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An integrative analysis of the short-term effects of tail autotomy on thermoregulation and dehydration rates in wall lizards

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ABSTRACT

Maintaining body temperature is essential for the optimal performance of physiological functions. Ectotherms depend on external heat sources to thermoregulate. However, thermoregulation may be constrained by body condition and hydration state. Autotomy (i.e., the voluntary shed of a body part) evolved in various animal lineages and allowed surviving certain events (such as predator attacks), but it may affect body condition and volume/surface ratios, increase dehydration and constrain thermoregulation. In the framework of a general analysis of the evolution of autotomy, here we assessed the effects of tail loss on the thermal preferences and evaporative water loss rates (EWL) in the lizard *Podarcis bocagei*, integrating the thermal and hydric factors. We did not observe shifts in the thermal preferences of experimentally autotomized lizards when compared to the controls, which contradicted the hypothesis that they would raise preferred temperature to increase metabolic rates and accelerate regeneration. Evaporative water loss rates were also similar for tailed and tailless individuals, suggesting negligible increase of water loss through the injury and no specific ecophysiological responses after autotomy. Therefore, the changes observed in autotomized lizards in the field are to be considered primarily behavioural, rather than physiological, and thermoregulation could be secondarily affected by behavioural compensations for an increased predation risk after autotomy. Functional studies are necessary to understand how lizards' interaction with the environment is altered after autotomy, and further studies including different dehydration levels would be useful to fully understand the effect of water shortage on lizards' performance after caudal autotomy.

1. Introduction

Most physiological functions of animals (e.g., locomotion, digestion, osmoregulation) are strongly affected by body temperature (Hillman et al., 2009), so that their performance is maximized within an optimal range of temperatures (Huey and Stevenson 1979). External heat sources are essential for the thermoregulation of ectothermic animals, in order to adjust their body temperature to an optimal thermal range. Shuttling heliotherms, such as many lizards, do so by adjusting the frequency and duration of basking events and selecting thermally optimal microhabitats (Huey 1982; Angilletta 2009). However, thermoregulatory behaviour may be costly in terms of predation exposure and time budgets (Verwaijen and Van Damme 2007; Herczeg et al., 2006). Thermoregulation is constrained by climate and the physical environment (Carrascal et al., 1992; Aguado and Braña 2014; Sannolo et al., 2019), but also depends on organismal traits such as reproductive condition (Braña 1993; Rodríguez-Díaz et al., 2010), feeding state (Brown and Griffin 2003; Gilbert and Miles 2016), colour (Clusella-Trullas et al., 2009), metabolic and cardiovascular rates (Seebacher and Franklin 2005; Brown and Au 2009), body size (Stevenson 1985; Carrascal et al., 1992), or hydration state (Sannolo and Carretero 2019; Rozen-Rechels et al., 2020). In particular, dehydrated ectotherms select lower temperatures, spend more time hidden, use shaded microhabitats, decrease activity and therefore exhibit a poorer thermoregulation, which will ultimately have repercussions on their survival and fitness (Crowley 1987; Ryan et al., 2016; Sannolo and Carretero 2019).

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Consequently, a physiological trade-off between thermoregulation (i.e., maintaining an optimal body temperature) and hydration (i.e., avoiding water loss) may be expected (Angilletta 2017).

Autotomy, meaning "self-detachment", is an adaptation present in both vertebrate and invertebrate groups that implies the self-induced loss of a body part as a strategy that allows escape from predators, solve molting complications, survive physical damages or eliminate toxins (Maginnis 2006). In many lizards, tail autotomy is a widespread antipredator strategy (McConnachie and Whiting 2003). In the lizard groups with most efficient mechanisms, autotomy occurs along predefined fracture planes within the vertebrae and surrounding tissues and is usually followed by the regeneration of the lost tail, which will be constituted by an unsegmented cartilaginous axis instead of osseous vertebrae (Bellairs and Bryant 1985; Arnold 1988). Tail loss can constrain locomotion, shift activity patterns and microhabitat use, affect feeding capacity and immune system, or decrease social status (Fox et al., 1981; Fox and Rostker 1982; Ramsay et al., 2001; Barrios et al., 2008; Kuo et al., 2013; Fernández-Rodríguez and Braña 2020). More importantly, recently autotomized lizards become more vulnerable to predation (Fox and McCoy 2000). Hence, the evolution of regeneration allowed the restoration of the lost functionality (Goss 1969; Lin et al., 2017; Fernández-Rodríguez and Braña 2020), but this requires time and a strong energy input that may compromise other highly demanding processes, such as reproduction or growth (Barrios et al., 2008; Bateman and Fleming 2009).

