RESEARCH ARTICLE

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

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ABSTRACT

To cope with limited availability of drinking water in their environment, terrestrial animals have developed numerous behavioral and physiological strategies including maintaining an optimal hydration state through dietary water intake. Recent studies performed in snakes, which are generalist carnivorous reptiles, suggest that the benefits of dietary water intake are negated by hydric costs of digestion. Most lizards are generalist insectivores that can shift their prey types, but firm experimental demonstration of dietary water intake is currently missing in these organisms. Here, we performed an experimental study in the common lizard *Zootoca vivipara*, a keystone mesopredator from temperate climates 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 indicate that common lizards cannot improve their hydration state through prey consumption, 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, potentially helping lizards to conserve a better body condition during periods of limited water availability. These findings have important implications for understanding the physiological responses of ectotherms to water stress, and highlight the complex interactions between hydration status, energy metabolism and feeding behavior in insectivorous lizards.

KEY WORDS: Physiology, Lizards, Water balance, Dietary water, Metabolites, Reptiles

INTRODUCTION

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 water sources in wild animals. Optimal hydration state can be maintained by drinking free-standing water (Karasov and del Rio, 2007), by food consumption (dietary water intake; Nagy and Knight, 1994) or cutaneous drinking (e.g. Jørgensen, 1994), or by internal metabolic production (metabolic water; Bailey, 1923; Jindra and Sehnal,

1990). Free-standing water is the most widespread, primary source of water intake in animals, but it is not continuously available in many terrestrial ecosystems and most organisms have developed strategies to cope with limited water availability. For example, water requirements can be met through dietary shifts (Leinbach et al., 2019), utilizing internal water reservoirs (e.g. urinary bladder; Davis and DeNardo, 2007) or even harvesting water from fog (Seely, 1979). Conversely, water loss can be minimized through behavioral changes in activity patterns (Bissonette, 1978) or microhabitat use (Dupoué et al., 2015; Dezetter et al., 2022), but also through internal osmoregulatory strategies (e.g. nitrogen excretion via increased uric acid production; Braun, 1999). Finally, some species can tolerate hyperosmolality and deviate from homeostatic set-points to cope with water constraints (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 investigated in non-arid-adapted species.

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 (mammals: Nagy and Gruchacz, 1994; birds: Tieleman and Williams, 2000; turtles: Nagy and Medica, 1986; lizards: Minnich and 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 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) suggest that they do not rely on dietary water intake because of the lower water content of their prey compared with plant material, and the significant protein content that increases the hydric cost of digestion and leads to more nitrogenous waste (Wright et al., 2013; Lillywhite, 2017). Even in arid environments, these species do not receive net hydric benefits from their diet (Murphy and DeNardo, 2019). Indeed, fluid secretion is required during digestion to aid in the transport and absorption of nutrients, and this hydric cost can be particularly important when carnivorous reptiles are already dehydrated (Murphy and DeNardo, 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 between food acquisition and water loss (Gregory et al., 1999). In addition, 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 water content (Lillywhite, 2017).

Currently, there is limited knowledge about dietary water intake and hydric costs of digestion in insectivorous reptiles, which include

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the vast majority of lizards (see Table 1; Meiri, 2018). In addition, it remains unknown whether 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 and macro- and micro-nutrient content, including water content (McCluney and Date, 2008). 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 life stages (Bestion et al., 2019). Previous work has focused on the energetic quality of food and its adequacy in relation to optimal diet models based on energetic constraints (Stamps et al., 1981; Dearing and Schall, 1992), but very few studies have investigated dietary shifts due to water constraints in reptiles (but see Mautz and Nagy, 1987). Yet, when prey have different water and protein content or different digestibility, this may influence dietary water intake, leading to dietary shifts caused by water balance instead of energy balance. For 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 population dynamics and trophic ecology of these prey species (Bestion et al., 2015). Thus, an 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 standard diet. To our knowledge, no study has examined whether different prey types are more or less effective at maintaining water balance in these organisms.

Water deprivation induces multiple physiological responses to restore hydration state, including lipolysis, reduced hepatic acid synthesis, or protein catabolism to release bound water, as previously demonstrated in birds, lizards and snakes (Gerson and Guglielmo, 2011; Brusch et al., 2018, 2020). These physiological responses are known to induce energy and structural costs with reduced body reserves and muscle condition (Dezetter et al., 2021; Brusch et al., 2022). Therefore, besides the direct effect of diet on the water budget, we need to consider the whole-organism metabolic response according to different prey types, as food might be important to recoup the energetic and structural costs of water deprivation; for example, through metabolic water or bound water released during the breakdown of fat or protein structures (McCue et al., 2017). Here, we designed a laboratory experiment to test whether consumption of different prey types can compensate for water deprivation. We investigated these responses in the common lizard, *Zootoca vivipara*, a generalist mesopredator with a widespread distribution across Eurasia, which is dependent on cool and moist environments inside humid grasslands and bogs. In these natural habitats, the common lizard is a keystone mesopredator because of its great diversity of invertebrate prey and its abundance (densities as high as 1000 lizards per hectare; 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 (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, proximal tail width loss (where lipids are stored; Chapple and Swain, 2002) and limb thickness reduction (due to muscle wasting; Brusch et al., 2018). However, no previous study has investigated the link between diet, water balance and body condition.

