Time Changes Everything: Analysing Temporal Patterns of Evaporative Water Loss

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Abstract

Higher air temperatures and drier conditions may create stronger water vapour pressure and increase rates of cutaneous water loss, while elevated body temperatures may in turn directly speed up metabolic rates that lead to higher respiratory water loss. Therefore, water budgets are an important organismal trait for understanding their responses to climate change. The most common method of water loss estimation combines respiratory and cutaneous pathways by measuring body weight loss over a defined period of time. Currently, obtained values are often summed or averaged for population or species comparisons. We warn about potential statistical problems using average or summed values of water loss due to emerging temporal patterns. In this study we used a model dataset of lizards and to investigate temporal patterns in water loss datasets. We found that temporal patterns strongly vary across datasets and often deviate from the summed/average profile. Also, the duration of the experiment needs to remain long enough to detect the temporal patterns and produce representative results, while averages at different end-points of the experiment will also vary with temporal patterns. We propose that a simple statistical approach including hour of the experiment as non-linear explanatory variable in GAMM is used to investigate and adequately account for temporal patterns, which will ensure comparability of studies using meta-analyses in the future. Found signal of temporal variation in water loss also suggests that it holds significant biological relevance, potentially mostly connected to behavioural but also physiological adjustments and needs research attention in the future.

Introduction

Evaporative water loss (EWL) is an important physiological parameter, as it may account for the majority of an animal's water loss and has implications for heat balance. Therefore, it is no surprise that in recent years, studies on hydric physiology have become increasingly important in the light of climate change research. Increases in air temperature as a result of global warming may affect all organisms. In terrestrial habitats, vertebrates will undergo higher air temperatures which may create stronger water vapour pressure gradients that may increase rates of cutaneous water loss. This has been reported in reptiles (e.g. Dmi'el 2001), while latest research in birds and mammals implies that some species may have developed a form of acute physiological control of water loss rates in response to environmental hydric conditions (e.g. Eto et al. 2020), which may also be the case in other groups of organisms.

Much of our understanding to date of water budgets comes from measurements obtained with different methodologies and this may hamper multispecies comparative studies because of incompatibility of comparing different approaches or because an averaged or summed value of the experimental dataset is used to unify across datasets and methodologies. Such later practices are often applied to testing hypothesis of climate change impacts (e.g. Le Gaillard et al. 2021). Methodologies of assessing water loss vary from sensitive measures of water vapour flux (respirometry, metabolic cages), skin resistance and loss of body weight done in the laboratory or tracing water budgets and loss in the field (doubly-marked water) (for example of lizards see review of methodologies in Le Gaillard et al. 2021). Using a single measure of water loss (either average or accumulated) implicitly assumes that hydoregulation trend remains constant throughout the monitoring period and may suffer from oversimplification, hiding both organismal responses and vulnerabilities. Not considering temporal variation in water loss also assumes that there is no inter-specific variation in patterns of water loss and to date has not been properly investigated.

The skin of an organism has some intrinsic level of resistance to water loss that is inversely related to water loss. For example, in reptiles, the resistance to water loss reflects physical properties of the skin, with epidermal lipids constituting the main barrier to water loss in lizards and snakes (Dmi'el 2001; Lillywhite 2006). It has also been shown that dynamic skin resistance may facilitate water regulation (e.g. Dmi'el 2001) and hydoregulation may also occur on the ocular level, where minimization of time spent with the eyes open may be a form of hydoregulatory behaviour (Lanham and Bull 2004; Mathews et al., 2000). Moreover, behavioural modulation of activity and habitat use will have consequences on the hydric exposure conditions thus impact water loss in natural conditions (e.g. Mautz 1980). However, under laboratory conditions, basal values of water loss are obtained (similar to resting metabolic rates), since individuals are placed in individual chambers with limited activity options and lack of other stimulus (predators, food, mates, rivals, etc.). The potential artefact connected to behaviour and activity under such experiments may be connected with hyperventilation due to stress which increases respiratory water loss (e.g. Robertshaw 2006) and this will be realized by initial higher values of water loss followed with a decrease. Mentioned behavioural and physiological adjustments linked with hydoregulation could be displayed as temporal patterns of water loss but are only rarely addressed in water loss studies.

