https://doi.org/10.1007/s00442-022-05166-z

Time changes everything: A multispecies analyses of temporal patterns in evaporative water loss

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Author 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.

Abstract

Increasing trends in environmental temperatures and changes in the hydrological cycle caused by climate change are affecting organisms around the world. Water balance is an important feature of organisms as warmer and drier environments increase water vapour pressure deficit and increase cutaneous water loss. The aim of our study was to understand the hourly kinetics of losses under water-restricted conditions. In general, this kinetics are thought to be negligible, and values from water loss studies have often been summed or averaged for population and species comparisons. We compiled a cross-species dataset of instantaneous water loss (EWL_i) of lizards in which weight was recorded every hour for 12 hours (a period considered ecologically relevant for lizards) under standardised conditions with minimal disturbance. We statistically tested whether the duration of the experiment affected the values and found that duration affected 83% of the datasets. In most cases, water loss was significantly higher during the first hour. This is likely a result of increased activity due to acclimation to the experimental conditions. However, as the experiment progressed, other temporal patterns of EWL_i occurred that were not consistent between species. In addition, the average value and the first and last values of EWL_i changed significantly for most species as the experiment progressed. These results suggest that hourly kinetics of water loss should not be ignored, while experimental procedures in which EWL is recorded continuously rather than only at the beginning and end of the experiment should be used to find finer temporal variations.

Keywords: hydric physiology, climate change, temporal variation, behaviour, physiology

Introduction

Water deficits in the environment that lead to stress strongly affect organisms during their life cycle, so maintaining adequate water balance is critical to their survival (Anderson et al. 2021). Current climate change projections show drastic changes in the water cycle in many regions of the world (Masson-Delmotte Vr et al. 2021). Increases in air temperature and shifts in precipitation regimes as a result of climate change may have negative impacts on water conditions for all organisms. In terrestrial habitats, many organisms will be exposed to higher air temperatures and drier conditions for at least part of the year, which will increase water vapour pressure deficit and increase cutaneous water loss (e.g., Hillman et al. 2008). In addition, projected increases in the severity and frequency of droughts will also negatively affect the water balance of terrestrial animals by reducing the availability of water for drinking or increasing the energy required to find water or food and shelter from the sun (Bradshaw 2003; Huey and Kingsolver 2019). Therefore, it is not surprising that studies of the hydric physiology of terrestrial fauna have become increasingly important in recent years in light of climate change research.

However, modelling water balance requires an understanding of the regulatory mechanisms. In squamate reptiles, most water loss is due to cutaneous gas exchange and a small portion to transpiration (Pirtle et al. 2019). Accordingly, the main role in maintaining the homeostatic state of water in the internal environment is determined by the properties of the skin. In reptiles, resistance to water loss reflects the physical properties of the skin, with epidermal lipids being the main barrier to water loss in lizards and snakes (Dmi'el 2001; Lillywhite 2006). In addition, it has been shown in lizards that dynamic skin resistance can facilitate water regulation (e.g., Dmi'el 2001). Hydroregulation may also occur at the ocular level, where minimising time spent with eyes open may be a form of hydroregulatory behaviour (Lanham and Bull 2004; Mathews et al., 2000). In addition, hyperventilation due to stress or increased physical activity increases respiratory water loss (e.g., Robertshaw 2006). Therefore, behavioural modulation of activity and habitat use affects hydric exposure conditions and thus influences water loss under natural conditions (e.g., Mautz 1980). Because a variety of physiological, behavioural, and hormonal mechanisms are involved in vertebrate water regulation, using the concept of functional traits controlling total water loss may be the best way to assess water balance.

Measuring total water loss is one of the simplest and most general methods of accounting for individual differences in resistance to water loss. Evaporative water loss (EWL), which accounts for the majority of an animal's water loss, is widely used in terrestrial animal groups (insects: e.g., Chown et al., 2011; amphibians: e.g., Hillman et al. 2008, reptiles: e.g., Dmi'el 2001, birds and mammals: e.g., Eto et al. 2020). In the experimental procedure, an individual is typically housed in a single chamber with limited activity opportunities and no other stimuli (predators, food, mates, rivals, etc.) to obtain baseline water loss rates (similar to resting metabolic rates). During the experiment, water loss rates can be measured using a variety of methods, such as water vapour flux (respirometry, metabolic cages), tracking water budgets and water loss with doubly labelled water, or measuring body weight loss (for a recent review of methods used in squamate reptiles, see Le Gaillard et al. 2021). In addition, water loss values can be recorded continuously or at different endpoints of the experiment, and the duration of the experiment can also vary considerably depending on the study organism and method (e.g., Mautz 1982; Lillywhite 2006; Lillywhite et al. 2009). Because we know that there are flexible water regulatory mechanisms and potential diurnal or context-dependent cycles in water loss (see above), we should expect that hydroregulatory mechanisms may influence measurements of water loss rates during the experiment. In addition, the wide range of methodological approaches used to assess total water loss rates may make it difficult to compare results. Therefore, it would be beneficial to propose a standardised measurement of total water loss using consistent protocols to compare data between studies.