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 invertebrate prey with contrasted nutrient and water content (crickets, spiders and woodlice). 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 the experimental treatment in a temperature-controlled room. They were compared with two groups of unfed lizards: one 'hydrated control' group with no food but *ad libitum* access to free-standing water and one 'dehydrated control' group without food or water. Using this experimental design, we tested the following three hypotheses. If lizards are able to extract dietary water resulting in a net water gain, we predict that lizards held with food would have responses closer to those of hydrated versus dehydrated control lizards. Alternatively, if eating imposes a net hydric cost, lizards held with food would exhibit responses closer to those of dehydrated rather than hydrated control lizards. Finally, prey characteristics should have an impact on the response; notably, digestively 'costly' prey (low energy content and hard exoskeleton) such as woodlice should provide limited energy and water benefits, and could induce a shift from hypothesis one to two.

MATERIALS AND METHODS
Study species, sampling and acclimation
The common lizard, *Zootoca vivipara* (Lichtenstein 1823), is a small lacertid lizard (Reptilia: Lacertidae) with a wide Eurosiberian distribution (Surget-Groba et al., 2006). It inhabits cold and wet habitats, such as humid grasslands and bogs, and is highly sensitive to water deprivation because of its high standard water loss rates (Lorenzon et al., 1999; Massot et al., 2002; Dupoué et al., 2017). This lizard is a mesopredator in its food web with a diet of various invertebrates (hexapods, spiders, isopods, annelids) ranging in size from 1 to 9 mm (Avery, 1966), which includes decomposer, 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 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 the French Ministry of the Environment (permit APAFIS#25281-2020042915424095).

We captured 128 lizards by hand in June 2020 and brought them back to a laboratory located nearby. We selected only sexually mature males based on external morphology, body size and known age from previous population monitoring. Upon lizard capture, one person measured snout–vent length (SVL, mean±s.d. 59.3 ±6.5 mm) and total length (TL) to the nearest millimeter with a plastic ruler as well as body mass (BM) to the nearest milligram using an electronic balance (mean±s.d. 4.19±1.62 g). Animals were then kept separated in 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, all lizards were provided with the same thermal conditions in a temperature-controlled room (23°C for 9 h per day 09:00–18:00 h, and 16°C at night) in addition to a heating lamp and UV source above their terrarium during daytime (33°C below the heating lamp). Lizards were fed with live crickets (*Acheta domesticus*) every 2 days and had water sprayed on the sides of their cages 3 times a day during acclimation.

After 1 week of acclimation, we collected morphology data of hydrated and fed lizards (referred to as T0 measurements). During the same day, they were weighed with an electronic balance and

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

Species	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 et al., 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 and 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 et al., 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 mostly provided from diet during the summer but not in other seasons. Free-standing water consumption unknown.	Green et al., 1991
Angolosaurus skoogi	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 et al., 1991
Dipsosaurus dorsalis	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 and Shoemaker, 1970
Dipsosaurus dorsalis	Herbivorous	Xeric	Direct test. Food consumption allows lizards 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
Agama impalearis	Insectivorous	Xeric	Indirect test. Field measures of water influx rates suggest that water is mostly provided from diet (termites) during the breeding season. Lizards apparently did not drink water.	Znari and Nagy, 1997
Callisaurus draconaides	Insectivorous	Semi-arid	Direct test. Food consumption allows lizards 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
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 lizards 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
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 and Shoemaker, 1972
Uma scoparia	Omnivorous	Semi-arid	Direct test. Food consumption (insects) allows lizards 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
Varanus caudolineatus	Omnivorous	Semi-arid	Indirect test. Field and laboratory measures of water influx rates suggest that water is significantly provided from diet. Free-standing water consumption unknown.	Thompson et al., 1997

photographed with a digital scanner (ventral view, CanoScan LiDE 700F, Canon) to measure tail width, a trait linked to fat and muscle storage in the tail. Tail width was measured at the 7th and 10th subcaudal scales by processing the digital images with the software ImageJ (NIH, Bethesda, MD, USA). We also assessed the transverse muscle thickness of the right hindleg at the mid-region of the upper limb with digital calipers (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 the whole study. We also took a blood sample from the post-orbital sinus using 1–2 micro-capillary tubes (ca. 20–40 µl whole blood). In the laboratory, samples were centrifuged at 11,000 rpm for 5 min to separate plasma from red blood cells. Plasma samples (~10 µl) were immediately frozen at −28°C until osmolality assays (see 'Parameters considered and plasma assays', below).

