain, as well
497 as the CEREEP Ecotron IleDeFrance staff.

498 **6. Competing interests**

499 No competing interests declared.

500 **7. Author contributions**

501 Chloé Chabaud: Conceptualization, Methodology, Investigation, Formal analysis, Writing-
502 Original Draft ; George A. Bruschi: Conceptualization, Methodology, Writing-
503 Review&Editing ; Anouk Pellerin: Investigation ; Olivier Lourdaux: Conceptualization,
504 Methodology, Writing-Review&Editing ; Jean-François Le Galliard: Conceptualization,
505 Methodology, Investigation, Writing-Review&Editing.

506 **8. Funding**

507 This study received the financial and technical support from the Centre National de la
508 Recherche Scientifique (CNRS) and was funded by the Agence Nationale de la Recherche
509 AQUATHERM project (ANR-17-CE02-0013 to JFLG and OL).

510 **9. Data availability**

511 Data will be deposited in Zenodo after acceptance.

512 **10. References**

513 **Albright TP, Mutiibwa D, Gerson Alexander R, Smith EK, Talbot WA, O'Neill JJ,**
514 **McKechnie AE & Wolf BO. 2017.** Mapping evaporative water loss in desert passerines
515 reveals an expanding threat of lethal dehydration. *Proceedings of the National Academy of*
516 *Sciences* **114**: 2283–2288.

- 517 **Anderson RA & Karasov WH. 1981.** Contrasts in energy intake and expenditure in sit-and-
518 wait and widely foraging lizards. *Oecologia* **49**: 67–72.
- 519 **Avery RA. 1966.** Food and feeding habits of the Common lizard (*Lacerta vivipara*) in the
520 west of England. *Journal of Zoology* **149**: 115–121.
- 521 **Avery RA. 1971.** Estimates of Food Consumption by the Lizard *Lacerta vivipara* Jacquin.
522 *The Journal of Animal Ecology* **40**: 351.
- 523 **Bailey V. 1923.** Sources of Water Supply for Desert Animals. *The Scientific Monthly* **17**: 66–
524 86.
- 525 **Bestion E, Cucherousset J, Teyssier A & Cote J. 2015.** Non-consumptive effects of a top-
526 predator decrease the strength of the trophic cascade in a four-level terrestrial food web. *Oikos*
527 **124**: 1597–1602.
- 528 **Bestion E, Soriano-Redondo A, Cucherousset J, Jacob S, White J, Zinger L, Fournelle L,
529 Di Gesu L, Teyssier A & Cote J. 2019.** Altered trophic interactions in warming climates:
530 consequences for predator diet breadth and fitness. *Proceedings of the Royal Society B:*
531 *Biological Sciences* **286**: 20192227.
- 532 **Bissonette JA. 1978.** The Influence of Extremes of Temperature on Activity Patterns of
533 Peccaries. *The Southwestern Naturalist* **23**: 339–346.
- 534 **Bradshaw SD. 1997.** Thermal Homeostasis. In: Bradshaw SD, ed. *Homeostasis in Desert*
535 *Reptiles*. Berlin, Heidelberg: Springer, 123–165.
- 536 **Braun EJ. 1999.** Integration of organ systems in avian osmoregulation. *Journal of*
537 *Experimental Zoology* **283**: 702–707.
- 538 **Brusch IV, G. A., Lourdais, O., Kaminsky, B., & DeNardo, D. F. 2018.** Muscles provide
539 an internal water reserve for reproduction. *Proceedings of the Royal Society B*, 285(1881),
540 20180752
- 541 **Brusch GA, Gavira RSB, Viton R, Dupoué A, Leroux-Coyau M, Meylan S, Le Galliard
542 JF & Lourdais O. 2020.** Additive effects of temperature and water availability on pregnancy
543 in a viviparous lizard. *The Journal of Experimental Biology* **223**: jeb228064.
- 544 **Brusch IV GA, Le Galliard JF, Viton R, Gavira RSB, Clobert J & Lourdais O. 2022.**
545 Reproducing in a changing world: combined effects of thermal conditions by day and night
546 and of water constraints during pregnancy in a cold-adapted ectotherm. *Oikos*: e09536.
- 547 **Capehart GD, Escallón C, Vernasco BJ, Moore IT & Taylor EN. 2016.** No drought about
548 it: Effects of supplemental hydration on the ecology, behavior, and physiology of free-ranging
549 rattlesnakes. *Journal of Arid Environments* **134**: 79–86.
- 550 **Carreira BM, Segurado P, Orizaola G, Gonçalves N, Pinto V, Laurila A & Rebelo R.
551 2016.** Warm vegetarians? Heat waves and diet shifts in tadpoles. *Ecology* **97**: 2964–2974.
- 552 **Carreira BM, Segurado P, Laurila A & Rebelo R. 2017.** Can heat waves change the
553 trophic role of the world's most invasive crayfish? Diet shifts in *Procambarus clarkii*. *PLOS*
554 *ONE* **12**: e0183108.

