Comparative ecophysiology of two sympatric lizards. Laying the groundwork for mechanistic distribution models

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Abstract. Distribution modelling usually makes inferences correlating species presence and environmental variables but does not take biotic relations into account. Alternative approaches based on a mechanistic understanding of biological processes are now being applied. Regarding lacertid lizards, physiological traits such as preferred body temperature (Tp) are well known to correlate with several physiological optima. Much less is known about their water ecology although body temperature and evaporative water loss (Wl) may trade-off. Two saxicolous lacertids, *Algyroides marchi* and *Podarcis hispanica* ss are sympatric in the Subbetic Mountains (SE Spain) were they can be found in syntopy. Previous distribution modelling indicates the first species is associated with mountains, low temperatures; high precipitation and forest cover whereas the second one is more generalistic. Here, we perform two ecophysiological tests with both species: a Tp experiment in thermal gradient and a Wl experiment in sealed chambers. Although both species attained similar body temperatures, *A. marchi* lost more water and more uniformly in time than *P. hispanica* ss that displayed an apparent response to dehydration. These results suggest that water loss rather temperature is crucial to explain the distribution patterns of *A. marchi* in relation to *P. hispanica* ss, the former risking dehydration in dry areas no matter what temperature is. Ecophysiological traits represent a promising tool to build future mechanistic models for (lacertid) lizards. Additionally, the implications for their biogeography and conservation are discussed.

Keywords. Water loss, preferred body temperature, mechanistic models, *Algyroides marchi*, *Podarcis*.

INTRODUCTION

Thermal ecology is central in ecophysiological studies on lizards, and lacertids are not an exception to this rule (Castilla et al., 1999). Past decades gave rise to a plethora of detailed studies describing the proximate mechanisms linking temperature to physiology, life history, and behaviour (Angilletta, 2010). Preferred body temperature in the absence of thermoregulatory constraints (Tp) constitutes an important trait in lizard studies correlating with several physiological optima (Huey and Bennet, 1987; Bauwens et al., 1995; Angilletta et al., 2002; Carretero, 2008a).

In contrast, lizard water ecology has been neglected. Although protocols for testing water loss rate (Wl) already

exist (Lillywhite, 2006) and even when morphological proxis have been found (Caslbeek et al., 2006) no comparative results are really available. Nevertheless, preliminary evidence suggests that body temperature and evaporative water loss may trade-off (Bruce, 1990; Bowker, 1993). Those species capable to reduce Wl while remaining mobile have the best opportunities for a precise control of temperature (Tracy, 1976; Buttemer, 1990). An animal with low Wl may move to regulate its temperature precisely without experiencing the cooling and dehydrating effects of evaporation (Tracy and Christian, 2005).

Nonetheless, both types of variables (Tp and Wl) are essential to develop mechanistic models of potential distribution of species (Adolph and Porter, 1993; Fei et al.,

2012). Species distribution modeling is currently dominated by correlation analyses. That is, to infer the factors restricting the range of an organism, its presence is correlated to the environmental variables prevailing in its range. This procedure produces good results (Peterson and Kitchell, 2001; Martínez-Freiria et al., 2008; Santos et al., 2009), especially when the species have low dispersal abilities (Sillero et al., 2009). However, other external variables can be involved namely, biotic factors which can keep species absent from environmentally suitable areas (Kearney and Porter, 2004). Among these factors, competitive interactions between ecologically similar species may restrict species ranges (Begon, 2006). Even if species interactions can be modeled based on correlation approaches (Costa et al., 2008), the determination of physiological traits may provide solid evidence (Porter et al., 2002,).