In the light of these observations, the goal of our meta-analysis was to examine temporal patterns in water loss datasets using a model group. We used an available dataset of lizards, and one of the most commonly used methodology to experimentally assess respiratory and cutaneous water loss rates using body weights (Le Gaillard et al. 2021). We investigated 1) whether temporal variation in water loss rates exist, 2) if we can categorize emerging temporal patterns, 3) how patterns influence outcomes of traditional methods and 4) provided guidelines how to statistically analyse them. Specifically, we aimed to inform future research on the possibility that the average or the accumulated value may mask the underlying temporal pattern and could be driven by physiological or behavioural background mechanisms.

Materials And Methods

Data Sources

We used data of instantaneous evaporative water loss rates (EWL_i) from multiple published sources comprising a data set from 23 populations of lizards, belonging to three different families and 16 different species (Table 1). A list of data sources used in the study is provided in the Data sources section and full data sets are available in the data repository listed in the Data Availability Statement. In all experiments used in the dataset, the experiments were designed to minimize activity, by 1) keeping temperatures at the resting level (the same as when lizards come out from the refuge), which minimizes the activity during the experiment; 2) having no light, sound, smell or other stimulus; and 3) there was no predation pressure, interaction with conspecifics or prey (for more information see sources and Table 1).

Table 1

Specifications of the data included in the meta-analysis with information on the species, sample size, sex and mean size of individuals (SVL = snout-to-vent length and Weight), and experimental conditions.

Experimental conditions									
Family	Species	N	Sex	SVL (mm)	Weight (g)	T (°C)	Relative humidity (%)	Period (hours)	Source
Lacertidae	<i>Algyroides fitzingeri</i>	6	M	37.65	1.16	~ 24	20–30	0700–1800	Carneiro et al. 2017
Lacertidae	<i>Algyroides marchi</i>	12	M	42.9	1.59	~ 24	20–30	0700–1800	García-Muñoz et al. 2013
Lacertidae	<i>Algyroides moreoticus</i>	5	M	47.93	3.18	~ 24	20–30	0700–1800	Carneiro et al. 2017
Lacertidae	<i>Algyroides nigropunctatus</i>	9	M	62.07	5.62	~ 24	20–30	0700–1800	Carneiro et al. 2017
Scincidae	<i>Chioninia stangeri</i>	10	M and F	73.9	9.01	~ 24	20–30	0800–1900	Carretero et al. 2016
Lacertidae	<i>Iberolacerta horvathi</i>	17	M and F	54.85	3.51	~ 25	25–35	0800–2000	Osojnik et al. 2013
Lacertidae	<i>Lacerta schreiberi</i>	8	M	95.99	23.58	~ 24	20–30	0800–2000	Ferreira et al. 2016
Lacertidae	<i>Podarcis bocagei</i>	10	M	54.06	3.58	~ 24	20–30	0800–2000	Ferreira et al. 2016
Lacertidae	<i>Podarcis guadarramae lusitanica</i>	9	M	53.73	3.07	~ 24	20–30	0800–2000	Ferreira et al. 2016
Lacertidae	<i>Podarcis liolepis</i>	16	M and F	57.17	3.65	~ 24	~ 35	0700–1900	Carneiro et al. 2015
Lacertidae	<i>Podarcis muralis ES</i>	13	M and F	59.34	7.33	~ 24	~ 35	0700–1900	Carneiro et al. 2015
Lacertidae	<i>Podarcis muralis SI</i>	16	M and F	54	3.72	~ 25	25–35	0800–2000	Osojnik et al. 2013
Lacertidae	<i>Psammodromus algirus</i>	8	M	74.38	11.57	~ 24	20–30	0800–2000	Ferreira et al. 2016
Phyllodactylidae	<i>Tarentola mauritanica DONANA</i>	15	M	70.45	11.79	~ 25	~ 25	1300–2400	Rato and Carretero 2015
Phyllodactylidae	<i>Tarentola mauritanica EVORA</i>	10	M	60.49	7.55	~ 25	~ 25	1300–2400	Rato and Carretero 2015
Phyllodactylidae	<i>Tarentola mauritanica JAÉN</i>	8	M	65.07	8.96	~ 25	~ 25	1300–2400	Rato and Carretero 2015
Phyllodactylidae	<i>Tarentola mauritanica MALCATA</i>	11	M	68.82	10.81	~ 25	~ 25	1300–2400	Rato and Carretero 2015
Phyllodactylidae	<i>Tarentola mauritanica MURCIA</i>	12	M	49.11	4.19	~ 25	~ 25	1300–2400	Rato and Carretero 2015
Phyllodactylidae	<i>Tarentola mauritanica PORTIMÃO</i>	13	M	69	10.24	~ 25	~ 25	1300–2400	Rato and Carretero 2015
Phyllodactylidae	<i>Tarentola mauritanica SÃO LOURENÇO</i>	15	M	61.5	7.82	~ 25	~ 25	1300–2400	Rato and Carretero 2015
Phyllodactylidae	<i>Tarentola substituta</i>	10	M	56.55	5.85	~ 24	20–30	0800–1900	Carretero et al. 2016
Lacertidae	<i>Timon lepidus ibericus</i>	6	M	140.72	69.77	~ 24	20–30	0800–2000	Ferreira et al. 2016