Experimental design
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
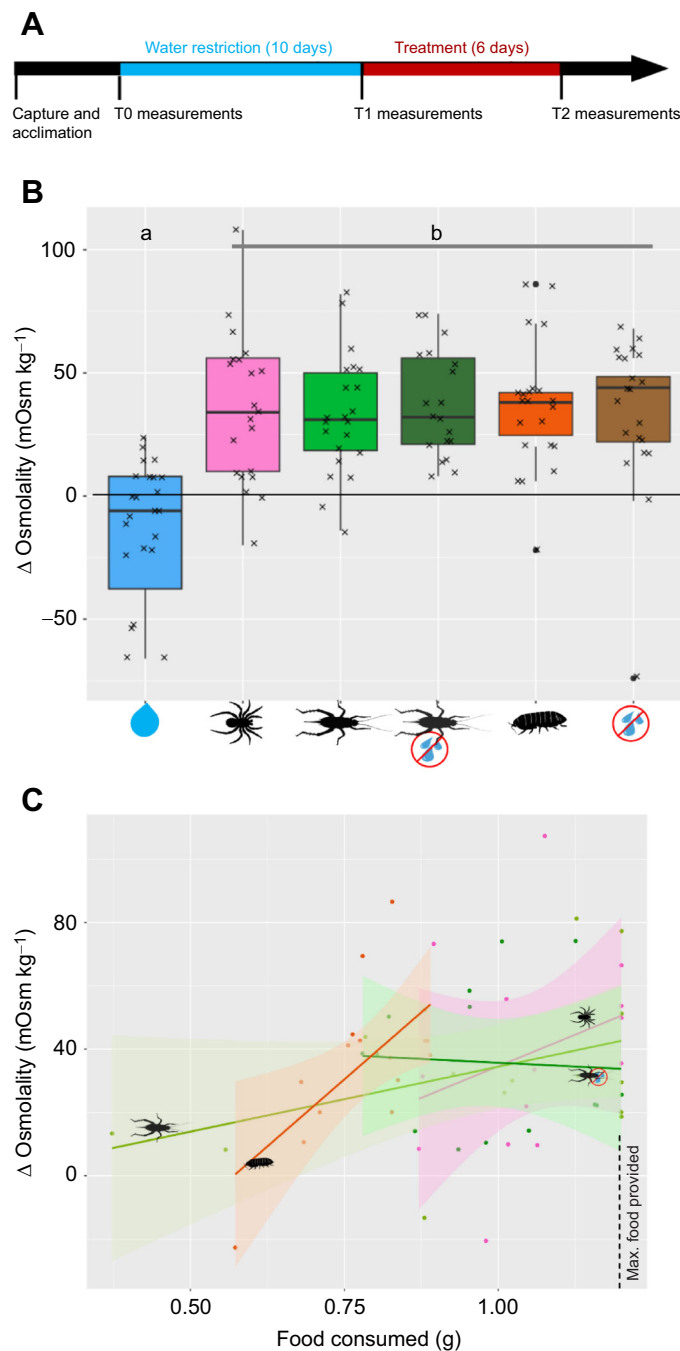
Fig. 1. Experimental design and changes in plasma osmolality.
(A) Timeline of the experimental design and of measurements performed between the start and the end of the manipulation in the six different treatment groups: hydrated control (water only); food only (one prey type among spiders, hydrated crickets, dehydrated crickets and woodlice); dehydrated control (no water, no food). (B) Boxplots of the change in osmolality. The lower and upper hinges correspond to the first and third quartiles, the middle bar is the median, whiskers are 1.5× the interquartile range and circles are outliers. Treatment groups are (from left to right): hydrated control, spiders, hydrated crickets, dehydrated crickets, woodlice and dehydrated control (N=21 per group). Significant differences between groups are indicated by different lowercase letters, based on *post hoc* tests with Tukey correction. (C) Scatterplot of the change in osmolality against the amount of food consumed (P=0.05) for each treatment group with access to prey. Data shown are raw values and linear smooths for each group, with shading indicating the 95% confidence interval.

to once every 2 days while feeding lizards every 2 days. Water spray only provided lizards with a few milliliters of water, which could be ingested by licking, but that dried in less than 30 min (no water at all during 6 days is fatal for adults in this species). At the end of the 10th day, we weighed lizards and measured tail and muscle width as indicated above (referred to as T1 measurements). We collected blood samples using 2–3 micro-capillary tubes (ca. 30–40 µl whole blood) and stored plasma samples as indicated above until osmolality and metabolite assays (see 'Parameters considered and plasma assays', below).

