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Chloé Chabaud, George Brusch, Anouk Pellerin, Olivier Lourdais, 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-04189871

Submitted on 14 Nov 2023  $\,$ 

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

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

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#### 21 Abstract

22 To cope with **limited availability of drinking water** in their environment, terrestrial animals have developed numerous behavioral and physiological strategies including maintaining an 23 24 optimal hydration state through dietary water intake. Recent studies performed in snakes, which are carnivorous reptiles with a specialized diet, suggest that benefits of dietary water 25 26 intake are negated by hydric costs of digestion. Most lizards are generalist insectivores that 27 can shift their prev types, but firm experimental demonstration of dietary water intake is currently missing in these organisms. Here, we performed an experimental study in the 28 common lizard Zootoca vivipara, a keystone mesopredator from temperate climates 29 30 exhibiting a great diversity of prey in its mesic habitats, in order to investigate the effects of food consumption and prey type on physiological responses to water deprivation. Our results 31 indicate that common lizards cannot improve their hydration state through prey consumption, 32 33 irrespective of prey type, suggesting that they are primarily dependent upon drinking water. Yet, high-quality prey consumption reduced the energetic costs of water deprivation, 34 35 potentially helping lizards to conserve a better body condition during periods of limited water availability. These findings have important implications for understanding the physiological 36 responses of ectotherms to water stress, and highlight the complex interactions between 37 38 hydration status, energy metabolism, and feeding behavior in insectivorous lizards. 39

Keywords: physiology - lizards – water balance – dietary water – metabolites - reptiles
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#### 42 **1. Introduction**

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

A strong dependence of water budgets on dietary water intake has been shown in some animals living in semi-arid or arid environments characterized by low annual rainfall, high ambient temperatures, and recurrent drought episodes (Nagy, 2004). For example, many herbivorous desert vertebrates feed on plant tissues or seeds to sustain their standard activity 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 advantage in these environments and some species display adaptive shifts in food preferences 69 70 toward resources with higher water content during the dry season (Karasov, 1983). However, recent studies in carnivorous squamate reptiles (i.e., lizards, snakes, and amphisbaenians), 71 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; Lillywhite, 2017). Even in arid environments, these species do not receive net hydric benefits 75 76 from their diet (Murphy & DeNardo, 2019). Indeed, fluid secretion is required during digestion to aid in transport and absorption of nutrients, and this hydric cost can be 77 particularly important when carnivorous reptiles are already dehydrated (Murphy & DeNardo, 78 79 2019). Feeding may also involve behavioral changes that increase water loss rates, such as locomotor activity during foraging and basking during digestion, and trade-offs can occur 80 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 the hydric cost of digestion and reduce opportunities for dietary shifts toward prey with higher 83 84 water content (Lillywhite, 2017).

Currently, there is limited knowledge about dietary water intake and hydric costs of digestion in insectivorous reptiles, which include the vast majority of lizards (see Table 1; Meiri, 2018). In addition, it remains unknown if inferences drawn from studies of arid adapted species apply to species from moister tropical or temperate zones, where free-standing water is more regularly available. Most insectivorous lizards are generalist mesopredators with a non-intermittent diet dominated by invertebrates such as insects, spiders, or other arthropods (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 on prey availability and resource expenditures across the activity season or between different 94 95 life stages (Bestion et al., 2019). Previous work has focused on the energetic quality of food and its adequacy to optimal diet models based on energetic constraints (Stamps, Tanaka, & 96 97 Krishnan, 1981; Dearing & Schall, 1992), but very few studies have investigated dietary shifts due to water constraints in reptiles (but see Mautz & Nagy, 1987). Yet, when prey have 98 99 different water and protein content or different digestibility, this may influence the dietary water intake, leading to dietary shifts caused by water balance instead of energy balance. For 100 101 example, if free-standing water is limited in the environment, insectivorous lizards might shift their diet towards high-water content prey to maintain water balance, which can impact the 102 103 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 lizards should include the possibility of different prey types that are representative of their 105 106 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 water, as previously demonstrated in birds, lizards, and snakes (Gerson & Guglielmo, 2011; 110 111 Brusch 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 Brusch 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 food might be important to recoup the energetic and structural costs of water deprivation. 115 116 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 distribution across Eurasia, which is dependent on cool and moist environments inside humid 119 120 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 suggested that the species is dependent on free-standing water to maintain water balance 123 124 (Grenot et al., 1987; Lorenzon et al., 1999; Rozen-Rechels et al., 2020). Additionally, water deprivation causes a range of systemic responses associated with a reduction of body mass, 125 126 proximal tail width loss (where lipids are stored, Chapple & Swain, 2002), and limb thickness reduction (due to muscle wasting, Brusch et al., 2018). However, no previous study has 127 investigated the link between diet, water balance, and body condition. 128

129 We tested for the occurrence of dietary water intake by comparing changes in plasma osmolality of mildly dehydrated lizards following the consumption of three distinct types of 130 131 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., 2015). These lizards were fed but had no access to free-standing water during the 6 days of 133 134 the experimental treatment in a temperature-controlled room. They were compared to two groups of unfed lizards: one "hydrated control" group with no food but ad libitum access to 135 free-standing water and one "dehydrated control" group without food or water. Using this 136 137 experimental design, we tested the following three hypotheses:

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

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146 hypothesis one to two.

