Assessing Ecophysiological Traits and Distribution Patterns of Two *Podarcis* Species in NE Iberia

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List of manuscripts

This thesis is based on the following manuscripts:

I Carneiro, D., García-Muñoz, E., Kaliontzopoulou, A., Llorente, G. A., Carretero, M. A. (2011). Comparing ecophysiological traits in two *Podarcis* Wall lizards with overlapping ranges. *Manuscript*

II Carneiro, D., García-Muñoz, E., Carretero, M. A. (2012). Field body temperatures of two *Podarcis* species (Reptilia: Lacertidae) in sympatry. *Manuscript*

III Carneiro, D. & Carretero, M. A. (2012). Predicting current and future distribution patterns and putative sympatry areas of two *Podarcis* Wall lizards in north-eastern Iberian Peninsula. *Manuscript*

Abstract

Ecological factors are known to limit species geographical distribution. Lacertids, being ectotherms, are likely to be most influenced by thermal conditions but factors such as environmental humidity or species interactions may also be preponderant.

Podarcis liolepis and *P. muralis* are lacertid species overlapping at a small scale in north-eastern Iberian Peninsula (IP). While *P. liolepis* ranges from the north-east of IP to southern France, *P. muralis* has a wide range across Europe but in IP is restricted to the north.

Knowing and understanding the factors most likely to shape this pattern is the aim of this study. This has been assessed through three methodological approaches. Ecophysiological experiments in the laboratory were conducted to assess the thermal and hydric requirements of *P. liolepis* and *P. muralis* (manuscript I). In a sympatry area, field records of body temperature were taken and the influence of environmental factors on body temperatures were assessed (manuscript II). Correlative predictive models were performed in order to assess the putative current and future geographic distribution of both species (manuscript III). The ecophysiological data previously obtained was integrated in the interpretation of the modelling data.

The three approaches revealed that environmental humidity and not temperature is the main abiotic factor constraining these species. However, evidence of possible interaction between both species was detected in terms of thermal physiology since the preferred body temperature of *P. liolepis* shifted upwards in the presence of *P. muralis*. Combining this information with the prediction for future scenarios of climate changes, it is expected that *P. liolepis* may be the most affected if climate gets to hot and dry since it would shift northwards and would disappear from some areas due to aridification. On the other hand, *P. muralis*' range is likely to decrease but little fragmentation would occur.

Keywords

Podarcis, sympatry area, preferred temperature, water loss, Ecological Niche-based models, environmental humidity

“Much of the work that is done under the name of ecology is not ecology at all, but either pure physiology (…) or pure geology, meteorology, or some other science concerned primarily with the outer world.”

Charles Elton (1927, pp.33)

“As an ecological problem, the question of why a species has a restricted distribution, given its present physiological and other features, can be answered, even if with difficulty. But range limits pose an evolutionary problem that has not been solved.”

Futuyma 1998, p. 535.

Abbreviations

AUC	Area under the receiver operating characteristics curve
CCCma	Canadian Centre for Climate Modelling and Analysis
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CV	Climatic variable
EGM	Ecological Niche-based models
GIS	Geographical Information Systems
HadCM3	Hadley Centre Coupled Model, *version 3*
IP	Iberian peninsula
RH	Relative humidity of the air
ROC	Receiver operating characteristic
SVL	Snout-to-vent length
T_{air}	Temperature of the air
Tb	(Field) Body temperature
Tp	Preferred temperature
Ts	Temperature of the substrate
W_0	Initial weight
Wl	Water loss

1 Introduction

Distribution patterns of species are driven by an assemblage of factors (Gaston, 2009) including abiotic (climate, geography, geology) and biotic factors (dispersal limitations, interactions among species; Hardin, 1960; Araújo and Luoto, 2007), phylogeography (Oliverio et al., 2000; Carranza et al., 2004), and ultimately, physiological limitations. In order to understand the most preponderant driving force(s) of distribution patterns insights from a variety of fields of study should be integrated. Genetic (Pinho et al., 2009; Camargo et al., 2010), morphological (Kaliontzopoulou et al., 2012) and modelling (Santos et al., 2006; Martínez-Freiría et al., 2008) techniques, among others, are widely used and should be combined with ecophysiological studies. Ecophysiology relates physiological mechanisms of organisms to their physical and biological environment (Macnab 2002).

Lizards provide good models to conduct ecophysiological studies since many species are conspicuous, sedentary and abundant, short-living and small, hence being easy to handle and to keep during laboratory studies (Pianka and Vitt, 2003).

Lizards constitute a paraphyletic assemblage within the Squamata order which also includes snakes (suborder Serpentes) and amphisbaenians (suborder Amphisbaenia) (Townsend et al., 2004; Camargo et al., 2010). Their representatives encompass a wide range of sizes, morphologies and ecological patterns and trends (Pianka and Vitt, 2003). They occupy a wide range of habitats from the tropical forests to the most arid and warm deserts both in water and in land (Vitt and Caldwell, 2009). Their distribution ranges as far north as latitude 70º, after the Arctic Circle (Andersen, 1971). However, only a restricted number of species cross that barrier revealing that most terrestrial ectotherms are not able to thrive at high latitudes and high altitudes because they impose marked constraints such as low environmental temperatures, shortage of shelters and short reproductive periods (Sears & Angilletta Jr., 2003). Therefore, temperate lizards living in such areas are more constrained in terms of growth and activity levels than conspecifics in more mild climates (Sears and Angilletta Jr., 2003) because low air temperature and high relative humidity may restrict the time available to forage leading to lower growth rates and decreased reproductive output (Adolph and Porter, 1993). Such restrictions lead to changes in life histories strategies. For example, it has been reported that *Sceloporus* lizards hatchlings in high altitudes in southern Utah grow faster than the hatchlings at low elevations but attain the same body size by the following spring after hatching (Sears and Angilletta Jr., 2003).

Since evaporation increases with increasing temperature, it has been suggested a strong interaction between thermoregulation and water balance in ectotherms, particularly when water supplies are restricted (Mautz, 1982). Also, body size influences thermal and hydric requirements. Small-bodied organisms have low thermal inertia and lose water faster than bigger and heavier organisms (Sears and Angilletta Jr., 2003) because of the low surface/volume relation (Gans et al., 1968; Turk et al., 2010). When size increases mass increases faster than the surface area because the volume increases in proportion to the cube of the linear dimension while the surface area is proportional to the square of the linear dimension (mass is directly proportional to volume). Since heating and cooling rates are related to surface area it is common to find that heating rates of similarly-sized and phylogenetically related individuals do not differ (Stevenson, 1985; Tosini and Avery, 1993; Fei et al., 2012).

1.1 Thermal ecophysiology

The influence of temperature on physiology has been the main focus in reptile studies probably because empirical observations reveal that reptiles directly depend on the sun for their daily activities (Huey, 1976).

Physiological processes, behaviour and the performance of functions such as locomotion, reproduction, development, digestion and growth (Huey and Stevenson, 1979; Huey, 1982; Angilletta et al., 2002) depend on body temperature (Tb) because it constrains the rates of biochemical reactions (Hochochka and Somero, 2002). Some ectotherms have a wide range of optimal temperature and so, are named eurytherms. Conversely, stenotherms have a narrow range of optimal Tb (Huey, 1982).

Organisms show several responses on how they maintain Tb. Homoeothermic organisms such as birds and mammals are able to precisely maintain a constant and adequate Tb by regulating the rates of heat production and loss through high rates of metabolism. On the other hand, ectotherms (poikilotherms in old textbooks) cannot metabolically maintain a stable body temperature (Huey and Slatkin, 1976; Castilla et al., 1999) so Tb mainly depends on changes of environmental temperature. Some ectotherms are, however, able to maintain a fairly constant body temperature different from environmental temperature by thermorregulating (Huey, 1982) since it allows keeping the rate of heating similar to the rate of heat loss (Schmidt-Nielsen, 1997b).

Thermoregulation consists of a set of behavioural and physiological interconnected strategies used to reduce heat loss by conduction and evaporation and maximizing heat gain by radiation (for heliothermic species) or conduction (for thigmothermic species) in order to maximize heat storage (Fig. 1; Macnab, 2002). Conduction, evaporation and radiation are the three physical processes by which temperature is transferred between an organism and the surrounding environment. Conduction of heat occurs when there is direct contact between two surfaces; convection is the movement of a fluid, so it may complicate the conductive heat transfer. When there is no direct contact between two objects/surfaces, heat is transferred by radiation. Solar radiation is the only commonly available external source of radiation.

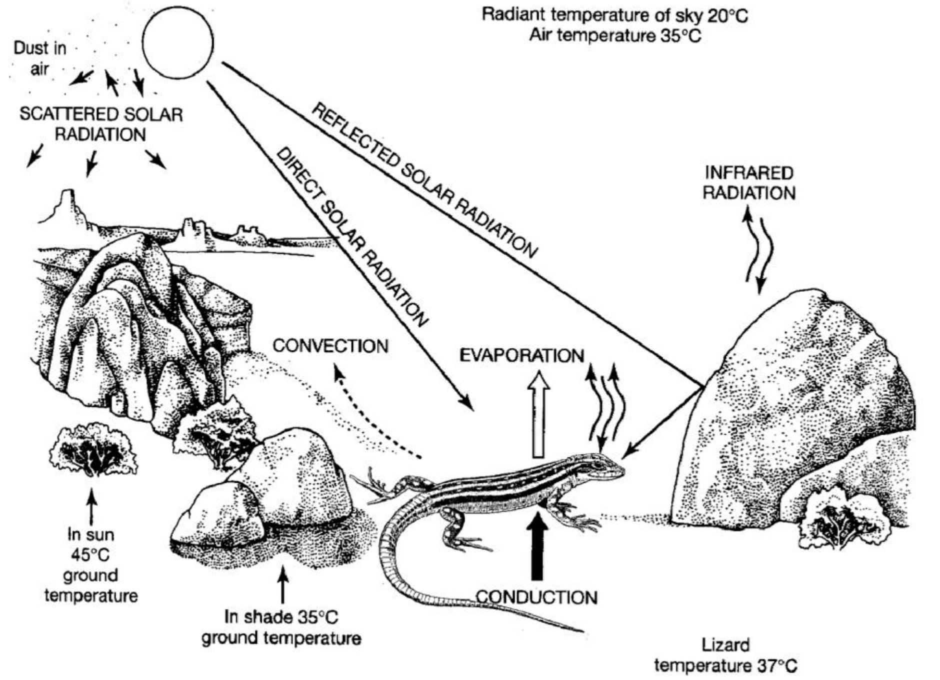

Fig. 1. Thermal transfer between a lizard and the surrounding environment. The animal heats by direct (sun) or indirect (soil, rocks) infrared radiation and conduction with the warm substrate and loses heat by evaporation, convection and conduction. Shifts between sun and shade also allow the animal to maintain a rather stable body temperature. Adapted from Ough et al., 2004.

Thermoregulatory behavioural strategies include i) habitat selection (changing heat flux by shuttling between hot and cold microenvironments), ii) exposing different surface areas by modifying posture (basking; see Glossary), and iii) regulating activity times (selective exploitation of environmental thermal flux) (Huey and Slatkin, 1976; Huey, 1982; Stevenson, 1985).