Determining the costs and benefits of autotomy and regeneration is essential for a proper understanding of the evolution of both biological functions. Among the potential costs of tail loss in lizards (see the review of Bateman and Fleming 2009 for more details), the possible effects on thermoregulation have been less studied and remain rather unclear. As mentioned above, thermoregulation is necessary for performance of other functions while is affected by the animal's condition (e.g., injuries, mutilations, or morphological changes). Overall lizard morphology, and likely the surface-to-volume ratio, change after tail loss, which could alter the heat exchange patterns (Chapple and Swain 2004). Besides, after tail loss some lizards increase their standard metabolic rate during regeneration up to 36% (Naya et al., 2007), which is probably associated to shifts in the protein metabolism (Sagonas et al., 2017). Since the regenerated tail restores the lost capacities after autotomy (e.g., sprint speed or jumping dynamics, see Zamora-Camacho et al., 2016 and Fernández-Rodríguez and Braña 2020), lizards could be expected to increase their body temperature after tail loss, in order to raise their metabolic rate and accelerate regeneration.

On the other hand, the loss of the tail can represent up to 19% decrease of lizards' body mass in some species (e.g., *Zootoca vivipara*, Herczeg et al., 2004), with a substantial decrease of fat storage (Chapple and Swain 2002) and loss of fluids contained in the tail (like blood). To date, dehydration has been neglected as a possible immediate consequence of tail loss, although it could account for the shifts in lizards' activity and behaviour observed in the field after tail autotomy, especially considering that hydration state may constrain thermoregulation and microhabitat selection (Ryan et al., 2016; Sannolo et al., 2018; Sannolo and Carretero 2019; Rozen-Rechels et al., 2020).

Under this framework, we aimed to assess the short-term effects of tail loss on the thermal and hydric physiology using Bocage's wall lizards (*Podarcis bocagei*) as a model organism, by investigating the thermal preferences and the evaporative water loss rates (EWL) of tailed and tailless lizards. We hypothesize that after tail loss lizards may either increase their preferred temperature to increase regeneration rate, or select lower temperatures to avoid an increase of the dehydration rates. As such, we designed two experiments to disentangle thermal and hydric factors. First, we recorded the temperature selected by lizards in a thermal gradient where water was provided *ad libitum*, so that thermoregulation was unrestricted by hydration state. Second, we measured the evaporative water loss at the preferred temperature of this species. With this design we were able to integrate thermoregulation and water balance in relation to tail autotomy under an ecophysiological framework in order to understand how the interaction of ectotherms with the thermal and hydric environment (Sannolo and Carretero 2019) is modified by autotomy.

2. Material and methods

Podarcis bocagei is a small lacertid lizard (Squamata: Lacertidae) endemic to north-western Iberian Peninsula, where it inhabits humid rocky and bushy areas (Galán 2014). Forty-five adult males (i.e., longer than 46 mm snout-vent-length) with complete tails (original or fully regenerated) were captured by noosing in July 2020, from Vila do Conde (41.33° N, 8.67° W) municipality (NW Portugal). Animals were transported to the laboratory and kept in individual terraria with food (*Tenebrio molitor* larvae) and water supplied *ad libitum*. Animals were kept in the natural photoperiod, supplied by natural light from a window, with supplemental heating provided daily by 150 W infrared reflector bulbs. Lizards were weighed in a precision balance (Sartorius M-Pact AX224, Sartorius AG, Goettingen, Germany) to the nearest 0.0001 g and their snout-vent-length (SVL), tail length (TL) and tail width (TW) were measured with digital callipers to the nearest 0.01 cm. Reproduction in this species occurs from April to July (Carretero et al., 2006), thus these experiments were conducted at the end of the reproductive season, when most of the lizards (including those participating in the experiments) had finished breeding.

Lizards were split into a control (tailed, N = 22) and experimental (tailless, N = 23) group. Every animal from each group underwent three trials of two consecutive experiments: first the preferred temperature (T_{pref}) experiment, followed by the desiccation (EWL) rates experiment in the following day. Using a before-after design, the two experiments were performed three times for each individual. The order of the individual was randomly selected to ensure a mix of animals from both groups tested simultaneously and to provide the necessary resting time between experiments (T_{pref} and EWL rates) and between trials.