Experimental conditions									
Lacertidae	<i>Zootoca (vivipara) camiolica</i>	11	M and F	55.32	4.29	~ 25	25–35	0800–1900	Žagar et al. 2017

Water loss values

Organisms weight is measured at the beginning of the experiment to record the initial weight (W_0) and usually after every hour to obtain hourly measures of weight (W_n). From the hourly weight data, the instantaneous evaporative water loss (EWL_i) is calculated using the formula $(W_n - W_{n+1} / W_0) \times 100$ and records the pattern of EWL along time. The accumulated water loss (EWL_a), and the average of all EWL_i values (mean EWL_i) are commonly calculated as described here (Osojnik et al. 2013). We included in our analysis the EWL_i values of 12 hours of experiment.

Meta-analysis of temporal patterns

Data was initially checked for normality, heteroscedasticity and dependence (Zuur et al. 2010). To find if there is a temporal pattern in the measurements, a general additive mixed model (GAMM) was used to analyse the temporal pattern per species. As covariates the SVL and the weight were included. The individual was included as a random effect. The analysis was performed in R (R Core Team, 2020) and the library “mgcv” (Wood 2017). To investigate if temporal patterns affect average values at different time points of the experiment, average EWL_i measurements after 2–12 hours of the experiment were compared with each other. For this a Friedman chi-square test was used; when the Friedman’s test was significant, there was a difference observed between hourly average EWL_i values per species and consequently the post hoc test called Conover test was used. The analysis was performed in R (R Core Team, 2020) and the library “PMCMRplus” (Pohlert 2021), “rcompanion” (Magniafico 2021) and “multcompView” (Graves et al. 2019).

Results

Out of 23 data sets (Table 1), 19 (83 %) lizard EWL_i values exhibited a temporal pattern (Table 2, Fig. 1). By statistically analysing EWL_i (factor “hour”, Table 2) and observing plots (Fig. 1), we could distinguish five significant temporal patterns in our data sets (Fig. 2). **1) Initial acclimatisation**; where the first value (EWL_8) of the experiment was significantly higher from all later values (Fig. 2). This was observed in 13 (68 %) data sets that exhibited a pattern. Initial acclimatisation is not exclusive and another temporal pattern may be found afterwards. **2) Fluctuation**; where EWL_i values at least twice significantly increased and decreased in time, discarding the initial value. This was observed in 7 (37 %) data sets. **3) Steady decline**; where EWL_i values show a steady decrease in time and the last value (EWL_{18}) was the lowest value (Fig. 2). Decline was observed in 5 (26 %) data sets. **4) Steady increase**; where EWL_i values show a steady increase in time and the last value (EWL_{18}) was the highest value (Fig. 2) and this was observed in 1 (5 %) data set. **5) Middle peak**; where EWL_i values significantly increased, peaked at mid-time of the experiment, and later significantly decreased in time. This was observed in 2 (11 %) data sets.

