

## RESEARCH ARTICLE

# Effects of food intake and hydration state on behavioral thermoregulation and locomotor activity in the tropidurid lizard *Tropidurus catalanensis*

Dylan J. Padilla Perez<sup>1,2,\*</sup>, Jose E. de Carvalho<sup>1</sup> and Carlos A. Navas<sup>1,3</sup>

## ABSTRACT

Theoretical models predict that lizards adjust their body temperature through behavioral thermoregulation as a function of food availability. However, behavioral thermoregulation is also governed by interactions among physiological and ecological factors other than food availability, such as hydration state, and sometimes it can even conflict with the locomotor activity of animals. Here, we aimed to investigate the role of food intake and hydration state on behavioral thermoregulation and voluntary locomotor activity in the lizard *Tropidurus catalanensis*. We hypothesized that food intake can influence behavioral thermoregulation via an interaction with hydration state. We also hypothesized that lizards should endeavor to spend as little time as possible to reach their preferred body temperature to defend other physiological and/or ecological functions. We collected lizards in the field and brought them to the laboratory to measure the preferred temperature selected in a thermal gradient and the total distance traveled by them in fed and unfed conditions and with variable hydration state. Our results showed that food consumption was the most important predictor of preferred temperature. In contrast, either the hydration state alone or its interaction with food consumption did not have important effects on the lizards' thermal preference. Also, we found that the total distance traveled by lizards was not affected by food intake and was barely affected by the hydration state. We provide an experimental approach and a robust analysis of the factors that influence behavioral thermoregulation and locomotor activity in a tropical lizard.

**KEY WORDS:** Energy balance, Dehydration, Heat, Water balance, Tropiduridae

## INTRODUCTION

Water and food availability vary widely in space and time, influencing the maintenance of water balance and energy budget of animals, which drives the evolution of traits required to cope with environmental changes (Rozen-Rechels et al., 2019). Thermal shifts, for example, have complex ecological and physiological implications for ectothermic vertebrates. In reptiles, changes in environmental temperature limit energetic input by constraining vital physiological

processes, such as digestion (Little and Seebacher, 2016). Therefore, patterns of food consumption, digestion and the conversion of food into biomass depend strongly on temperature (Angilletta, 2001; Greenwald and Kanter, 1979; Waldschmidt et al., 1986). In this context, thermoregulating lineages should maintain body temperatures compatible with digestion through behavioral adjustments, including the selection of microhabitats with appropriate thermal conditions (Cowles and Bogert, 1944; Huey and Slatkin, 1976). However, by selecting microhabitats with relatively high environmental temperatures, reptiles may face a trade-off with potential water loss, especially when free-standing water is temporally or spatially limited in the environment, or when intraspecific competition for water increases (Owen-Smith and Goodall, 2014; Rozen-Rechels et al., 2019; Valeix et al., 2008). For instance, basking lizards that inhabit open and dry areas are usually in contact with hot and dry air, which increases their metabolic rate (Christian and Bedford, 1995), thereby increasing the loss of pulmonary and cutaneous water, resulting in a considerable risk of desiccation.

The interaction between temperature and water is critical for life from the cellular level to whole-organism performance (Franks et al., 1990). Maintaining a relatively high body temperature through behavioral thermoregulation combined with readily available nutrients promotes growth in some lizards (Brett, 1971; Elliott, 1982; Jonassen et al., 1999; Schuler et al., 2011). Adjusting water balance is very important because water constitutes the solvent of biochemical reactions, it serves as a fluid for nutritional provisioning of cells (Chaplin, 2006) and it influences daily activity patterns, habitat selection and locomotor activity. However, lizards do not only thermoregulate and adjust their water balance but also evade predators, defend territories and attract mates. When thermoregulation mediated by food consumption conflicts with these other activities, an individual incurs a cost of missed opportunities, also known as non-energetic costs (Angilletta, 2009). For example, territorial lizards cannot bask uninterrupted while defending their territories (Angilletta, 2009). Also, the additional load associated with food consumption can produce significant decrements in locomotor activity in both sexes (Davis and DeNardo, 2007), leading to a reduced foraging ability. Thus, given non-energetic costs, individuals should endeavor to spend as little time as possible to reach their preferred body temperature (Angilletta, 2009). Accordingly, some lizards are able to detect differences in the thermal quality of microhabitats so that they can select the one that offers the faster rate of heating (Belliere and Carrascal, 2002).

Here, we investigated the effects of food intake and hydration state on behavioral thermoregulation and voluntary locomotor activity in the tropidurid lizard *Tropidurus catalanensis*. This is a tropical lizard that shows a remarkable ability to thermoregulate behaviorally, either by shuttling between sunny and shady spots (Piantoni et al., 2016) or by exploiting the thermal conductance of the microhabitat (Ribeiro et al., 2008). This lizard feeds on a variety of items ranging from

<sup>1</sup>Department of Ecology and Evolutionary Biology, Federal University of São Paulo (UNIFESP) – Diadema Campus, Rua Prof. Artur Riedel, 275, CEP 09972-270 Diadema, São Paulo, Brazil. <sup>2</sup>School of Life Sciences, Arizona State University, Tempe, AZ 85281, USA. <sup>3</sup>Department of Physiology, Biosciences Institute, University of São Paulo, Rua do Matão, Travessa 14, CEP 05508-900 São Paulo, São Paulo, Brazil.

\*Author for correspondence (dylan.padilla10@unifesp.br)

 D.J.P.P., 0000-0001-7276-1317; J.E.d.C., 0000-0001-7276-1317; C.A.N., 0000-0002-9859-0568

insects to other species of lizards (authors' personal observations), making it a convenient study system. We hypothesized that food consumption and the hydration state interact to influence behavioral thermoregulation. This interaction can result in two possible outcomes: (1) if food intake grants water to the animals (e.g. Lillywhite, 2017), then fed lizards regardless of the hydration state would select relatively higher temperatures than unfed ones to optimize digestion; or (2) if ingested food provides nutrients but not enough water (e.g. Lillywhite, 2016), then dehydrated fed lizards would select similar or relatively lower ambient temperatures than dehydrated unfed ones. Finally, lizards should spend as little time as possible to reach their target body temperature so they can evade a conflict with other activities associated with locomotor activity, such as foraging or escape behaviors. Therefore, (3) we also predict that lizards in fed and unfed conditions would have similar locomotor activity (see predictions in Fig. S1 for more details).