Podarcis and *Algyroides* are two Mediterranean lacertid genera with highly contrasting richness and distribution patterns. While 21 species of *Podarcis* wall lizards are currently recognized, most of them with broad continuous distributions (Arnold et al., 2007), *Algyroides* consist of only four species with separate relict ranges (Harris et al., 1999). Among them, *Algyroides marchi* is the most saxicolous species restricted to a very small range in the Subbetic Mountains, SE Spain (Carretero et al., 2010). This species may be found in sympatry with *Podarcis hispanica* ss a member of the *P. hispanica* species complex (Carretero, 2008b; Kaliontzopoulou et al., 2011, 2012), which also inhabits rocky habitats. Correlative distribution models (Rubio and Carrascal, 1994; Sillero et al., 2009; Carretero et al., 2010) indicate that, at large scale, the presence of *A. marchi* depends on mountains, low temperatures, high precipitation and abundant forest cover, whereas, at small scale, it is constrained by terrain roughness and closure. Although both species can be found in syntopy (Carretero et al., 2010), the general trend is that *A. marchi* is restricted to the more humid spots, on the contrary *P. hispanica* ss inhabits drier and warmer rocky habitats, therefore we can say that *P. hispanica* ss is more tolerant than *A. marchi*. To elucidate to what extent this is due to ecophysiological differences or to (mutual) exclusion, we here analyse Tp and Wl experimentally in both species to determine whether these traits 1) differ intrinsically between them; and 2) are affected by intraspecific and interspecific interference.

MATERIALS AND METHODS

Five adult males of each species were collected in a syntopic area (Rambla los Vaquerizos, 38°3′N, 2°29′E). *P. hispanica* ss was assessed following Kaliounzoupoulou et al. (2011) where this samples were genetically analyzed. All individuals were captured by hand or noose (García-Muñoz and Sillero, 2010) in July 2010, and brought to the laboratory. Individuals were marked using a marker pen and kept in individual terraria for a total of eight days with food (*Tenebrio molitor* larvae and grasshoppers) and water provided *ad libitum* and under light (14 h light – 10 h darkness) and temperature (23.3 °C ± 1 °C) conditions similar to the natural regime. Each lizard was subjected to two experiments (see below). After each set of experiments, lizards were supplied with water and food *ad libitum* until they recovered the initial weight, and at the end of all experiments lizards were released in the capture site.

Lizards were exposed to a **c**lassic Tp experimental design of thermal gradient (~25-45 °C, $0.3 \times 0.4 \times 1.0$ m length experimental terrarium, Veríssimo and Carretero, 2009) produced by a 100 W infrared reflector bulb fixed 15 cm above the substrate and maintaining natural photoperiod. The walls of the terrarium were covered with white panels to prevent external stimuli influencing the lizard's thermoregulatory behaviour. Tp was measured with k-thermocouple digital thermometer (HIBOK 14; manufactured by HIBOK, precision 0.01 °C) by inserting a probe in the cloaca every hour during 10 hours (from 9:00 to 18:00 h local time). Snout ventral length (SVL) of each lizard was also registered to the nearest 0.01 mm using a digital calliper (model: Mitutoyo Digimatic Caliper).

Evaporative water loss experiment were developed using sealed chambers (~25 °C controlled temperature chamber, ~30-35 %RH generated by 100 g of silica gel). Wl was measured with a precision balance (precision 0.0001 mg; CPA model 224S, Sartorius Bohemia, New York). Initial weight (W_0) at the start of the evaporative water loss experiment was recorded. Lizards were individually placed in transparent plastic boxes covered with a perforated lid and floor placed inside another box with 5 g of silica gel at the bottom that allowed free air exchange between the individual box and sealed chambers. Five small boxes with lizards were placed in a sealed chamber at the same time. Lizards inside the boxes were removed from the chamber hourly, weighed together with the box on the precision balance and put back into the chamber, over a period of 12 hours. The whole operation did not take longer that 20 sec. The sealed chambers were maintained in obscurity and an undisturbed room in order to avoid stressing the animals.