Table 2

Results of GAMM models of EWL_i for factors hour of the experiment (Hour), body length (SVL), and body weight (Weight). Significant factor hour confirms a temporal pattern in the dataset and patterns were categorized based on plotted values (see Fig. 1) as: IA = initial acclimatisation, F = fluctuation, SD = steady decline, SI = steady increase and MP = middle peak.

Species	Hour			SVL				Weight				Pattern
	Edf	F	P	Estimate	SE	t	P	Estimate	SE	t	P	
<i>Algyroides fitzingeri</i>	8.118	85.53	< 0.0001	-0.0004	0.0006	-0.639	0.524	0.0013	0.0016	0.782	0.435	IA
<i>Algyroides marchi</i>	6.307	18.69	< 0.0001	0.0054	0.1542	0.035	0.972	-0.3214	0.6444	-0.499	0.620	IA + F
<i>Algyroides moreoticus</i>	3.994	73.38	< 0.0001	0.0075	0.0343	0.217	0.829	-0.0262	0.0612	-0.428	0.669	IA + SD
<i>Algyroides nigropunctatus</i>	4.469	5.312	< 0.001	0.0058	0.0051	1.132	0.261	-0.0153	0.0209	-0.733	0.465	IA + F
<i>Chioninia stangeri</i>	1	10.16	0.002	0.0050	0.0143	0.348	0.729	-0.0204	0.0297	-0.685	0.495	SD
<i>Iberolacerta horvathi</i>	1	0.295	0.587	0.0021	0.0066	0.324	0.746	-0.0196	0.0522	-0.375	0.708	/
<i>Lacerta schreiberi</i>	3.805	10.18	< 0.0001	0.0137	0.0051	2.685	0.009	-0.0264	0.0090	-2.938	0.004	MP
<i>Podarcis bocagei</i>	1	0.93	0.337	-0.0092	0.0153	-0.601	0.549	0.0017	0.0970	0.017	0.986	/
<i>Podarcis guadarramae lusitanica</i>	3.771	4.17	0.008	-0.0252	0.5843	-1.646	0.103	0.1121	0.1024	1.096	0.276	F
<i>Podarcis liolepis</i>	1	7.71	0.006	0.0080	0.0100	0.792	0.429	-0.0245	0.0496	-0.493	0.622	SD
<i>Podarcis muralis ES</i>	1	0.08	0.775	0.0083	0.0033	2.498	0.014	-0.0464	0.0066	-6.992	< 0.0001	/
<i>Podarcis muralis SI</i>	1	3.12	0.079	-0.0125	0.0100	-1.248	0.214	0.0968	0.0541	1.790	0.075	/
<i>Psammmodromus algirus</i>	4.361	5.27	< 0.001	0.0190	0.0042	4.552	< 0.0001	-0.0295	0.0051	-4.828	< 0.0001	F
<i>Tarentola mauritanica. DOÑANA</i>	8.295	60.51	< 0.0001	0.0058	0.0028	2.093	0.038	-0.0075	0.0058	-1.288	0.200	IA + F
<i>Tarentola mauritanica EVORA</i>	2.178	32.18	< 0.0001	0.0039	0.0024	1.617	0.109	-0.0114	0.0076	-1.502	0.136	IA + SD
<i>Tarentola mauritanica JAÉN</i>	2.565	34.08	< 0.0001	-0.0014	0.0011	-1.338	0.185	0.0047	0.0024	1.961	0.053	IA + SD
<i>Tarentola mauritanica PORTIMÃO</i>	5.035	31.56	< 0.0001	-0.0004	0.0012	-0.318	0.751	0.0020	0.0032	0.625	0.533	IA
<i>Tarentola mauritanica MALCATA</i>	5.382	84.08	< 0.0001	-0.0025	0.0013	-2.020	0.046	0.0056	0.0028	2.012	0.047	IA
<i>Tarentola mauritanica MURCIA</i>	7.925	100	< 0.0001	0.0006	0.0008	0.686	0.494	-0.0011	0.0024	-0.463	0.644	IA + F
<i>Tarentola mauritanica SÃO LOURENÇO</i>	8.118	85.53	< 0.0001	-0.0004	0.0006	-0.639	0.524	0.0013	0.0016	0.782	0.435	IA + F
<i>Tarentola substituta</i>	5.404	9.051	< 0.0001	0.0100	0.0092	1.084	0.281	-0.0173	0.0283	-0.611	0.543	IA
<i>Timon lepidus ibericus</i>	1	6.402	0.014	0.0015	0.0032	0.476	0.636	-0.0020	0.0025	-0.797	0.429	SI