## MATERIALS AND METHODS

### Study species

The tropidurid lizard *Tropidurus catalanensis* Gudynas and Skuk 1983 is a tropical ground-dwelling, diurnal species, within the Torquatus complex. Its distribution range includes southwestern São Paulo and southeastern Mato Grosso do Sul, Brazil, but it reaches northwestern Uruguay and northeastern Argentina (Kunz and Borges-Martins, 2013). This is a small species relative to other congeners (according to the species description, the largest male and female within the type series had a snout–vent length, SVL, of 127.9 and 98.8 mm, respectively) and predominantly insectivorous. Individuals typically remain active during the entire photophase. The species inhabits open areas, mainly rocks, termite nests and logs, but has also been observed climbing walls and trees (Bergallo and Rocha, 1993; Kunz and Borges-Martins, 2013; Rodrigues, 1987). Rodrigues (1987) notes that this species is a good colonizer able to expand its distribution as a result of deforestation (at least for the Torquatus populations in the State of São Paulo, Brazil). In the municipality of São Paulo, this species has colonized several urban areas, including natural parks with small streams and rocky outcrops originally covered with seasonal forests. Regarding its thermoregulatory behavior, the South American *Tropidurus* of the Torquatus group (*sensu* Frost et al., 2001) comprises mainly heliothermic species, although heating strategies may change seasonally. Ribeiro et al. (2008) showed that, during the wet season, the substrate temperature explained the variation in lizard body temperature better than air temperature, whereas this pattern is inverted during the dry season.

### Conditioning regime

We collected 28 lizards (14 females and 14 males) from January to August 2018 in two different localities: the Parque Continental and the Parque Estadual Fontes do Ipiranga, municipality of São Paulo, Brazil. Both localities correspond to urban areas colonized by populations of the study species. We transported the lizards to the laboratory at the Biosciences Institute, University of São Paulo, where they were maintained in an ambient-temperature room with a 12 h:12 h day:night cycle. We housed the lizards individually in plastic boxes (51×40×13 cm), with water *ad libitum* and food offered according to treatment. We used a 100 W halogen lamp as a heat source in each box and all boxes were perforated to allow ventilation and avoid overheating. Maintenance of the animals and experiments were authorized by the Ethics Committee on Animal Use of the Federal University of São Paulo (CEUA/UNIFESP #5791280218) and the ICMBio (Chico Mendes Institute #56472-3).

### Description of treatments

We first weighed and measured the SVL of all lizards using a precision measuring tape. At the beginning of the first week, we randomly separated them into two groups of 14 individuals: in one of these groups, lizards were regularly fed every 3 days with a diet composed of enough cockroaches to produce a meal equivalent to 5% of their body mass (referred herein as to 'fed lizards'), while lizards in the other group did not receive any food (referred herein as to 'unfed lizards'). At the start of the second week, we manipulated the hydration level of the lizards in both groups. Accordingly, we randomly selected half of the lizards ( $n=7$ ) in each group and moderately dehydrated them by denying them access to food and water for a period of time sufficient to reduce their body mass by at least 10–15%. We denied regular food as lizards could probably rehydrate through dietary intake and by producing metabolic water. We maintained the other half of the lizards in both groups (fed and unfed) with water *ad libitum* and regular food. Therefore, our experimental design consisted of two groups, each with 14 individuals that varied in the time they were exposed to dehydration and with different body conditions. The lizards could not be considered *a priori* as 'hydrated' and 'dehydrated' because, even if we had quantified plasma osmolality, there is no information about the dehydration tolerance for our study species in the literature, and it would be difficult to define the lizards' hydration state based solely on plasma osmolality values (see 'Statistical analyses', below, for a description of the models that allowed us to handle these data).

As rates of dehydration vary between individuals, we were unable to standardize the dehydration period to result in an equal percentage loss of body mass for all individuals. Unfed lizards were dehydrated in 10 days on average and fed ones in 15 days. At the end of the conditioning regime, we weighed the lizards again to make sure that we accurately controlled the lizards' body mass in each group.

### Preferred temperature selection and locomotor activity experiments

To measure behavioral thermoregulation and locomotion of lizards, we used a Bioseb operator-independent Thermal Gradient Test (model TGT2-2/1). We measured the preferred ambient temperatures selected by the lizards inside the thermogradient as a proxy for behavioral thermoregulation. Similarly, we used the total distance traveled by the lizards along the gradient as the operational variable for voluntary locomotor activity. The preferred temperature selected in the thermal gradient represents a good measure of behavioral thermoregulation as microclimate selection is the dominant mechanism by which lizards and other reptiles adjust their body temperature (Cowles and Bogert, 1944). The thermogradient comprised a rectangular enclosure made of aluminium (133×17×14 cm), with a perforated acrylic top to allow ventilation and observation of the lizard. We placed this device in a temperature-controlled room set at 25°C and 50% relative humidity. We used the software provided by the manufacturer to establish a quasilinear thermal gradient composed of 20 temperature zones, with the coldest temperature set at 10°C and the hottest at 40.7°C, through which the animals were free to move in an unrestrained manner. This temperature range was met by thermal conduction and it corresponds to the environmental temperature that our study species may experience yearly in São Paulo. Although tropidurids lizards are known to be primarily heliotherms, previous studies have shown that lizards within the torquatus group can also warm up through thermal conduction (Ribeiro et al., 2008), making this gradient an appropriate device to measure behavioral thermoregulation.