Values of Tp (untransformed) did not deviate from normality (Shapiro-Wilk's test, $P > 0.05$ in all cases), were homoscedastic (univariate Levene's tests and multivariate Box M, $P > 0.05$ in all cases) and variances and means were uncorrelated. Repeat measures (rm) ANOVA, and rmANCOVAs (with LogSVL; and with LogSVL and $LogW_0$ (initial weight) as covariables) were performed in order to evaluate differences in Tp (hourly Tp; °C) between both species (*SP*; *A. marchi* and *P. hispanica* s.s.) A rmANOVA, and two rmANCOVAs (with LogSVL, and with logSVL and initial weight, $LogW_0$ as covariables) were performed to evaluate differences on accumulative water loss ($Wl = Log \{[(W_o-W_n)/W_o]*100\}$). A post-hoc Duncan´s test was performed after rmANOVA and rmANCOVA in order to detect differences (with and without body size/mass effects) in Wl between species.

In addition, Pearson correlation and linear least-squares were performed (for both species) in order to determine if there

is a relationship between Tp (Mean Tp for the 10 h, ºC) and total Wl (for the total accumulative water loss 12 h; Wl = Log (W8-W20)).
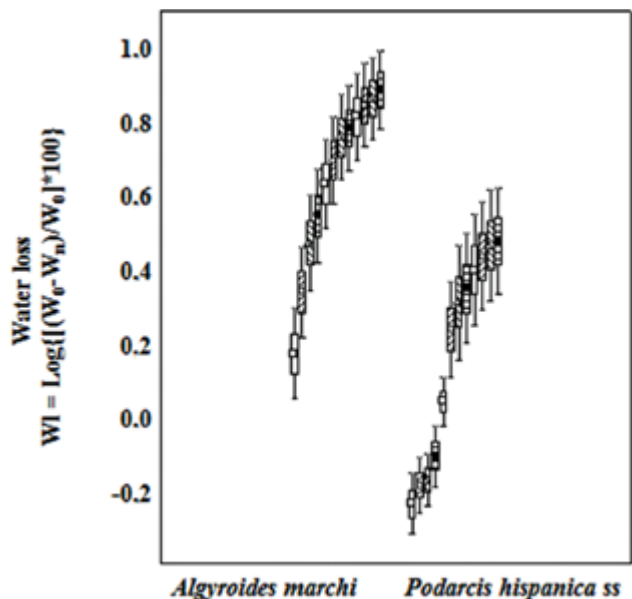

Fig. 1. Cumulative water loss in both species tested (*A. marchi; P. hispanica ss*), different whiskers denote different cumulative water loss intervals and their 95% IC, Wl = Log{[(W_0-W_n)/W_0]*100}, for both species in an experiment conducted during 12 hours.

RESULTS

Lizards of both species showed similar preferred body temperatures (*Algyroides marchi*, mean ± SD Tp = 31.5 ± 0.5; *P. hispanica* ss, mean ± SD Tp = 31.8 ± 1.5) and repeated measures AN(C)OVA showed no statistical differences, also after size-mass correction (rmANOVA, $F_{9, 72}$ = 1.702, P = 0.104; rmANCOVA, $F_{9, 63}$ = 0.394, P = 0.933, LogSVL = 3.830; rmANCOVA, $F_{9, 54}$ = 0.293, P = 0.973, LogSVL = 3.830, $LogW_0$ = 0.776).