- 555 **Chapple DG & Swain R. 2002.** Distribution of energy reserves in a viviparous skink: Does
556 tail autotomy involve the loss of lipid stores? *Austral Ecology* **27**: 565–572.
- 557 **Cloudsley-Thompson JL. 1975.** Adaptations of Arthropoda to Arid Environments. *Annual*
558 *Review of Entomology* **20**: 261–283.
- 559 **Davis JR & DeNardo DF. 2007.** The urinary bladder as a physiological reservoir that
560 moderates dehydration in a large desert lizard, the Gila monster *Heloderma suspectum*.
561 *Journal of Experimental Biology* **210**: 1472–1480.
- 562 **Dearing MD & Schall JosJ. 1992.** Testing Models of Optimal Diet Assembly by the
563 Generalist Herbivorous Lizard *Cnemidophorus Murinus*. *Ecology* **73**: 845–858.
- 564 **Dezetter M, Dupoué A, Le Galliard JF & Lourdais O. 2021.** Additive effects of
565 developmental acclimation and physiological syndromes on lifetime metabolic and water loss
566 rates of a dry-skinned ectotherm. *Functional Ecology*.
- 567 **Dezetter M, Le Galliard JF & Lourdais O. 2022.** Behavioural hydroregulation protects
568 against acute effects of drought in a dry-skinned ectotherm. *Oecologia*.
- 569 **Dupoué A, Stahlschmidt ZR, Michaud B & Lourdais O. 2015.** Physiological state
570 influences evaporative water loss and microclimate preference in the snake *Vipera aspis*.
571 *Physiology & Behavior* **144**: 82–89.
- 572 **Dupoué A, Rutschmann A, Le Galliard JF, Miles DB, Clobert J, DeNardo DF, Bruschi**
573 **GA & Meylan S. 2017.** Water availability and environmental temperature correlate with
574 geographic variation in water balance in common lizards. *Oecologia* **185**: 561–571.
- 575 **Dupoué A, Le Galliard J, Josserand R, DeNardo DF, Decencièrè B, Agostini S, Haussy C**
576 **& Meylan S. 2018.** Water restriction causes an intergenerational trade-off and delayed
577 mother–offspring conflict in a viviparous lizard (J Grindstaff, Ed.). *Functional Ecology* **32**:
578 676–686.
- 579 **Edwards M, Sheehy CM, Fedler MT & Lillywhite HB. 2021.** Thirst and drinking in North
580 American watersnakes (*Nerodia* spp.). *Journal of Experimental Biology* **224**: jeb241414.
- 581 **Emerson DN. 1967.** Preliminary Study on Seasonal Liver Lipids and Glycogen, and Blood
582 Sugar Levels in the Turtle *Graptemys pseudogeographica* (Gray) from South Dakota.
583 *Herpetologica* **23**: 68–70.
- 584 **Gerson AR & Guglielmo CG. 2011.** House sparrows (*Passer domesticus*) increase protein
585 catabolism in response to water restriction. *American Journal of Physiology-Regulatory,*
586 *Integrative and Comparative Physiology* **300**: R925–R930.
- 587 **Gettinger RD. 1984.** Energy and Water Metabolism of Free-Ranging Pocket Gophers,
588 *Thomomys Bottae*. *Ecology* **65**: 740–751.
- 589 **Gienger CM, Tracy CR & Nagy KA. 2014.** Life in the Lizard Slow Lane: Gila Monsters
590 Have Low Rates of Energy Use and Water Flux. *Copeia* **2014**: 279–287.
- 591 **Gleeson TT. 1982.** Lactate and glycogen metabolism during and after exercise in the lizard
592 *Sceloporus occidentalis*. *Journal of Comparative Physiology ? B* **147**: 79–84.