The first trial of the two experiments (T_{pref} and EWL) was performed a week after capture, before inducing caudal autotomy to the experimental group (i.e., all the lizards were tailed, so these were the control or baseline measurements). Afterwards, tail loss was induced in the experimental group by grasping the animals from the base of the tail, until the lizards self-detached it (according to the methodologies used by Fernández-Rodríguez and Braña 2020), leaving a tail stub (TS) of around 1 cm (mean ± SD: 1.23 ± 0.25 cm). There was no remaining regenerated tissue in the tail stub of the autotomized lizards (experimental group), and all those belonging to the control (tailed) group had completely intact tails (i.e., without regenerated portions). We decided not to include regenerated lizards in the control group because one of our hypotheses was that regenerated tails might behave differently regarding dehydration. As such, we maintained a completely homogeneous control group in order to compare autotomized lizards (experimental group) to completely intact individuals. However, such design implies that some lizards of the experimental group would lose the tail for the first time while others would autotomize it for the second time, which could have an effect on how they face the effects after autotomy. Therefore, to confirm the robustness of our results and conclusions, we repeated the analyses to test the thermal and hydric variables measured pooling tailed and tailless lizards, but using only the lizards from the experimental group and considering their initial tail condition (intact or regenerated) at the moment of capture. These results are included in Suppl. 1 and showed that intact and previously regenerated lizards in our experiment responded equally (for dehydration rates and preferred temperatures) after tail autotomy, regardless of their previous tail condition.

Immediately following autotomy, lizards were weighed and measured for TS and the tails were weighed and measured for TL and TW. After one day of rest, both the experimental and control groups underwent a second trial of experiments (T_{pref} and EWL), to evaluate the immediate effects of tail loss. We also aimed to test the possible shifts on temperature selection and EWL during the first stages of regeneration, so all the lizards performed the third trial once the tailless ones had started tail regeneration (i.e., 1 week after autotomy). After tail loss, the wound is rapidly closed by the surrounding tissues and muscle fibres, which will dry within the next hours and form a scab that will cover the injury during the first days (Bellairs and Bryant 1985). The scab detaches within approximately a week in this species (authors' personal observation), leaving the blastema (which is covered only by a very thin epithelium) exposed to environmental conditions. Therefore, one week was deemed sufficient resting period between the second and third trial. After finishing the third trial, all the lizards were released in their capture site.

2.1. *Preferred temperature*

Animals were placed individually in acrylic terraria (100 L x 30 W × 40 H cm) without refuges, with a 0.5 cm layer of a mixture of sand and vermiculite on the bottom and a 150 W infrared reflector bulb placed 25 cm high at one end. The thermal gradient in the terrariums ranged from ±20–50 °C, according to the methodology by Carretero (2012). The animals were then allowed to freely thermoregulate for a 10-h period (from 9:00 to 19:00 h). Lizards were not fed during the trials, but had water available in a small dish throughout all the experiment (in order to prevent any possible confounding effects from any potential gradual water stress caused by prolonged exposure to the thermal gradient, Sannolo and Carretero 2019). To record the thermoregulatory behaviour, a thermal (InfraRed) photograph was taken every hour with a thermographic camera FLIR T335 (sensitivity: < 0.05 °C; accuracy: ± 2%; IR image resolution: 320 × 240 pixels; Flir Systems Inc., Wilsonville, Oregon, USA), at a distance of around 30 cm from the animal.

Thermal photographs were later analysed using the software FLIR Tools 2.1 (Copyright, 2014 FLIR Systems, Inc; http://www.flir.com). For this post-processing, skin emissivity was established at 0.96 and a correction for the reflective temperature was performed by taking the mean temperature of thermal photographs of a crinkled aluminium surface (taken from inside the gradients when the lizards were photographed). Following the methodology of Barroso et al. (2016), the Spotmeter tool was used to measure eye temperature in order to estimate the temperature of the lizard, as this has been shown to be an accurate proxy of internal temperature (calibrated for *Podarcis* sp., Barroso et al., 2016). At the end of the test, lizards were returned to their holding terraria where they were fed, provided water *ad libitum* and sprayed in order to re-establish any potential water losses.