Species	Hour		SVL				Weight				Pattern	
<i>Zootoca (vivipara) carniolica</i>	3.758	4.067	0.015	0.0004	0.0102	0.040	0.968	-0.0261	0.0548	-1.024	0.308	IA + MP

Comparisons between average EWL_i values after 2–12 hours of the experiment confirmed that with the duration of the experiment the average value significantly changes if a temporal pattern was detected with GAMM (Table 3, Fig. 3). There was only one case where GAMM did not detect a significant effect of “hour”, while Friedman chi-square test detected significant effect on average EWL_i values across the duration of the experiment. This was for *Podarcis muralis* SI, where P value of GAMM was close to significance; $P = 0.079$ (Table 1).

Table 3
Results of Friedman chi-square comparisons between average EWL_i values after 2–12 hours of the experiment.

Species	Friedman χ^2	df	P
<i>Algyroides fitzingeri</i>	59.00	10	<0.0001
<i>Algyroides marchi</i>	49.00	10	<0.0001
<i>Algyroides moreoticus</i>	111.52	10	<0.0001
<i>Algyroides nigropunctatus</i>	31.68	10	0.0005
<i>Chioninia stangeri</i>	57.00	10	<0.0001
<i>Iberolacerta horvathi</i>	10.00	10	0.4377
<i>Lacerta schreiberi</i>	51.00	10	<0.0001
<i>Podarcis bocagei</i>	17.00	10	0.0656
<i>Podarcis guadarramae lusitanica</i>	24.97	10	0.0054
<i>Podarcis liolepis</i>	19.07	10	0.0394
<i>Podarcis muralis ES</i>	12.69	10	0.2416
<i>Podarcis muralis SI</i>	23.00	10	0.0106
<i>Psammmodromus algirus</i>	18.00	10	0.0573
<i>Tarentola mauritanica DOÑANA</i>	148.16	10	<0.0001
<i>Tarentola mauritanica EVORA</i>	98.00	10	<0.0001
<i>Tarentola mauritanica JAÉN</i>	78.25	10	<0.0001
<i>Tarentola mauritanica PORTIMÃO</i>	124.95	10	<0.0001
<i>Tarentola mauritanica MALCATA</i>	108.55	10	<0.0001
<i>Tarentola mauritanica MURCIA</i>	120.00	10	<0.0001
<i>Tarentola mauritanica SÃO LOURENÇO</i>	148.30	10	<0.0001
<i>Tarentola substituta</i>	63.00	10	<0.0001
<i>Timon lepidus ibericus</i>	27.00	10	0.0029
<i>Zootoca (vivipara) carniolica</i>	25.00	10	0.0045

Discussion

Our meta-analysis showed that water loss not only rarely remains constant throughout time but also that temporal profile strongly varies across species often deviating from the summed/average profile. We could distinguish five different temporal patterns in our dataset. Majority exhibited initial acclimation and alternating drops and rises in water loss across 12 hours of the experiment. Few cases exhibited a steady decline or increase or a middle peak of EWL_i values. We emphasize that all temporal patterns are likely to cause statistical problems in comparative studies where summed or average values of water loss rates are used. Moreover, the length of experiment in the tested dataset show that 12 hours is sufficient to detect temporal patterns but shorter experiments might hamper recovering fine variation trends. A high occurrence of such complex temporal patterns also supports the possibility that they hold biological relevance that needs future research endeavours.