These behavioural strategies are controlled at neuronal level. Two sorts of hypothalamic receptors sensitive to temperature determine the upper and lower set point temperatures (Tosini and Avery, 1996). The upper and lower thermal set points are temperatures at which animals change behaviour in order to termorregulate more precisely, keeping Tb within the optimal range. Whenever Tb reaches the lower set point the animal makes the Tb rise by starting to bask. They can also place themselves in direct contact with warm substrate and increase heat gain by conduction with the temperature of the substratum. When an animal reaches the upper set point after basking, it starts a cooling behaviour, such as lighten the skin colour, positioning more parallel to the sun or shifting to the shade (Tosini and Avery, 1993). Skin colour is empirically expected to influence heating rates: darker colour would heat faster because it increases the amount of solar radiation absorbed rather than reflected (Schmidt-Nielsen, 1997b). However, in lacertid lizards no relevant differences in heating rates have been observed between melanic and non-melanic individuals (Tosini and Avery, 1993). Conversely, it appears to be a physiological by-product of other evolutionary pressures on behaviour (Raia et al., 2010).

Dorsal skin receptors trigger behavioural thermoregulatory mechanisms that are mediated by the described neural control. If no information reaches the skin receptors animals cannot properly control thermoregulatory behaviour hence spending more time in the heated area of a testing terraria and show a lower upper set point (Tosini et al., 1995). If Tb rises above the upper set point, performance rapidly decreases until the critical thermal maximum while below the lower thermal set point performance decreases slower until the critical thermal minimum (Huey and Stevenson, 1979). The critical temperatures are determined when an animal cannot respond to external stimuli, that is, it cannot turn on its feet when placed on its back (Yang et al., 2008). When animals are exposed to exceptionally high or low temperatures and are unable to prevent reaching a Tb near the critical thermal set points several factors may lead to death: (i) denaturation of proteins, thermal coagulation; (ii) inactivation of temperature-sensitive enzymes at rates that exceed formation rates; (iii) inadequate oxygen supply (because increased temperature accelerates the demand of oxygen; Niewiakowski and Waldschmidt, 1992); (iv) temperature effects on membrane structure (particularly in molecular structures that depend on weak interactions that are easily changed by temperature); (v) different Q_{10} (see Glossary) on interdependent metabolic reactions (leading to depletion or accumulation of intermediary metabolic products) (Schmidt-Nielsen, 1997b). Usually reptiles cease activity much before reaching such critical values except in emergency situations (Huey, 1976).

By thermorregulating, reptiles use environmental conditions to reach a Tb close to the preferred body temperature (Tp) which is reached when there are no thermoregulatory constraints (Van Damme et al., 1990). This only happens in laboratorial controlled conditions since in the natural habitat biotic and abiotic constraints are present.

According to Tosini et al., (1994), the Tb of species within the lacertids genera *Zootoca* and *Podarcis* can be affected by the feeding status. After feeding, most lizards select higher mean Tb and higher upper and lower set point temperatures than before feeding or after defecating (Macnab, 2002; Tosini et al., 1994). The overall time spent basking and shuttling frequency also increased after feeding. This response is probably due to an increase of the rate and/or efficiency of digestion since higher Tb seems to facilitate the mechanical processing of food and the catabolic action of enzymes speeding the digestive process (Macnab, 2002; Tosini et al., 1994). However, the opposite pattern was observed in nocturnal geckos in which starved lizards selected higher Tp than fed lizards (Autumn and De Nardo, 1995). This may be related with the different diel cycles of diurnal and nocturnal species.

Daily activity patterns may change along the year for temperate lizards (Fig. 2). With increasing number of light hours activity periods are longer and Tb rises (Lorenzon et al., 1999). In contrast, with less light hours the lower and upper set points decrease (Rismiller and Heldmaier, 1982; Tosini and Avery, 1996) and lizards become inactive.

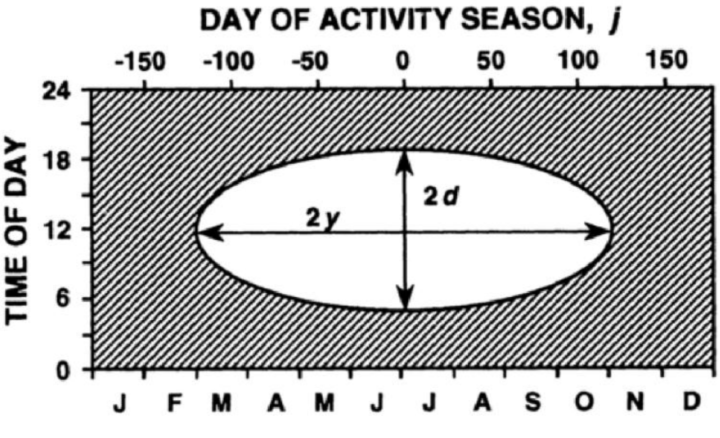

Fig. 2. Seasonal variation in diel activity of temperate diurnal lizards. White region indicates times when thermal conditions allow activity; shaded region indicates period of inactivity. Adapted from Adolph and Porter (1993).

During periods of inactivity Tb may fall outside the set-point range with no harm for the inactive individuals (Christian and Weavers, 1996). Tb changes caused by photoperiod are probably the result of its influence on different factors, namely on environmental temperature. For instance, photoperiod seems to affect thermal set points through the parietal eye (see Glossary) of lizards (Tosini and Avery, 1996). Infra-red irradiance also changes along the year or in a day-period hence influencing the levels of activity of lizards and selected Tb. When irradiation increases the upper and lower set point temperatures also increase (Tosini et al., 1995). In areas where fast changes of irradiance occur, as when cloud cover increases, responding rapidly to such changes is adaptive for lizards.

Several techniques have been widely used for measuring Tb of medium to small-sized lizards. The thermographic technique (infra-red thermography) is a non-invasive method adequate for measuring the temperatures of small and medium-sized lizards (Jones and Avery, 1989; Tosini and Avery, 1993). On the other hand, the contact thermometer allows measuring the core Tb by inserting a probe inside the cloaca (Rismiller and Heldmaier, 1982). Temperature records given by these two techniques are not equivalent: the infrared thermography provides with higher values of Tb and increasing bias with temperature which may occur because the infrared thermometer is not small enough and measures the background temperature (Carretero, 2012). With an infrared camera this bias would be prevented. Additionally, radiotelemetry is used to measure Tb in free-ranging reptiles of bigger size, as monitor lizards for example (Christian and Weavers, 1996) although it is an invasive technique, is not adequate for small species and it is expensive (Carretero, 2012).

1.2 Hydric ecophysiology

Ecophysiological studies regarding hydric requirements in adult reptiles are scarce. They are mainly focused on water loss (Wl) rates based on physiological studies on skin resistance to Wl and scales (Roberts and Lillywhite, 1980; Tu et al., 2002; Calsbeek et al., 2006).

Wl rates are correlated with the habitat occupied by many species. As a general trend, reptiles inhabiting dry habitats have higher skin resistance to Wl (Munsey, 1972; Bentley and Schmidt-Nielsen, 1966; Tu et al., 2002). This has been demonstrated for snakes (Gans et al., 1968; Dmi’el, 1998; Tu et al., 2002; Moen et al., 2005), agamid lizards (Eynan and Dmi'el, 1993), anoles (Sexton and Heatwole, 1968; Dmi'el et al.,1997), skinks (Shoemaker et al., 1967), *Sphaerodactylus* geckos (Steinberg et al., 2007) tegu lizards (Christian et al., 1999), among others. Terrestrial reptiles usually have Wl rates much smaller than aquatic reptiles (Lillywhite and SanMartino, 1993; Moen et al., 2005).

Munsey (1972) showed similar Wl rates for different lizard species belonging to distinct genera that occupy the same habitat and have similar activity patterns (Turk et al., 2010). Nevertheless, closely related species living in similar habitats may have different Wl rates which may be explained by differences in skin resistance to dessication (Munsey, 1972).

Lizards mostly use free-water available in the environment through drinking (Benabib and Congdon, 1992). However, in dry and arid habitats where free-water is scarce, some species persist only using the water available in food, such as ingested insects (Benabib and Congdon, 1992), although some may also drink water from condensation in their own skin (Withers, 1993).

Lizards lose water mainly through skin (Bentley and Schmidt-Nielsen, 1966; Dmi'el, 1972a; Dmi'el et al., 1997; Blamires and Christian, 1999) but also through the respiratory and the excretory tracts, and the eyes (Mautz, 1982; Blamires and Christian, 1999). Cutaneous Wl rates depend on the surface area exposed, on its resistance to Wl and on the water differential between the organism and the surrounding environment. The amount of water lost through respiration is determined by the ventilation rate and Tb (Thompson and Withers, 1998). Respiratory water loss is directly proportional to metabolic rate, assuming that the volume of expired air is saturated with water vapour (Gans et al., 1968; Munsey, 1972) and that the efficiency of oxygen extraction is constant but this may not be true in all reptiles (Dmi'el, 1972). The amount of water lost by excretion is usually not significant for most lizards because, in contrast with mammals, they excrete uric acid (solid) and not urea (soluble in water). Ocular water loss may be an important mean of water loss in the absence of barriers such as spectacled eye lids (Mautz, 1982). However, the cutaneous water loss exceeds the respiratory and ocular water loss by a factor of two or more (Schmidt-Nielsen, 1997a). This is true even for desert lizards which lose two thirds of body water through skin and only one third through the respiration.

Wl increases with increasing temperature and wind speed and decreasing barometric pressure because they cause an increase in water vapour density difference between the epidermis and the surroundings (Blamires and Christian, 1999; Gans et al., 1968). Also, humidity conditions of the environment have an important role in determining rates of WI (Blamires and Christian, 1999). In humid conditions, the difference of water vapour pressure between the epidermis and the surrounding air is smaller, and the water vapour diffusing force from the water surface into the air is correspondingly reduced (Schmidt-Nielsen, 1997a). On the contrary, if the atmosphere is dry, the water vapour density decreases in the environment so evaporation increases (Mautz, 1982).WI rates influence Tb (Warburg, 1965) since lizards provided with more water select higher Tb (Lorenzon et al., 1999). On the contrary, water scarcity may lead to a decrease of Tb because lizards tend to spend more time in refuges. This reveals that hydric conditions of individuals may prevent reaching Tp (Grant and Dunham, 1988). Shoemaker, Licht and Dawson (1967) reported that skinks placed at an air temperature above Tp lost 25 times more water than at lower Tb. The same was observed for resting and active snakes: a difference in 13ºC caused by activity doubled total WI (Dmi'el, 1972) revealing that activity levels also influence the rates of WI (Gans et al., 1968). Even so, higher rates of WI are detected in resting desert iguanas shedding skin possibly due to a temporary decrease of skin resistance to WI (Minnich, 1971). Therefore, higher activity levels are prone to indirectly influence other organismal processes. For instance, growth rate is positively related to higher activity levels so it can be limited by water availability even if lizards are provided with food and heat (Sears & Angilletta Jr., 2003). During laboratory experiments it has been observed that individuals submitted to desiccation conditions had lower activity levels and lower growth rate. Conversely, lizard hatchlings supplemented with additional water grow faster than those with restricted access to water (Lorenzon et al., 1999). This is also verified in natural conditions: tropical lizards grow faster in the wet season rather than in the dry season (Stamps and Tanaka, 1981).