In the light of these observations, temporal variation in water loss rates can lead to problems when comparative studies use different endpoints or average values because variability cannot be successfully captured. Furthermore, the discovery of temporal variation in water loss data sets will support the potentially important role of hydroregulatory mechanisms in achieving and maintaining an organism's water balance under desiccation conditions. For this reason, we decided to perform a comparison of evaporative water loss datasets for several species. The study was conducted on lizards because a large number of datasets were available (16 different species and 23 populations) and they were studies that used the same experimental methodology. All measurements were taken hourly over a 12-hour period (a period that the literature suggests is ecologically relevant for lizard activity, Rodda, 2020).Our goal was to test different types of temporal patterns in water loss rates: 1) the constant null model, which assumes no temporal variation in water loss rates; 2) the initial acclimation model, with an initial elevated value due to stress and followed by a decline in hourly water loss rates; and 3) more complicated models with hourly changes associated with intrinsic rhythms or responses to experimental conditions. We also examined how temporal variations statistically affect the use of water loss rates calculated at different endpoints. With the results of this study, future research should be better informed about the possibility that there may be temporal variation in water loss rates, which needs to be considered when conducting comparative studies and meta-analyses.

Materials and methods

Data Sources

We compiled a data set on water loss from 23 lizard populations belonging to 16 different species and three different families that were generated by our team (Table 1). A list of data sources used in the study is provided in the Data Sources section, and the full data sets are available in the data repository listed in the Data Availability Statement. Data from these studies included 12 hourly measurements of lizard body weight. The same and standardized protocol was used in all experiments. Only adult lizards with intact or fully regenerated tails were used. After capture, lizards were first acclimated to laboratory conditions (acclimation period of 1-2 days) and first used in preferred temperature experiments, followed by a water loss experiment. They were not fed for at least 24 hours before the water loss experiment. Their activity during the experiments was minimized by 1) maintaining temperatures at resting levels (the same when the lizards come out of refugium), which minimizes activity during the experiment; 2) there was no light, sound, odor, or other stimuli; and 3) there was no predation pressure, interaction with conspecifics, or prey. During weight measurement, each box of resting lizards was carefully removed from the chamber and weighed, a protocol that took an average of 30 seconds. For more information, see the sources listed in Table 1.

Water loss values

Instantaneous water loss (EWL_i) values were calculated from weight measurements taken hourly for 12 hours. The weight measured at the beginning of the experiment was recorded as the initial weight (W_0). The weights measured after each hour gave the hourly weight measurements (W_n). We calculated the EWL_i using the formula (W_n - W_{n+1} / W_n) × 100. We calculated the average water loss at different end points of the experiment (EWL_{s-e}) for an increasing duration of the experiment, obtaining 11 EWL_{s-e} values (calculated from 2, 3, 4 ... 12 EWL_i values).

Analysis of temporal patterns

Data was initially checked for normality, heteroscedasticity, and dependence (Zuur et al. 2010). To determine if there was a temporal pattern of EWL_i across the experiment, a general additive mixed model (GAMM) was used. Duration of the experiment was used as a non-linear variable. Body size (length from snout to vent, SVL) was included as a covariate. Individual was included as a random effect. The analysis was performed in R (R Core Team, 2020) and the library "mgcv" (Wood 2017).

Second, to investigate how increasing duration of the experiment affects the rate of water loss, the values obtained at the beginning and end of the experiment (EWL_{s-e}), calculated independently for each individual, were compared. To account for the non-independence of the data, we performed a Friedman repeated measures test. If it was significant, it means that a significant difference was observed between the compared values. Consequently, the Conover post hoc test was used and the values over the increasing duration of the experiment were plotted to show the significantly different groups. These analyses were repeated in data sets where the first EWL_i value was deleted when initial acclimation was detected. The analysis was performed in R (R Core Team, 2020) and the library "PMCMRplus" (Pohlert 2021), "rcompanion" (Mangiafico 2021), and "multcompView" (Graves et al. 2019).

Results

Of 23 data sets of EWL_i (Table 1), 15 (65%) data sets had a significantly elevated first value defined as initial acclimation (IA) (GAMM, factor "duration", Edf not close to 1, and $P < 0.05$, Table 2 and Figure 1). Apart from IA, we detected other temporal variations in EWL_i values in later hours of the experiment (Table 2, Figure 1) or in data sets where the initial value was removed due to IA and the analysis was repeated (Table 2, Figure 2). In one data set – *Algyroides fitzingeri*, IA pattern remained also after first two hours of the experiment (Table 2, Figure 2). Other temporal patterns were observed in a total of 17 (74%) data sets (Table 2).

We were able to describe four different forms of temporal variation (Figure 1 and 2) as: 1) Steady decrease (SD); here, EWL_i values showed a steady decrease over time and the last value (EWL_{i-12}) was the lowest value (GAMM, Edf close to 1 and $P < 0.05$, Table 2, Figure 1 and 2). 2) Steady increase (SI); here, EWL_i values show a steady increase over time and the last value (EWL_{i-12}) was the highest value (GAMM, Edf close to 1 and $P < 0.05$, Table 2, Figure 1 and 2). 3) Mid-peak (MP); here, EWL_i values significantly increased, peaked, and later significantly decreased (GAMM, Edf not close to 1, and $P < 0.05$, Table 2, Figures 1 and 2). 4) Middle valley (MV); here, EWLi values significantly decreased, were lowest, and later increased with time (GAMM, Edf not close to 1, and $P < 0.05$, Table 2, Figure 2). Overall, SD was observed in 9 (39%) data sets, MP in 4 (17%) data sets, SI and MV, both in 2 (9%) data sets (Table 2). Size of individuals (SVL) was a significant factor in only one complete data set (Table 3) and when the first value was removed in data sets with IA, no significant effects of SVL were observed (Table 3).