We connected a video camera to the thermogradient to continuously film the lizards. Thereby, we measured the time spent by the lizards in each temperature zone together with the total distance traveled along the gradient. We introduced one lizard at a time from either the fed or the unfed group (see ‘Description of treatments’, above) inside the gradient, then we recorded the position of the animal every minute after the first 30–60 min to minimize errors associated with handling. The preferred temperature of a lizard was the weighted mean temperature among the zones in which it spent time during 5 h of observation. It is important to mention that all the experiments began at 09:00 h and lizards selected from the fed group had been fed 10 min before the beginning of the experiment. In lizards, feeding generates a characteristic rapid increase in metabolic rate, which correlates with body temperature. The increase in metabolic rate (i.e. specific dynamic action) is governed by body size and meal size (for reviews, see Andrade et al., 2005; Secor, 2009). Given the body size of the lizards in this study and the amount of food we fed them, we believe that by performing a 5 h experiment we were able to cover the period between ingestion and peak metabolic rate (Brown and Griffin, 2005). Wall and Shine (2008) showed that thermal gradient data may sometimes be misleading. They demonstrated that the selection of body temperature by the lizard *Lialis burtonis* is influenced by the position of the gradient in which the lizards were introduced (cold end or warm end). However, in this study, we randomized the starting position of the lizards in the gradient (cold, middle or hot zone of the thermogradient).

#### Blood sample collection and plasma osmolality measurements

We used the individual’s plasma osmolality and body condition index to produce a proxy for hydration state (see ‘Statistical analyses’, below). After measuring preferred temperature and locomotor activity, we drew approximately 0.1 ml blood from each lizard by cardiocentesis (e.g. Tousignant and Crews, 1995; Mendoza-Roldan et al., 2019), using a heparinized 1 ml syringe. Total time for blood sampling was typically less than 5 min. We immediately centrifuged blood samples at 4000 g for 3 min to separate the plasma from blood cells. We aliquoted the plasma (30 µl) into a separate vial and froze it at –80°C for no longer than 1 week to measure plasma osmolality. We measured the osmolality of 3 aliquots (10 µl each) of plasma per lizard by using a water vapor osmometer (model 5600, Wescor Inc.). We avoided collecting blood samples from the caudal vein because lymphatic contamination is possible by this method (Hernandez-Divers, 2006). By performing cardiocentesis, we were able to collect a larger volume of blood and none of the lizards were injured even when no anesthetic was used. All lizards survived and exhibited good health after blood sampling.

#### Statistical analyses

We modeled (linear models) the preferred temperature of lizards as a function of food intake and hydration index. Accordingly, we adopted the preferred ambient temperature selected by the lizards as our response variable. Similarly, we adopted food consumption (factor) and the hydration index (continuous variable) as our explanatory variables. We did not include sex as a predictor because the number of males and females was the same in the two groups so that the effect of one sex would be offset by the other. We created a hydration index given that the manipulation of the hydration state could alter the lizards’ body mass and plasma osmolality. To do this, we first calculated the body condition index proposed by Peig and

Green (2009), the scaled mass index  $\hat{M}_i$ . This index standardizes body mass at a fixed value of a linear body measurement based on the scaling relationship between mass and length, according to Eqn 1:

$$\hat{M}_i = M_i \left[ \frac{L_0}{L_i} \right]^{b_{\text{SMA}}}, \quad (1)$$

where  $M_i$  and  $L_i$  are the body mass and SVL of individual  $i$ , respectively;  $b_{\text{SMA}}$  is the scaling exponent estimated by the standardized major axis regression of  $\ln M$  on  $\ln L$ ;  $L_0$  is an arbitrary value of  $L$  (the arithmetic mean length for the study population in this case); and  $\hat{M}_i$  is the predicted body mass for individual  $i$  when the linear body measure is standardized to  $L_0$ . In a variety of vertebrate species, including small mammals, birds, reptiles and amphibians,  $\hat{M}_i$  performed better than the ordinary least squares residual index  $R_i$  as a predictor of variations in fat and protein reserves as well as other body components (Peig and Green, 2009; MacCracken and Stebbings, 2012). We then performed a linear regression model of plasma osmolality on  $\hat{M}_i$  and used the residuals as the hydration index. By using the residuals, we could account for the effects of both the individuals’ body condition and plasma osmolality on the preferred ambient temperature.

We used an information-theoretic approach to evaluate model goodness of fit. Information-theoretic criteria such as Akaike’s information criterion (AIC) and AIC corrected for small sample size (AIC<sub>c</sub>) are not a ‘test’ in any sense, and there are no associated concepts such as test power or  $P$ -values or  $\alpha$ -levels (Burnham and Anderson, 2002). Statistical hypothesis testing represents a very different, and generally inferior, paradigm for the analysis of data in complex settings. Therefore, we avoided the use of the word ‘significant’ in reporting our results under an information-theoretic paradigm. In this context, we fitted a set of candidate models (including the null model, in which preferred temperature was described by a single parameter, the overall mean) and performed a conditional model averaging by running the function `model.avg` available in the R package `MuMIn` (<https://CRAN.R-project.org/package=MuMIn>). Models used for inference were those with  $\Delta\text{AIC}_c$  values lower than 5 in relation to the best model (lowest AIC<sub>c</sub>). Then, we averaged over all models within this cutoff point ( $\Delta\text{AIC}_c < 5$ ). In addition, we estimated the relative importance of predictor variables  $x_j$  by summing the Akaike weights across all the models in the set where variable  $j$  occurs. Thus, the relative importance of variable  $j$  is reflected in the sum  $w^+(j)$ . The larger the  $w^+(j)$ , the more important variable  $j$  is, relative to the other variables. Using the  $w^+(j)$ , all the variables can be ranked in their importance. Finally, the direction and magnitude of the effect size should be based on model-averaged parameter estimates with appropriate measures of precision. Accordingly, the results presented in this study are the model-averaged parameter estimates, instead of parameters calculated directly from the raw data, which can be found in Figs S2, S3 and S4.