Preventing desiccation is vital for whole-organism processes and lizards have several adaptations to preserve water and prevent dehydration. They include behavioural and physiological changes such as skin resistance, uric acid excretion, production of dry faeces, salt gland excretion (Munsey, 1972) and vasomotor changes (Eynan and Dmi'el, 1993).

Behavioural strategies adopted by reptiles include: i) increasing the inactivity period which reduces the respiratory WI during the driest months (Christian et al., 1999); ii) selecting highly humid microenvironments when they are inactive; iii) changing thermoregulatory behaviour by spending less time at high temperatures thereby reducing the cutaneous water loss (Mautz, 1982; Lorenzon et al., 1999). These strategies can be adopted simultaneously (Christian et al., 1999).

Physiological strategies to prevent WI are associated with skin resistance to WI which is related to the outer layers of epidermis, including beta (β, hard and inflexible corneous layers), mesos, alpha (α, soft-pliable corneous layers) and germinative layers (from the outside inwards) (Tu et al., 2002; Alibardi, 2003). Skin resistance is conferred by dead, keratin-filled cells inserted in a matrix of lamellar lipids and increases with increasing beta-keratin thickness and deposition of lamellar lipids in the mesos layer (Bennet and Licht, 1975; Tu et al., 2002). In fact, lipids are the main barrier to water in terrestrial life (plants, arthropods and vertebrates) (Lillywhite, 2006).

The higher resistance to WI of reptile's skin in comparison to other vertebrates (Bennet and Licht, 1975) has been commonly addressed to the presence of scales. Among anoles species it has been reported a correlation between scalation and habitat type: scale numbers increased with increasing precipitation and decreasing temperature (Calsbeek et al., 2006). Such trend may have its explanation on the fact that the number and size of scales influence the surface of skin exposed to the atmosphere. Although higher area of exposed skin may not be directly responsible to higher WI rates due to keratinization of the skin, it may influence local water flux. The general pattern of scalation in desert reptiles consists on large overlapping scales while in mesic or tropical species it is usually consistent with small and granular scales with more exposed skin area (Calsbeek et al., 2006). These patterns may be correlated with ecological variables: when humidity and elevation increase while temperature decreases, scale number increase (Horton, 1972). Even so, they may instead be under maternal effect, they may be pleiotropically linked to other trait(s) under selection or due to phenotypic plasticity (Calsbeek et al., 2006). Such patterns are verified both inter- and intra-specifically although exceptions are known to exist (*Sphaerodactylus*; MacLean, 1985; anoles; Malhotra and Thorpe, 1991). Yet, it has been observed that scaleless snake individuals registered the same, or even lower, cutaneous water loss than normal individuals with scales (Bennet and Licht, 1975). This may reflect that reptilian scales may not be closely associated with preventing WI. Scalation may not be a good predictor of the skin resistance to WI also because species with similar rates of WI have opposite scalation patterns (Dmi'el et al., 1997).

Among reptiles, phylogenetic differences concerning the efficiency of retaining water are detected. For instance, agamids are more effective than geckos which in turn are more effective than skinks (Sexton and Heatwole, 1968), colubrids also evaporate more than viperids for the same experimental temperatures (Dmï'el, 1972).

Studies on hydric physiology of reptiles are needed (simultaneously with thermal requirements) because evidence show their physiology, distribution and life-histories to be influenced by water availability (García-Muñoz et al, 2011; Carretero et al., 2012).

1.3 Ecological Niche Models

Modelling species' ecological niche under a geographic context allows not only predicting the effects of climate changes on their distribution but also the expansion of potentially invasive species, the biogeographic relationships between them, the presence of hotspot areas and high diversity areas or areas adequate for reintroductions (Guisan and Zimmermann, 2000; Peterson, 2006; Sillero et al., 2009). Ecological niches of species (and not their geographic distribution; cf. Sillero; 2011, Soberón & Peterson, 2005) are inferred by Ecological Niche Models (ENMs). ENMs are based on the relation species-environment since it is known that i) abiotic conditions, ii) biotic factors like competition or prey availability (Santos et al., 2006), iii) historical processes like vicariant speciation (Busack, 1986) and isolation of species by glaciations events (Avise et al., 1998), iv) dispersal factors and v) evolutionary history, may play a preponderant role in determining the distributions of some species (Costa et al., 2008) by influencing their distribution patterns (Soberón and Peterson, 2005; Peterson, 2006). ENMs relate ecogeographical variables with the observed distribution of species.

Correlative distribution models have been the most commonly used to predict species distributions and the impacts of climate change (Kearney et al., 2010). These models predict the realized niche of a species (Guisan and Zimmermann, 2000; Pearson and Dawson, 2003) by associating spatial environmental data (slope, aspect, elevation, topographic position, habitat type, geology) with species' abundance or presence records (presence-true absence records; presence-pseudoabsence; presence-only) to provide values of suitability for each point in space (Sillero, 2011). The realized niche model thus generated, being a subset of the fundamental niche, reflects that the species' absence may be influenced not only by competition processes as suggested by Hutchinson (1957), but also by other biotic interactions (mutualism, predation, parasitism, symbiosis) or by dispersal limitations or historical reasons (Holt, 2009) (Fig. 3). Biotic interactions have the potential to be directly accounted for in the model by including the distribution of other species (Soberón and Peterson, 2005).

Correlative models assume distributional equilibrium (or pseudo-equilibrium) of species with the variables in the model (Monahan, 2009). Since climate changes are expected to create non-equilibrium distributions, extrapolations for changing scenarios are limited between species' patterns and the environment (Lischke et al., 1998), although for organisms reacting slowly to environmental variability this limitation is less restrictive.

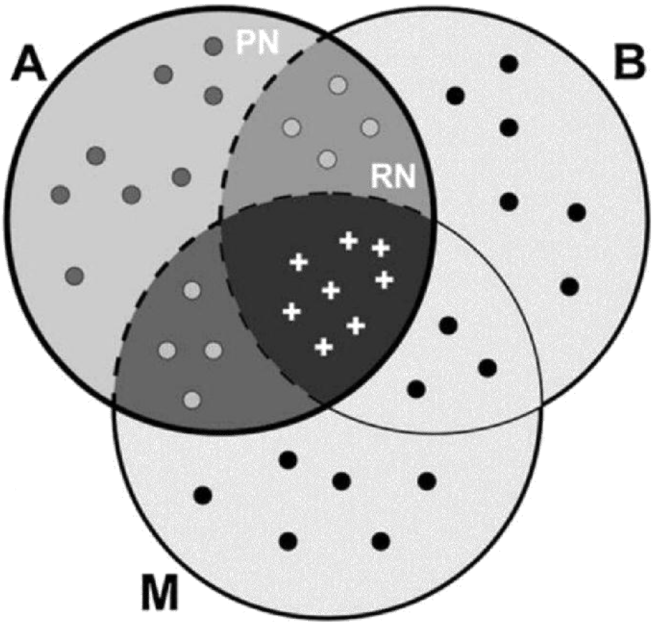

Fig. 3. Representation of a geographic area with the main factors limiting species distributions: abiotic (A), biotic (B) and historical or dispersal (M). Outside the area of intersection of the three factors habitat is unsuitable for species. The realized niche (RN) is the area common to A and B while the fundamental niche (PN) is located in A. Circles are species absences and pluses are species presences. Adapted from Sillero (2009).

Correlative models also assume that physiological limits and indirect influences from climate remain constant over time and space and that their distributional patterns depend mostly on major climatic variables. In addition, historical factors (such as past geological or climatic events), or physical barriers, may influence the present distribution of biological entities therefore limiting this type of modelling (Araújo and Guisan, 2006). Correlative models do not clarify if a species (apparent) absence in a certain area is determined by its physiology or by extrinsic limitations (Monahan, 2009). There are three categories of correlative models depending on the type of data used: Presence-Absence, Presence-Pseudoabsence and Presence-only models (Table 1).

Presence-Absence models relate the presence or the absence of a species with a set of ecogeographical variables hence inducing the suitable conditions for the presence of a species and unsuitable for its absence. Presence-Pseudoabsence models use only presence records and attribute suitable conditions to areas where a species record exists and pseudoabsence areas where no records exist (Phillips et al., 2006). Presence-only models also use only presence records and overlap them with maps of the ecogeographical variables deducing the range of conditions suitable for survival.Presence-only models may be advantageous over Presence-Absence models because the latter are prompted to introduce errors since absences may have traces of biotic interactions, dispersal constraints and disturbances which may impede the accurate modelling of distributions although presence-only may also bring some inaccuracy as species may be absent from an area with suitable conditions due to past events that caused local extinctions (Elith et al., 2011).

Table 1. The three types of correlative methods according to the type of data used and most common techniques used to produce Ecological Niche-based models.

Method type	Modelling technique
Presence - Absence	GLM (General Linear Models) GAM (General Additive models) Logistic regression
Presence - Pseudoabsence	Maxent (Phillips et al., 2004; Phillips et al., 2006) GARP(Genetic Algorithm for Rule-set Prediction)
Presence – only	Multidimensional Model Envelope (Busby, 1986; Nix, 1986) HABITAT (Walker and Cocks, 1991) DOMAIN (Carpenter et al., 1993) ENFA (Ecological-Niche Factor Analysis)

Models produced according to the type of data and software selected should be calibrated and then their performance evaluated. This is done by selecting a percentage of the data set for training (used to calibrate the model) and other for testing (to evaluate the quality of the predictions (Guisan and Zimmermann, 2000; Phillips, Anderson and Shappire, 2006). The model should be calibrated and then evaluated by crossvalidation (Van Houwelingen and Le Cessie, 1990), subsampling or bootstrap (Efron, 1979; Fielding and Bell, 1997). The crossvalidation technique randomly splits the occurrence data in equal-sized subsets which are compared in order to validate the test results. The goal is to assess if the result is replicable or just a product of arbitrary fluctuations. This technique uses all the data for validation so small data sets are better used. However, in the case of a small data set only bootstrap methods should be applied (Phillips et al., 2006). They determine the variability (accuracy) of a statistics between subsamples (Shao and Tu, 1995). Bootstrap is a Monte Carlo statistical method that takes sampling with replacement from the original sample, hence being more accurate than sampling without replacement in terms of simulating chance as it samples the impacts of the real sample size (Wintle et al., 2005). Each sample is created independently from the other samples and the number of samples is equal to the total number of presence points.

The importance of the variables for the prediction of the model can be assessed by Jackknife (Miller, 1974) which is a resampling method that uses subsets of the original sample to estimate the bias and standard error of a statistic. This is determined by leaving one observation out from the sample set each time it recalculates so each model is built using *n*-1 observations (Miller, 1974; Pearson et al., 2006).