2.2. *Evaporative water loss*

Evaporative water loss rate trials were performed the day after from the preferred temperature trials. According to Sannolo et al. (2018), lizards were placed in individual plastic containers (10 H × 9 W cm), with ventilation holes on the top and the bottom, and kept in an incubator for 8 h (from 9:00 to 5:00 h) fitted with 125 g of silica gel to ensure a dry environment (relative humidity < 20%) inside the incubator throughout the experiment. Since the preferred temperature for *P. bocagei* has been shown to range between 29.4 and 32.0 °C (Sannolo et al., 2018), the incubator was set to 32 °C. During the trial period, lizards were weighed to the nearest 0.0001 g every hour in a precision balance (Sartorius M-Pact AX224, Sartorius AG, Goettingen, Germany). Lizards that reached 5% decrease in mass (i.e., water lost) were removed from the incubator and the trial ceased for that individual, as this has been previously used as safety cut-off to prevent exaggerated hydric stress (Sannolo et al., 2018). Faeces found in the plastic containers during the trials were not removed, according to previous studies (Carneiro et al., 2015), but its presence was recorded and accounted for.

2.3. *Statistical analysis*

All the variables were checked for normality and homogeneity of variance of the residuals (Kolmogorov-Smirnov and Levene tests). Homogeneity of sizes (SVL) between tailed and tailless groups was tested by a one-way ANOVA with SVL as the response variable and tail group as a factor.

For the preferred temperature data, mean and median temperatures were calculated for each lizard in each of the three trials. To test for possible differences between tailed and autotomized lizards in the mean and median temperature of each individual through the three trials, repeated measures ANOVAs were carried out with tail group as intersubject factor and SVL as the covariate. Besides, a general linear mixed model was used to check if there were differences between tailed and tailless lizards in the variation of temperature preference of each individual through the 10 h of the trials, and among the three different trials. In this model, absolute temperatures were the response variable while tail group (tailed/tailless), hour (1–10), trial (1–3) and SVL were fixed factors and the individual was a random factor.

As lizards of the experimental group have less mass and volume due to tail loss, they were expected to have less surface to lose water through, which could mask differences in desiccation rates. To determine whether the possible effects of tail loss on desiccation rates would be due to physiological shifts and not just to differences in morphology (i.e., the lack of the tail), the theoretical water loss expected for the removed tail was estimated and included in the analysis. As such, we applied the following correction to the desiccation data for each tailless individual: $1 + TM/M$; were TM is the mass of the shed tail, and M is the lizard's mass (before losing the tail). A general linear mixed model was fitted to analyse differences between tailed and tailless individuals in the desiccation rate through time in each trial and through the different trials. Accumulated absolute mass loss (in each measure) was the response variable, tail group (tailed/tailless), hour (1–8), trial (1–3), SVL and initial mass (at the beginning of each trial) were fixed factors, and the individual was a random factor. A Pearson's Chi-squared test was run to determine if there were differences in the presence of faeces between tailed and tailless lizards.

Statistical analyses were performed in SPSS (version 20) and in R software version 3.6.0 (R Core Team, https://www.r.project.org) where the nlme package (Pinheiro et al., 2017) was used to fit the general linear mixed models and the graphs produced with the ggplot2 package (Wickham, 2009).

3. Results

All variables were normally distributed and fit the assumption of residual homoscedasticity (Kolmogorov-Smirnov and Levene tests: $p > 0.05$ in all cases). Tailed and tailless groups were homogeneous regarding snout-vent-length (mean ± SD SVL Tailed: 62.3 ± 4.3 mm; Tailless: 60.4 ± 4.0 mm; one-way ANOVA: $F_{1,43} = 0.456$, $p = 0.503$) and mass before autotomy (mean ± SD mass before autotomy Tailed: 4.53 ± 0.84 g; Tailless: 4.26 ± 0.83 g; one-way ANOVA: $F_{1,43} = 1.149$, $p = 0.290$). Tailless individuals lost up to around 15% of their weight after autotomy (mean ± SD: 15.25 ± 3.11%).

3.1. *Preferred temperature*

The pattern of temperature variation across the 10 h did not vary through the three different trials between tailed and tailless lizards neither among the trials, nor during the 10 h (general linear mixed model, tail group factor, tail group*trial and tail group*time interactions, $p > 0.05$ in all cases; Fig. 1, Table 1). However, preferred temperature fluctuated significantly through time and through the three trials (general linear mixed model, trial and time factors, $p < 0.001$ in both cases; Fig. 1, Table 1). Moreover, both the mean and the median preferred temperature did not vary between tailed and tailless groups