The high share of temporal patterns found in the diverse dataset clearly suggests time must be correctly considered in water loss studies. However, traditionally, the average or the sum of hourly water loss are used for comparative purposes. We showed that this might cause statistical problems (Fig. 2) and consequently results' interpretation. Comparing the averages across different experimental intervals (2–12 hours) showed averages calculated at different time-points will be significantly affected by temporal patterns. Specifically, the “initial acclimatisation” with the high initial value gives a non-normal distribution, which makes the average value meaningless and artificially inflates the total water loss value. The “steady decline” pattern could pull down the sum and the average value of the water loss and oppositely, the “steady increase” pattern would pull them up. For the “fluctuation” pattern, the sum is potentially going up or down, while for the “middle peak” it may go up. The normality of the data set could be compromised in all datasets with temporal patterns. With non-normal distribution it is more difficult to interpret the average value. Therefore, we suggest that in the future average and sum value of EWL_i should systematically be avoided in comparative studies, unless no temporal pattern is statistically proven and normality of data is achieved.

Moreover, when comparing temporal patterns, it is of high importance that the measurement durations are equal between datasets. It is for example recommended that if initial acclimatisation period is omitted, this should be done for all datasets in the comparison, even if not showing initial acclimatisation. Furthermore, with this analysis we show that there is a high chance that temporal variation will be missed (83% chance detected in our study data set) if the duration of the experiment is too short. In the light of refinement principle (3R principles; Russel and Burch 1959) it would be beneficial to shorten the duration of the experiment, however, this cannot be recommend based on the present evidence. We have shown that even if initial stabilisation in the data is observed after the first 6-hours of the experiment, temporal patterns may emerge later in the second part of the 12-hour experiment. Some studies of water loss follow experimental procedure where the weight is recorded only at the beginning and the end of the experiment (e.g. after 12 hours). We highly recommend to avoid such procedure because information obtained will be very limited and inferences about temporal variation will not be possible, while the animal will be used in the experiment.

On the other hand, the absence of patterns detected in approximately 17 % of analysed datasets should also not be neglected. Especially high share of datasets exhibited the “initial acclimatisation” pattern. One potential explanation for found absence of “initial acclimatisation” pattern that is likely caused by higher rates of activity at the beginning of the experiment, followed by lower rates of activity due to individual variability in susceptibility to handling stress (e.g. Rodríguez-Prieto et al. 2011). Thus these patterns may not be relevant to the population/species, but are more an artefact of taking hourly measurements over the course of a controlled experiment. Tested individuals may also have wet surface of the body due to housing conditions that dries up in the first hour of the experiment. Therefore, tested individual should always be allowed to calm down and dry the surface of the skin of any excessive humidity.

Found temporal patterns, “fluctuation”, “steady decline”, “steady increase” and “middle peak”, may be connected to several mechanisms that modulate skin/ocular resistance to water loss and respiration rates. Known behavioural mechanisms in reptiles for coping with dehydration are decreasing activity and hiding to burrows, shutting down eyes to prevent excessive evaporation through ocular surface, and decreasing respiratory rate (Mautz 1980, 1982; Araya-Donoso et al. 2021). Eyes have been shown to contribute significantly to water loss, since their surface is very permeable and minimization of time spent with the eyes open may be a form of hydroregulatory behaviour (Lanham and Bull 2004; Mathews et al. 2000). The permeability of the eyes may be important especially for species with relatively big eye surfaces compared to body. Furthermore, it is known that the variability of lipid content in the skin of reptiles is supposed to be the most responsible for the rate of cutaneous water loss (Roberts and Lillywhite 1980). Also, different fluid repartition among body compartments may occur as a response to dehydration (Nose et al. 1983; Arad et al. 1989). Overall, these existing evidence of behavioural and physiological hydroregulation not only provide potential explanations for findings of temporal patterns of water loss but also supports their significance.