Models thereby produced and validated can be projected to past and future climate scenarios considering also the relevance of the variables for the current distribution of species. Assessing past distribution renders further understanding on species origin and dispersal while assessing the impacts of future climate changes allows predicting how species distributions are likely to be affected, namely in terms of range decrease or expansion, or even extinctions and how such climate changes may affect their life-histories and physiology (Hijmans and Graham, 2006; Bernstein et al., 2007).

Some enterprises provide coupled atmosphere-ocean general circulation models commonly used to predict the impacts of future climate changes (CCCma, HadCM3 and CSIRO). Environmental scenarios are used to simulate global or regional climate changes although they are not real predictions (Nakicenovic et al., 2001). They are based on a series of assumptions on water-air circulation (including production of greenhouse gases and aerosol precursor emissions) but also on demographic, social, economic, technological, and environmental developments (Bernstein et al., 2007).

There are four main groups of scenarios (A1, B1, A2 and B2) and each is a logical and consistent portrayal of a possible future state of the world (Fig. 4) (Bernstein et al., 2007). Within A1 group there are three groups that describe alternative directions of technological change (fossil intensive (A1FI), non-fossil energy resources (A1T) and a balance across all sources (A1B) (Bernstein et al., 2007). The A1 group describes a world with rapid human population growth until mid-century, very rapid economic growth and rapid introduction of new and more efficient technologies. The A2 scenarios reflect a very heterogeneous world with the largest population size and the slowest economic development of all scenarios along with the highest emissions of CO^2 (Fig. 4A). The B1 predicts rapid changes in economic structures and the introduction of clean and resource-efficient technologies namely a smooth transition to alternative energy systems and the decline of conventional oil and gas resources being the one with lower CO^2 emissions and lower temperature increase (Fig. 4A and B). B2 scenarios describe a continuously increasing population (lower than A2) and intermediate economic development but it is oriented toward environmental protection but globally the energy system remains predominantly hydrocarbon-based to 2100.

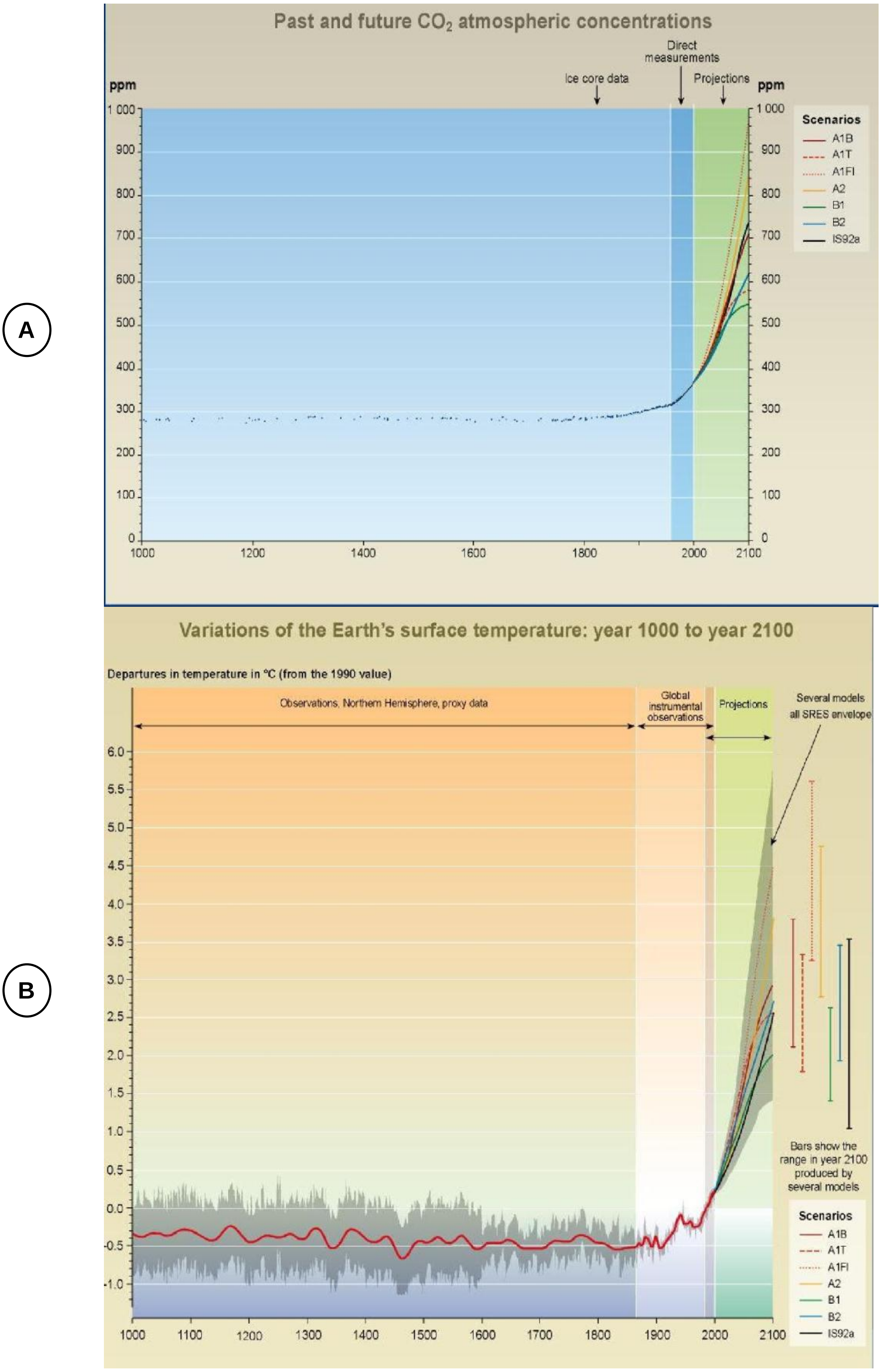

Fig. 4. (A) Past and future CO^2 atmospheric concentrations; (B) Variations of the Earth's surface temperature: years 1000 to 2100. Adapted from IPCC, 2003.

1.4 Model species

Podarcis species are lacertid lizards usually found in parapatry, but sympatry or syntopy are not uncommon (Carretero, 2008).

In the north-east of Iberian Peninsula *P. liolepis* and *P. muralis* have overlapping ranges in restricted areas (Fig. 5).

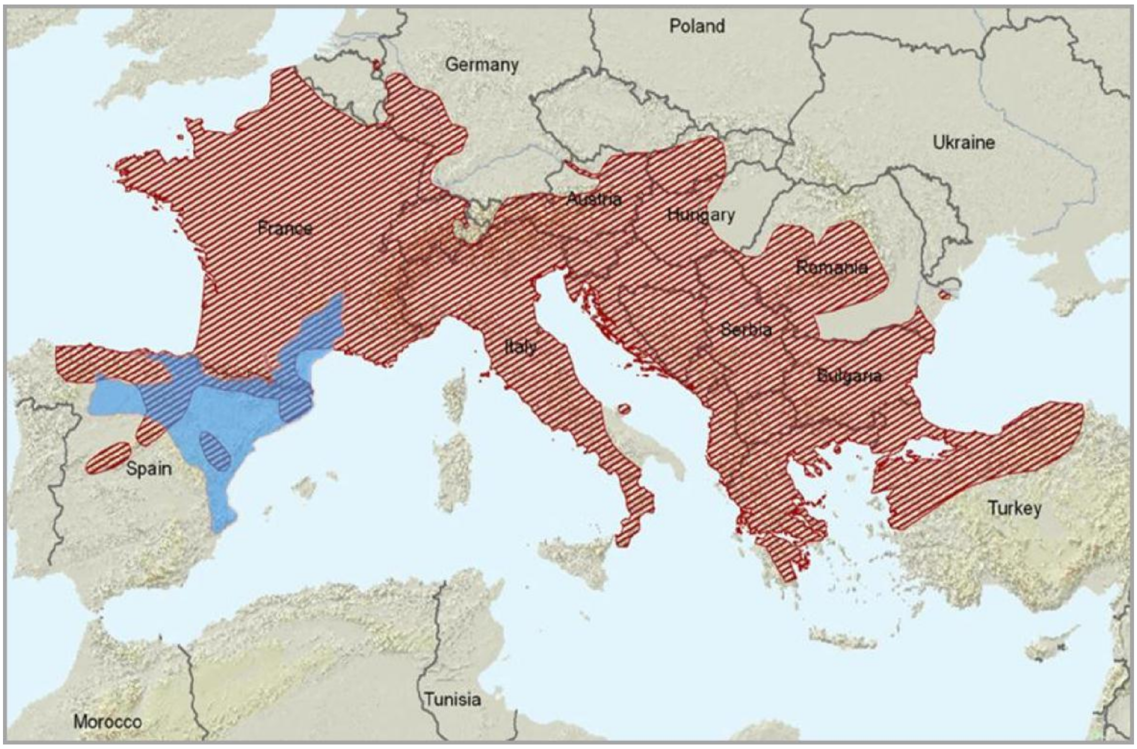

Fig. 5. General distribution of *P. liolepis* (blue) and *P. muralis* (red). The two species overlap in north-eastern Iberian Peninsula. After IUCN and Renoult et al., 2010.

The two species are not sister taxa and diverged more than 10.09 Mya (Carretero, 2008; Kaliontzopoulou et al., 2011). They are morphologically and ecologically similar (Fig. 6; Diego-Rasilla, 2009; Renoult et al., 2010).

P. liolepis occurs in north-eastern Spain and some areas of southern France (Geniez and Deso, 2009; Renoult et al., 2010) in a wide variety of habitats, except those of Eurosiberian characteristics, from the sea level to 1700 m but it is most abundant in plains and open habitats. *P. muralis* has the widest distribution among *Podarcis*, ranging from eastern Iberian Peninsula to north-western Turkey (Gasc et al., 1997). *P. muralis* occupies a great variety of non-Mediterranean habitats in Iberia including pine, beech and fir forests, riparian woodland, meadows and crops although it can be found in Mediterranean environments in the Italian Peninsula and in the Balkans (Llorente et al., 1995; Gasc et al., 1997; IUCN, 2012).

Fig. 6. *Podarcis liolepis* captured in Barcelona city on April 2011: males (1- dorsal surface; 2- ventral surface) and females (3 and 4). *P. muralis* captured in the Montseny Natural Park, North-East Iberia: males (5 and 6) and females (7 and 8). Fotos: Antigoni Kaliontzopoulou. The images are not at scale.

They are found in sympatry in the northern part of *P. liolepis*' distribution in Iberia, in a restricted area in the Montseny Natural Park (Fig. 7) and in mountain areas of Navarra and coastal areas of País Vasco (Gosá, 1985, 2003; Gosá et al., 1986; Llorente et al., 1995; Diego-Rasilla, 2009).

In addition, they are in contact with other lacertid species. *P. liolepis* may be in contact with *P. hispanica* type 1a to the west and with *P. hispanica* type 1b to the south. In the southernmost part of the distribution, in the area around the town of Valencia, it is found in sympatry with *P. hispanica* sensu stricto (Renoult 2010). To the west of Valencia there are contact zones with *P. hispanica* type 2.