The average water loss values (EWL_{s-e}) calculated at different endpoints of the experiment were significantly different from each other in a total of 20 (87%) complete data sets (Table 4, the graphs in Online Resource 1 contain the results of the Conover post hoc test showing the significantly different groups). When the initial EWL_i value in the data sets with IA was excluded and the analyses were repeated, the EWLs-e calculated at the different endpoints of the experiment still differed significantly in all but two data sets (Table 4, plots with Conover post hoc tests are in Online Resource 2).

Discussion

Environmental physiologists studying animal water loss using gravimetric methods of weighting animals often collect data over a longer (more ecologically relevant) period of time and calculate the overall average or mean from the values obtained at the beginning and end of the experiment. However, regardless of the methodology used, continuously collected data have rarely been analysed to examine temporal variation in water loss data sets. Here, we compared temporal variation in instantaneous water loss (EWL_i) values from experiments in which animal weight was recorded continuously for 12 hours using the same protocol. This allowed analysis of temporal variation in instantaneous water loss over the duration of the experiment in a cross-species data set from comparable studies. Elevated initial values were observed in a large proportion of the data sets examined (65%), suggesting that this was an artefact due to the initial disturbance. Initial habituation, manifested as a very high first value, is often due to the stress of placing an animal in a chamber for the first time at the beginning of the experiment, resulting in increased activity followed by lower activity rates (e.g., Rodríguez-Prieto et al. 2011). After such initial acclimation, one would expect the measurements to stabilise or gradually decrease, which could be associated with a steady decrease in activity in the subsequent period. Such a temporal pattern of steady decrease was found in about 40% of our data sets. In addition, our analysis also showed that the instantaneous water loss may exhibit other temporal variations, such as a steady increase and a unimodal pattern of values over the duration of the experiment.

In addition, we tested whether the temporal variation of water loss affected the average water loss calculated at different endpoints of the experiment. This was confirmed for a large proportion of the data sets (87%). In particular, "initial acclimation" artificially inflates the average values. For example, when averages are calculated for *Algyroides marchi* from the first three hours of the experiment, the average value is overestimated by > 300% compared to the value calculated after 12 hours of the experiment. Next, the pattern of "steady decrease" found in nine data sets will gradually pull down the average values. For example, in the case of the steady decline of EWL_i in *Tarentola mauritanica* from Évora, we found a significantly lower average value every 1-2 hours of the experiment. Conversely, the pattern of "steady increase" gradually pulls up the average values. This was found in *Timon lepidus*, where the calculated average EWL_i values increased significantly throughout the duration of the experiment, except in the last 3 hours of the experiment, where the increase seemed to gradually level off. In addition, other unimodal patterns may inflate or deflate the average values depending on the time points in the experiment at which we compare the values. For example, in *Psammodromus algirus*, the total value calculated at 3 or 12 hours is the same, but it is significantly different from the values calculated at all other endpoints. In summary, our results indicate a bias in the measurements depending on the calculation period (a deviation from the average value when we use different endpoints). Consequently, this may also complicate the interpretation of the results. We suggest that EWL_i averages should be used with caution in the future and that continuous data collection should be encouraged with analyses that use the duration of the experiment as a factor.

During the experiments in our study, the lizards were never touched, but the boxes containing the individually housed lizards were carefully removed from the chamber and weighted to avoid disturbing the lizards. This procedure minimised the stress felt by the animals during the measurements, but we cannot rule out the possibility that the animals were disturbed to some degree when they were measured every hour. If the temporal patterns we found were simply an artefact of "manipulation," we would expect to see a linear effect over time and across species. In fact, in about 40% of our data sets, we recorded a linear response in which EWL_i values gradually decreased. For all species that showed a steady decrease in water loss, it is likely that this was due to a decrease in their activity or other unknown behavioural changes, such as body posture, eye behaviour, etc., and stress-induced changes in ventilation. The other patterns, a "steady increase" or modal patterns with a "peak" or "valley", were noted in a few species, which again may be due to differences in species responses to handling stress. Nevertheless, we cannot be certain that other behavioural or physiological mechanisms involved in hydroregulation may have contributed to the observed hourly kinetics.

One possibility is that the different ecology of the lizards used in our data set could also play a role. Specifically, we found that four species of lacertids, all of which are diurnal and generally found in mesic habitats and active at the ground surface (Arnold and Oveden 2004), exhibited a middle spike in EWL_i values. In contrast, all populations of geckos that are cathemeral or nocturnal and inhabit dry, rocky environments (Rodda, 2020) showed a steady or middle decline in EWL_i values. In addition, several mechanisms that modulate water loss through evaporation and respiration rate have already been described in lizards. For example, differential distribution of fluid between body compartments in response to dehydration has already been noted (Nose et al. 1983; Arad et al. 1989), which may contribute to the observed changes in water loss rates over time. Physiological mechanisms that can change rapidly include vascular mechanisms, but these mainly influence heat exchange rather than water loss (Hill et al. 2004). Another potential influencing factor is changes in ventilation, which can affect water loss through reparation, and changes in body posture, which can affect the surface area exposed to cutaneous water loss (e.g., Robertshaw 2006, Pirtle et al. 2019). Another important surface through which water evaporates in a reptile's body is the eyes, as their surface is highly permeable, and minimising time spent with eyes open may be a form of hydroregulatory behaviour (Mautz 1980, 1982; Lanham and Bull 2004; Mathews et al. 2000).