- 593 **Gleeson TT. 1991.** Patterns of metabolic recovery from exercise in amphibians and reptiles.
594 *Journal of Experimental Biology* **160**: 187–207.
- 595 **Green B, Dryden G & Dryden K. 1991.** Field energetics of a large carnivorous lizard,
596 *Varanus rosenbergi*. *Oecologia* **88**: 547–551.
- 597 **Gregory PT, Crampton LH & Skebo KM. 1999.** Conflicts and interactions among
598 reproduction, thermoregulation and feeding in viviparous reptiles: are gravid snakes anorexic?
599 *Journal of Zoology* **248**: 231–241.
- 600 **Grenot C, Heulin B, Pilorge T, Khodadoost M, Ortega A & Mou YP. 1987.** Water Budget
601 in Some Populations of the European Common Lizard, *Lacerta vivipara* Jacquin. *Functional*
602 *Ecology* **1**: 131.
- 603 **Harlow HJ, Hillman SS & Hoffman M. 1976.** The effect of temperature on digestive
604 efficiency in the herbivorous lizard, *Dipsosaurus dorsalis*. *Journal of Comparative*
605 *Physiology ? B* **111**: 1–6.
- 606 **Hothorn T, Bretz F, Westfall P, Heiberger RM, Schuetzenmeister A, Scheibe S &**
607 **Hothorn MT. 2016.** Package ‘multcomp’. Simultaneous inference in general parametric
608 models. *Project for Statistical Computing, Vienna, Austria*.
- 609 **IPCC (2019)** Special Report on Climate Change and Land. An IPCC Special Report on
610 climate change, desertification, land degradation, sustainable land management, food security,
611 and greenhouse gas fluxes in terrestrial ecosystems.
612
- 613 **Jindra M & Sehnal F. 1990.** Linkage between diet humidity, metabolic water production and
614 heat dissipation in the larvae of *Galleria mellonella*. *Insect Biochemistry* **20**: 389–395.
- 615 **Jørgensen CB. 1994.** Water economy in a terrestrial toad (*Bufo bufo*), with special
616 reference to cutaneous drinking and urinary bladder function. *Comparative Biochemistry*
617 *and Physiology Part A: Physiology* **109**: 311–324.
- 618 **Karasov WH. 1983.** Water Flux and Water Requirement in Free-living Antelope Ground
619 Squirrels *Ammospermophilus leucurus*. *Physiological Zoology* **56**: 94–105.
- 620 **Karasov WH & Diamond JM. 1988.** Interplay between Physiology and Ecology in
621 Digestion. *BioScience* **38**: 602–611.
- 622 **Karasov WH & del Rio CM. 2007.** *Physiological Ecology: How Animals Process Energy,*
623 *Nutrients, and Toxins*. Princeton University Press.
- 624 **Le Galliard JF, Chabaud C, de Andrade DOV, Brischoux F, Carretero MA, Dupoué A,**
625 **Gavira RSB, Lourdais O, Sannolo M & Van Dooren TJM. 2021.** A worldwide and
626 annotated database of evaporative water loss rates in squamate reptiles. *Global Ecology and*
627 *Biogeography* **30**: 1938–1950.
- 628 **Leinbach IL, McCluney KE & Sabo JL. 2019.** Predator water balance alters intraguild
629 predation in a streamside food web. *Ecology* **100**: e02635.
- 630 **Lillywhite HB. 2017.** Feeding begets drinking: insights from intermittent feeding in snakes.
631 *The Journal of Experimental Biology* **220**: 3565–3570.

- 632 **Lillywhite HB, Heatwole H & Sheehy CM. 2014.** Dehydration and Drinking Behavior of
633 the Marine File Snake *Acrochordus granulatus*. *Physiological and Biochemical Zoology* **87**:
634 46–55.
- 635 **Lorenzon P, Clobert J, Oppliger A & John-Alder H. 1999.** Effect of water constraint on
636 growth rate, activity and body temperature of yearling common lizard (*Lacerta vivipara*).
637 *Oecologia* **118**: 423–430.
- 638 **Massot M, Clobert J, Lorenzon P & Rossi JM. 2002.** Condition-dependent dispersal and
639 ontogeny of the dispersal behaviour: an experimental approach. *Journal of Animal Ecology*
640 **71**: 253–261.
- 641 **Mautz WJ & Nagy KA. 1987.** Ontogenetic Changes in Diet, Field Metabolic Rate, and
642 Water Flux in the Herbivorous Lizard *Dipsosaurus dorsalis*. *Physiological Zoology* **60**: 640–
643 658.
- 644 **McCluney KE & Date RC. 2008.** The effects of hydration on growth of the house cricket,
645 *Acheta domesticus*. *Journal of Insect Science* **8**: 32.
- 646 **McCue MD. 2008.** Fatty acid analyses may provide insight into the progression of starvation
647 among squamate reptiles. *Comparative Biochemistry and Physiology Part A: Molecular &*
648 *Integrative Physiology* **151**: 239–246.
- 649 **McCue MD, Lillywhite HB & Beaupre SJ. 2012.** Physiological Responses to Starvation in
650 Snakes: Low Energy Specialists. In: McCue MD, ed. *Comparative Physiology of Fasting,*
651 *Starvation, and Food Limitation*. Berlin, Heidelberg: Springer, 103–131.
- 652 **Meiri S. 2018.** Traits of lizards of the world: Variation around a successful evolutionary
653 design. *Global Ecology and Biogeography* **27**: 1168–1172.
- 654 **Minnich JE & Shoemaker VH. 1970.** Diet, Behavior and Water Turnover in the Desert
655 Iguana, *Dipsosaurus dorsalis*. *The American Midland Naturalist* **84**: 496–509.
- 656 **Minnich JE & Shoemaker VH. 1972.** Water and Electrolyte Turnover in a Field Population
657 of the Lizard, *Uma scoparia*. *Copeia* **1972**: 650–659.
- 658 **Munsey LD. 1972.** Water loss in five species of lizards. *Comparative Biochemistry and*
659 *Physiology Part A: Physiology* **43**: 781–794.
- 660 **Murphy MS & DeNardo DF. 2019.** Rattlesnakes Must Drink: Meal Consumption Does Not
661 Improve Hydration State. *Physiological and Biochemical Zoology* **92**: 381–385.
- 662 **Nagy KA, Clarke BC, Seely MK, Mitchell D & Lighton JRB. 1991.** Water and Energy
663 Balance in Namibian Desert Sand-Dune Lizards *Angolosaurus skoogi* (Andersson, 1916).
664 *Functional Ecology* **5**: 731–739.
- 665 **Nagy KA. 2001.** Food requirements of wild animals: predictive equations for free-living
666 mammals, reptiles, and birds. *Nutrition Abstracts and Reviews, Series B*.
- 667 **Nagy KA. 2004.** Water economy of free-living desert animals. *International Congress Series*
668 **1275**: 291–297.