We used the same procedure mentioned above to model the effects of food consumption and hydration state on the locomotor activity of lizards. In this case, our explanatory variables were the same as above, but we adopted the total distance traveled by individuals in the thermal gradient as our response variable. We fitted generalized linear models (GLM) using a Gamma distribution given the residual structure of our data. Finally, as a descriptive analysis, we calculated the mean and standard deviation values for preferred temperature, plasma osmolality and total distance traveled by lizards to provide data to compare with those in the existing literature. For further comparisons, we conducted an intention-to-treat (ITT) analysis

**Table 1. Most likely models predicting the preferred temperature of lizards based on the second-order Akaike information criterion (AIC<sub>c</sub>)**

Model	<i>k</i>	loglik	AIC <sub>c</sub>	ΔAIC <sub>c</sub>	Weight
Food consumption	3	-66.025	139.049	0.000	0.646
Hydration index+Food consumption	4	-65.992	141.723	2.674	0.170
Null model	2	-68.976	142.432	3.383	0.119
Hydration index+Food consumption+Food consumption×Hydration index	5	-65.463	143.654	4.605	0.065

A multiplication sign within a model represents an interaction term.

where all lizards that were randomly selected for each group were included in the statistical analysis and analyzed according to the group they were originally preassigned (i.e. 'hydrated' and 'dehydrated'; see 'Description of treatments', above), regardless of what value of plasma osmolality we obtained from the blood samples. This method enabled us to compare the results obtained from an information-theoretic criterion (AIC) with those based on a null hypothesis testing approach. This way, we were able to draw accurate conclusions regarding the effectiveness of the approach used for the analysis of the data. We performed all the analyses using the free software R 4.0.2 for Mac OS (<https://www.R-project.org/>).

## RESULTS

### Effects of food intake and plasma osmolality on preferred temperature

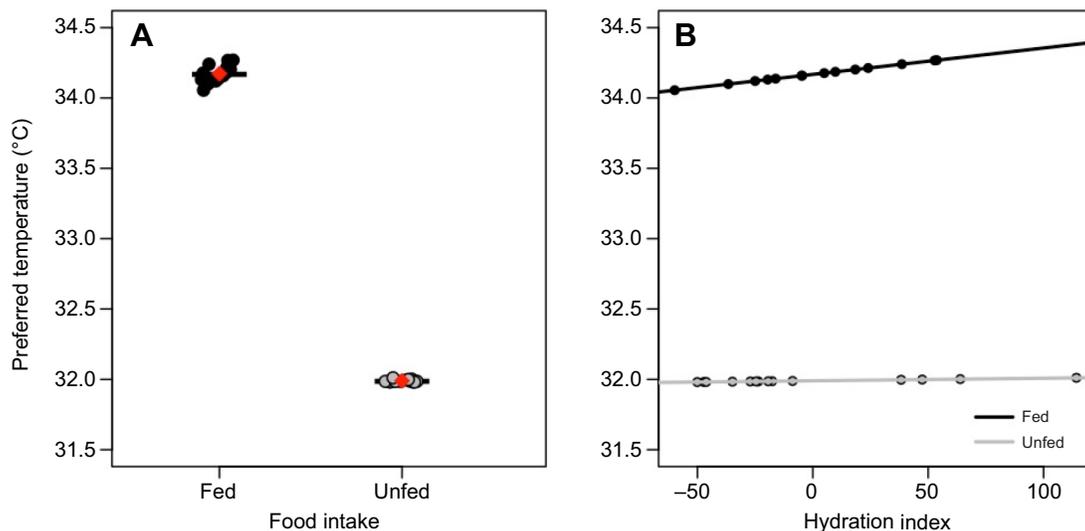
The lizards we used in this study ranged in SVL from 78 to 132 mm ( $n=28$ ), with a mean value of 103 mm and a standard deviation of 17.0 mm. The preferred temperature selected by fed lizards ( $n=14$ ) in the thermal gradient ranged from 29.2 to 37.3°C, with a mean±s.d. of 34.3±2.3°C. Similarly, the temperature selected by the unfed lizards ( $n=14$ ) ranged from 28.6 to 37.7°C, with a mean±s.d. value of 31.8±2.9°C. The difference in the preferred temperature between the experimental groups was 2.4°C. Furthermore, plasma osmolality of fed lizards ( $n=14$ ) in captivity conditions ranged from 291 to 420 mOsm kg<sup>-1</sup>, with a mean±s.d. value of 370±36.5 mOsm kg<sup>-1</sup>. In contrast, plasma osmolality of the unfed lizards ( $n=14$ ) ranged from 332.3 to 523 mOsm kg<sup>-1</sup>, with a mean±s.d. value of 381.7±53.8 mOsm kg<sup>-1</sup>. The difference in plasma osmolality between the experimental groups (fed versus unfed) was 10.9 mOsm kg<sup>-1</sup>.

We found a set of three different models primarily based on food consumption to be the most likely predictor of the preferred ambient temperature selected by the lizards. Also, two of these models showed a slight effect of hydration index (Table 1). The probability that the lizards' food intake determines their preferred temperature was relatively high (0.88) given the sum of Akaike weights (model probabilities) of models in which this variable was included. Fed lizards selected higher ambient temperatures than unfed ones (Fig. 1A). In addition, the hydration index had a 0.23 probability of affecting the lizards' preferred temperature. We found a barely detectable positive linear relationship between preferred temperature and the hydration index, especially in fed lizards, but this was not sufficient to observe an important difference in the slope for the unfed ones (Fig. 1B), which explains the low probability that an interaction between food consumption and hydration index affects the preferred ambient temperature (0.06).

### Effects of food intake and plasma osmolality on the total distance traveled

The total distance traveled by fed lizards ( $n=14$ ) ranged from 1.6 to 44.9 m, with a mean±s.d. of 22.3±13.7 m. In unfed lizards ( $n=14$ ), this variable ranged from 6.1 to 55.5 m, with a mean±s.d. of 25.0±16.3 m. The difference in means between the two groups was 2.7 m.