Because the studies used in our data set involved repeated handling of animals, it is particularly difficult to distinguish handling stress from other hydroregulatory mechanisms. Indeed, many available and published datasets on water loss are gravimetric, particularly in lizards and snakes (e.g., Gaillard et al., 2021). Therefore, we suggest that in the future, other methods of measuring water loss rates, such as repeated measurements with respiratory hygrometry, could be used with continuous measurements without disturbing the animals to further investigate temporal variation in water loss rates. This would allow us to better understand whether hydroregulatory mechanisms play a role in the temporal variation of water loss rates. However, in practise, when working with species that are difficult to access or with many individuals within a limited time period, logistics prevent extensive use of respirometers. When gravimetric methods are used, temporal variations due to handling stress should be adequately accounted for when comparing populations or species.

In conclusion, one of the recommendations of our study is to encourage the use of a period of acclimation (ideally 2 hours) before measuring the initial mass of the lizard and to perform the experiments during the time when the animals are resting to avoid high activity. Although it would be advantageous in terms of the refinement principle (3R principles-replacement, reduction, and refinement; Russel and Burch 1959) to shorten the duration of the experiment, this cannot be recommended on the basis of the present findings. We have shown that temporal patterns can emerge in the second part of our 12-h experiments, always taking care that animals did not lose too much water and always recovered easily from mild dehydration. If there is a finer temporal regulation of water loss and we find that it has biological relevance, this should be the main focus of future water loss studies. Overall, we recommend that future water loss studies focus on methods that can record water loss continuously and without disturbing the animals over time. As a next step, we should dive deeper into understanding why and how these patterns emerge to improve our understanding of the functional responses of organisms to dehydrating conditions. This knowledge is also important in light of the projected impacts of global climate change, which will bring higher ambient temperatures and dry conditions to many habitats around the world.

Declarations:

Funding: AŽ and MdG were supported by the Slovenian Research Agency (grant J1-2466 and AŽ: ARRS, Programme P1-0255, and MdG: Programme P4-0107). AŽ was also supported by the project 28014/02/SAICT/2017 granted by Fundação para a Ciência e a Tecnologia (FCT, Portugal). Most data were generated in the framework of the projects PTDC/BIA-BEC/101256/2008 and 28014/02/SAICT/2017 also from FCT.

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 Zenodo repository (https:// https://zenodo.org/).

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.

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Table 1. Details of data included in the analysis with information on species, sample size, sex, and average size of individuals (SVL = length from snout to-vent and weight) and experimental conditions.

						Experimental conditions			
Family	**Species**	**N**	**Sex**	**SVL (mm)**	**Weight (g)**	**T (ºC)**	**Relative humidity (%)**	**Period (hours)**	**Source**
Lacertidae	*Algyroides fitzingeri*	6	M	37.65	1.16	~ 24	20–30	0700–1800	Carneiro et al. 2017
Lacertidae	*Algyroides marchi*	12	M	42.9	1.59	~ 24	20–30	0700–1800	García-Muñoz et al. 2013
Lacertidae	*Algyroides moreoticus*	5	M	47.93	3.18	~ 24	20–30	0700–1800	Carneiro et al. 2017
Lacertidae	*Algyroides nigropunctatus*	9	M	62.07	5.62	~ 24	20–30	0700–1800	Carneiro et al. 2017
Scincidae	*Chioninia stangeri*	10	M and F	73.9	9.01	~ 24	20–30	0800–1900	Carretero et al. 2016
Lacertidae	*Iberolacerta horvathi*	17	M and F	54.85	3.51	~ 25	25–35	0800–2000	Osojnik et al. 2013
Lacertidae	*Lacerta schreiberi*	8	M	95.99	23.58	~ 24	20–30	0800–2000	Ferreira et al. 2016
Lacertidae	*Podarcis bocagei*	10	M	54.06	3.58	~ 24	20–30	0800–2000	Ferreira et al. 2016
Lacertidae	*Podarcis guadarramae lusitanica*	9	M	53.73	3.07	~ 24	20–30	0800–2000	Ferreira et al. 2016
Lacertidae	*Podarcis liolepis*	16	M and F	57.17	3.65	~ 24	~ 35	0700–1900	Carneiro et al. 2015
Lacertidae	*Podarcis muralis ES*	13	M and F	59.34	7.33	~ 24	~ 35	0700–1900	Carneiro et al. 2015
Lacertidae	*Podarcis muralis SI*	16	M and F	54	3.72	~ 25	25–35	0800–2000	Osojnik et al. 2013
Lacertidae	*Psammodromus algirus*	8	M	74.38	11.57	~ 24	20–30	0800–2000	Ferreira et al. 2016
Phyllodactylidae	*Tarentola mauritanica DOÑANA*	15	M	70.45	11.79	~ 25	~ 25	1300–2400	Rato and Carretero 2015
Phyllodactylidae	*Tarentola mauritanica EVORA*	10	M	60.49	7.55	~ 25	~ 25	1300–2400	Rato and Carretero 2015
Phyllodactylidae	*Tarentola mauritanica JAÉN*	8	M	65.07	8.96	~ 25	~ 25	1300–2400	Rato and Carretero 2015
Phyllodactylidae	*Tarentola mauritanica MALCATA*	11	M	68.82	10.81	~ 25	~ 25	1300–2400	Rato and Carretero 2015
Phyllodactylidae	*Tarentola mauritanica MURCIA*	12	M	49.11	4.19	~ 25	~ 25	1300–2400	Rato and Carretero 2015
Phyllodactylidae	*Tarentola mauritanica PORTIMÃO*	13	M	69	10.24	~ 25	~ 25	1300–2400	Rato and Carretero 2015
Phyllodactylidae	*Tarentola mauritanica SÃO LOURENCO*	15	M	61.5	7.82	~ 25	~ 25	1300–2400	Rato and Carretero 2015
Phyllodactylidae	*Tarentola substituta*	10	M	56.55	5.85	~ 24	20–30	0800–1900	Carretero et al. 2016
Lacertidae	*Timon lepidus ibericus*	6	M	140.72	69.77	~ 24	20–30	0800–2000	Ferreira et al. 2016
Lacertidae	*Zootoca (vivipara) carniolica*	11	M and F	55.32	4.29	~ 25	25–35	0800–1900	Žagar et al. 2017