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*, a herbivorous insect that can be purchased at reptile breeding shops. Crickets were kept with dry

food, with or without water, in a climatic chamber at 25°C based on earlier studies showing that such water restriction conditions can trigger dehydration (McCluney and Date, 2008). We also wanted to compare the 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' crickets (held with access to water) or 'dehydrated' crickets (held for at least a week without access to water). Second, we provided other lizards with woodlice (several species including *Armadillum vulgare*, *Porcellio scaber*, *Philloscia muscorum* and *Oniscus asellus*). Woodlice are detritivorous isopods and were collected in the same outdoor enclosures as the lizards. They were kept in a dark, humid box with leaves and compost until being provided to the lizards. Third, we provided some lizards with spiders (several species of Lycosidae or

wolf spiders), which are carnivorous invertebrates. All arachnids were collected by hand in the nearby forest plot as well as in outdoor enclosures the day before feeding the lizards and 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 Reeves et al., 2021) and the differences in these ratios (spiders having higher protein:lipid ratio than crickets and woodlice) can inform us about the hydric cost of digestion depending on prey type.

To measure prey water content, invertebrates were first weighed alive individually with a high-precision scale (±0.01 mg). Then, they were killed by placing them in the freezer at −20°C for a few hours, before being dried in a laboratory oven at 60°C for 24 h and weighed again to determine dry mass. Water content was calculated as the difference between wet mass and dry mass. Despite the absence of a statistically significant difference in cricket water content (Table 2), we chose to maintain the two groups as distinct treatments. In addition, energy content was determined with dry aliquots (0.5–1.1 g) by using an isoperibol bomb 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 similar to each other (Table 2).

We separated lizards into six different treatments groups for 6 days (Fig. 1A) including a 'hydrated control' where lizards had access to *ad libitum* water but no food; four groups of lizards that did not have access to *ad libitum* water but were provided with 200 mg per day of one of the following: hydrated crickets, dehydrated crickets, woodlice or spiders; and a 'dehydrated control' group where lizard did not have access to *ad libitum* water or food. 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).

Parameters considered and plasma assays

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 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, triglycerides stored in adipose tissue or gluconeogenesis, and also ketone bodies produced by the breakdown of fatty acids in the liver during periods of food restriction (McCue et al., 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 during food

restriction (Price, 2017). We also monitored morphology over time (tail and leg width) as indicators of structural mobilization. We measured the body mass of lizards every 2 days, and we weighed any remaining food in the terrarium to assess how much was eaten by each individual (Table 2). On the 7th day, we measured body mass, leg and muscle width (T2 measurement), and collected a blood sample to measure plasma osmolality and metabolites. All lizards were then removed from the experiment, fed and provided with water *ad libitum* for 2–3 days, and released into the outdoor enclosures.

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 plasma volumes (Dupoué et al., 2017). Before analyses, plasma was diluted (1:3) in standard saline solution (280 mOsm kg^{-1}) to obtain duplicates for each sample (coefficient of variation, CV<0.4%). Normosmic plasma osmolality values (280–320 mOsm kg^{-1}) indicate that lizards are hydrated, whereas hyperosmotic values (>350 mOsm kg^{-1}) indicate that lizards are moderately to sub-clinically dehydrated (Dupoué et al., 2018). Second, we measured plasma concentrations of the ketone body β-hydroxybutyrate (β-HB) with a colorimetric method (β-Hydroxybutyrate Ketone Body Colorimetric Assay Kit, 700190, Cayman Chemical, Ann Arbor, MI, USA). β-HB is a carbohydrate that is produced in the liver as a byproduct of the catabolism of fatty acid β-oxidation during fasting periods in a process known as ketogenesis. β-HB can be used as an energy source for the brain during periods of starvation and thus provides information on nutritional status (McCue, 2008; McCue et al., 2012). Here, plasma samples were diluted 1:8 (using 8 µl of plasma) in the dilution buffer of the assay kit. Absorbance was measured at 450 nm and then converted to concentration values using the dilution range provided by the kit. We performed duplicates on a few samples to measure the coefficient of variation within plates (CV$_{within}$=7%) and between plates (CV$_{between}$=11%) Third, we measured levels of circulating triglycerides in plasma by a colorimetric assay (Triglyceride Colorimetric Assay kit, 10010303, Cayman Chemical) using 2.5 µl of plasma diluted 1:4 in the provided dilution buffer. This method quantifies the total triglycerides using a chain of three enzymatic reactions resulting in hydrogen peroxide production, which is converted into quinoneimine dye quantified by absorbance at 540 nm (CV$_{within}$=12%, CV$_{between}$=13%). Finally, we measured glucose concentration in the plasma with a colorimetric assay (Glucose Colorimetric Assay Kit, 10009582, Cayman Chemical). We used 1.5 µl of plasma diluted 1:13 in the provided dilution buffer as the glucose concentrations were very high compared with kit sensitivity. The three chemical reactions in the glucose assay lead to the production of a pink dye, which was quantified by absorbance at 500 nm (CV$_{within}$=4%, CV$_{between}$>30%, so we only compared samples measured on the same plate).