We found that the null model, in which the total distance traveled is described by a single parameter (the overall mean) was the most likely model that fitted the data (Table 2). Still, the hydration index showed a relatively slight probability of affecting the total distance traveled by lizards (0.30). The distance traveled by lizards decreased linearly as their hydration index increased (Fig. 2). Also, food



**Fig. 1. Preferred ambient temperature of the lizard *Tropidurus catalanensis*.** (A) The most important predictor of preferred ambient temperature was food intake. Dots represent model-averaged predicted values, red diamonds represent mean values and the bold horizontal lines represent median values. (B) The lizards' preferred temperature was barely affected by the hydration index.

**Table 2. Most likely models predicting the total distance traveled by lizards based on the second-order Akaike information criterion (AIC<sub>c</sub>)**

Model	<i>k</i>	loglik	AIC <sub>c</sub>	ΔAIC <sub>c</sub>	Weight
Null model	2	-112.624	229.727	0.000	0.693
Hydration index	3	-112.418	231.835	2.108	0.241
Hydration index+Food consumption	4	-112.348	234.434	4.707	0.066

consumption was of little importance in determining the total distance traveled (0.06).

## DISCUSSION

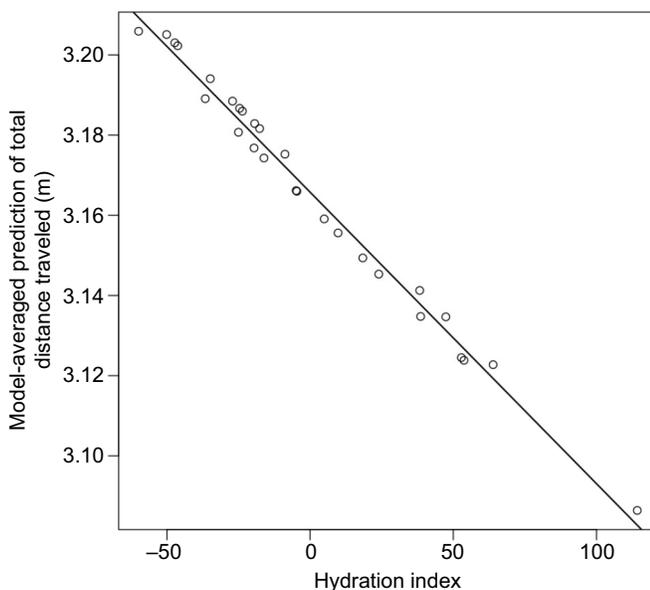
Models of optimal thermoregulation based on energetic costs and benefits predict that ectothermic animals that thermoregulate will adjust their body temperature as a function of food availability (e.g. Elliot, 1982; Huey, 1982). Accordingly, several studies have shown that reptiles select higher body temperatures after feeding either in the laboratory or in the field (Cowles and Bogert, 1944; Regal, 1966; Moll and Legler, 1971; Cogger, 1974; Schall, 1977; Bradshaw et al., 1980). Consistent with these findings, we found in this study that fed *T. catalanensis* lizards preferred higher temperatures than unfed ones, indicating that different physiological processes have different optimal temperatures (Bustard, 1967b; Pough, 1974, 1980; Lang, 1979). The rate at which food is processed increases with temperature but then becomes steady at some value (Hainsworth and Wolf, 1978; Huey, 1982); the higher the digestive rate, the higher the gross energy gained per unit time. The combination of readily available food and the capacity for the lizards to reach relatively high body temperatures may favor growth rates by increasing the opportunities an individual has for keeping its body temperature near mean activity temperature, thus increasing foraging time, and, as a consequence, its rate of digestion (Ballinger, 1977; Sinervo, 1990; Grant and Dunham, 1990), which favors higher growth rates. Contrary to our predictions, we found a slight effect of hydration state by itself and a very low importance of the interaction between hydration state and food

consumption. A fasting period of around 1 or 2 weeks is probably longer than what a frequent feeder, such as our study species, may experience in the wild. Perhaps a chronic exposure to fasting conditions has a stronger effect on lizards' thermal preferences than an acute exposure to dehydration conditions.

Behavioral observations in several species of reptiles suggest that the preferred body temperature increases during digestion (Huey, 1982; Peterson et al., 1993; Dorcas et al., 1997; Sievert and Andreadis, 1999; Andrade et al., 2005; Secor, 2009). However, thermal preference during post-prandial periods is highly variable among reptile species and depends on many factors, such as the energetic and non-energetic costs of high body temperature (Schuler et al., 2011; Angilletta, 2009). While we found that meal consumption drove *T. catalanensis* individuals to thermoregulate, Schuler et al. (2011), found that both the mean and maximal body temperature (33 and 35°C, respectively) were unaffected by metabolic state in the lizard *Sceloporus jarrovi*. This finding suggests that the benefits of foraging effectively, evading predators and defending territory sometimes outweigh the energetic cost of a high body temperature during fasting. Similarly, Van Berkel and Clusella-Trullas (2018) showed that behavioral thermoregulation is highly repeatable, but unaffected by digestive status in the lizard *Agama atra*.

Feeding is a requisite life process that sometimes conflicts with locomotor activity. The interactions between load carrying and locomotor activity provide a familiar model of such trade-offs, which benefit one process at the expense of the other (Davis and DeNardo, 2008). In this context, the additional load associated with the consumption of meals can alter voluntary locomotor activity, also resulting in reduced foraging ability or increased predation risk or both (Garland and Arnold, 1983; Huey and Hertz, 1984; Mehta, 2006). However, this trade-off is most likely to occur in species that have the capacity to intake prey with more than 25% of their body mass, which is not the case of the lizard *T. catalanensis*. Our results support the idea mentioned above as we found in this study that the null model was the most likely model describing the locomotor activity in our study species. Fed lizards probably spent as little time as possible in reaching their preferred body temperature so they could offset the cost of missed opportunity. In this context, the fact that the two groups exhibited the same locomotor activity probably reflects behavioral adjustments to look for appropriate temperatures along the gradients.