Table 2. Results of GAMM models of EWL_i for the duration of experiment (Duration) factor for complete data sets and for data sets without the initial EWL_i value where initial acclimation was detected. The significant Duration factor confirms a significant change in EWL_i values over the duration of the experiment due to initial acclimation (IA) or other temporal variation in the data sets, which were categorized based on the plotted values (see Figure 1) as follows: SD = steady decline, SI = steady increase, MP = mid-peak, and MV = mid-valley.

Species	**Complete data set**					**Without initial value**			
	Edf	**F**	**P**	**Initial acclim-ation**	**Other temporal patterns**	**Edf**	**F**	**P**	**Other temporal patterns**
Algyroides fitzingeri	6.406	40.85	**<0.0001**	**IA**		1	22.97	**<0.0001**	**IA-2****
Algyroides marchi	6.307	19.05	**<0.0001**	**IA**		1	23.45	**<0.0001**	**SD**
Algyroides moreoticus	3.994	73.95	**<0.0001**	**IA**		3.674	42.26	**<0.0001**	**SD**
Algyroides nigropunctatus	4.469	5.37	**<0.001**	**IA**		1	1.802	0.183	
Chioninia stangeri	1	10.26	**0.002**		**SD**				
Iberolacerta horvathi	1.656	0.81	0.343						
Lacerta schreiberi	3.805	10.30	**<0.0001**		**MP**				
Podarcis bocagei	1	0.94	0.335						
Podarcis guadarramae lusitanica	3.771	4.21	**0.007**	**IA**		2.694	3.03	**0.022**	**MP**
Podarcis liolepis	1	7.75	**0.006**		**SD**				
Podarcis muralis ES	1	0.08	0.774						
Podarcis muralis SI	1	3.14	**0.078***		**SI***				
Psammodromus algirus	4.253	5.19	**<0.001**	**IA**		4.371	5.60	**0.001**	**MP**
Tarentola mauritanica. DOÑANA	8.295	60.89	**<0.0001**	**IA**		1.762	25.36	**<0.0001**	**SD**
Tarentola mauritanica EVORA	2.178	32.48	**<0.0001**	**IA**		1.708	30.63	**<0.0001**	**SD**
Tarentola mauritanica JAÉN	2.565	34.48	**<0.0001**	**IA**		1	37.12	**<0.0001**	**SD**
Tarentola mauritanica PORTIMÃO	5.035	31.78	**<0.0001**	**IA**		2.728	10.66	**<0.0001**	**MV**
Tarentola mauritanica MALCATA	5.382	84.79	**<0.0001**	**IA**		4.092	39.58	**<0.0001**	**MV**
Tarentola mauritanica MURCIA	7.925	100.80	**<0.0001**	**IA**		1	83.71	**<0.0001**	**SD**
Tarentola mauritanica SÂO LOURENCO	8.118	86.06	**<0.0001**	**IA**		1.864	39.65	**<0.0001**	**SD**
Tarentola substituta	5.393	9.11	**<0.0001**	**IA**		1	0.40	0.530	
Timon lepidus ibericus	1	6.50	**0.013**		**SI**				
Zootoca (vivipara) carniolica	3.758	4.10	**0.015**	**IA**		2.451	3.65	**0.015**	**MP**

* For *Podarcis muralis* SI the duration factor is close to significance, the Edf value is 1 and the plot in Figure 1 shows an increase in values, so we classify it as a steady increase (SI).

**For *Algyroides marchi*, the pattern defined as initial acclimation persisted in the data set without the initial value.

Table 3. Results of the body size factor (SVL = snout-vent length) included in the GAMM models of EWL_i (see Table 1) for complete data sets and for data sets without the initial EWL_i value where initial acclimation was detected.