In the Pyrenees, *P. muralis* is in syntopy with *Iberolacerta bonnali* in the highest altitudinal limit and in the western Pyrennes it is in parapatry with *I. aranica* and *I. aurelioi* (Rica, 1983; Palanca et al., 1997). It is also in parapatry with *P. bocagei* (Galán, 1986) in the Northwest of Iberia, and with *I. galani* in the north of León (Delibes and Salvador, 1986).

Fig. 7. Map of the Montseny Natural Park with the main regions. Yellow circle - the collection site of *P. muralis* in April 2011 (Santa Fé); Blue circle – collection site of *P. liolepis* and *P. muralis* (sympatry area; Turó del Home).

Objectives

The main goal of this thesis is to understand what shapes the distribution patterns of *P. liolepis* (Llorente et al., 1995; Renoult et al., 2010) and *P. muralis* (Schulte, 2008; Diego-Rasilla, 2009) (Fig. 5). Specifically, the ecological requirements of each species are assessed by:

- determining the thermal preferrence and optimal thresholds in adult individuals of the two *Podarcis* species;
- estimating the water loss rates in adults;
- testing for species interactions in terms of thermal requirements;
- assessing field body temperatures in a sympatry area;
- elaborating correlative models of distribution in NE Iberia;
- extrapolating the models to the future;
- developing a new methodology that allows determining, simultaneously, Tp and Wl.

Manuscript I

Comparing ecophysiological traits in two *Podarcis* Wall lizards with overlapping ranges

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Abstract

Environmental factors directly constrain the distribution of ectotherms. Most ecophysiological studies on reptiles focus on temperature but environmental humidity may also be a relevant factor. Here, we assess the ecophysiological traits of two lizard species with different but partially overlapping distributions in the Iberian Peninsula: *Podarcis liolepis* (Southern, Mediterranean) and *P. muralis* (Northern, Atlantic). Preferred body temperatures (Tp) and water loss rates (Wl) under realistic environmental conditions were measured in the lab. Tp was determined in a photothermal gradient during ten consecutive hourly intervals and Wl was measured during twelve hourly intervals inside dry sealed chambers. Also, putative thermal interactions with conspecifics and heterospecifics were tested for both species by placing two lizards in a termogradient.

Surprisingly, results revealed no differences of Tp between species or sexes. Conversely, interspecific differences in the time profiles of Wl were found. More significantly, an asymmetrical interaction was detected regarding body temperature: *P. liolepis* shifted Tb upwards in the presence of an inter-specific individual while Tp of *P. muralis* remained unchanged. Implications on species distribution patterns are discussed and the importance of hydric ecophysiology for reptilian taxa is highlighted.

Keywords: Ecophysiology, thermal preference, water loss, *Podarcis*, sympatry

Introduction

A species' geographic range is conditioned by both biotic and abiotic factors, once the effects of geographic barriers are excluded (Díaz et al. 1996; Gvoždík 2002; Kearney and Porter 2004). Lizards, being ectotherms, are highly constrained by abiotic conditions because they follow more closely the temporal and spatial variations of the environment, especially regarding temperature and humidity.

Body temperature (Tb) certainly has a major influence on lizard physicochemical processes (Huey 1991; Castilla et al. 1999). The highest performance of those processes is attained when the preferred body temperature (Tp) is reached. By definition, Tp is attained in the absence of thermoregulatory constraints (Van Damme, Bauwens, & Verheyen, 1990; Bauwens et al., 1995; Castilla, Van Damme, & Bauwens, 1999; Carretero, Roig, & Llorente, 2005; Carretero, 2008). In fact, when Tb comes close to Tp, performance of many physiological, biochemical and other organismal processes is enhanced (Bauwens et al., 1995; Huey and Slatkin, 1976; Van Damme, Bauwens, Castilla, and Verheyen, 1989). Nevertheless, Tp may change within the lifespan of individuals (Van Damme et al. 1986) while it is conserved within species (Van Damme et al. 1990) for individuals in the same sex, reproductive state and body condition (Carretero et al. 2005). Although studies on thermal ecology of reptiles dominate the ecophysiological literature other environmental variables may be acting. Namely, several studies suggest water constraints as important in limiting reptiles' geographic range (Flatt et al, 2001; Packard, 1999; Packard, 1991). Particularly for the genus *Podarcis* ecological niche models suggest that humidity to be more relevant than temperature (Sá-Sousa, 2000; Herkt, 2007; Kaliontzopoulou et al., 2008; Carretero et al., 2010).

In addition to abiotic factors, inter-specific interaction with ecologically similar species may shape a species' distribution (Arntzen and Espregueira Themudo 2008; Costa et al. 2008). In such cases, thermal and hydric physiology may be particularly relevant, as they constitute environmental features for an ectotherm. When in sympatry, individuals may have to spend time in activities that otherwise would not be part of their daily time budget such as behavioral interactions with either conspecifics or heterospecifics. This poses extra constraints on the mechanisms of thermoregulation. In fact, the presence of another species may shift body temperatures below (or above) the optimum (García-Muñoz and Carretero *submitted*; Osojnik et al. 2010). For instance, the presence of another species may result in more time spent moving instead of basking preventing it from reaching a suitable Tb hence decreasing lizard's performance. This is exacerbated in high populations densities because social interactions are expected to be more frequent (Avery 1982; Diego-Rasilla and Pérez-Mellado 2000). Although studies on hydric constraints in sympatry are scarce, similar patterns may be predicted, where the presence of a heterospecific competitor may entangle the acquisition of an optimal hydric balance (see García-Muñoz et al., *submitted*). From a different perspective, lizards selecting high temperatures may increase their water loss rates (Wl) risking desiccation (Mautz, 1982; Bowker, 1993). Hence, if water availability is restricted, a trade-off between Tp and Wl may be predicted. Because of that, restrictions in water availability may lead to changes in activity patterns which may cause changes in body temperature, binding together both physiological traits (Andrews, 1982; Adolph and Porter, 1993; Lorenzon et al., 1999).

The congeneric lacertids *Podarcis muralis* and *P. liolepis* provide a good model system to study how species coexistence influences thermal and hydric requirements, because a) their geographical ranges overlap; b) they are phylogenetically related although not sister taxa (Harris and Arnold 1999) and roughly similar morphologically and ecologically. *P. muralis* is widespread across Europe but restricted in Iberia, while *P. liolepis* is abundant but restricted to NE Iberia and Southern France). Both can be sympatric and syntopic. *P. liolepis* is a member of the Iberian and North-African clade of the genus *Podarcis*, while *P. muralis* is thought to belong to the Italian clade (Harris and Arnold 1999).

There are indications that the two species occupy different habitats in terms of temperature and water availability (Gosá 1985; Llorente et al. 1995). This suggests that both species may diverge in their physiological traits, which has not been assessed yet. In this study we aim at 1) assessing intraspecific (sex and time) and interspecific variation in Tp; 2) determining water loss rates (Wl) and their temporal variation for both species and sexes; 3) testing for the existence of a trade-off between Tp and Wl; and 4) experimentally evaluating whether intra- and interspecific thermal interaction may affect Tps.

Material and Methods

Study area and sample size

Only adult individuals were used in all the experiments (minimum adult snout-vent lengths, SVL, 46mm for *P. muralis*, Diego-Rasilla, 2009; 38mm for *P. liolepis*, Carretero, Llorente, 1993; Kaliontzopoulou, et al., 2006). Twenty one *P. liolepis* (9 males and 12 females) were collected in a site (Palau Reial, 41°23'15''N 2°07'04''E; 76 m altitude) within Barcelona (NE Spain) dominated by *Pinus pinea*, *Pinus halepensis*, *Cedrus deodara* and *Cupressus sempervirens*. Fifteen *P. muralis* (7 males and 8 females) were collected from Santa Fé del Montseny (Montseny Natural Park, NE Iberia, 41°46′26″N 2°27′43″E; 1136 m) dominated by *Quercus ilex* and *Fagus sylvatica* forests.

All specimens were captured by noose (García-Muñoz and Sillero 2010) in April 2011 and transported to the facilities of the University of Barcelona, where they were kept in 20 x 10 x 15cm individual terraria under natural light conditions. Food (*Achaeta domestica* and larval *Tenebrio molitor*) and water were provided *ad libitum* while lizards were not involved in experiments. In accordance with the phenology of both species in the region (Rivera et al., 2011), all females appeared to be pregnant after observing the belly size, copulation marks and conducting an egg palpation (Arnold 2004).

For each lizard, we measured SVL to the nearest 0.01 mm using digital calipers. Lizards were first subjected to an experiment to characterize preferred temperature, followed by an experiment to quantify water loss in the following day. No food or water were provided during and in-between both experiments. Male lizards were still allowed to recover from previous experiments for nine days, providing them food and water *ad libitum*, and then underwent interaction experiments. Only lizards with unbroken or fully regenerated tails were used. When accidental autotomy occurred during the experiments, subsequent Tp and Wl measurements were excluded from the analyses since tail loss may interfere with thermoregulation and rate of water loss since it causes changes in the shape and the surface area exposed (Chapple and Swain 2004).

Thermal gradient experiment

Lizards of both species were individually exposed to a thermal gradient (ranging between 25 and 45^{0}C) produced by a 150-W infra-red reflector bulb fixed 25 cm above the substrate in the extreme of a 100 x 40 x 30 cm terrarium. Experimental procedures followed Carretero et al. (2006). The natural photoperiod was maintained by exposing the terraria to external light. The preferred body temperature (Tp) within the gradient (Bauwens et al., 1995) was recorded during a single day at ten consecutive hourly intervals distributed throughout the period of daily activity observed in the field (7-16 h, solar time). The bulb was switched on one hour in advance (6h, solar time) in order to avoid outliers created by the early-morning warm-up (Bauwens et al., 1995). Body temperature was recorded using a digital k-termocouple thermometer HIBOK® 18 (precision 0.1 ºC) by inserting a probe of 1.5 mm in the cloaca. No more than 10 seconds elapsed between the capture of the animal from the terrarium and the measurement of body temperature, to minimize lizard stress and manipulation time in recorded Tps. Even if some disturbance is produced during this procedure, effects are much smaller than the measurement bias of alternative methods like infrared thermometers (Carretero 2012).

Water loss experiment

This experiment was conducted in sealed chambers (40x30x20cm) at ~24^{0}C and ~35% relative humidity created by 100g of silica gel on the bottom side of the lid. Temperature and humidity inside this chamber were monitored using a Fluke®-971 hygrothermometer to the nearest 0.1ºC and 0.1%, respectively. Each individual was introduced in a sealed chamber with a false bottom with five grams of silica gel. Water loss (Wl) was quantified by weighing the individuals (inside the individual plastic box in order to avoid stress during handling) using a digital balance (precision 0.0001g) each hour for 12 consecutive hours, overlapping with the activity time of the lizards (see above). The weight difference observed between measurement intervals and water loss rates directly reflect the amount of cutaneous, pulmonary and ocular water loss. Although defecation and urination have a relatively low contribution to total water loss (Munsey 1972), these products were not removed from the individual plastic box during the experimental process.