Physiological processes can have different sensitivities even when optimal temperatures are similar (Huey, 1982). By maintaining high body temperatures during digestion, *T. catalanensis* may be able to increase its capacity to grow, as predicted by life-history theory (Brett, 1971; Elliott, 1982; Jonassen et al., 1999). At the same time, lizards should endeavor to spend as little time as possible to reach their preferred body temperature even when individuals are in fed or unfed conditions to defend their capacity to perform other critical functions, such as locomotion. Thus, we might expect a similar locomotor activity in fed as well as in unfed lizards. Furthermore, that hydration state may influence thermal preference has been well documented in amphibians (Preest and Pough, 1989; Malvin and Woods, 1991; O'Connor and Tracy, 1992), but direct evidence is still lacking for a



**Fig. 2. Total distance traveled by the lizard *Tropidurus catalanensis*.** The null model was the one with the highest probability of describing the total distance traveled. Although the hydration index slightly affected the distance traveled by the lizards, the importance of this variable was relatively low and so was the weight of evidence of the models that included this variable (see Table 2).

similar relationship among reptiles (Ballinger et al., 1977; Mautz, 1982), especially for tropical and subtropical species. In particular, behavioral thermoregulation in the lizard *T. catalanensis* was barely affected by the individuals' hydration state, but it was greatly affected by food intake. Fed individuals would have better body condition and, hence, they may be more successful at exploiting their thermal environment than individuals with poor body condition. An ITT analysis confirmed our conclusions, revealing unbiased results and providing support for the use of information theory in evolutionary physiology studies.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: D.J.P.P., J.E.d.C., C.A.N.; Formal analysis: D.J.P.P.; Writing - original draft: D.J.P.P., J.E.d.C., C.A.N.; Writing - review & editing: D.J.P.P., J.E.d.C., C.A.N.; Supervision: J.E.d.C., C.A.N.; Funding acquisition: D.J.P.P.

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#### Data availability

The dataset and R script are available from the Dryad digital repository (Padilla Perez et al., 2021): dryad.59zw3r269

#### Supplementary information

Supplementary information available online at <https://jeb.biologists.org/lookup/doi/10.1242/jeb.242199.supplemental>