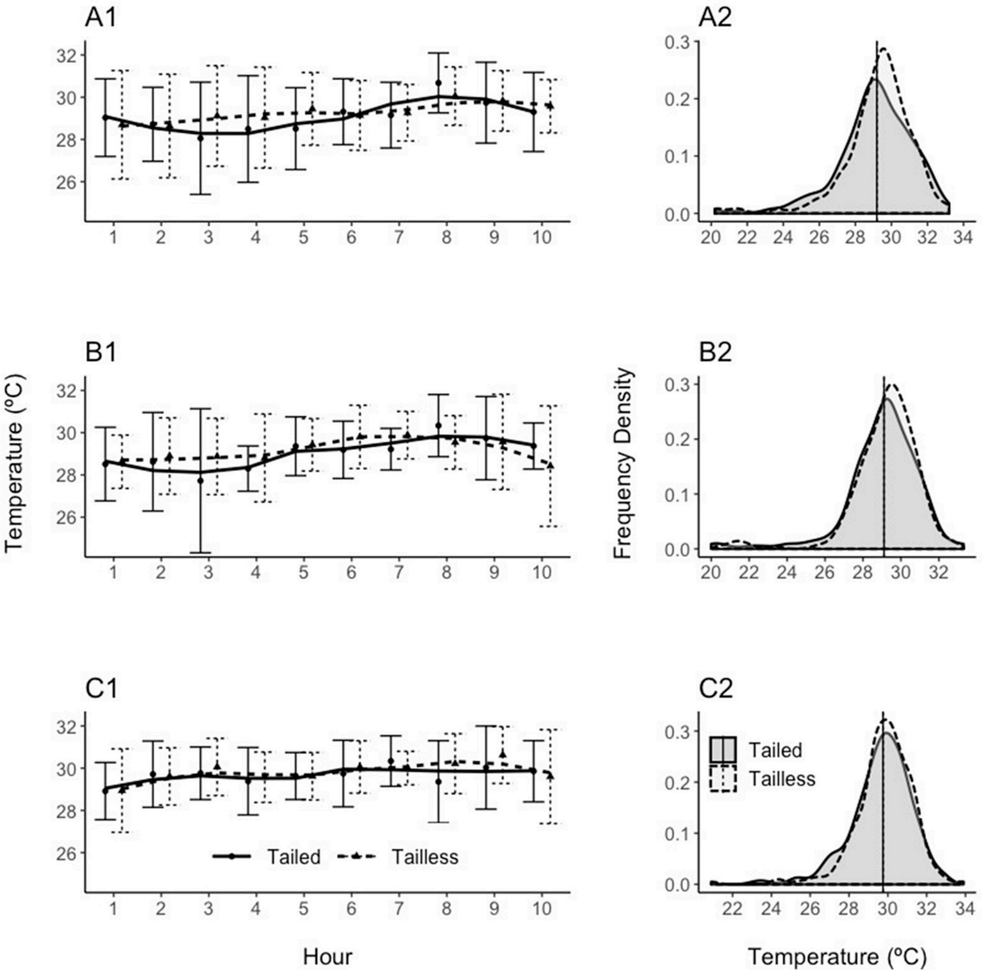

Fig. 1. A1, B1, C1. Body temperatures of tailed (circles, continuous line) and tailless (triangles, discontinuous line) lizards during the tested 10-h period. Values are means ± SD with a LOESS curve fitted to demonstrate the general pattern. **A2, B2, C2.** Frequency density plot of body temperatures registered for tailed (grey, continuous line) and tailless (white, discontinuous line) individuals during all the trial. Vertical lines represent the mean values for tailed (continuous line) and tailless (discontinuous line), which overlap as they are very similar for both groups. The A graphs correspond to the first trial before inducing tail autotomy to the experimental (tailless group); the B graphs represent the second trial, a day after autotomy on tailless group; the C graphs correspond to the third trial, a week after inducing autotomy to the experimental group.

Table 1

Statistical results of the general linear mixed model of the preferred temperature variation between tailed and tailless lizards through the 10 h of the trials, among the three different trials. The absolute temperatures were the response variable of the model, tail group (tailed/tailless), time (1–10 h), trial (1–3) and SVL were fixed factors, and the individual was a random factor.

	DF	F-value	p-value
Trial	2	19.40	<0.001
Tail group	1	0.61	0.439
Time	1	57.00	<0.001
SVL	1	2.59	0.115
Trial*Tail group	2	0.00	0.999
Trial*Time	2	1.41	0.244
Tail group*Time	1	1.59	0.207
Trial*Tail group*Time	2	1.92	0.147

(Fig. 2), through the three different trials (repeated measures ANOVAs with tail group as inter-subject factor and SVL as covariate, mean temperature: $F_{1,42} = 0.003$, $p = 0.997$; median temperature: $F_{1,42} = 0.015$, $p = 0.985$).