In conclusion, our analysis of water loss data sets using diverse dataset shed a light on problematics of global comparative studies using average and sum values of water loss. All organisms today are suffering from impacts of global climate change that involve experiencing higher ambient temperatures and drying conditions, therefore, for improving our understanding of water loss in connection with complex impacts of global climate change we need to first fully understand properties of this physiological and behavioural functional trait. Simple and straightforward statistical approach and reporting should be used and the length of experiments should maintain long enough and comparable to be able to detect temporal patterns. Overall, our main recommendation for future is performing 12-hour experiments, collecting hourly measurements and providing raw data discriminated by time and individual. A standardized framework for analysing and reporting temporal patterns of water loss may follow the same procedure as used in this study: 1) comparing hourly values of water loss (EWL_i) with GAMM, using “hour” as factor, 2) plotting fitted hourly EWL_i values, 3) if “initial acclimatisation” pattern is identified, the first value should be removed and the remaining dataset should again be tested following steps 1) and 2). Reporting should include results of GAMM – non-linear explanatory variable “hour”, size and weight as covariates, individual as random effect. Plots should use fitted values for each hour and made per species/population. We believe that in the future, especial care should be taken not to ignore temporal patterns when using water loss data in order to improve our understanding of functional responses of organisms to dehydrating conditions.

Declarations

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Conflict of interest: The authors declare that they have no conflict of interest.

Ethics approval: This article does not directly contain studies with animals performed by any of the authors but includes already published datasets. Authors of all source datasets stated in their publications that all applicable institutional and/or national guidelines for the care and use of animals were followed during water loss experiments.

Availability of data and material: Data and Supporting Information (with DOI) will be available upon acceptance on the Pangaea repository (<https://www.pangaea.de/>).

Authors' contributions: AŽ, MAC and MdG conceived the ideas and designed methodology; AŽ and MAC compiled the datasets used in analysis; MdG analysed the data; AŽ led the writing of the manuscript, MdG and MAC wrote part of the manuscript, and all authors contributed critically to the drafts and gave final approval for publication.

References

1. Arad Z, Horowitz M, Eylath U, Marder J (1989) Osmoregulation and body fluid compartmentalization in dehydrated heat-exposed pigeons. *American Journal of Physiology-Regulatory Integrative Comparative Physiology* 257:R377–R382. doi:10.1152/ajpregu.1989.257.2.R377
2. Araya-Donoso R, San Juan E, Tamburrino I, Lamborot M, Veloso C, Véliz D (2021) Integrating genetics, physiology, and morphology to study desert adaptation in a lizard species. *Journal of Animal Ecology* doi:abs/. 10.1111/1365-2656.13546
3. Carneiro D, García-Muñoz E, Žagar A, Pafilis P, Carretero MA (2017) Is ecophysiology congruent with the present-day relictual distribution of a lizard group? Evidence from preferred temperatures and water loss rates. *Herpetological Journal* 27:47–56
4. Carretero MA, Lopes EP, Vasconcelos R (2016) An ecophysiological background for biogeographic patterns of two island lizards? *The Science of Nature* 103:1–10 <http://dxdoiorg/101007/s00114-016-1422-8>
5. Dmi'el R (2001) Skin resistance to evaporative water loss in reptiles: a physiological adaptive mechanism to environmental stress or a phylogenetically dictated trait? *Israel Journal of Zoology* 47:56–67. doi:abs/10.1560/ENQ9-KD7R-WFGW-KUQW
6. Eto E, Withers P, Cooper C (2020) Can birds do it too? Evidence for convergence in evaporative water loss regulation for birds and mammals. *Proceedings of the Royal Society B: Biological Sciences* 284:20171478 doi:10.1098/rspb.2017.1478
7. Ferreira C, Santos X, Carretero MA (2016) Does ecophysiology mediate reptile responses to fire regimes? Evidence from Iberian lizards. *PeerJ* <http://dxdoiorg/107717/peerj2107>
8. García-Muñoz E, Carretero MA (2013) Comparative ecophysiology of two sympatric lizards Laying the groundwork for mechanistic distribution models. *Acta Herpetologica* 8:123–128
9. Graves S, Piepho HP, Selzer L, Dorai-Raj S (2019) multcompView: visualizations of paired comparisons. R package version 0.1-8
10. Huey RB, Kingsolver JG (2019) Climate warming, resource availability, and the metabolic meltdown of ectotherms. *Am Nat* 194:E140–E150. doi:10.1086/705679
11. Lanham EJ, Bull CM (2004) Enhanced vigilance in groups in *Egernia stokesii*, a lizard with stable social aggregations. *J Zool* 263:95–99. doi:10.1017/S0952836904004923
12. Le Galliard JF et al (2021) A global and annotated database of evaporative water loss rates in squamate reptiles of the world. *Global Ecology and Biogeography*
13. Lillywhite HB (2006) Water relations of tetrapod integument. *J Exp Biol* 209:202–226. doi:10.1242/jeb.02007
14. Mangiafico S (2021) rcompanion: Functions to support extension education program evaluation. R package version 2.3.27
15. Mathews CG, Amlaner CJ (2000) Eye states and postures of the western fence lizard (*Sceloporus occidentalis*), with special reference to asynchronous eye closure and behavioral sleep. *Journal of Herpetology* 34:472–475
16. Mautz WJ (1980) Factors influencing evaporative water loss in lizards. *Comparative Biochemistry Physiology Part A: Physiology* 67:429–437. doi:10.1016/S0300-9629(80)80019-3
17. Mautz WJ (1982) Patterns of evaporative water loss. In: Gans C, Pough FH (eds) *Biology of the Reptilia* 12, Physiology C. Academic Press, London, pp 443–481
18. Nose H, MoRitmo T, Ogura K (1983) Distribution of water losses among fluid compartments of tissues under thermal dehydration in the rat. *Jpn J Physiol* 33:1019–1029. doi:10.2170/jjphysiol.33.1019
19. Osojnik N, Žagar A, Carretero MA, García-Muñoz E, Vrezec A (2013) Ecophysiological dissimilarities of two sympatric lizards. *Herpetologica* 69:445–454. doi:10.1655/HERPETOLOGICA-D-13-00014