Table 2. Water content and nutritional quality of invertebrates used in the experiment

	Hydrated crickets	Dehydrated crickets	Spiders	Woodlice
Water content (%)	75.25±3.99 N=20	72.24±2.27 N=20	73.27±4.83 N=20	65.58±1.74* N=20
Energy content (kJ g^{-1})	6.23±0.29 N=4	6.52 N=1	6.34±0.51 N=2	3.92±0.11* N=3
Quantity of food eaten (g)	0.98±0.23 N=21	1.02±0.14 N=22	1.01±0.15 N=21	0.78±0.08* N=21

Data are means±s.d.; N refers to the number of individual prey for water content, the number of pools used for energy content (where each pool contains tens of individuals) and the 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. Asterisks indicate that the group is significantly different from the others (*P<0.05).

Statistical analyses

All statistical analyses were performed using R statistical software (version 3.6.3; http://www.R-project.org/). 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 intra-individual change in plasma osmolality from T0 to T1. There was no difference among groups ($F_{5,120}$=0.89, P=0.49) as all lizards had similar increases in plasma osmolality (mean±s.e.m. 41.08±3.86 mOsm kg^{-1}). Then, to assess the effects of treatments on morphology, plasma osmolality and metabolites over the course of the experiment, we performed independent linear models for each response variable. We calculated intra-individual changes as the difference between T1 and T2 measurements and included the fixed effects of 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 the model assumptions with regard to normality and homogeneity of variance of residuals (Heumann et al., 2016). *Post hoc* tests of differences among groups were performed using the *multcomp* package (Hothorn et al., 2016). All results are reported as means ±s.e.m. unless otherwise stated. As we noticed a difference in the amount of food 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 manipulation period as a covariate.

RESULTS

Dehydration and water balance

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

Morphological changes

Mean initial body mass was 3.81±0.05 g with no difference among treatment groups ($F_{5,123}$=0.64, P=0.67), but the change in body mass between T1 and T2 differed strongly among treatment groups ($F_{5,128}$=34.23, P<0.0001; Fig. 2A). Lizards in the hydrated control group lost less body mass than those in other treatment groups (Tukey *post hoc* tests, all P<0.005). Lizards 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 body mass and their body mass loss was not statistically different from that of lizards deprived of free-standing water and food (P=0.29; Fig. 2A). Tail width did not differ between hydrated control lizards and 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 dehydrated control and lizards fed with woodlice (respectively, contrasts: β=−0.24, P=0.01 and β=−0.26, P=0.02; Fig. 2B). At the same time, hindlimb width decreased on average in all lizards (−0.15±0.009 mm; Fig. 2C). Hindlimb width loss was similar and low in hydrated control lizards and those provided with spiders or hydrated crickets (Tukey *post hoc* tests: all P>0.2, Fig. 2C). The change in hindlimb width was

greatest in lizards from the dehydrated control group and in those 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 the morphological changes (change in: body mass $F_{1,82}$=0.66, P=0.42; tail width $F_{1,76}$=1.65, P=0.21; hindlimb width $F_{1,80}$=0.0004, P=0.98).

Plasma metabolites

There was a strong effect of food treatment on changes in plasma triglyceride concentration ($F_{5,88}$=37.25, P<0.0001; Fig. 3A), as well as an effect of initial concentration ($F_{1,88}$=111.20, P<0.0001). *Post hoc* analyses indicated three different treatment group categories. First, triglyceride concentrations on average decreased slightly in control lizards with no food irrespective of water availability. Second, triglyceride concentrations did not change on average in lizards provided with woodlice. Third, lizards fed with hydrated or dehydrated crickets and spiders had higher concentrations of plasma triglycerides (respectively, contrasts: β=420.8, β=413.4 and β=286.5, all P<0.0001; Fig. 3A) compared with the other groups. Across all treatment groups with access to food, the amount of food consumed was positively correlated with the change in triglyceride concentration ($F_{1,53}$=7.83, P=0.007). At the same time, plasma β-HB concentrations slightly increased with a low variability among lizards and 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). In contrast, intra-individual changes of glucose concentration were highly variable and differed among treatment groups ($F_{5,73}$=4.04, P=0.0027; $F_{1,73}$=5.99, P=0.017; Fig. 3B). In the dehydrated control treatment, lizard glucose concentrations increased (estimate±s.e. 67.83±18.94, P=0.0006) above the levels observed in the hydrated control treatment, whereas lizards provided with food had intermediate values. We found no effect of food consumption on glucose concentration changes ($F_{1,45}$=0.01, P=0.92).