Interaction experiments

This consisted of a variant of the thermal gradient experiment conducted by monitoring, simultaneously, two adult males thermorregulating in the same thermal gradient terrarium, to determine the Tb they selected. To minimize the possible effects of body size or mass, pairs of males with similar SVL and mass were placed together.
Two different tests were performed: intra- and interspecific interaction plus the control tests provided by the standard thermal gradient experiment conducted before. The following combinations of male couples were set: five *P. muralis*/*P. muralis* and five *P.*

liolepis/*P. liolepis* intra-specific experiments as well as ten combinations for the inter-specific experiments (*P. muralis*/*P. liolepis*).

Statistical analyses

All data were log-transformed to meet the assumptions of normality, homoscedasticity and sphericity as confirmed through Shapiro-Wilk's test ($P>0.05$ in all cases), multivariate Box M and univariate Levene's tests ($P>0.05$ in most cases), and Mauchley's tests ($P>0.05$ in all cases), respectively.

To test whether significant variation in selected temperatures existed between the studied species and sexes, while also examining temporal variation throughout the day, we performed Repeated Measures ANOVAs (ANOVAR) with sex and species as between-subject factors and time interval as within-subject factor. To investigate if there were statistical differences along time and between species and sexes concerning the amount of Wl, ANOVAR were performed for instantaneous Wl (relative to initial weight, W_0; ($Wl=(W_n-W_{n+1})/W_0$)) using sex and species as between-subject factors and time interval as a within-subject factor. ANOVAs for each time interval of cumulative Wl, ($Wl= (W_0-W_n)/W_0$) were performed to determine putative differences between species and sexes (between-subject factors). We examined both descriptors because instantaneous Wl captures the amount of Wl in each time interval, while cumulative Wl provides a quantitative measure of the rate at which individuals lose water.

The possible trade-off between Tp and Wl was investigated through correlations between Mean Tp and total Wl (calculated as $Wl=(W_0-W_{12})/W_0$) for each sex by species group separately.

In order to investigate whether interaction between intra- or inter-specific individuals may lead to a selection of Tb different from Tp, ANOVARs were performed using species and test (with classes "alone", "intraspecific" and "interspecific") as the between-subject factors and time as the within-subject factor. ANCOVAs using SVL, initial weight (W_0) and SVL and W_0 as covariates were also employed for the three experiments to test whether size and shape had an influence on the two ecophysiological characteristics. Duncan *post-hoc* tests were used for multiple comparisons between species and sexes for each experiment.

Statistica 7.1 (StatSoft 2005) was used to perform all analyses. Significance was evaluated at *a*-value of 0.05.

Results

ANOVA revealed significant sexual dimorphism in SVL in both species, with opposite patterns between species; *P. liolepis* males were longer than females while the opposite was observed for *P. muralis*. Males and females of *P. muralis* also differed in initial weight, females being heavier (Appendix 1). Males of both species only differed in SVL while females also differed in W_0 (Duncan post-hoc test $p<0.05$ for all significant differences). ANCOVA performed on W_0 with sex and species as within-subject factor and SVL as covariate revealed that *P. muralis* is relatively heavier than *P. liolepis* ($F_{1,29}= 32.54$; $p=4*10^{-6}$).

Preferred temperature

Overall Tp of *P. liolepis* females was 31.22±0.32ºC while *P. liolepis* males selected 31.97±0.51ºC. Mean Tp of *P. muralis* females was 31.28±0.26ºC and mean Tp of *P. muralis* males was 31.76±0.17ºC (Fig. 1).

ANOVAR revealed statistical differences along time intervals but not between sexes or species (Table 1). Subsequent ANCOVAR using W_0 and SVL as covariates did not reveal any statistical difference either between species or sexes (Table 1). No significant interactions were detected.

Water loss

ANOVAR showed statistical significance for time and time*species, indicating that species have different Wl patterns along time, even when variation due to SVL is taken into account (Table 1). By contrast, using W_0 as the covariate rendered the time*species effect non-significant, but species, sex and species*sex presented a statistically significant effect on Wl, indicating that sexes of each species lose water differently. When using both SVL and W_0 as covariates, sex and species were the only significant effects indicating differences in the patterns of Wl only between species and sexes.

Duncan post-hoc tests between species and sexes using W_0 as the covariate only revealed significant differences between *P. muralis* females and *P. liolepis* males ($p=0.02$). When no covariates were considered, no statistical differences were found.

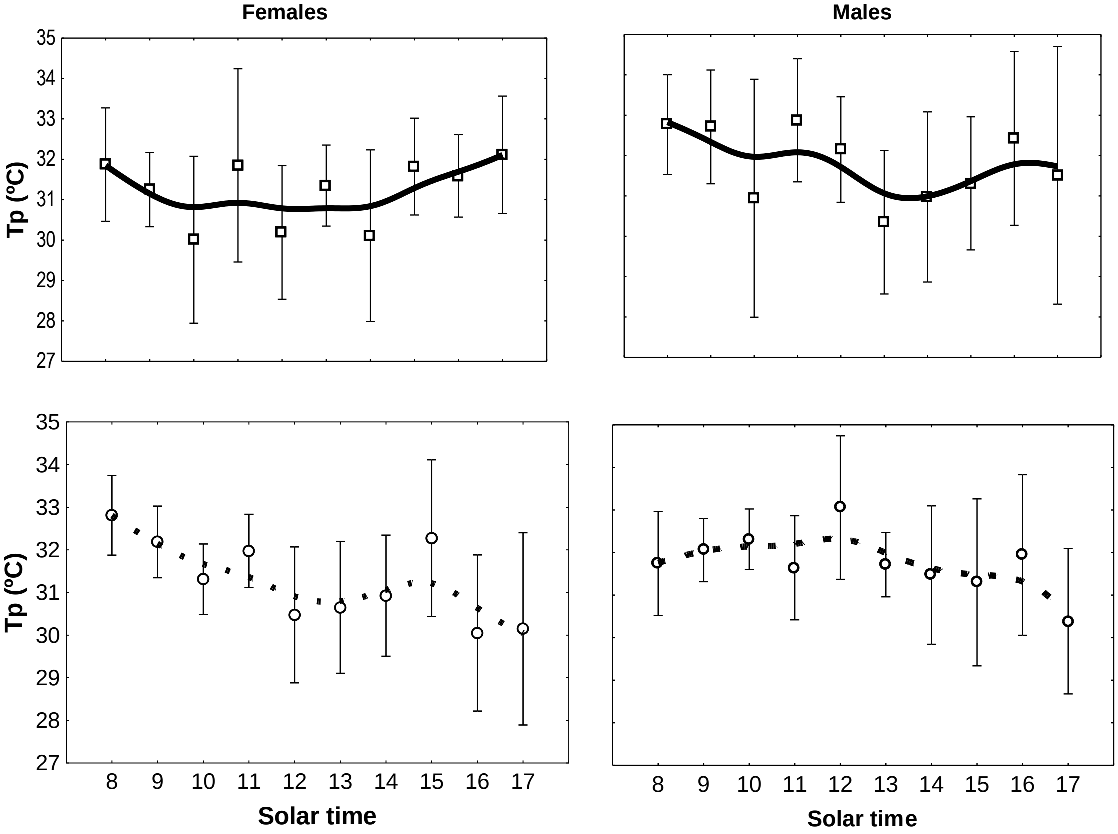

Fig. 1. Daily Tp variation pattern of females and males of *P. liolepis* (top, continuous line) and *P. muralis* (bottom, dashed line). Whiskers represent ± 0.95 confidence intervals.

Examination of the daily patterns of WI (Fig. 2) revealed that *P. liolepis* males lost more water after the 3-4 time interval while *P. muralis* only started losing more after the second half of the experiment (6-7 time interval). Differences in cumulated WI between species were detected in the last three hours of the experiment once corrected for SVL and W_0 (Appendix 2).

Tp vs. water loss

No significant correlation between mean Tp and total WI was detected for any sex and species revealing no trade-off between both variables (*P. liolepis* males: $r^2 = 0.0376$; $p = 0.6171$; *P. liolepis* females: $r^2 = 0.0194$; $p = 0.7009$; *P. muralis* males: $r^2 = 0.0614$; $p = 0.5922$; *P. muralis* females: $r^2 = 0.0009$; $p = 0.9423$).

Table 1. ANOVA and ANCOVAs results of Tp and instantaneous Wl data using SVL, W_0 or both W_0 and SVL as covariates. Significant effects are marked with an asterisk (*). Data are -transformed.

	Tp experiment results			**Wl experiment results**		
ANOVAR	**df**	**F**	***p***	**df**	**F**	***p***
Species	1,30	0.0	0.89	1,21	1.82	0.19
Sex	1,30	2.5	0.12	1,21	1.41	0.25
Species*Sex	1,30	0.0	0.90	1,21	0.32	0.58
Time	9,270	2.3	0.02*	11,231	2.54	0.01*
Time*Species	9,270	1.1	0.33	11,231	1.89	0.04*
Time*Sex	9,270	1.6	0.12	11,231	0.89	0.55
Time*Species*Sex	9,270	1.0	0.42	11,231	1.36	0.19
ANCOVARs						
Covar. (SVL)	1,29	0.22	0.64	1,20	10^{-4}	0.99
Species	1,29	0.01	0.91	1,20	1.69	0.21
Sex	1,29	2.43	0.13	1,20	1.33	0.26
Species*Sex	1,29	0.03	0.87	1,20	0.21	0.65
Time	9,261	0.33	0.96	11,220	0.38	0.96
Time*(SVL)	9,261	0.35	0.96	11,220	0.38	0.96
Time*Species	9,261	1.13	0.34	11,220	1.94	0.04*
Time*Sex	9,261	1.55	0.13	11,220	0.87	0.58
Time*Species*Sex	9,261	0.55	0.84	11,220	1.00	0.45
Covar. (W_0)	1,29	0.18	0.68	1,20	32.48	0.00*
Species	1,29	0.04	0.84	1,20	6.49	0.02*
Sex	1,29	2.19	0.15	1,20	6.76	0.02*
Species*Sex	1,29	0.02	0.90	1,20	4.80	0.04*
Time	9,261	0.81	0.60	11,220	0.48	0.92
Time*(W_0)	9,261	1.00	0.43	11,220	0.66	0.78
Time*Species	9,261	1.43	0.18	11,220	1.26	0.25
Time*Sex	9,261	1.57	0.12	11,220	0.93	0.51
Time*Species*Sex	9,261	1.10	0.36	11,220	1.26	0.25
Covar. (SVL)	1,28	0.11	0.74	1,19	3.99	0.06
Covar. (W_0)	1,28	0.07	0.80	1,19	41.32	$<10^{-6}$*
Species	1,28	0.01	0.91	1,19	8.56	0.01*
Sex	1,28	2.17	0.15	1,19	6.99	0.02*
Species*Sex	1,28	0.06	0.81	1,19	1.61	0.22
Time	9,252	0.41	0.93	11,209	0.49	0.91
Time*(SVL)	9,252	0.47	0.89	11,209	0.54	0.87
Time*(W_0)	9,252	1.11	0.36	11,209	0.81	0.63
Time*Species	9,252	1.44	0.17	11,209	1.20	0.29
Time*Sex	9,252	1.52	0.14	11,209	0.90	0.54
Time*Species*Sex	9,252	0.71	0.70	11,209	0.96	0.49