Species	**Complete data set**				**Without initial value**			
	Estimate	**SE**	**t**	**P**	**Estimate**	**SE**	**t**	**P**
Algyroides fitzingeri	0.0184	0.013	1.456	0.151	-0.0576	0.080	-0.717	0.477
Algyroides marchi	-0.0594	0.085	-0.702	0.486	*			
Algyroides moreoticus	-0.0065	0.011	-0.574	0.567	-0.0046	0.011	-0.405	0.687
Algyroides nigropunctatus	0.0025	0.002	1.020	0.311	0.0032	0.002	1.834	0.070
Chioninia stangeri	-0.0037	0.007	-0.546	0.586				
Iberolacerta horvathi	-0.0003	0.004	-0.089	0.929				
Lacerta schreiberi	-0.0009	0.002	-0.546	0.587				
Podarcis bocagei	-0.0090	0.005	-1.720	0.088				
Podarcis guadarramae lusitanica	-0.0111	0.009	-1.262	0.210	-0.0114	0.009	-1.212	0.299
Podarcis liolepis	0.0037	0.005	0.731	0.466				
Podarcis muralis ES	-0.0008	0.007	-0.113	0.910				
Podarcis muralis SI	0.0026	0.006	-1.248	0.655				
Psammodromus algirus	0.0014	0.004	0.371	0.711	0.0011	0.004	0.265	0.792
Tarentola mauritanica. DOÑANA	0.0025	0.001	2.111	**0.036**	0.0023	0.001	1.965	0.051
Tarentola mauritanica EVORA	0.0004	0.001	0.570	0.570	0.0005	0.001	0.695	0.489
Tarentola mauritanica JAÉN	0.0006	0.0004	1.640	0.105	0.0006	0.0004	1.502	0.137
Tarentola mauritanica PORTIMÃO	0.0002	0.001	0.363	0.717	0.0003	0.001	0.409	0.684
Tarentola mauritanica MALCATA	-0.0002	0.0005	-0.323	0.748	-0.0002	0.0005	-0.526	0.600
Tarentola mauritanica MURCIA	0.0002	0.0002	0.918	0.360	0.0002	0.0002	1.190	0.236
Tarentola mauritanica SÂO LOURENCO	0.0001	0.0002	0.418	0.677	0.0001	0.0002	0.634	0.527
Tarentola substituta	0.0046	0.003	1.657	0.101	0.0036	0.003	1.297	0.198
Timon lepidus ibericus	-0.0007	0.002	-0.485	0.630				
Zootoca (vivipara) carniolica	-0.0066	0.008	-0.839	0.403	-0.0067	0.005	-1.302	0.196

* Due to the small sample size, we had to omit the SVL in the GAMM analysis in the dataset without initial value of *Algyroides marchi*.

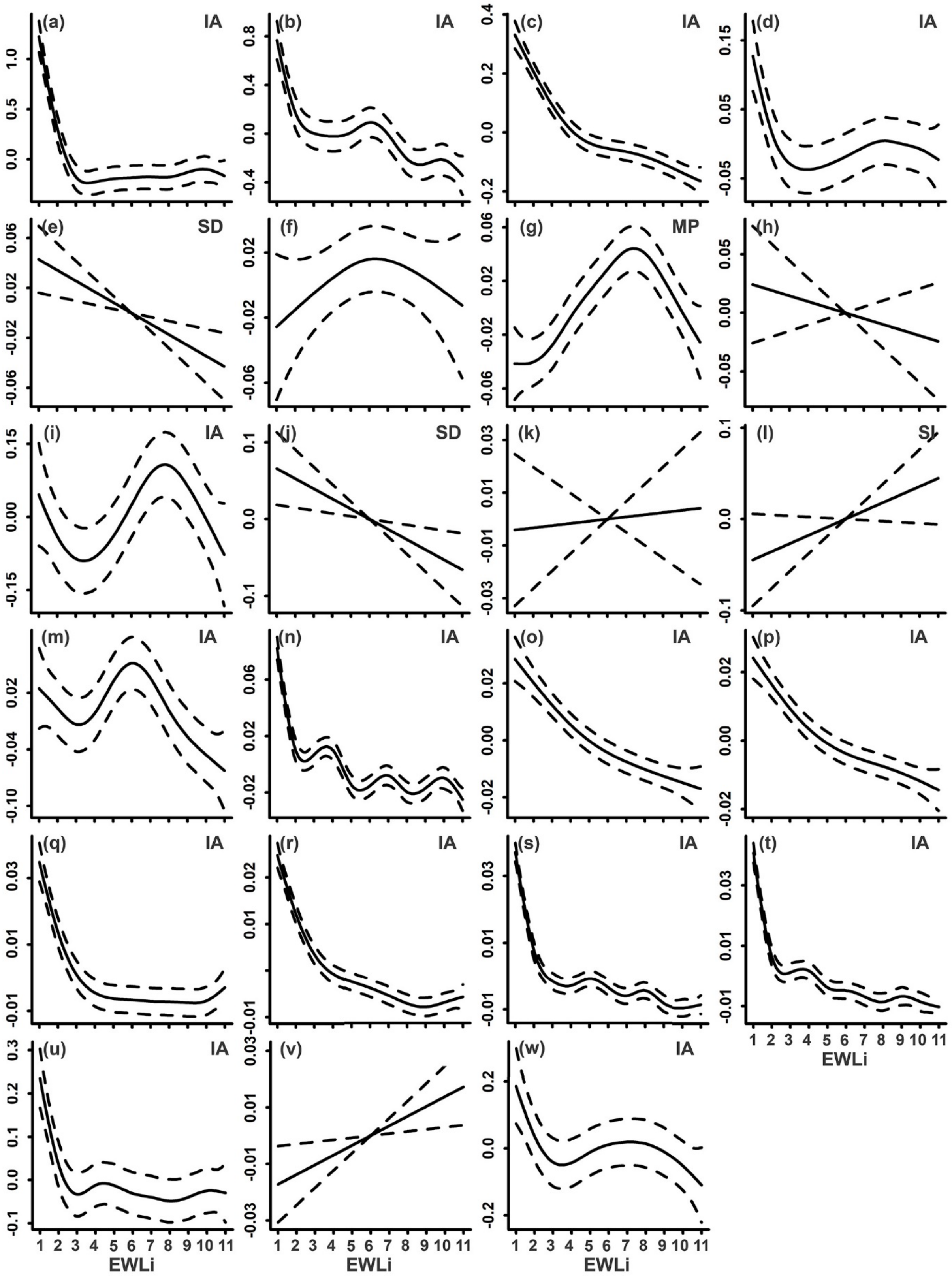
Table 4. Results of Friedman test comparisons between average water loss values (EWL_{s-e}) calculated at different endpoints of the experiment for complete data sets and for data sets without the initial EWL_i value where initial acclimation was detected.