3.2. Evaporative water loss

No differences were found in the desiccation rates between tailed and autotomized lizards, nor in their desiccation rates through time and through the three trials (general linear mixed model, tail group factor, tail group*trial and tail group*time interactions, $p > 0.05$ in all cases; Fig. 3, Table 2). Nonetheless, desiccation rates of all individuals

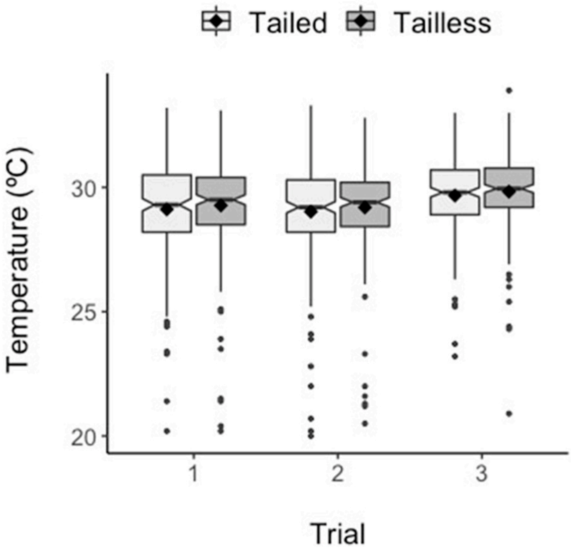

Fig. 2. Temperature boxplots of tailed (light grey) and tailless (dark grey) for the three trials (1, first trial, before inducing tail autotomy to the tailless group; 2, second trial, a day after autotomy; 3, third trial, a week after autotomy). Rhombuses represent the mean values, horizontal black lines inside the boxes represent the median values, and box notches gives visual indication of whether boxplots are significantly different or not (if notches overlap, they may not be significantly different).

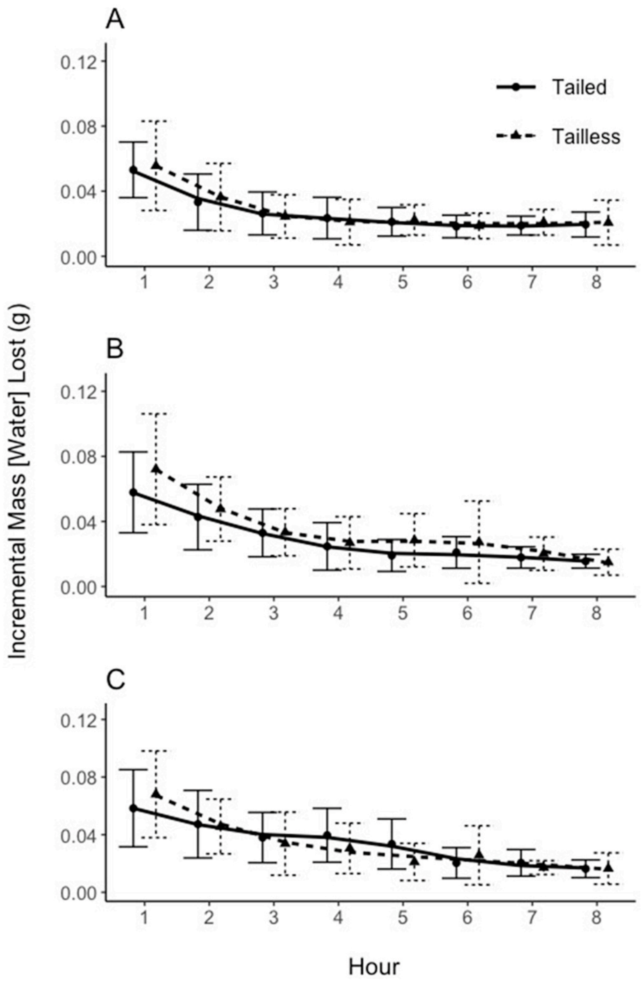

Fig. 3. Evaporative Water Loss (EWL) rates of tailed (circles, continuous lines) and tailless (triangles, discontinuous lines) individuals at each of the 8 h tested, in the three trials done (A, first trial, before inducing tail autotomy to the tailless group; B, second trial, a day after autotomy; C, third trial, a week after autotomy). Values are means ± SD, and curves were fitted with LOESS to illustrate the general trend.