DISCUSSION

In this study, we tested whether prey consumption by a generalist mesopredator lizard could compensate for water deprivation via dietary water intake. Notably, our results revealed that 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 access to free-standing water was associated with the restoration of an optimal hydration status as 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 to respiratory and cutaneous evaporative water losses (Le Galliard et al., 2021). At the same 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 a significant 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 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).
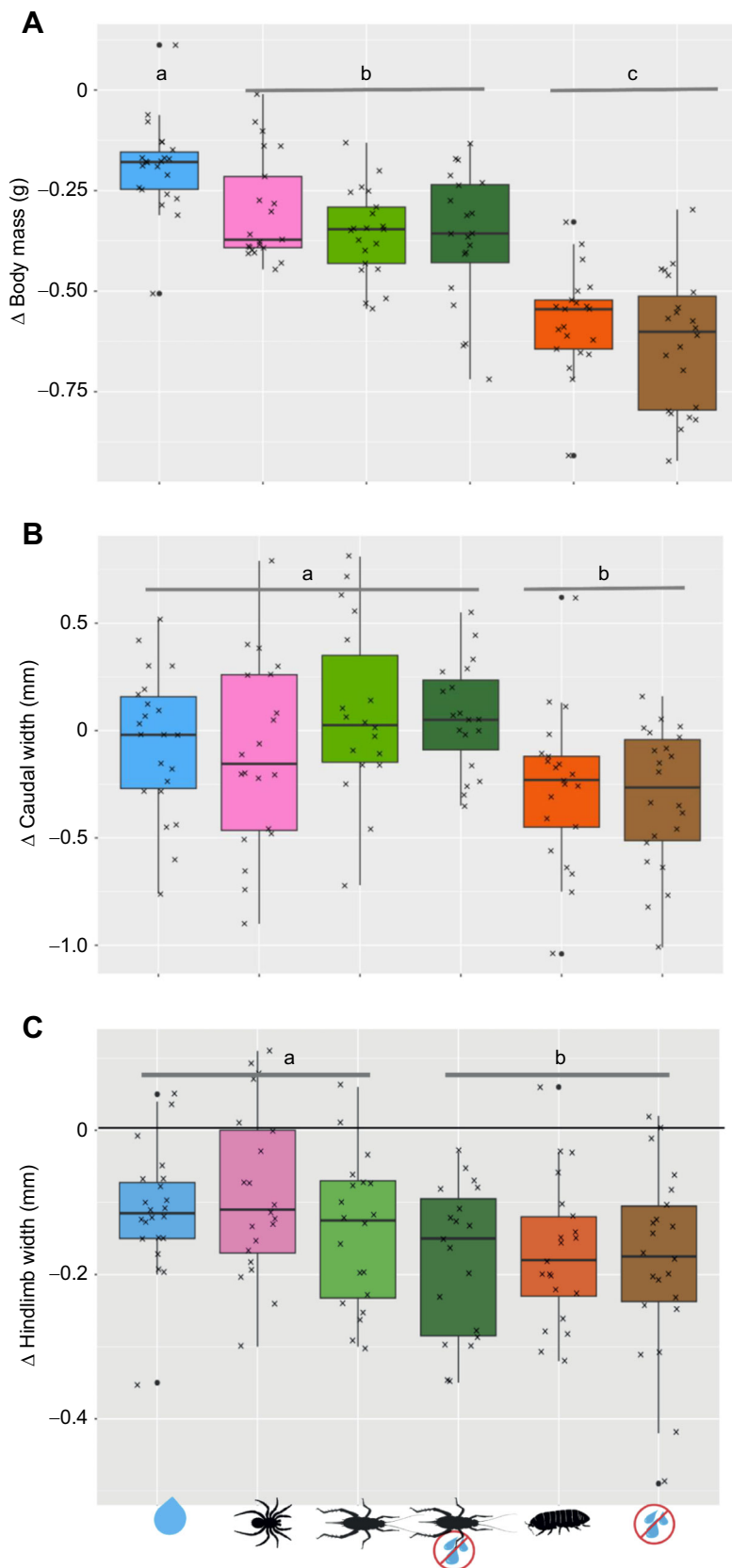
Fig. 2. Morphological changes. (A) Change in body mass (mean±s.e.m. initial value: 3.81±0.05 g) between the start and the end of the manipulation in the different treatment groups. (B) Change in caudal width (mean±s.e.m. initial width 6.46 ±0.04 mm) between the start and the end of the manipulation in the different treatment groups. (C) Change in hindlimb width (mean±s.e.m. initial width 1.98±0.01 mm) between the start and the end of the manipulation in the different treatment groups. Treatment groups are (from left to right): hydrated control, spiders, hydrated crickets, dehydrated crickets, woodlice, dehydrated control (N=21 per group). The lower and upper hinges of the box plots correspond to the first and third quartiles, the middle bar is the median, whiskers are 1.5× the interquartile range and circles are outliers. Significant differences between groups are indicated by different lowercase letters it is not the case right now, I have asked for changes from G1 to a, G2 to b, G3 to c, based on *post hoc* tests with Tukey correction.