In water loss experiments, *A. marchi* showed the highest Wl rates, reaching at the end of the experiment a water loss of approximately 10% of the initial weight. In contrast, *P. hispanica* ss showed much lower rates, especially during the first hours, final water loss attaining 3% of the initial weight. In addition, both species showed different Wl rates though time (*Time*Sp*, rmANOVA, Fig. 1) and post-hoc analyses revealed that both species responded in different ways to Wl in the first hours (Duncan test: Wl_{1-4}, P = 0.037; Wl_{1-5}, P = 0.035). These differences between both species were not SVL- or weight dependent, and these interactions remained significant (*Time*Sp*; rmANCOVAs, Table 2) after size/body mass was accounted for. In addition, another post-hoc test was performed after including covariables to test if the differences between both species were SVL- or weight dependent or intrinsic (Duncan test: Cov LogSVL, Wl_{1-3}, P =
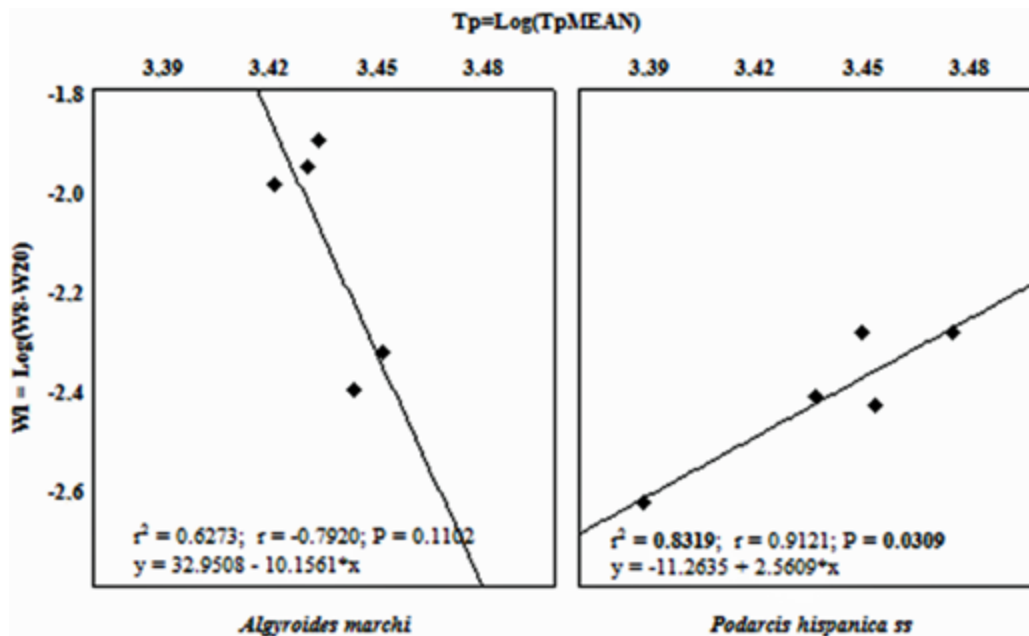

Fig. 2. Relationship among preferred temperature (mean; Log transformed) and total water loss (Log transformed) in both species

0.046; Wl_{1-4}, P = 0.021; Wl_{1-5}, P = 0.020; Wl_{1-6}, P = 0.029; Duncan test: $CovW_0$, Wl_{1-4}, P = 0.029, Wl_{1-5}, P = 0.027, Wl_{1-6}, P = 0.039).

Regression between Tp (mean Tp 10 hours, ºC) and Wl (12 hours, total accumulative water loss) varied between species. While in *A. marchi* no significant relations were detected, *P. hispanica* ss showed an inverse relationship between Tp and Wl (Fig. 2).

DISCUSSION

Our results indicate that both sympatric lizards differ in some ecophysiological traits. Concerning the thermal traits, both species appeared to have similar preferred temperatures which remained not significant after variation in body mass/size was accounted for. As other lacertid lizards, *A. marchi* and *P. hispanica* ss kept their body temperatures within a narrow range when free of thermal constraints (reviewed in Castilla et al., 1999; Carretero et al., 2005; Carretero at al., 2006; Veríssimo and Carretero, 2009). It is well documented that behavioural adjustments are the primary means by which lizards buffer fluctuations in ambient heat loads to maintain their Tp within the range that is conducive to optimal performance (Castilla et al., 1999).