	Complete data set			**Without initial value**		
Species	**Friedman**	**df**	**P**	**Friedman**	**df**	**P**
Algyroides fitzingeri	36.97	10	**<0.0001**	26.36	9	**0.002**
Algyroides marchi	40.73	10	**<0.0001**	33.87	9	**<0.0001**
Algyroides moreoticus	74.26	10	**<0.0001**	33.87	9	**<0.0001**
Algyroides nigropunctatus	20.51	10	**0.025**	7.836	9	0.551
Chioninia stangeri	25.46	10	**0.005**			
Iberolacerta horvathi	14.26	10	0.162			
Lacerta schreiberi	41.50	10	**<0.0001**			
Podarcis bocagei	18.98	10	**0.040**			
Podarcis guadarramae lusitanica	75.90	10	**<0.0001**	75.90	10	**<0.0001**
Podarcis liolepis	26.00	10	**0.004**			
Podarcis muralis ES	14.86	10	0.137			
Podarcis muralis SI	11.18	10	0.344			
Psammodromus algirus	42.59	10	**<0.0001**	42.59	10	**<0.0001**
Tarentola mauritanica DOÑANA	104.76	10	**<0.0001**	81.15	9	**<0.0001**
Tarentola mauritanica EVORA	74.91	10	**<0.0001**	61.11	9	**<0.0001**
Tarentola mauritanica JAÉN	67.43	10	**<0.0001**	57.38	9	**<0.0001**
Tarentola mauritanica PORTIMÃO	85.55	10	**<0.0001**	65.34	9	**<0.0001**
Tarentola mauritanica MALCATA	77.47	10	**<0.0001**	60.40	9	**<0.0001**
Tarentola mauritanica MURCIA	74.50	10	**<0.0001**	53.40	9	**<0.0001**
Tarentola mauritanica SÂO LOURENCO	91.58	10	**<0.0001**	64.89	9	**<0.0001**
Tarentola substituta	26.84	10	**0.003**	7.68	9	0.567
Timon lepidus ibericus	36.06	10	**<0.0001**			
Zootoca (vivipara) carniolica	103.62	10	**<0.0001**	23.99	9	**0.004**

Figure 1. Plotted fitted values of EWL_i over time for 23 lizard data sets: a) *Algyroides fitzingeri*, b) *Algyroides marchi*, c) *Algyroides moreoticus*, d) *Algyroides nigropunctatus*, e) *Chioninia stangeri*, f) *Iberolacerta horvathi*, g) *Lacerta schreiberi*, h) *Podarcis bocagei*, i) *Podarcis guadarramae lusitanica*, j) *Podarcis liolepis*, k) *Podarcis muralis* ES, l) *Podarcis muralis* SI, m) *Psammodromus algirus*, n) *Tarentola mauritanica* DOÑANA, o) *Tarentola mauritanica* EVORA, p) *Tarentola mauritanica* JAÉN, q) *Tarentola mauritanica* PORTIMÃO, r) *Tarentola mauritanica* MALCATA, s) *Tarentola mauritanica* MURCIA, t) *Tarentola mauritanica* SÂO LOURENCO, u) *Tarentola substitute*, v) *Timon lepidus ibericus*, and w) *Zootoca* (*vivipara*) *carniolica*. Temporal patterns were assigned to data sets with significant results from the GAMM analysis for the duration factor (see Table 2) and are labelled as follows: IA = initial acclimation, SD = steady decline, SI = steady increase, and MP = mid-peak. The black line represents the predicted line, while the dotted line indicates the standard error.

Figure 2. Plotted fitted values of EWL_i over time for 15 data sets where the first EWL_i measurement was removed because of initial acclimation (see Table 2): a) *Algyroides fitzingeri*, b) *Algyroides marchi*, c) *Algyroides moreoticus*, d) *Algyroides nigropunctatus*, e) *Podarcis guadarramae lusitanica*, f) *Psammodromus algirus*, g) *Tarentola mauritanica* DOÑANA, h) *Tarentola mauritanica* EVORA, i) *Tarentola mauritanica* JAÉN, j) *Tarentola mauritanica* PORTIMÃO, k) *Tarentola mauritanica* MALCATA, l) *Tarentola mauritanica* MURCIA, m) *Tarentola mauritanica* SÂO LOURENCO, n) *Tarentola substitute*, and o) *Zootoca* (*vivipara*) *carniolica*. Temporal patterns were assigned to data sets with significant results from the GAMM analysis for the duration factor (see Table 2) and are labelled as follows: IA = initial acclimation, SD = steady decline, SI = steady increase, MP = mid-peak, and MV = mid-valley. The black line represents the predicted line, while the dotted line indicates the standard error.