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#### 148 **2. Material and methods**

#### 149 <u>2.1. Study species, sampling, and acclimation</u>

150 The common lizard Zootoca vivipara is a small lacertid lizard (Reptilia: Lacertidae) with a wide Eurosiberian distribution (Surget-Groba et al., 2006). It inhabits cold and wet habitats, 151 such as humid grasslands and bogs, and is highly sensitive to water deprivation due to high 152 standard water loss rates (Lorenzon et al., 1999; Massot et al., 2002; Dupoué et al., 2017). 153 This lizard is a mesopredator in its food web with a diet of various invertebrates (hexapods, 154 spiders, isopods, annelids) ranging in size from 1 to 9 mm (Avery, 1996), which includes 155 156 decomposers, herbivorous, and predatory species (Bestion et al., 2019). Common lizards used in this study were captured in semi-natural populations maintained in 25 outdoor enclosures in 157 158 CEREEP-Ecotron IleDeFrance in Saint Pierre lès Nemours, France (48°17'N, 2°41'E). Experiments were carried out in accordance with institutional guidelines, under license from 159 the French Ministry of the Environment (permit APAFIS#25281-2020042915424095). 160 We captured 128 lizards by hand in June 2020 and brought them back to a laboratory 161 located nearby. We selected only sexually mature males based on external morphology, body 162 size, and known age from previous population monitoring. Upon capture, one person 163 measured snout-vent length (SVL, mean=59.3  $\pm$  6.5 mm SD) and total length (TL) to the 164 nearest millimeter with a plastic ruler as well as body mass (BM) to the nearest milligram 165

using an electronic balance (mean= $4.19 \pm 1.62$  g SD). Animals were then kept separated in 166 167 individual terraria (18×11×12 cm) with sterilized peat substrate, a basking rock, a shelter buried in the substrate and a water cup with *ad libitum* access to water. During captivity, we 168 169 provided all lizards with the same thermal conditions in a temperature-controlled room (23°C for 9h per day 09:00-18:00, and 16°C at night) in addition to heating lamp and UV above their 170 171 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 times a day during acclimation. 173

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

187 <u>2.2. Experimental design</u>

In order to investigate the effects of food consumption on water and energy balance in mildly dehydrated lizards, we first exposed all lizards to a moderate water restriction for 10 days (Fig 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  $10^{th}$  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 µl 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 trophic roles. First, we provided some lizards with crickets Acheta domesticus, an herbivorous 199 insect that can be purchased at reptile breeding shops. Crickets were kept with dry food and 200 with or without water in a climatic chamber at 25°C based on earlier studies showing that 201 such water restriction conditions can trigger dehydration (McCluney & Date, 2008). We also 202 203 wanted to compare a same type of prey but with different water content, in order to test the quality of prey in addition to prey type. To do so, we provided lizards either with "hydrated" 204 205 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 including Armadillum vulgare, Porcellio scaber, Philloscia muscorum and Oniscus asellus). 207 208 Woodlice are detritivorous isopods and were collected in the same outdoor enclosures as the lizards. They were kept in a dark, humid box with leaf and compost until being provided to 209 the lizards. Third, we provided some lizards with spiders (several species of Lycosidae or 210 211 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 are different in their macronutrient components (proteins, carbohydrates and lipids, see 214 Reeves et al., 2021) and differences in those ratios (spiders having higher protein: lipid ratio 215

than crickets and woodlice) can inform us about the hydric cost of digestion depending onprey type.