Regarding the water ecophysiology, much less is known in lizards in general, and only two very recent studies analysed lacertid species (Carneiro et al., in press; Osojnik et al., 2013). Nevertheless, physiological studies have provided abundant information on osmoregulation and on mechanisms of water conservation of other lizards (Minnich, 1982; Nagy, 1982). Thus, the resistance to water loss reflects the combined effect of two integumentary components on the rate of water loss. The first one is a structural component, including differences in skin micro-structures and lipid content. The second component is more dynamic, representing physiological, vasomotor responses to short-term variations in the environment. This physiological response enables better and more immediate control of Wl (Eynan and Dmi´el, 1993). Our results not only show intraspecific differences in the magnitude of absolute water loss rate but also provide evidence for a dissimilar pattern of water loss throughout a normal diel activity period. Under the same conditions, *A. marchi* lost more water and at a more even rate than *P. hispanica* ss. In fact, *P. hispanica* ss displayed an apparent response to dehydration within the first four hours while *A. marchi* did not. In addition, results for *P. hispanica* ss supported a trade-off between Tp and Wl at the individual level while those for *A. marchi* were inconclusive. Future studies should evaluate such a relationship in others species.

Altogether, our ecophysiological results suggest that water should be more important than temperature to explain the distribution patterns of *A. marchi* vs. *P. hispanica* ss, the former risking dehydration in dry areas whatever the temperature. This is accordance with high resolution distribution models (Sillero et al., 2009; Carretero et al., 2010) indicating that *A. marchi* is restricted to mountains, high precipitation and good forest cover at a large scale and rugged and steep terrains at a small scale. These humid environments (allow to minimise lizard evaporative loss) are scarce in Mediterranean areas, which are characterised by midday and aestival draught, particularly in SE Iberia. Hence, it is not surprising that *A. marchi* displays such a restricted distribution. Also, taking into account the episodes of aridification that the whole Mediterranean Basin suffered periodically since the Pliocene (Cavazza and Wezel, 2003; Jiménez-Moreno et al., 2010), it would be expectable that *A. marchi* became gradually replaced by *P. hispanica* ss in this region. Going further, it is tempting to associate the climate transition, from subtropical to Mediterranean, since the Miocene to the spread and diversification of *Podarcis* sp. (Carretero, 2008b) in contrast to the retraction of *Algyroides* ssp. in southern Europe (Harris et al., 1999). This, however, needs confirmation by further comparative studies with other species of both genera. Last but not least, if the scenarios for climate change (IPCC, 2007) are confirmed, the vulnerability of *A. marchi* to dehydration and competitive displacement by sympatric *P. hispanica* ss put the former species at a serious extinction risk, in fact higher than suggested by correlation models (Carvalho et al., 2010). Overall, ecophysiological traits like preferred temperatures and water loss rates represent a promising tool to build future mechanistic models for lacertids and likely for other lizard groups.

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REFERENCES

Adolph, S.C., Porter, W.P. (1993): Temperature, activity, and lizard life histories. Am. Nat. **142:** 273-295.

Angilletta, M.J., Niewiarowski, P.H., Navas C.A. (2002): The evolution of thermal physiology in ectotherms. J. Therm. Biol. **27:** 249-268.

Angilletta, M.J. Jr. (2009): Thermal adaptation. Oxford University Press, Oxford.

Arnold, E.N., Arribas, O.J., Carranza, S. (2007): Systematics of the palaearctic and oriental lizard tribe lacertini (squamata: lacertidae: lacertinae), with descriptions of eight new genera. Zootaxa **1430:** 1-8.

Bauwens, D., Garland, T. Jr., Castilla, A.M., Van Damme, A. (1995): Evolution of sprint speed in lacertid lizards: morphological, physiological, and behavioural covariation. Evolution **49:** 848-863.

Begon, M., Townsend, C., Harper, J.L. (2006): Ecology. From individuals to ecosystems. Blackwell Publishing Ltd., Oxford.

Bowker, R.G. (1993): The thermoregulation of the lizards *Cnemidophorus exanguis* and *C. velox*: some consequences of high body temperature. In: Biology of Whiptail Lizards (genus *Cnemidophorus*), pp. 117-132. Wrigth, J.W., Vitt, L.J., Eds, Oklahoma Museum of Natural History, Norman, Oklahoma.

Bruce, G.W. (1990): Trade-offs in activity time and physiological performance for thermoregulating desert lizards, *Sceloporus merriami*. Ecology **71:** 2323-2333.