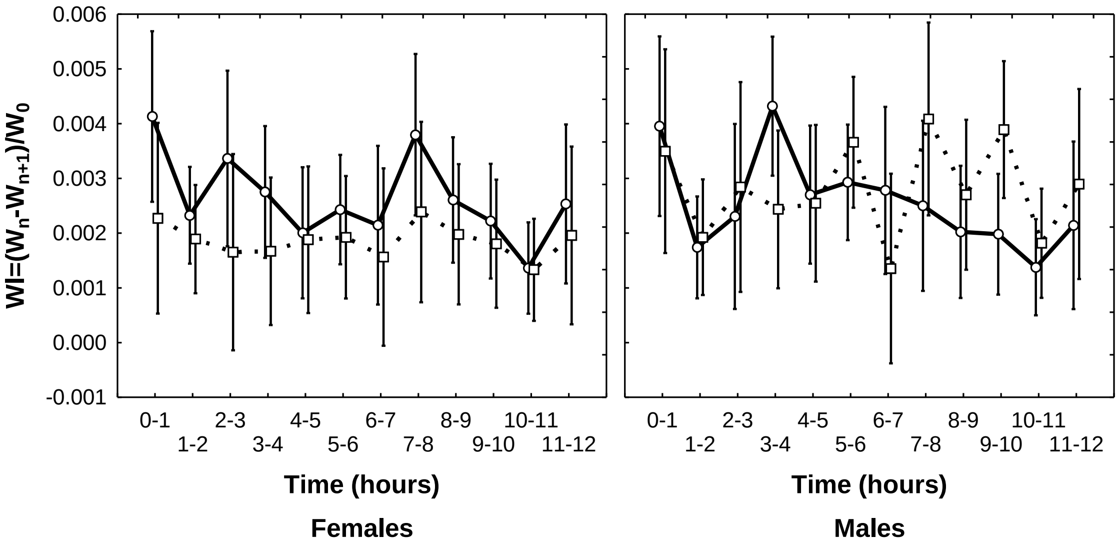

Fig. 2. Variation patterns of instantaneous water loss of females and males of *P. liolepis* (full line) and *P. muralis* (dashed line) during 12h in sealed chambers.

Interaction experiments

When alone in the thermogradient males of both species selected similar Tb (Table 2) which differed by 0.21±0.34 ºC. The same was true for the intra-specific test in which species' Tb differed 0.98±0.23ºC (Appendix 3).

Test type, species and time had a significant effect on Tp of lizards sharing the same terrarium (Appendix 3). When introducing SVL as a covariate, the species*test interaction remained significant. When using W_0 as covariate, only species and test remained significant (Appendix 3). Finally, when the ten Tb measurements taken along the day were averaged by individual, not only species and test, but also the interaction between them, were significant (Fig. 3).

Duncan post-hoc tests after the ANCOVA using W_0 as covariate, showed significant differences for *P. liolepis* between alone and interspecific (p=0.002) tests. Also, the results obtained for *P. liolepis* when alone in the thermogradient were statistically different from those of *P. muralis* in the alone experiment (p=0.003), in the interspecific (p=0.008) and intraspecific (p=0.003) experiments. No differences were found among tests of *P. muralis* (p>0.05). Essentially, the Tbs of *P. muralis* remained similar in the three experiments while *P. liolepis* selected higher Tp in intra- and interspecific experiments (32.87±0.25ºC and 33.69±0.23, respectively).

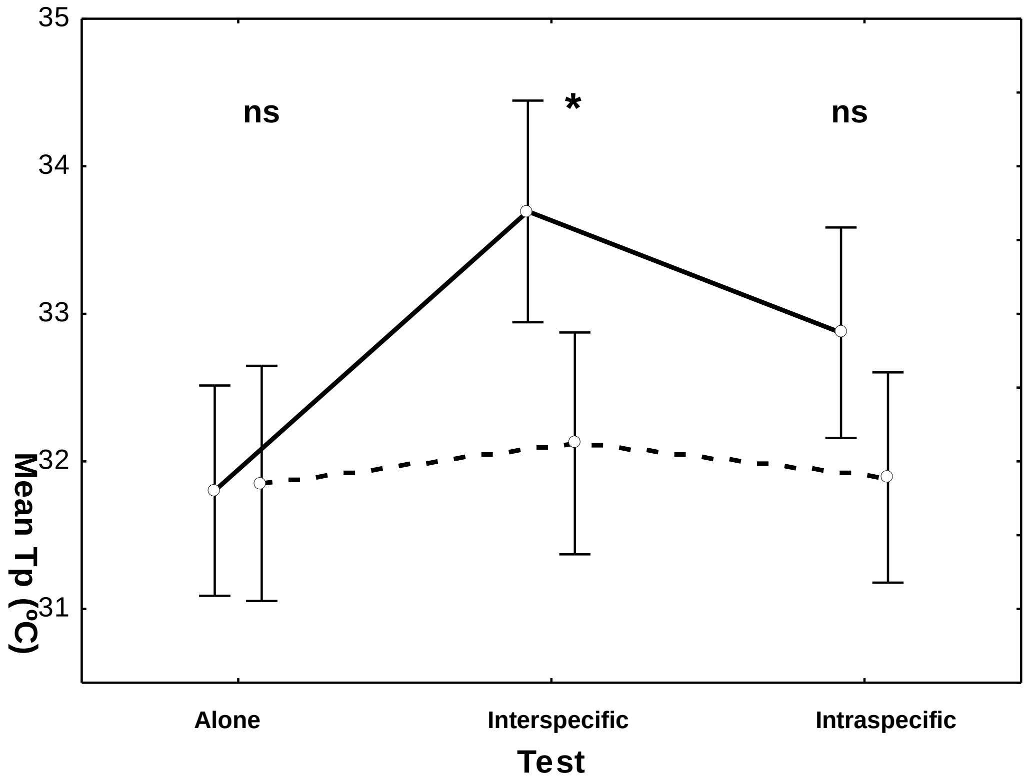

Fig. 3. Preferred body temperatures of *Podarcis* liolepis (full line) and *P. muralis* (dashed line) in three experiments: Alone- one individual per termogradient; Interspecific- one individual of each species per termogradient; Intraspecific- two individuals of the same species per termogradient (Post-hoc Duncan test; ns, p> 0.05; * p< 0.01).

Discussion

The results obtained here confirm previous findings on the variation of Tp in lacertid lizards. Most importantly, they provide new insights concerning water ecology and highlight the interactive nature of physiological traits. Namely, this work provides experimental evidence that asymmetric interactions between and within both species have an influence on their thermal ecology. Both aspects have important implications, not only for determining the life history mechanisms and demonstrating competitive interactions, but also for elucidating the biogeographical patterns of these lacertid species.

Concerning thermal physiology, both species select similar Tps. Since *P. muralis* and *P. liolepis* are quite divergent phylogenetically, belonging to different clades within the genus *Podarcis* (Harris and Arnold, 1999; Harris et al., 2005) and since phylogenetically intermediate *Podarcis* forms display different Tp (Veríssimo and

Carretero 2009), this observation suggests that thermal physiology of both species has converged. A phylogenetically-informed comparative analysis including more species of the genus is, however, needed in order to confirm this hypothesis, as other factors such as habitat use and historical biogeography of both species in question may have molded the observed patterns.

Nonetheless, Tp may vary within a lifespan of individuals (Braña, 1993; Castilla et al., 1999; Carretero et al., 2005) as our results seem to suggest in terms of Tp of the females and along time-period. Females analyzed (pregnant) selected lower Tps than males as reported for other populations of the same species (Braña 1993) and for other lacertid lizards (Van Damme, et al., 1987; Castilla, et al., 1999; Carretero et al., 2005; Carretero, 2006). Such a trend may reflect the physiological requirements of the embryos rather than those of the female herself (Van Damme et al. 1986; Castilla et al. 1999; Gvoždík and Castilla 2001; Carretero et al. 2005; Carretero, Marcos and de Prado 2006; Veríssimo and Carretero 2008). However, extrapolating these differences between the sexes over time is risky since lizards were caught during the beginning of the breeding season and seasonal variation is expected (Harris et al., 1998; Huey, 1976; Van Damme et al., 1987).

Also, patterns of Tp along time indicate different putative thermal requirements along the day (Fig. 1). In the early hours most individuals select around the same Tp since the SE is narrow. This supports the idea that selecting Tp is more important in early hours of the day when habitat temperatures are low and the need of thermoregulation is a priority (Rismiller and Heldmaier, 1982; Tosini, Jones, and Avery, 1994; Diego-Rasilla and Pérez-Mellado, 2000; Grbac and Bauwens, 2001). By contrast, from the middle until the end of the daily experiment a high SE is observed suggesting less thermal restrictions during that period so both species select a wider range of Tp. Differences in Tp along time disappear after correcting for body length and mass suggesting that previous differences were due to loss of physical condition throughout time inside the terrarium, namely in terms of body water. This is also quite realistic in field conditions and may explain why the bimodal summer activity contains a higher morning peak when compared with the afternoon (Braña 1991; Galán 1995).

Differences in WI between species and sexes when corrected for initial weight suggest differences in robustness between classes. In addition, divergent trends in the temporal patterns of WI between species were found, likely arising from different compensatory mechanisms. *P. liolepis* males apparently trigger some response to hydric stress on the first time interval (1-2 in the graph; Fig. 2A) because the rate of WI reduced drastically during that time. In fact, if the WI rate was similar for each time interval, the cumulative

Wl profile would be linear. This suggests that water already lost had a negative feedback on the water loss rate of lizards at the beginning of the experiment because the conditions of external temperature and humidity remained constant. Nevertheless, after this initial period, *P. liolepis* individuals were not capable of maintaining such initial compensatory mechanism and started losing water at a more constant rate (Fig. 2). Conversely, *P. muralis* showed a higher Wl rate on the second half of the experiment, suggesting more long-term compensatory mechanisms in this species.

Differences in dynamic physiological properties (pulmonary respiration rate, ocular evaporation) rather than static (skin permeability) are to be tested as proximate compensatory mechanisms (Bentley and Schmidt-Nielsen 1966; Roberts 1968; Dmi'el 1972; Eynan and Dmi'el 1993).

Some studies suggest that Tp and Wl may trade-off intra- and interspecifically between closely related species (Warburg 1965; Tracy and Christian 2005; Tracy et al. 2008). Yet, this is not the case here. Indeed, thermal rigidity has been reported for many lacertids (Van Damme et al. 1989; Gvoždík and Castilla 2001; Carretero et al. 2005; Díaz et al. 2006) including *P. muralis* (Tosini and R. Avery 1993), and is likely to also occur in *P. liolepis*. On the other hand, SVL variation was certainly lower within a given population than across populations within the same species (i.e. Kaliontzopoulou, Carretero, and Llorente, 2012). Both factors make unlikely the detection of a trade-off, at least in the current samples, while a wider interpopulation analysis is recommended.