Only a few previous studies have investigated dietary water intake in squamate reptiles using experimental approaches. Direct tests on herbivorous and insectivorous species have found similar results, with food consumption reducing body mass loss due to water deprivation (Table 1; Munsey, 1972). However, osmolality was not measured, so previous knowledge of the effect of food consumption
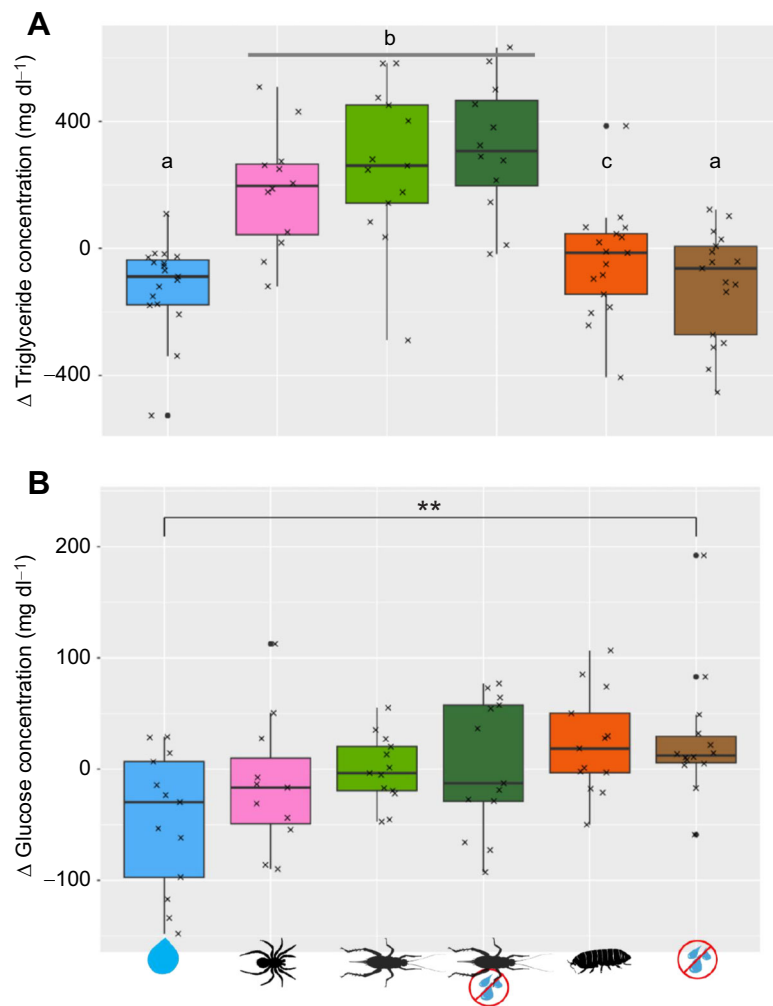
Fig. 3. Changes in blood parameters. (A) Change in plasma triglyceride concentration between the start and the end of the manipulation in the different treatment groups. (B) Change in plasma glucose concentration between the start and the end of the manipulation in the different treatment groups. Treatment groups are (from left to right): hydrated control, spiders, hydrated crickets, dehydrated crickets, woodlice, dehydrated control (N=21 per group). The lower and upper hinges of the box plots correspond to the first and third quartiles, the middle bar is the median, whiskers are 1.5× the interquartile range and circles are outliers. Significant differences between groups are indicated by different lowercase letters, based on *post hoc* tests with Tukey correction (**P<0.005).

on animal physiological hydration state is lacking. Studies focusing on carnivorous species explain the water costs of digestion through intermittent feeding and consumption of protein-rich prey, two traits associated with a greater water use during digestion, metabolism and excretion (Wright et al., 2013; Lillywhite, 2017). Thus, food consumption and metabolism, as well as the elimination of metabolic waste, can increase the requirement for water, potentially exacerbating dehydration during periods of drought in carnivorous reptiles, such as snakes (Lillywhite, 2017). In our study, the lizards were insectivores that fed continuously, but increased dehydration due to feeding could be the result of increased activity and body temperature due to the thermal and energetic requirements of foraging and digestion (Reilly et al., 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 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 determine whether this was due to a digestion constraint or was the result of a behavioral choice of woodlice-fed lizards to thermoregulate more, as we had no data on locomotor activity and thermoregulation. If locomotion for foraging and thermoregulation to reach an optimal temperature for digestion are mostly responsible for increased water losses and negate the 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 environments than in captivity (Anderson and Karasov, 1981). In addition, previous studies have shown that snakes could cease feeding when dehydration becomes moderate (e.g. Edwards et al., 2021; Lillywhite et al., 2014), which supports the existence of complex interactions between water and energy balance, and suggest that poor body condition in wild populations could be correlated with dehydration status (Sandfoss and Lillywhite, 2019). Therefore, complementary studies are needed to quantify the hydric costs of digestion and the behavioral trade-offs between feeding and hydration.