Buttemer, W.A. (1990): Effect of temperature on evaporative water loss of the australian tree frogs *Litoria caerulea* and *Litoria chloris*. Physiol. Zool. **63:** 1043-1057.

Carneiro, D., García-Muñoz, E., Kalionzopoulou, A., Llorente, G.A., Carretero, M. A. (in press): Comparing ecophysiological traits in two Podarcis Wall lizards with overlapping ranges. Amphibia-Reptilia.

Carretero, M.A., Roig, J.M., Llorente, G.A. (2005): Variation in preferred body temperature in an oviparous population of *Lacerta* (*Zootoca*) *vivipara*. Herpetol J. **15:** 51-55.

Carretero, M.A., Marcos, E., de Prado, P. (2006): Intraspecific variation of preferred temperatures in the NE form of *Podarcis hispanica*. In: Mainland and insular lacertid lizards: a Mediterranean perspective, pp. 55-64. Corti, C., Lo Cascio, P., Biaggini, M., Eds, Firenze University Press, Florence.

Carretero, M.A. (2008a): Preferred temperatures of *Tarentola mauritanica* in spring. Acta Herpetol. **3:** 57-64.

Carretero, M.A. (2008b): An integrated assessment of the specific status in a group with complex systematics: the Iberomaghrebian lizard genus *Podarcis* (Squamata, Lacertidae). Integr. Zool. **4:** 247-266.

Carretero, M.A., Ceacero, F., García-Muñoz, E., Sillero, N., Olmedo, M.I., Hernández-Sastre, P.L., Rubio, J.L. (2010): Seguimiento de *Algyroides marchi*. In: Seguimiento de *Algyroides marchi*. Informe final, Monografías SARE. Pp. 1-51. Asociación Herpetológica Española - Ministerio de Medio Ambiente, y Medio Rural y Marino. Madrid.

Carvalho, S., Brito, J.C., Crespo, E.G., Possingham, H.P. (2010): From climate change predictions to actions - conserving vulnerable animal groups in hotspots at a regional scale. Glob. Change Biol. **16:** 3257-3270.

Caslbeek, R., Knouft, J.H., Smith T.B. (2006): Variation in scale numbers is consistent with ecologically based natural selection acting within and between lizard species evolution. Ecology **20:** 377-394

Castilla, A.M., Van Damme, R., Bauwens, D. (1999): Field body temperatures, mechanisms of thermoregulation and evolution of thermal characteristics in licertid lizards. Nat. Croat. **8:** 253-274

Cavazza, W., Wezel, F.C. (2003): The Mediterranean region - A geological primer. Episodes **26:** 160-168.

Costa, G.C., Wolfe, C., Shepard, D.B., Caldwell, J.P., Vitt, L.J. (2008): Detecting the influence of climatic variables on species distributions: a test using GIS niche-based models along a steep longitudinal environmental gradient. J. Biogeogr. **35:** 637-646.

Eynan, M., Dmi´el, R. (1993): Skin resistance to water loss in agamid lizards. Oecologia. **95:** 290-294.

Fei, T., Skidmore, A.K., Venus, B., Wang, T., Schlerf, M., Toxopeus, B., van Overjijk, S., Bian, M., Lui, Y. (2012): A body temperature model for lizards as estimated from the thermal environment. J. Therm. Biol. **31:** 56-64.

García-Muñoz, E., Sillero, N. (2010): Two new types of noose for capturing herps. Acta Herpetol. **5:** 259-263.

Harris, D.J., Arnold, E.N., Thomas, R.H. (1999): A phylogeny of the european lizard genus *Algyroides* (Reptilia: Lacertidae) based on DNA sequences, with comments on the evolution of the group. J. Zool. Lond. **249:** 49-60.

Huey, R.B., Bennett, A.F. (1987): Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. Evolution **41:** 1098-1115.

Huey, R.B., Stevenson, R.D. (1979): Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. Am. Zool. **19:** 357-366.