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

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

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#### 236 <u>2.3. Parameters considered and plasma assays</u>:

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

associated behavioral changes) has a non-negligible hydric cost (Wright et al., 2013). We also 241 242 tracked plasma circulating metabolites associated with catabolism and fasting in reptiles. We considered glucose which is liberated in the liver during fasting from stored glycogen, 243 244 triglycerides stored in adipose tissue or neoglucogenesis, and also ketone bodies produced by the breakdown of fatty acids in the liver during periods of food restriction (McCue, 245 246 Lillywhite, & Beaupre, 2012; Price *et al.*, 2013). In particular, the brain, unlike other organs, can only use glucose and ketone bodies to function, which makes them particularly essential 247 during food restriction (Price, 2017). We also monitored morphology over time (tail and leg 248 width) as indicators of structural mobilization. We measured the body mass of lizards every 249 250 two days, and we weighed any remaining food in the terrarium to assess how much was eaten by each individual (Table 2). On the 7<sup>th</sup> day, we measured body mass, leg and muscle width 251 (T2 measurement), and collected a blood sample to measure plasma osmolality and 252 253 metabolites. All lizards were then removed from the experiment, fed and provided water ad *libitum* for 2-3 days, and released into the outdoor enclosures. 254 255 First, plasma osmolality was determined using a vapor pressure osmometer (Vapro

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

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

#### 283 <u>2.4. Statistical analyses</u>

All statistical analyses were performed using R statistical software (version 3.6.3; R Core Team 2020). First, we checked plasma osmolality values during the first phase of our manipulation to verify that lizards were mildly dehydrated and dehydration was similar among treatment groups. This test was performed with an analysis of variance of the intraindividual change in plasma osmolality from T0 to T1. There was no difference among groups ( $F_{5,120}$ =0.89, p=0.49) since all lizards had similar increases in plasma osmolality (mean ± SE: 41.08 ± 3.86 mOsm kg<sup>-1</sup>). 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 difference between T1 and T2 (referred to as  $\Delta$ variable) and included the fixed effects of 293 294 treatment groups and initial value of the response variable in a linear regression model fitted with the *lm* procedure. Prior to statistical tests, we used diagnostic plots of residuals to verify 295 296 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 performed using the *multcomp* package (Hothorn *et al.*, 2016). All results are reported as 298 means  $\pm$  SE unless otherwise stated. As we noticed a difference in the amount of food 299 300 consumed among treatment groups with access to food ( $F_{3,83}=10.22$ , p<0.0001), we conducted a second set of analyses with fed lizards using the total amount of food consumed during the 301 302 manipulation period as a covariate.

#### 303 **3. Results**

#### 304 <u>3.1. Dehydration and water balance</u>

305 Treatment groups had a significant effect on  $\Delta$ osmolality (F<sub>5,120</sub>=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 <u>3.2. Morphological changes</u>
- 313 Mean initial body mass was  $3.81 \pm 0.05$  g with no difference among treatment groups
- 314 (F<sub>5,123</sub>=0.64, p=0.67), but body mass change between T1 and T2 ( $\Delta$ BM) differed strongly
- 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 dehydrated control (p<0.0001). Of the fed lizards, those provided with woodlice lost the most 318 319 body mass and their body mass loss was not statistically different than in lizards deprived of free-standing water and food (p=0.29; Fig 2A). With regard to tail width, it did not change on 320 321 average in hydrated control lizards or those provided with spiders or crickets (contrast: p>0.3; Fig 2B), whereas a significant and similar reduction in tail width was observed in the 322 dehydrated control and lizards fed with woodlice (respectively, contrasts:  $\beta$ =-0.24, p=0.01 and 323  $\beta$ =-0.26, p=0.02; Fig 2B). At the same time, hindlimb width decreased on average in all 324 325 lizards (mean  $\Delta$ hindlimb=-0.15  $\pm$  0.009 mm, Fig 2C). Hindlimb width loss was similar and low in hydrated control lizards and those provided with spiders or hydrated cricket (Tukey 326 *post-hoc* tests : all p>0.2, Fig 2C). Meanwhile,  $\Delta$ hindlimb width was **greatest** in lizards from 327 328 dehydrated control and in lizards provided with woodlice and dehydrated crickets (Tukey *post-hoc* tests : p<0.05). The amount of food consumed by each lizard did not correlate with 329 330 the morphological changes ( $\Delta$ BM F<sub>1,82</sub>=0.66, p=0.42;  $\Delta$ tail F<sub>1,76</sub>=1.65, p=0.21;  $\Delta$ hindlimb 331 F<sub>1.80</sub>=0.0004, p=0.98).

332 <u>3.3. Plasma metabolites</u>

333 There was a strong effect of food treatment on triglyceride concentration changes in plasma ( $F_{5.88}$ =37.25, p<0.0001; Fig 3A), as well as an effect of initial concentration ( $F_{1.88}$ =111.20, 334 p<0.0001). Post-hoc analyses indicated three different treatment group categories. First, 335 336 triglyceride concentrations on average decreased slightly in control lizards with no food irrespective of water availability. Second, triglyceride concentrations did not change on 337 338 average in lizards provided with woodlice. Third, lizards fed with hydrated or dehydrated crickets and spiders had higher concentrations of plasma triglycerides (respectively, contrasts: 339  $\beta$ =420.8,  $\beta$ =413.4 and  $\beta$ =286.5, all p<0.0001; Fig 3A) compared to the other groups. Across 340