- 669 **Nagy KA & Gruchacz MJ. 1994.** Seasonal Water and Energy Metabolism of the Desert-
670 Dwelling Kangaroo Rat (*Dipodomys merriami*). *Physiological Zoology* **67**: 1461–1478.
- 671 **Nagy KA & Knight MH. 1994.** Energy, Water, and Food Use by Springbok Antelope
672 (*Antidorcas marsupialis*) in the Kalahari Desert. *Journal of Mammalogy* **75**: 860–872.
- 673 **Nagy KA & Medica' PA. 1986.** Physiological Ecology of Desert Tortoises in Southern
674 Nevada. *Herpetologica*, 73-92.
- 675 **Naulleau G. 1983.** The Effects of Temperature on Digestion in *Vipera aspis*. *Journal of*
676 *Herpetology* **17**: 166–170.
- 677 **Pekár S, Líznarová E & Řezáč M. 2016.** Suitability of woodlice prey for generalist and
678 specialist spider predators: a comparative study. *Ecological Entomology* **41**: 123–130.
- 679 **Peterson CC. 1996.** Anhomeostasis: Seasonal Water and Solute Relations in Two
680 Populations of the Desert Tortoise (*Gopherus agassizii*) during Chronic Drought.
681 *Physiological Zoology*.
- 682 **Pontes RDQ, Cartaxo ACL & Jonas R. 1988.** Concentrations of ketone bodies in the blood
683 of the green lizard *Ameiva ameiva* (Teiidae) in different physiological situations. *Comparative*
684 *Biochemistry and Physiology Part A: Physiology* **89**: 309–311.
- 685 **Price ER, Jones TT, Wallace BP & Guglielmo CG. 2013.** Serum triglycerides and β-
686 hydroxybutyrate predict feeding status in green turtles (*Chelonia mydas*): Evaluating a single
687 blood sample method for assessing feeding/fasting in reptiles. *Journal of Experimental*
688 *Marine Biology and Ecology* **439**: 176–180.
- 689 **Price ER. 2017.** The physiology of lipid storage and use in reptiles. *Biological Reviews* **92**:
690 1406–1426.
- 691 **Reeves JT, Fuhlendorf SD, Davis CA & Wilder SM. 2021.** Arthropod prey vary among
692 orders in their nutrient and exoskeleton content. *Ecology and Evolution* **11**: 17774–17785.
- 693 **Reilly SM, McBrayer LB & Miles DB (Eds.). 2007.** *Lizard Ecology*. Cambridge:
694 Cambridge University Press.
- 695 **Riddell EA, Iknayan KJ, Wolf BO, Sinervo B & Beissinger SR. 2019.** Cooling
696 requirements fueled the collapse of a desert bird community from climate change.
697 *Proceedings of the National Academy of Sciences* **116**: 21609–21615.
- 698 **Rozen-Rechels D, Badiane A, Agostini S, Meylan S & Galliard JFL. 2020.** Water
699 restriction induces behavioral fight but impairs thermoregulation in a dry-skinned ectotherm.
700 *Oikos*.
- 701 **Schulte PM. 2015.** The effects of temperature on aerobic metabolism: towards a mechanistic
702 understanding of the responses of ectotherms to a changing environment (JE Podrabsky, JH
703 Stillman, and L Tomanek, Eds.). *Journal of Experimental Biology* **218**: 1856–1866.
- 704 **Seely MK. 1979.** Irregular fog as a water source for desert dune beetles. *Oecologia* **42**: 213–
705 227.