IPCC (2007): Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change. Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J., Hanson, D.E., Eds, Cambridge University Press, Cambridge.

Jiménez-Moreno, G., Fauquette, S., Suc, J.P. (2010): Miocene to Pliocene vegetation reconstruction and climate estimates in the Iberian Peninsula from pollen data. Rev. Palaeobot. Palyno. **162:** 403-415.

Kaliontzopoulou, A., Pinho, C., Harris, D.J., Carretero, M.A. (2011): When cryptic diversity blurs the picture: a cautionary tale from Iberian and North African Podarcis wall lizards. Biol. J. Linn. Soc. **103:** 779-800.

Kaliontzopoulou, A., Carretero, M.A., Llorente, G.A. (2012): Morphology of the Podarcis wall lizards

(squamata: lacertidae) from the Iberian Peninsula and North Africa: patterns of variation in a putative cryptic species complex. Zool. J. Linn. Soc. **164:** 173-193.

Kearney, M., Porter, W.P. (2004): Mapping the fundamental niche: physiology, climate, and the distribution of a nocturnal lizard. Ecol. **85:** 3119-3131.

Lillywhite, H.B. (2006): Water relations of tetrapod integument. J. Exp. Biol. **209:** 202-226.

Martínez-Freiría, F., Sillero, N., Lizana, M., Brito, J.C. (2008): GIS-based niche models identify environmental mechanisms sustaining the location of a contact zone between European vipers (*Vipera aspis*, *V. latastei and V. seoanei*). Divers. Distrib. **14:** 452-461.

Minnich, J.E. (1982): The use of water. In: Biology of the Reptilia, Volume 12, pp. 325-395. Gans, C., Pough, F.H., Eds, Academic Press, London and New York.

Nagy, K.A. (1982): Field studies of water relations. In: Biology of the Reptilia, Volume 12, pp. 483-501. Gans, C., Pough, F.H., Eds, Academic Press, London and New York.

Osojnik, N., Žagar, A., Carretero, M. A., García-Muñoz, E., Vrezec, A. (2013): Eco-physiological dissimilarities of two sympatric lizards. Herpetologica **69**: 445-454.

Petersen, J.H., Kitchell, J.F. (2001): Climate regimes and water temperature changes in the Columbia River: bioenergetic implications for predators of juvenile salmon. Can. J. Fish. Aquat. Sci. **58:** 1831-1841.

Porter, W.P., Sabo, J.L., Tracy, C.M., Reichman, O.J., Tamankutti, N. (2002): Physiology on a Landscape Scale: Plant-Animal Interaction. Integr. Comp. Biol., **42:** 431-453.

Rubio, J.L., Carrascal, L.M. (1994): Habitat selection and conservation of an endemic Spanish lizard *Algyroides marchi* (Reptilia: Lacertidae). Biol. Conserv. **70:** 245-250.

Santos, X., Brito, J.C., Caro, J., Abril, A.J., Lorenzo, M., Sillero, N., Pleguezuelos, J.M. (2009): Ecological modelling predicts habitat suitability and extinction proneness in isolated populations of endangered species. Biol. Conserv. **142:** 344-352.

Sillero, N., Brito, J.C., Skidmore, A.K., Toxopeus, A.G. (2009): Biogeographical patterns derived from remote sensing variables: the amphibians and reptiles of the Iberian Peninsula. Amphibia-Reptilia **30:** 185-206

Tracy, C.R. (1976): A model of the dynamic exchanges of water and energy between a terrestrial amphibian and its environment. Ecol. Monogr. **46:** 293-326.

Tracy, C.R., Christian, K.A. (2005): Preferred Temperature Correlates with Evaporative Water Loss in Hylid Frogs from Northern Australia. Physiol. Biochem. Zool. **78:** 839-846.

Veríssimo, C.V., Carretero, M.A. (2009): Preferred temperatures of *Podarcis vaucheri* from Morocco: intraspecific variation and interspecific comparisons. Amphibia-Reptilia **30:** 17-23.