#### References

- Andrade, D. V., Cruz-Neto, A. P., Abe, A. S. and Wang, T. (2005). Specific dynamic action in ectothermic vertebrates: a review of the determinants of postprandial metabolic response in fishes, amphibians, and reptiles. In *Physiological and Ecological Adaptations to Feeding in Vertebrates*, (ed. J. M. Strack and T. Wang) pp. 305-324. Science Publishers. ISBN 1-57808-246-3
- Angilletta, M. J. (2001). Thermal and physiological constraints on energy assimilation in a widespread lizard (*Sceloporus undulatus*). *Ecology* **82**, 3044-3056. doi:10.1890/0012-9658(2001)082[3044:TAPCOE]2.0.CO;2
- Angilletta, M. J., Jr. (2009). *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford: Oxford University Press.
- Ballinger, R. E. (1977). Reproductive strategies: food availability as a source of proximal variation in a lizard. *Ecology* **58**, 628-635. doi:10.2307/1939012
- Belliure, J. and Carrascal, L. M. (2002). Influence of heat transmission mode on heating rates and on the selection of patches for heating in a Mediterranean lizard. *Physiol. Biochem. Zool.* **75**, 369-376. doi:10.1086/342768
- Bergallo, H. G. and Duarte Rocha, C. F. (1993). Activity patterns and body temperatures of two sympatric lizards (*Tropidurus torquatus* and *Cnemidophorus ocellifer*) with different foraging tactics in southeastern Brazil. *Amphib-Reptilia* **14**, 312-315. doi:10.1163/156853893X000525
- Bradshaw, S. D., Gans, C. and Saint Girons, H. (1980). Behavioral thermoregulation in a pygopodid lizard, *Lialis burtonis*. *Copeia* **1980**, 738-743. doi:10.2307/1444452
- Brown, R. P. and Griffin, S. (2005). Lower selected body temperatures after food deprivation in the lizard *Anolis carolinensis*. *J. Therm. Biol.* **30**, 79-83. doi:10.1016/j.jtherbio.2004.07.005
- Brett, J. R. (1971). Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of Sockeye salmon (*Oncorhynchus nerka*). *Am. Zool.* **11**, 99-113. doi:10.1093/icb/11.1.99
- Burnham, K. P. and Anderson, D. R. (2002). *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. New York, New York, USA: Ecological Modelling. Springer Science & Business Media.
- Bustard, H. R. (1967b). Activity cycle and thermoregulation in the Australian gecko *Gehyra variegata*. *Copeia*, **1967**, 753-758. doi:10.2307/1441885
- Chaplin, M. (2006). Do we underestimate the importance of water in cell biology? *Nat. Rev. Mol. Cell Biol.* **7**, 861-866. doi:10.1038/nrm2021
- Christian, K. A. and Bedford, G. S. (1995). Seasonal changes in thermoregulation by the frillneck lizard, *Chlamydosaurus kingii*, in tropical Australia. *Ecology* **76**, 124-132. doi:10.2307/1940636
- Cogger, H. G. (1974). Thermal relations of the Malle fragon *Amphibolurus fordii* (Lacertilia: Agamidae). *Aust. J. Zool.* **22**, 319-339. doi:10.1071/ZO9740319
- Cowles, R. B. and Bogert, C. M. (1944). A preliminary study of the thermal requirements of desert reptiles. *Bulletin of the American Museum of Natural History* **83**. <http://hdl.handle.net/2246/1237>
- Davis, J. R. and DeNardo, D. F. (2007). The urinary bladder as a physiological reservoir that moderates dehydration in a large desert lizard, the gila monster *Heloderma suspectum*. *J. Exp. Biol.* **210**, 1472-1480. doi:10.1242/jeb.003061
- Davis, J. R. and DeNardo, D. F. (2008). Water storage compromises walking endurance in an active forager: evidence of a trade-off between osmoregulation and locomotor performance. *J. Comp. Physiol. A* **194**, 713-718. doi:10.1007/s00359-008-0342-2
- Dorcas, M. E., Peterson, C. R. and Flint, M. E. T. (1997). The thermal biology of digestion in rubber boas (*Charina bottae*): physiology, behavior and environmental constraints. *Physiol. Zool.* **70**, 292-300. doi:10.1086/639601
- Elliott, J. M. (1982). The effects of temperature and ration size on the growth and energetics of salmonids in captivity. *Comp. Biochem. Physiol. Part B: Comp. Biochem.* **73**, 81-91. doi:10.1016/0305-0491(82)90202-4
- Franks, F., Mathias, S. F. and Hatley, R. H. M. (1990). Water, temperature and life. *Phil. Trans. R. Soc. Lond. B* **326**, 517-533. doi:10.1098/rstb.1990.0029
- Frost, D. R., Rodrigues, M. T., Grant, T. and Titus, T. A. (2001). Phylogenetics of the lizard genus *Tropidurus* (Squamata: Tropiduridae: Tropidurinae): direct optimization, descriptive efficiency, and sensitivity analysis of congruence between molecular data and morphology. *Mol. Phylogenet. Evol.* **21**, 352-371. doi:10.1006/mpev.2001.1015
- Garland, T. and Arnold, S. J. (1983). Effects of a full stomach on locomotor performance of juvenile garter snakes (*Thamnophis elegans*). *Copeia* **1983**, 1092-1096. doi:10.2307/1445117
- Grant, B. W. and Dunham, A. E. (1990). Elevational covariation in environmental constraints and life histories of the desert lizard *Sceloporus merriami*. *Ecology* **71**, 1765-1776. doi:10.2307/1937584
- Greenwald, O. E. and Kanter, M. E. (1979). Effects of temperature and behavioral thermoregulation on digestive efficiency and rate in corn snakes (*Elaphe guttata guttata*). *Physiol. Zool.* **52**, 398-408. doi:10.1086/physzool.52.3.30155760
- Hainsworth, F. R. and Wolf, L. L. (1978). The economics of temperature regulation and torpor in nonmammalian organisms. In *Strategies in Cold: Natural Torpidity and Thermogenesis* (ed. c. H. Wang and J. W. Hudson), pp. 147-184. New York and London: Academic Press.
- Hernandez-Divers, S. J. (2006). Diagnostic techniques. In *Reptile Medicine and Surgery*, 2nd edn. (ed. D. Mader), pp. 490-532. Saunders Elsevier.
- Huey, R. B. (1982). Temperature, physiology, and the ecology of reptiles. In *Biology of the Reptilia*, Vol. 12 (ed. C. Gans and F. Pough), pp. 25-91. New York: Academic Press.
- Huey, R. B. and Hertz, P. E. (1984). Is a jack-of-all-temperatures a master of none? *Evolution* **38**, 441-444. doi:10.1111/j.1558-5646.1984.tb00302.x
- Huey, R. B. and Slatkin, M. (1976). Cost and benefits of lizard thermoregulation. *Q. Rev. Biol.* **51**, 363-384. doi:10.1086/409470
- Jonassen, T. M., Imsland, A. K. and Stefansson, S. O. (1999). The interaction of temperature and fish size on growth of juvenile halibut. *J. Fish Biol.* **54**, 556-572. doi:10.1111/j.1095-8649.1999.tb00635.x
- Kunz, T. S. and Borges-Martins, M. (2013). A new microendemic species of *Tropidurus* (Squamata: Tropiduridae) from southern Brazil and revalidation of *Tropidurus catalanensis* Gudynas & Skuk, 1983. *Zootaxa* **3681**, 413-439. doi:10.11646/zootaxa.3681.4.6
- Lang, J. W. (1979). Thermophilic response of the American alligator and the American crocodile to feeding. *Copeia* **1979**, 48-59. doi:10.2307/1443728
- Lillywhite, H. B. (2016). Behavior and physiology: an ecological and evolutionary viewpoint on the energy and water relations of ectothermic amphibians and reptiles. In *Amphibian and Reptile Adaptations to the Environment: Interplay Between Physiology and Behavior* (ed. D. Andrade, C. Bevier and J. de Carvalho), Ch. 1, pp. 1-25. CRC Press.
- Lillywhite, H. B. (2017). Feeding begets drinking: insights from intermittent feeding in snakes. *J. Exp. Biol.* **220**, 3565-3570. doi:10.1242/jeb.163725
- Little, A. G. and Seebacher, F. (2016). Acclimation, acclimatization, and seasonal variation in amphibians and reptiles. In *Amphibian and Reptile Adaptations to the Environment: Interplay Between Physiology and Behavior* (ed. D. Andrade, C. Bevier and J. de Carvalho), Ch. 2, pp. 41-51. CRC Press.
- MacCracken, J. G. and Stebbings, J. L. (2012). Test of a body condition index with amphibians. *J. Herpetol.* **46**, 346-350. doi:10.1670/10-292
- Malvin, G. M. and Woods, S. C. (1991). Behavioral thermoregulation of the toad, *Bufo marinus*: effects of air humidity. *J. Exp. Zool.* **258**, 322-326. doi:10.1002/jez.1402580307
- Mautz, W. J. (1982). Patterns of evaporative water loss. In *Biology of the Reptilia* (ed. C. Gans and F. H. Pough), pp. 443-481. London: Academic Press.