- 706 **Stamps J, Tanaka S & Krishnan VV. 1981.** The Relationship between Selectivity and Food
707 Abundance in a Juvenile Lizard. *Ecology* **62**: 1079–1092.
- 708 **Storey KB. 1996.** Metabolic adaptations supporting anoxia tolerance in reptiles: Recent
709 advances. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular*
710 *Biology* **113**: 23–35.
- 711 **Surget-Groba Y, Heulin B, Guillaume CP, Puky M, Semenov D, Orlova V, Kupriyanova**
712 **L, Ghira I & Smajda B. 2006.** Multiple origins of viviparity, or reversal from viviparity to
713 oviparity? The European common lizard (*Zootoca vivipara*, Lacertidae) and the evolution of
714 parity. *Biological Journal of the Linnean Society* **87**: 1–11.
- 715 **Thompson GG, Bradshaw SD & Withers PC. 1997.** Energy and Water Turnover Rates of a
716 Free-living and Captive Goanna, *Varanus caudolineatus* (Lacertilia: Varanidae). *Comparative*
717 *Biochemistry and Physiology Part A: Physiology* **116**: 105–111.
- 718 **Tieleman, B. I., & Williams, J. B. (2000).** The adjustment of avian metabolic rates and water
719 fluxes to desert environments. *Physiological and Biochemical Zoology*, 73(4), 461-479
- 720 **Urban MC, Bocedi G, Hendry AP, Mihoub JB, Pe'er G, Singer A, Bridle JR, Crozier**
721 **LG, De Meester L, Godsoe W, Gonzalez A, Hellmann JJ, Holt RD, Huth A, Johst K,**
722 **Krug CB, Leadley PW, Palmer SCF, Pantel JH, Schmitz A, Zollner PA & Travis MJJ.**
723 **2016.** Improving the forecast for biodiversity under climate change. *Science* **353**: aad8466.
- 724 **Vacheva E & Naumov B. 2019.** Diet of the Viviparous lizard *Zootoca vivipara*
725 (Lichtenstein, 1823) (Reptilia: Lacertidae) from its southern range. *ARPHA Conference*
726 *Abstracts* **2**: e46484.
- 727 **Wright CD, Jackson ML & DeNardo DF. 2013.** Meal consumption is ineffective at
728 maintaining or correcting water balance in a desert lizard, *Heloderma suspectum*. *Journal of*
729 *Experimental Biology* **216**: 1439–1447.
- 730 **Zain-ul-Abedin M & Katorski B. 1967.** Gluconeogenesis in the kidney and liver slices of a
731 lizard, *Uromastix hardwickii*. *The Biochemical Journal* **102**: 189–193.
- 732 **Zhang P, van Leeuwen CHA, Bogers D, Poelman M, Xu J & Bakker ES. 2020.**
733 Ectothermic omnivores increase herbivory in response to rising temperature. *Oikos* **129**:
734 1028–1039.
- 735 **Zimmerman LC & Tracy CR. 1989.** Interactions between the Environment and Ectothermy
736 and Herbivory in Reptiles. *Physiological Zoology* **62**: 374–409.
- 737 **Znari M & Nagy KA. 1997.** Field Metabolic Rate and Water Flux in Free-Living Bibron's
738 Agama (*Agama impalearis*, Boettger, 1874) in Morocco. *Herpetologica* **53**: 81–88.
- 739

Table 1: Summary of empirical studies of dietary water intake in squamate reptiles.

Species name	Standard diet	Climate zone	Link between diet and water balance	Reference
<i>Acrochordus granulatus</i>	Carnivorous	Marine	Direct test. Dietary water intake is insufficient for water balance	Lillywhite, Heatwole, & Sheehy, 2014
<i>Agkistrodon conanti</i>	Carnivorous	Semi-aquatic	Direct test. Food consumption increases post-prandial drinking in a laboratory experiment.	Lillywhite, 2017
<i>Crotalus atrox</i>	Carnivorous	Semi-arid	Direct test. Eating compromises water balance (osmolality) in chronic laboratory experiments of food consumption under water deprivation.	Murphy & DeNardo, 2019
<i>Heloderma suspectum</i>	Carnivorous	Xeric	Direct test. Eating does not correct water balance (osmolality) in acute and chronic laboratory experiments of food consumption.	Wright <i>et al.</i> , 2013
<i>Lampropeltis californiae</i>	Carnivorous	Semi-arid	Direct test. Food consumption increases post-prandial drinking in a laboratory experiment.	Lillywhite, 2017
<i>Nerodia fasciata</i>	Carnivorous	Semi-aquatic	Direct test. Food consumption increases post-prandial drinking in a laboratory experiment.	Lillywhite, 2017
<i>Nerodia clarkii</i>	Carnivorous	Marine	Indirect test. Moderate dehydration with access to food (fish) inhibits feeding and leads to body mass loss.	Edwards <i>et al.</i> , 2021
<i>Nerodia fasciata</i>	Carnivorous	Semi-aquatic	Indirect test. Moderate dehydration with access to food (fish) inhibits feeding and leads to body mass loss.	Edwards <i>et al.</i> , 2021
<i>Pantherophis alleghaniensis</i>	Carnivorous	Mesic	Direct test. Food consumption increases post-prandial drinking in a laboratory experiment.	Lillywhite, 2017
<i>Varanus rosenbergi</i>	Carnivorous	Mesic	Indirect test. Field measures of water influx rates suggest that water is mostly provided from diet during the summer but not in other seasons. Free-standing water consumption unknown.	Green, Dryden, & Dryden, 1991
<i>Angolosaurus skoogi</i>	Herbivorous	Xeric	Indirect test. Field measures of water influx rates suggest that water is mostly provided from a diet including succulent plants. Lizards apparently did not drink water but lost body mass.	Nagy <i>et al.</i> , 1991
<i>Dipsosaurus dorsalis</i>	Herbivorous	Xeric	Indirect test. Field measures of water influx rates suggest that water is mostly provided from diet and lizards maintain a constant hydration state during the year, but shift to different plant materials during the summer.	Minnich & Shoemaker, 1970
<i>Dipsosaurus dorsalis</i>	Herbivorous	Xeric	Direct test. Food consumption allows to recoup part of the body mass loss induced by a chronic water deprivation experiment in the laboratory. Osmolality and body hydration were not measured.	Munsey, 1972