Table 2
Statistical results of the general mixed model of the evaporative water loss rates variation between tailed and tailless lizards through time, in each trial and through the different trials. Accumulated absolute mass loss (in each measure) was the response variable, tail group (tailed/tailless), time (1–8 h), trial (1–3), SVL and the initial mass (at the beginning of each trial) were fixed factors, and the individual was a random factor.

	DF	F-value	p-value
Trial	2	53.626	<0.001
Tail group	1	0.313	0.579
Time	7	83.647	<0.001
Initial mass	1	39.462	<0.001
SVL	1	2.159	0.149
Trial*Tail group	2	7.256	<0.001
Trial*Time	14	1.421	0.137
Tail group*Time	7	1.314	0.241
Initial mass*SVL	1	1.879	0.171
Trial*Tail group*Time	14	0.784	0.688

increased significantly with time within each trial and all lizards lost significantly more water during the second and third trial than during the first one (general linear mixed model, trial and time factors, $p < 0.001$ in both cases, Table 2). Defecation during the experiment was not included in the model, as there were no differences between tailed and tailless lizards after tail loss (Pearson's Chi-squared tests, second trial: $X^2 = 0.178$, $p = 0.673$; third trial: $X^2 = 2.002$, $p = 0.157$).

4. Discussion

Regulation of thermal and hydric balances and the interaction between these two processes is a cornerstone in the lizard ecophysiology (Sannolo and Carretero 2019). Its examination is essential for a proper understanding of how they interact with the environment. The thermal and hydric priorities on one side, and the individual's body condition (poorer after caudal autotomy) on the other, may both have contributed towards a shift in thermoregulation and water balance. Nonetheless, our results showed that neither the hydric nor the thermal physiology were intrinsically shifted either immediately after caudal autotomy or a week after tail loss.

Our first experiment was designed to remove the effect of hydration on thermal preferences by eliminating the water constraint. As such, lizards were hydrated before the trial started and they had water available throughout the whole test. Not surprisingly, the time pattern of preferred body temperatures during the trials was horizontal (Fig. 1), which, according to Sannolo and Carretero (2019), confirmed that lizards were not under hydric stress during the tests (otherwise they would probably have adopted a curvilinear strategy of temperature selection, or a descending pattern as reported by S'khifa et al., 2020).

Our thermal results are consistent with previous studies reporting no change on the selected body temperatures after tail loss on other lizard species (Martín and Salvador 1993; Wilson 1994 in Chapple and Swain 2004; Chapple and Swain 2004; Herczeg et al., 2004; Cromie and Chapple 2012; Zamora-Camacho et al., 2015). The selected body temperatures were also individually consistent during the three different trials, which agrees with previous results that showed that short-term captivity does not influence temperature selection (Díaz-Ricaurte and Serrano 2020). As suggested by Herczeg et al. (2004) for *Zootoca vivipara*, the role of the tail in heat exchange seems to be insignificant for small lizards (like *P. bocagei*). Regardless, it could be a relevant heating and cooling organ in large lizards by adjusting the blood flow inside the tail (Dzialowsky & O'Connor 1999). Our results also contradict the hypothesis that lizards would select higher temperatures after tail loss to increase metabolic rates and accelerate tail regeneration (Zamora-Camacho et al., 2015). Considering the trade-off between thermoregulation (i.e., maintaining an optimal body temperature) and hydroregulation (i.e., avoiding dehydration), increasing body temperature would probably result in higher EWL rates, which would also increase the risk of overheating and compromise other processes (such as activity rates, growth, or reproduction; Andrews 1982; Lorenzon et al., 1999; Wang et al., 2016; Sannolo and Carretero 2019; Rozen-Rechels et al., 2020). This suggests that maintaining the preferred body temperatures (rather than increasing them) would be the most plausible and less costly situation after tail loss, provided that water loss rates are not affected by tail loss.