Figure 1


(a) IA
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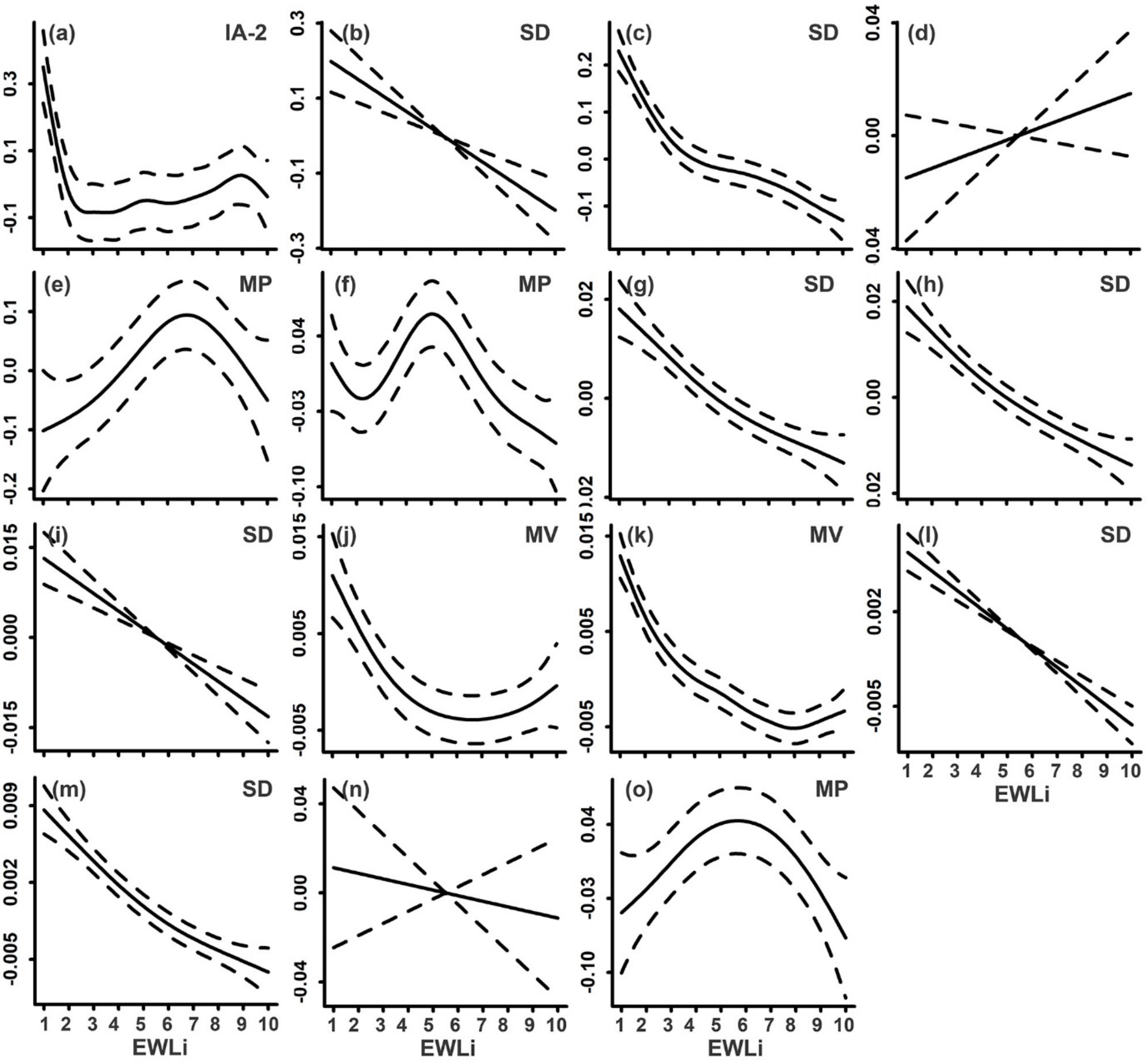
0.5
0.0
(b) IA
0.8
0.4
0.0
-0.4
(c) IA
0.4
0.2
0.0
-0.2
(d) IA
0.15
0.05
-0.05
(e) SD
0.06
0.02
-0.02
-0.06
(f)
0.02
-0.02
-0.06
(g) MP
0.06
0.02
-0.02
-0.06
(h)
0.05
0.00
-0.05
(i) IA
0.15
0.00
-0.15
(j) SD
0.1
0.0
-0.1
(k)
0.03
0.01
-0.01
-0.03
(l) SI
0.1
0.0
-0.1
(m) IA
0.02
-0.04
-0.10
(n) IA
0.06
0.02
-0.02
(o) IA
0.02
0.00
-0.02
(p) IA
0.02
0.00
-0.02
(q) IA
0.03
0.01
-0.01
(r) IA
0.02
0.01
-0.01
(s) IA
0.03
0.01
-0.01
(t) IA
0.03
0.01
-0.01
1 2 3 4 5 6 7 8 9 10 11
EWLi
(u) IA
0.3
0.2
0.1
0.0
-0.1
1 2 3 4 5 6 7 8 9 10 11
EWLi
(v)
0.03
0.01
-0.01
-0.03
1 2 3 4 5 6 7 8 9 10 11
EWLi
(w) IA
0.2
0.0
-0.2
1 2 3 4 5 6 7 8 9 10 11
EWLi

Figure 2


(a)
IA-2
0.3
0.1
-0.1
(b)
SD
0.3
0.1
-0.1
-0.3
(c)
SD
0.2
0.1
0.0
-0.1
(d)
0.04
0.00
0.04
(e)
MP
0.1
0.0
-0.1
-0.2
(f)
MP
0.04
-0.03
-0.10
(g)
SD
0.02
0.00
0.02
(h)
SD
0.02
0.00
0.02
(i)
SD
0.015
0.000
-0.015
(j)
MV
0.015
0.005
-0.005
(k)
MV
0.015
0.005
-0.005
(l)
SD
0.002
-0.005
(m)
SD
0.009
0.002
-0.005
(n)
0.04
0.00
-0.04
(o)
MP
0.04
-0.03
-0.10
1 2 3 4 5 6 7 8 9 10
EWLi