<i>Agama impalearis</i>	Insectivorous	Xeric	Indirect test. Field measures of water influx rates suggest that water is mostly provided from diet (termites) during breeding season. Lizards apparently did not drink water.	Znari & Nagy, 1997
<i>Callisaurus draconoides</i>	Insectivorous	Semi-arid	Direct test. Food consumption allows to recoup the body mass loss induced by a chronic water deprivation experiment in the laboratory. Osmolality and body hydration were not measured.	Munsey, 1972
<i>Cnemidophorus tigris</i>	Insectivorous	Semi-arid	Direct test. Food consumption reduced the body mass loss induced by a chronic water deprivation experiment in the laboratory. Osmolality and body hydration were not measured.	Munsey, 1972
<i>Sceloporus occidentalis</i>	Insectivorous	Mesic	Direct test. Food consumption allows to recoup part of the body mass loss induced by a chronic water deprivation experiment in the laboratory. Osmolality and body hydration were not measured.	Munsey, 1972
<i>Uma scoparia</i>	Omnivorous	Semi-arid	Indirect test. Field measures of water influx rates indicate stronger flux rates in juveniles feeding on insects than in adults feeding on dry plant materials. Lizards maintain water balance despite limited evidence that they drink free-standing water.	Minnich & Shoemaker, 1972
<i>Uma scoparia</i>	Omnivorous	Semi-arid	Direct test. Food consumption (insects) allows to recoup part of the body mass loss induced by a chronic water deprivation experiment in the laboratory. Osmolality and body hydration was not measured.	Munsey, 1972
<i>Varanus caudolineatus</i>	Omnivorous	Semi-Arid	Indirect test. Field and laboratory measures of water influx rates suggest that water is importantly provided from diet. Free-standing water consumption unknown.	Thompson, Bradshaw, & Withers, 1997

Table 2: Water content and nutritional quality (mean \pm SD) of invertebrates used in the experiment. N=20 individuals for water content, N = number of pools used for energy content, with each pool containing tens of individuals, and N = number of lizards in each food treatment for quantity of food eaten. The total quantity of food provided during the 6 days of manipulation was 1.2 g on average. *** indicates that the group is significantly different from the others.

	Hydrated Crickets	Dehydrated Crickets	Spiders	Woodlice
Water content	75.25 \pm 3.99 % N=20	72.24 \pm 2.27 % N=20	73.27 \pm 4.83 % N=20	65.58 \pm 1.74 % *** N=20
Energy content	6.23 \pm 0.29 kJ/g N=4	6.52 kJ/g N=1	6.34 \pm 0.51 kJ/g N=2	3.92 \pm 0.11 kJ/g *** N=3
Quantity of food eaten	0.98 \pm 0.23 g N=21	1.02 \pm 0.14 g N=22	1.01 \pm 0.15 g N=21	0.78 \pm 0.08 g *** N=21

Caption to figures

Figure 1: A) Timeline of the experimental design and of measurements performed between the start and the end of the manipulation in the different treatment groups. B) Boxplots of Δ osmolality. Lower and upper hinges correspond to the first and third quartiles and middle bar is the median. Differences between groups are indicated based on *post hoc* tests with Tukey correction (from left to right: Hydrated control, Spiders, Hydrated crickets, Dehydrated crickets, Woodlice, and Dehydrated control). C) Scatterplot of Δ osmolality against the amount of food consumed (g) for each treatment group with access to prey. Data shown are raw values and linear smooths for each group with a confidence interval in grey.

Figure 2: Morphological changes A) Change in body mass (g, mean initial value: $3,81 \pm 0,05$ g) between the start and the end of the manipulation in the different treatment groups. B) Change in caudal width (mm, mean initial width $6,46 \pm 0,04$) between the start and the end of the manipulation in the different treatment groups. C) Change in hindlimb width (mm, mean initial width $1,98 \pm 0,01$) between the start and the end of the manipulation in the different treatment groups. Treatment groups are represented from left to right: Hydrated control, Spiders, Hydrated crickets, Dehydrated crickets, Woodlice, Dehydrated control. Lower and upper hinges correspond to the first and third quartiles and middle bar is the median. Differences between groups are indicated based on *post hoc* tests with Tukey correction.