Contrary to our initial expectations, EWL rates remained unaffected by autotomy at different timeframes. Although some species of lizards can lose a considerable amount of water through the skin (Pirtle et al., 2019), a high percentage of water loss through evaporation occurs in the airways and the lungs, during breathing (Thompson and Withers 1997). In this context, our results suggest that autotomy might not provoke a relevant physiological shift in the overall EWL rates (although further physiological studies that measure metabolic rates in specific tissues are needed to disentangle the physiological responses after tail loss). The loss of water through the injury also seemed insignificant. This is likely due to its small surface, and the minimization mediated by the evolution of several adaptations allowed the reduction of the physical trauma of autotomy. Indeed, immediately after tail loss, there is a strong vasoconstriction and constriction of the muscle fibres surrounding the wound to prevent the lizard from losing much blood and internal fluids

(Bellairs and Bryant 1985). After a few hours, the external layer of muscles and tissue will dry and constitute a scab that protects the injury. Underneath the scab, the blastema will start growing. After approximately a week, in the case of *P. bocagei* (pers. obs.), but, for instance, 4–5 weeks in the leopard gecko *E. macularius* (see McLean and Vickaryous 2011), the scab will detach and the blastema will be in contact with the air (Bellairs and Bryant 1985; Bryant et al., 2002). Although the blastema is covered by a thin layer of epithelium without scales, water loss through that surface was insignificant, possibly due to its small area.

While there were no apparent consequences of tail loss on thermoregulation and water loss in the lab, the well documented differences observed between tailed and tailless lizards in the field (see below) must therefore have an underlying behavioural (rather than physiological) mechanism. Indeed, in our thermal preference experiment, we did provide water *ad libitum* yet we did not monitor if autotomized lizards drank more frequently, which could be compensating for a potential increase in water loss. Moreover, although our study assessed the preferred body temperatures, it did not include other thermal parameters, such as the mean basking time, which tailless lizards may increase to maintain body temperatures (Martín and Salvador 1993).

More importantly, the experiments performed here took place with no predation risk. Thermoregulation is costly in terms of time and exposure to predators (Withers and Campbell 1985; Alford and Lutterschmidt 2012) and tailless lizards often face higher predation risks and are more vulnerable to predation. This is because of shifts in locomotor dynamics, and because once their tails are lost, they cannot rely on autotomy during a new predator encounter until a long-enough tail has regenerated (Dial and Fitzpatrick, 1984; Fox and McCoy, 2000; Fernández-Rodríguez and Braña, 2020). As a consequence, it is expectable that lizards exhibit behavioural adjustments to minimize predation risk, such as decreased activity, different microhabitat use or adopt a more cryptic and wary behaviour (Formanowicz et al., 1990; Martín and Salvador 1993; Cooper 2003, 2007). In turn, these behavioural shifts may affect thermoregulatory performance and, hence, alter the body temperature of lizards in the wild. A poor thermoregulation would constrain locomotion and foraging performances (Avery and Mynott 1990; Angilletta et al., 2002), which are relevant tasks for survival and fitness (Garland and Losos 1994; Robson and Miles 2000).

4.1. Conclusions

Our study showed that, under controlled conditions, tail autotomy had no measurable short to mid-term effects on the thermal and hydric ecology of the small lizard *P. bocagei*. This seems to contradict observations in the field (Martín and Salvador 1993), thus suggesting that such compensatory mechanisms must have an underlying behavioural mechanism as opposed to a physiological one. Certainly, more divergence in ecophysiology is expected in the field than in the laboratory: while our animals had continuous water availability during the thermal preference experiments, lizards in the field would probably be more dehydrated throughout the day or after consecutive dry days. Furthermore, lizards in the field are exposed to a range of other pressures, such as risk of predation and unstable availability of food/water, which may push the animals' physiology to an extreme not contemplated by the "ideal" and stable laboratory conditions in which these animals were tested.

Our results suggest that the tested ecophysiological parameters are similar in tailed and tailless lizards. Nonetheless, autotomized lizards may incur additional metabolic costs due to the loss of both weight (e.g., our individuals had already lost up to around 15% of their weight after autotomy) and fat reserves (which are also a reservoir of metabolic water, Price 2017). These biochemical processes are expected to be important in the mid to long-term and not necessarily immediately after autotomy. Besides, as it has been shown before, the consequences of dehydration for thermoregulation could be more severe in those areas with drought periods, as it may happen in our studied population (Sannolo et al., 2018). Ultimately, future studies should aim to determine the medium to long-term effects of caudal autotomy on the thermal and hydric ecology of these animals while also investigating (or at least controlling) for potential effects of different hydration states or for any behavioural mechanisms which may be buffering underlying physiological differences.

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CRediT authorship contribution statement

Irene Fernández-Rodríguez: conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing an original draft, review and editing of the draft, visualization, project administration. **Frederico M. Barroso**: conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, review and editing of the draft, visualization, project administration. **Miguel A. Carretero**: conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, review and editing of the draft, visualization, supervision, project administration, funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2021.102976.

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