Despite no evidence for the hypothesis that water can be extracted from food from our 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 of chronic water deprivation, which included a general loss of body condition, muscle 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 chose three prey types corresponding to the natural diet of *Z. vivipara* (Vacheva and Naumov, 2019) and representing different trophic roles ranging from decomposers to insectivores (Bestion et al., 2019), with distinct mobility strategies, morphology, and 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, insects, isopods or gastropods (Vacheva and Naumov, 2019). For example,

Avery (1966) found mostly spiders and homopters in the stomach contents of common lizards at two localities but also rarer prey such as ants and woodlice. Here, lizards provided with woodlice ate less readily 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 digest, making them distasteful for lizards (see Pekár et al., 2016). The results on metabolites indicate that the concentration of plasma triglycerides was aligned with the 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 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 and little lipid transport and storage, which is consistent with previous results seen in other reptiles (Price et al., 2013). As a consequence, the general body condition of woodlice-fed and water-deprived lizards was much more impacted than that of lizards fed with higher quality food such as crickets and spiders: these lizards lost more body mass, more muscle and more fat reserves from their tail, and were virtually indistinguishable from those held without food or water. Thus, despite providing no significant net intake of water, high-quality food and strong food consumption were important to recoup some of the energy costs of water 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, including muscular mass and reserves that are essential for future survival. Thus, while food 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 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 more accurate assessment of the lizards' condition and improved tests with prey that are both hydrated and less hydrated as 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 prey would not allow this generalist lizard to avoid dehydration, but it could reduce the short-term costs of drought and long-term fitness consequences (Dezetter et al., 2022).

Unexpectedly, we found a significant difference between hydrated and dehydrated control groups in their plasma glucose concentration but no effect of food provisioning and water deprivation on plasma concentrations of ketone bodies (β-HB). It is predicted that the concentration of ketone bodies should elevate during the second stage of fasting when carbohydrate stores are depleted and animals mobilize lipid stores instead (McCue et al., 2012). However, previous studies have shown that plasma ketone concentrations do not 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, glucose in water-deprived lizards was maintained at relatively high levels, regardless of their feeding status. In addition, lizards with neither water nor food had much higher glucose concentrations at the end of the manipulation than those from the hydrated control group. In vertebrates exposed to periods of fasting or intense exercise, maintenance of high 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 and Katorski, 1967) and glycogenolysis (the degradation of glycogen stored in adipose tissues or muscles; Storey, 1996).

Gluconeogenesis involves glucose production in the liver from transported blood glycerol that is released by triglyceride hydrolysis in the tail or other 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 blood of food- and water-deprived lizards may represent a higher capacity for gluconeogenesis due to lipid catabolism induced by fasting and dehydration. Similarly, water deprivation in the absence of food led to stronger muscle wasting, which may result in muscle glycogen degradation and increased glucose production. Although glucose concentration could impact osmolality and introduce bias in our dehydration proxy, our findings showed similar osmolality in fed groups and the dehydrated control group. The differing glucose concentrations between these groups supports the notion that salt concentrations primarily drive osmolality variations. This conversion of muscle stored glycogen into plasma circulating glucose was previously demonstrated in turtles (Emerson, 1967) and in amphibians and lizards after vigorous exercise, which is close to simulated conditions of 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 with 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 has been well studied (Schulte, 2015) but we know little about the other abiotic factors involved in the regulation of metabolism and energetic functions of the organisms. If digestion is strongly dependent on body temperature regulation in reptiles (e.g. Harlow et al., 1976; Naulleau, 1983), how it is related to water balance and how water availability impacts nutrient assimilation is poorly studied. When body temperature increases, evaporative water loss also increases, and this loss is further accentuated by higher metabolism and respiration rates (Reilly et al., 2007), which are closely linked to food intake. It is therefore crucial to account for potential effects of food consumption and digestion on temperature and water balance regulation in reptiles. Changes in diet have been observed in other ectothermic 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 aquatic ectotherms, the impact of environmental factors on the diet of terrestrial ectotherms remains poorly known (Zimmerman and Tracy, 1989). Our findings indicate that insectivorous species face trade-offs between water and energy balance, and their survival in water-deprived 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 climate change (Zhang et al., 2020). Further research is needed to gain a deeper understanding of how digestive physiology affects various aspects of an individual's life history traits (Karasov and Diamond, 1988), as trade-offs between linked physiological traits are crucial in understanding organism responses to environmental constraints (Urban et al., 2016; Albright et al., 2017; Riddell et al., 2019).

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