Figure 3: Changes in blood parameters A) Change in triglyceride concentration in plasma between the start and the end of the manipulation in the different treatment groups. B) Change in glucose concentration in plasma between the start and the end of the manipulation in the different treatment groups. Treatment groups are represented from left to right: Hydrated control, Spiders, Hydrated crickets, Dehydrated crickets, Woodlice, Dehydrated control.

Lower and upper hinges correspond to the first and third quartiles and middle bar is the median. Differences between groups are indicated based on post hoc tests with Tukey correction.

Pre-print version

Figure 1

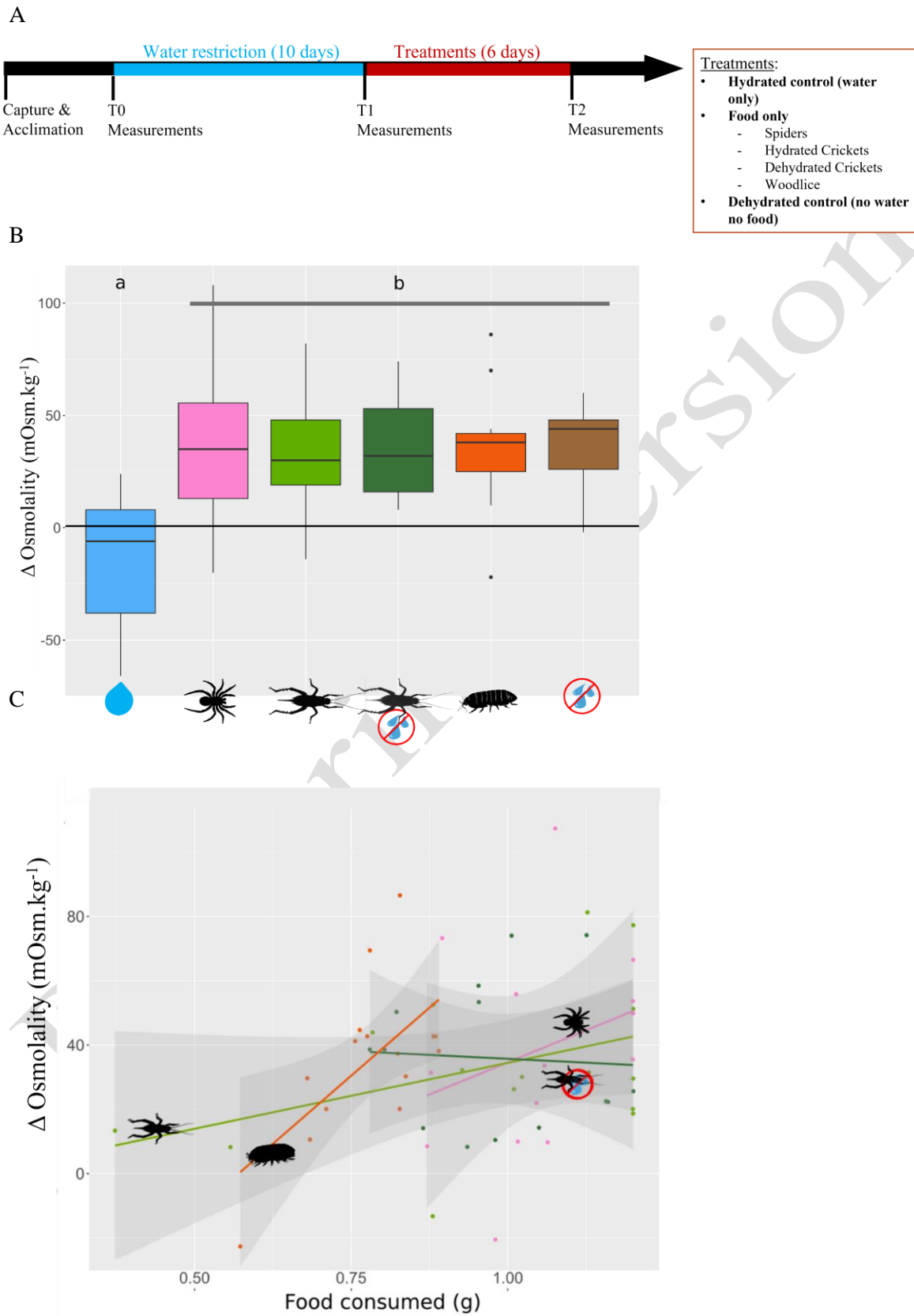


Figure 2

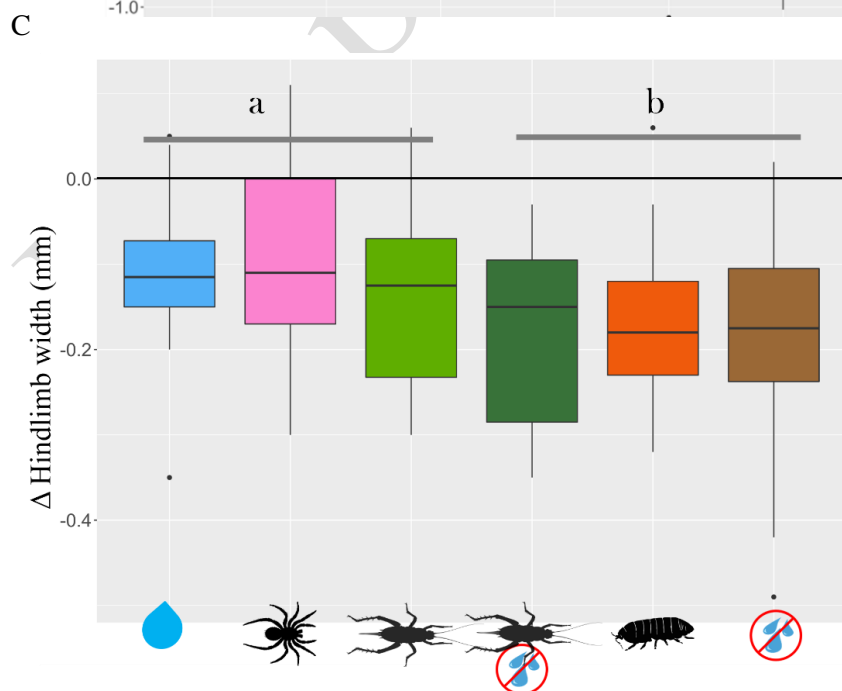
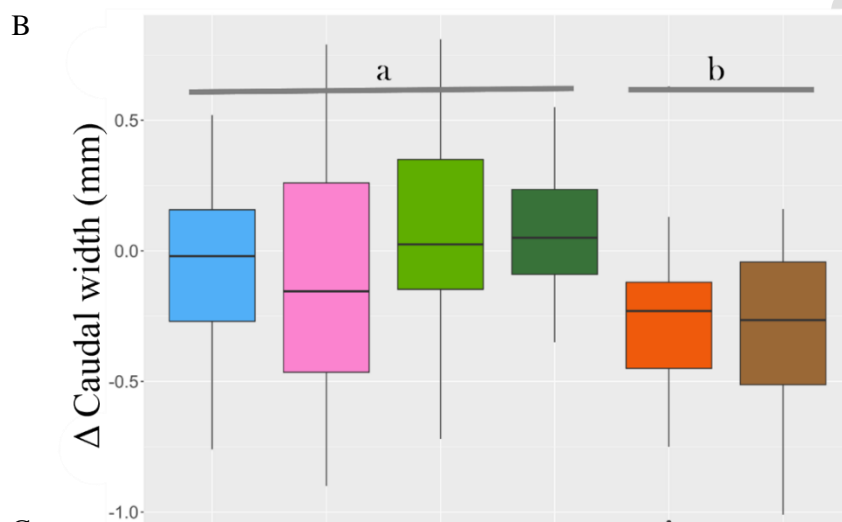
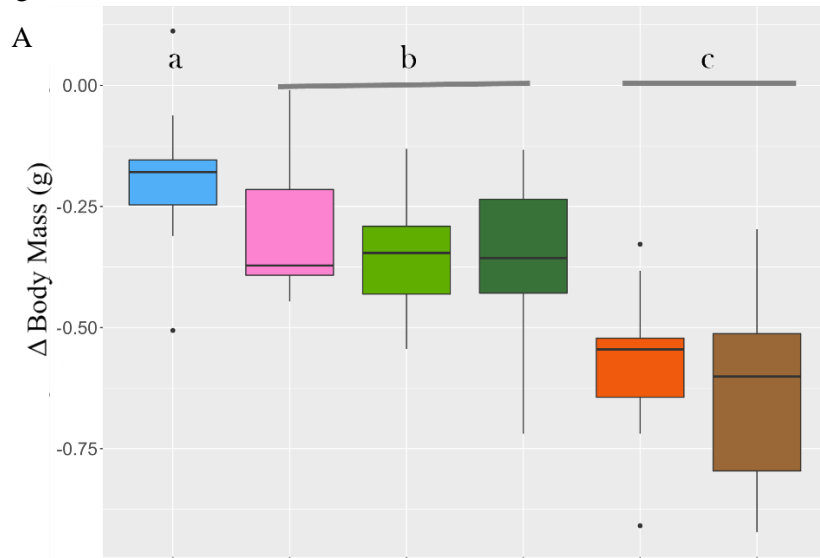


Figure 3