- Mehta, R. S.** (2006). Meal size effects on antipredator behavior of hatchling trinket snakes, *Elaphe helena*. *Ethology* **112**, 649-656. doi:10.1111/j.1439-0310.2006.01224.x
- Mendoza-Roldan, J. A., Colella, V., Lia, R. P., Nguyen, V. L., Barros-Battesti, D. M., Iatta, R., Dantas-Torres, F. and Otranto, D.** (2019). *Borrelia burgdorferi* (sensu lato) in ectoparasites and reptiles in southern Italy. *Parasit. Vectors*, **12**, 35. doi:10.1186/s13071-019-3286-1
- Moll, E. O. and Legler, J. M.** (1971). *The life history of a neotropical slider turtle, Pseudemys scripta (Schoepff) in Panama*, Vol. 11, pp. 1-102. Los Angeles, Bull. Co. Mus. Nat. Hist. (Sci.).
- O'Connor, M. P. and Tracy, C. R.** (1992). Thermoregulation by juvenile toads of *Bufo woodhousei* in the field and in the laboratory. *Copeia* **1992**, 865-876. doi:10.2307/1446164
- Owen-Smith, N. and Goodall, V.** (2014). Coping with savanna seasonality: comparative daily activity patterns of African ungulates as revealed by GPS telemetry. *J. Zool.* **293**, 181-191. doi:10.1111/jzo.12132
- Padilla-Perez, D. J., de Carvalho, J. E. and Navas, C. A.** (2021). Data from: Effects of food intake and hydration state on behavioral thermoregulation and locomotor activity in the tropidurid lizard *Tropidurus catalanensis*. Dryad. doi:10.5061/dryad.59zw3r269
- Peig, J. and Green, A. J.** (2009). New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos* **118**, 1883-1891. doi:10.1111/j.1600-0706.2009.17643.x
- Peterson, C. R., Gibson, A. R. and Dorcas, M. E.** (1993). Snake thermal ecology: the causes and consequences of body temperature variation. In *Snakes: Ecology and Behavior* (ed. R. A. Seigel and J. T. Collins), pp. 241-314. New York: McGraw-Hill.
- Piantoni, C., Navas, C. A. and Iburgüengoytia, N. R.** (2016). Vulnerability to climate warming of four genera of New World iguanians based on their thermal ecology. *Anim. Conserv.* **19**, 391-400. doi:10.1111/acv.12255
- Pough, F. H.** (1974). Preface to 'A Preliminary Study of the Thermal Requirements of Desert Reptiles' by R. B. Cowles and C. M. Bogert, facsimile reprint, pp. i-iv. Society for the Study of Amphibians and Reptiles. <https://ssarherps.org/publications/books-pamphlets/facsimile-reprints/>
- Pough, F. H.** (1980). The advantages of ectothermy for tetrapods. *Am. Natur.* **115**, 113-120. doi:10.1086/283547
- Preest, M. R. and Pough, F. H.** (1989). Interaction of temperature and hydration on locomotion of toads. *Funct. Ecol.* **3**, 693-699. doi:10.2307/2389501
- Regal, P. J.** (1966). Thermophilic response following feeding in certain reptiles. *Copeia* **1966**, 588-590. doi:10.2307/1441092
- Ribeiro, L. B., Gomides, S. C., Santos, A. O. and Sousa, B. M.** (2008). Thermoregulatory behavior of the saxicolous lizard, *Tropidurus torquatus* (Squamata: Tropiduridae), in a rocky outcrop in Minas Gerais, Brazil. *Herpetological Conserv. Biol.* **3**, 63-70.
- Rodrigues, M. T.** (1987). Sistemática, ecologia e zoogeografia dos *Tropidurus* do grupo torquatus ao Sul do Rio Amazonas (Sauria, Iguanidae). *Arq. Zoologia* **31**, 105-230. doi:10.11606/issn.2176-7793.v31i3p105-230
- Rozen-Rechels, D., Dupoué, A., Lourdaï, O., Chamailé-Jammes, S., Meylan, S., Clobert, J. and Le Galliard, J.-F.** (2019). When water interacts with temperature: Ecological and evolutionary implications of thermo-hydroregulation in terrestrial ectotherms. *Ecol. Evol.* **9**, 10029-10043. doi:10.1002/ece3.5440
- Schall, J. J.** (1977). Thermal ecology of five sympatric species of *Cnemidophorus* (Sauria: Teiidae). *Herpetologica* **33**, 261-272.
- Schuler, M. S., Sears, M. W. and Angilletta, M. J.** (2011). Food consumption does not affect the preferred body temperature of Yarrow's spiny lizard (*Sceloporus jarrovi*). *J. Therm. Biol.* **36**, 112-115. doi:10.1016/j.jtherbio.2010.12.002
- Secor, S. M.** (2009). Specific dynamic action: a review of the postprandial metabolic response. *J. Comp. Physiol. B* **179**, 1-56. doi:10.1007/s00360-008-0283-7
- Sievert, L. M. and Andreadis, P.** (1999). Specific dynamic action and postprandial thermophily in juvenile northern water snakes, *Nerodia sipedon*. *J. Therm. Biol.* **24**, 51-55. doi:10.1016/S0306-4565(98)00037-0
- Sinervo, B.** (1990). Evolution of thermal physiology and growth rate between populations of the western fence lizard (*Sceloporus occidentalis*). *Oecologia* **83**, 228-237. doi:10.1007/BF00317757
- Tousignant, A. and Crews, D.** (1995). Incubation temperature and gonadal sex affect growth and physiology in the leopard gecko (*Eublepharis macularius*), a lizard with temperature-dependent sex determination. *J. Morphol.* **224**, 159-170. doi:10.1002/jmor.1052240205
- Valeix, M., Fritz, H., Matsika, R., Matsvimbo, F. and Madzikanda, H.** (2008). The role of water abundance, thermoregulation, perceived predation risk and interference competition in water access by African herbivores. *Afr. J. Ecol.* **46**, 402-410. doi:10.1111/j.1365-2028.2007.00874.x
- Van Berkel, J. and Clusella-Trullas, S.** (2018). Behavioral thermoregulation is highly repeatable and unaffected by digestive status in *Agama atra*. *Integr. Zool.* **13**, 482-493. doi:10.1111/1749-4877.12325
- Waldschmidt, S. R., Jones, S. M. and Porter, W. P.** (1986). The effect of body temperature and feeding regime on activity, passage time, and digestive coefficient in the lizard *Uta stansburiana*. *Physiol. Zool.* **59**, 376-383. doi:10.1086/physzool.59.3.30156109
- Wall, M. and Shine, R.** (2008). Post-feeding thermophily in lizards (*Lialis burtonis* Gray, Pygopodidae): laboratory studies can provide misleading results. *J. Therm. Biol.* **33**, 274-279. doi:10.1016/j.jtherbio.2008.02.005