20. Pohlert T (2021) PMCMRplus: Calculate Pairwise Multiple Comparisons of Mean Rank Sums Extended. R package
21. R Core Team (2020) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. <https://www.R-project.org/>
22. Rato C, Carretero M (2015) Ecophysiology tracks phylogeny and meets ecological models in an Iberian gecko. *Physiological and Biochemical Zoology* 88:564–575 <http://dxdoi.org/101086/682170>
23. Roberts JB, Lillywhite HB (1980) Lipid barrier to water exchange in reptile epidermis. *Science* 207:1077–1079. doi:10.1126/science.207.4435.1077
24. Robertshaw D (2006) Mechanisms for the control of respiratory evaporative heat loss in panting animals. *J Appl Physiol* 101:664–668. doi:10.1152/jappphysiol.01380.2005
25. Rodríguez-Prieto I, Martín J, Fernandez-Juricic E (2011) Individual variation in behavioural plasticity: direct and indirect effects of boldness, exploration and sociability on habituation to predators in lizards. *Proceedings of the Royal Society B: Biological Sciences* 278:266–273 doi:10.1098/rspb.2010.1194
26. Russell WMS, Burch RL (1959) The principles of humane experimental technique. Methuen
27. Wood SN (2017) Generalized additive models: an introduction with R. CRC press
28. Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. *Methods Ecol Evol* 1:3–14. doi:10.1111/j.2041-210X.2009.00001.x
29. Žagar A, Vrezec A, Carretero MA (2017) Does thermal and hydric physiology of *Zootoca (vivipara) carniolica* reflect their selected microhabitat conditions? *Salamandra* 53:153–159

Figures

Figure 1

Plotted fitted values of EWLi over time for 23 lizard populations of 16 different lizard species used in the analysis.

Figure 2

Five temporal patterns in water loss (A), their potential statistical problems of using sum (Σ) and average values (normal distribution plot) (B), and possible background mechanisms (C).

Figure 3

Plotted average EWLi values across the duration of the experiment for 23 lizard populations of 16 different lizard species with results of pairwise Friedman chi-square comparisons and Conover post-hoc tests between all average EWLi values indicated with small letters.