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  $\beta$ -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 concentration:  $F_{1,118}=2.69$ , p<0.0001). Instead, intra-individual change of glucose 345 concentration was highly variable and differed among treatment groups ( $F_{5,73}$ =4.04, 346 p=0.0027; F<sub>1.73</sub>=5.99, p=0.017; Fig 3B). In the dehydrated control treatment, lizard glucose 347 concentrations increased (estimate= $67.83 \pm 18.94$ , p=0.0006) above the levels observed in the 348 hydrated control treatment, whereas lizards provided with food had intermediate values. We 349 found no effect of food consumption on glucose concentration changes ( $F_{1,45}=0.01$ , p=0.92). 350

351

#### 352 4. Discussion

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

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

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

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. Indeed, hunting for prey should have a much higher energetic and hydric cost in natural 395 396 environments than in captivity (Anderson & Karasov, 1981). Therefore, complementary studies are needed to quantify the hydric costs of digestion and the behavioral trade-offs 397 between feeding and hydration. 398

Despite no evidence for the hypothesis that water can be extracted from food from our 399 400 data on plasma osmolality, a relevant metric of whole-organism hydration status (Capehart et al., 2016), we found that food consumption can mitigate the structural and energetic costs 401 402 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 Brusch et al., 2018) and lipid catabolism from the reserves stored in the tail (Price, 2017). We 404 405 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 forage actively on a wide diversity of invertebrate species including larvae and adult spiders, 409 410 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 because these prey are less water and energy-rich (Table 2) and are less easy to handle and 414 digest, making them distasteful for lizards (see Pekár, Líznarová, & Řezáč, 2016). The results 415

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

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

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

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

- 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
- 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. Brusch: Conceptualization, Methodology, Writing-
- 503 Review&Editing ; Anouk Pellerin: Investigation ; Olivier Lourdais: 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.

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

| Agama impalearis        | Insectivorous | Xeric     | Indirect test. Field measures of water influx rates suggest that water is<br>mostly provided from diet (termites) during breeding season. Lizards<br>apparently did not drink water.   | Znari & Nagy, 1997                     |
|-------------------------|---------------|-----------|--|--|
| Callisaurus draconaides | Insectivorous | Semi-arid | Direct test. Food consumption allows to recoup the body mass loss<br>induced by a chronic water deprivation experiment in the laboratory.<br>Osmolality and body hydration were not measured.  | Munsey, 1972                           |
| Cnemidophorus tigris    | 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                           |
| Sceloporus occidentalis | Insectivorous | Mesic     | Direct test. Food consumption allows to recoup part of the body mass loss<br>induced by a chronic water deprivation experiment in the laboratory.<br>Osmolality and body hydration were not measured.  | Munsey, 1972                           |
| Uma scoparia            | 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 &<br>Shoemaker, 1972           |
| Uma scoparia            | Omnivorous    | Semi-arid | Direct test. Food consumption (insects) allows to recoup part of the body<br>mass loss induced by a chronic water deprivation experiment in the<br>laboratory. Osmolality and body hydration was not measured.   | Munsey, 1972                           |
| Varanus caudolineatus   | Omnivorous    | Semi-Arid | Indirect test. Field and laboratory measures of water influx rates suggest<br>that water is importantly provided from diet. Free-standing water<br>consumption unknown.  | Thompson, Bradshaw,<br>& Withers, 1997 |
|                         | g f           |           |  |  |

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          | $75.25 \pm 3.99$ %           | $72.24 \pm 2.27$ %  | 73.27 ± 4.83 %               | 65.58 ± 1.74 % ***                      |
|                        |                              |                     |                              |   |
|                        | N=20                         | N=20                | N=20                         | N=20                                    |
|                        |                              |                     |                              |   |
| Energy content         | $6.23 \pm 0.29 \text{ kJ/g}$ | 6.52 kJ/g           | $6.34 \pm 0.51 \text{ kJ/g}$ | $3.92 \pm 0.11$ kJ/g ***                |
|                        |                              |                     |                              | , i i i i i i i i i i i i i i i i i i i |
|                        | N=4                          | N=1                 | N=2                          | N=3                                     |
|                        |                              |                     | 1                            |   |
| Quantity of food eaten | $0.98 \pm 0.23$ g            | $1.02 \pm 0.14$ g   | $1.01 \pm 0.15$ g            | 0.78 ± 0.08 g ***                       |
|                        |                              |                     |                              |   |
|                        | N=21                         | N=22                | N=21                         | N=21                                    |
|                        |                              |                     |                              |   |

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#### **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  $\Delta$ 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  $\Delta$ 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 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.

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## Figure1

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