The Tp of these two species is probably more influenced by their biogeographic origin than by the current environment (Bauwens *et al.*, 1995). Given the overall distribution of *P. muralis* in Europe, which encompasses both Eurosiberian and Mediterranean habitat, this species could be expected to occupy a wider range within the Iberian Peninsula (Pérez-Mellado, 2002). By contrast, it is limited to the non-Mediterranean areas, namely the Atlantic coast and mountains with cooler and wetter climate (Llorente et al., 1995; Pérez-Mellado, 2002; Lanuza, Luna, and Lozano, 2012). This suggests that the presence of *P. liolepis* may play a role in confining the southernmost limit of the distribution of *P. muralis* in the Iberian Peninsula. In fact, in other areas of Iberia, this species is also coexisting with other *Podarcis* species but only locally: with *P. bocagei* in Asturias (Galán 1986) and with *P. hispanica* type 1b in the Central System, the later apparently leading to shifts in *P. muralis*' habitat use (Martín-Vallejo et al. 1995). Investigating the Tp of those species may further elucidate the role of thermal requirements in determining the present distribution of *P. liolepis.* If the latter modifies its selected Tb in the presence of other individuals, it would be taken as an indication of interference or competition. Nevertheless, other non-ecophysiological aspects (i.e. behavioural or trophic) may also be involved in a different Tb selection by this species.

Moreover, the current picture of the relationships between both species in the Iberian Peninsula may be altered if the predictions for climate change (IPCC 2007) have an effect on population densities and/or species ranges. A way of predicting future consequences for our findings is considering, for instance, a scenario where populational density increases. In the case of *P. muralis*, usually found in higher densities than *P. liolepis* in northern Iberian Peninsula, this factor would be expected to have a negligible effect on the species' physiology. Yet, for *P. liolepis*, it is likely that the increase in Tb is a consequence of an increased frequency of interactions among individuals. Diego-Rasilla (2000) recorded in the field for a population of *P. hispanica* type 1b (according to Kaliontzopoulou et al., 2012) that the time spent basking is positively correlated with the difference between body and air temperatures which would affect the amount of daily time used for other activities (see also Grbac and Bauwens, 2001). Because *P. liolepis* increase Tb when in the presence of *P. muralis* instead of decreasing, results point to an active response of this species in the presence of heterospecifics increasing the time devoted to thermoregulation. This pattern contrasts to that found by García-Muñoz and Carretero (*submitted*) for *Algyroides marchi*, which was displaced by syntopic *P. hispanica s.s* to a marginal position in the thermogradient resulting in a decreased Tp.

Studies such as this, encompassing several physiological traits, could be used as a baseline to understand the complexity of species' biogeographical patterns. For instance, if the climate becomes warmer and drier, *P. liolepis* would have fewer difficulties attaining an elevated Tp in the presence of *P. muralis* while long term compensatory mechanisms would provide same advantage to this species to remain active during prolonged drought periods. If climate shifts to colder, wetter conditions, *P. liolepis* would be thermally constrained to respond to the presence of *P. muralis* by increasing Tp. Conversely, *P. muralis* would not be influenced by *P. liolepis* regarding thermal requirements and would not be exposed to long periods of desiccation.

Hydric stress is also known to trigger changes in activity patterns leading to changes in body temperature, growth and metabolic rates, affecting whole-organism processes (Andrews, 1982; Adolph and Porter, 1993; Lorenzon, 1999). In addition, if such changes of thermal conditions occur in a short-time period, Species will not be able to reach their Tp and performance will probably be affected hence reducing the probability of survival (Diego-Rasilla, 2001). If an animal can keep its body temperature within a range around Tp, it will be favored in terms of natural selection (Van *Damme* et al., 1990).

In conclusion, although further comparative studies will determine to what extent the physiology of sympatric lizard species vary and interact across taxa and regions, results obtained here already highlight the importance of incorporating water ecology experiments (added to the classic thermal ones) and interaction tests in the framework of lizard ecophysiological studies. Also, biogeographical analyses and climate change forecasts should not ignore this evidence.

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Supplementary material

Appendix 1. Descriptive statistics of SVL, W_0, Tp and instantaneous WI at each time interval for each species and sex.

P. liolepis **males**

	N	Mean	Minimum	Maximum	SD	SE
SVL	9	60.60	56.87	63.65	2.21	0.74
W0	9	4.72	4.23	5.57	0.41	0.14
TP9	9	32.77	30.60	36.00	1.61	0.53
TP10	9	32.71	30.10	35.80	1.83	0.61
TP11	9	30.94	24.00	35.50	3.84	1.28
TP12	8	32.88	30.90	35.40	1.83	0.65
TP13	8	32.15	29.60	34.50	1.56	0.55
TP14	8	30.35	26.10	33.60	2.13	0.75
TP15	8	30.98	26.90	34.20	2.52	0.89
TP16	8	31.31	29.20	35.50	1.97	0.70
TP17	8	32.43	29.10	37.30	2.57	0.91
TP18	8	31.51	25.50	37.00	3.82	1.35
(W8-W9)/W8	9	0.004	0.002	0.01	0.001	0.0005
(W9-W10)/W8	9	0.002	0.0004	0.004	0.001	0.0004
(W10-W11)/W8	9	0.002	0.0001	0.01	0.002	0.0006
(W11-W12)/W8	9	0.004	0.002	0.01	0.003	0.0008
(W12-W13)/W8	9	0.003	0.0001	0.01	0.002	0.0008
(W13-W14)W8	9	0.003	0.001	0.01	0.002	0.0005
(W14-W15)/W8	9	0.003	-0.001	0.01	0.002	0.0007
(W15-W16)/W8	9	0.003	0.001	0.01	0.001	0.0004
(W16-W17)/W8	9	0.002	0.0002	0.004	0.001	0.0004
(W17-W18)/W8	9	0.002	-0.0001	0.004	0.001	0.0005
(W18-W19)/W8	9	0.001	-0.0003	0.003	0.001	0.0003
(W19-W20)/W8	9	0.002	-0.0005	0.005	0.001	0.0005

P. liolepis **females**

SVL	10	55.17	47.94	63.47	4.63	1.46
W0	10	3.08	1.95	4.05	0.67	0.21
TP9	10	31.87	29.10	35.20	1.96	0.62
TP10	10	31.25	30.00	34.10	1.28	0.41
TP11	10	30.01	24.70	34.40	2.89	0.91
TP12	10	31.85	25.40	35.70	3.34	1.06
TP13	10	30.19	24.90	33.40	2.31	0.73
TP14	10	31.35	28.20	33.10	1.40	0.44
TP15	10	30.11	26.20	34.60	2.97	0.94
TP16	10	31.82	29.70	34.70	1.68	0.53
TP17	10	31.59	28.60	33.70	1.42	0.45
TP18	10	32.11	29.50	36.40	2.03	0.64
(W8-W9)/W8	10	0.004	0.001	0.013	0.004	0.0010
(W9-W10)/W8	10	0.002	0.001	0.004	0.001	0.0004
(W10-W11)/W8	10	0.003	-0.004	0.010	0.004	0.0010
(W11-W12)/W8	10	0.003	0.0001	0.010	0.002	0.0006
(W12-W13)/W8	10	0.002	0.0002	0.004	0.001	0.0004
(W13-W14)W8	10	0.002	0.001	0.005	0.001	0.0005
(W14-W15)/W8	10	0.002	-0.005	0.005	0.003	0.0009
(W15-W16)/W8	10	0.004	0.002	0.010	0.002	0.0006
(W16-W17)/W8	10	0.003	0.001	0.010	0.002	0.0007
(W17-W18)/W8	10	0.002	0.0003	0.004	0.001	0.0004
(W18-W19)/W8	10	0.001	0.0002	0.003	0.001	0.0003
(W19-W20)/W8	10	0.003	-0.0004	0.009	0.003	0.0009

Appendix 1. *Continued*

***P. muralis* males**

	N	Mean	Minimum	Maximum	SD	SE
SVL	7	55.64	46.07	66.45	6.20	2.34
W0	7	6.09	3.55	9.43	2.31	0.87
TP9	7	31.74	29.50	33.40	1.32	0.50
TP10	7	32.06	30.80	33.30	0.80	0.30
TP11	7	32.31	30.70	32.90	0.77	0.29
TP12	7	31.64	30.50	34.40	1.32	0.50
TP13	7	33.06	30.00	35.20	1.82	0.69
TP14	7	31.71	30.90	33.20	0.82	0.31
TP15	7	31.47	29.20	34.40	1.76	0.66
TP16	7	31.30	28.60	34.70	2.12	0.80
TP17	7	31.94	29.20	35.40	2.04	0.77
TP18	7	30.39	26.60	32.10	1.85	0.70
(W8-W9)/W8	7	0.004	0.001	0.006	0.002	0.0007
(W9-W10)/W8	7	0.002	-0.0004	0.005	0.002	0.0007
(W10-W11)/W8	7	0.003	0.001	0.006	0.002	0.0006
(W11-W12)/W8	7	0.002	0.0005	0.005	0.002	0.0006
(W12-W13)/W8	7	0.003	0.001	0.006	0.002	0.0008
(W13-W14)W8	7	0.004	0.001	0.006	0.002	0.0008
(W14-W15)/W8	7	0.001	-0.002	0.004	0.003	0.0010
(W15-W16)/W8	7	0.004	0.001	0.012	0.004	0.0015
(W16-W17)/W8	7	0.003	0.0003	0.006	0.002	0.0008
(W17-W18)/W8	7	0.004	0.001	0.008	0.003	0.0010
(W18-W19)/W8	7	0.002	-0.002	0.005	0.003	0.0008
(W19-W20)/W8	7	0.003	0.0003	0.009	0.003	0.0012

***P. muralis* females**

SVL	8	61.06	57.42	64.82	3.19	1.13
W0	8	8.12	3.76	11.15	2.92	1.03
TP9	8	32.81	31.2	34.60	1.12	0.40
TP10	8	32.19	30.70	33.80	1.003	0.35
TP11	8	31.31	29.90	32.60	0.99	0.35
TP12	8	31.98	30.90	34.00	1.03	0.36
TP13	8	30.48	27.50	32.50	1.91	0.67
TP14	8	30.65	27.80	33.40	1.85	0.65
TP15	8	30.93	28.70	32.90	1.70	0.60
TP16	8	32.28	29.80	36.00	2.20	0.78
TP17	8	30.05	25.40	32.60	2.19	0.78
TP18	8	30.15	23.60	31.80	2.70	0.95
(W8-W9)/W8	8	0.002	0.0008	0.004	0.001	0.0004
(W9-W10)/W8	8	0.002	0.0005	0.004	0.001	0.0004
(W10-W11)/W8	8	0.002	0.0008	0.003	0.001	0.0002
(W11-W12)/W8	8	0.002	0.0003	0.003	0.001	0.0003
(W12-W13)/W8	8	0.002	0.0001	0.003	0.001	0.0004
(W13-W14)W8	8	0.002	0.0010	0.004	0.001	0.0004
(W14-W15)/W8	8	0.002	0.0003	0.003	0.001	0.0004
(W15-W16)/W8	8	0.002	0.0002	0.005	0.002	0.0006
(W16-W17)/W8	8	0.002	0.0002	0.004	0.001	0.0005
(W17-W18)/W8	8	0.002	0.0009	0.003	0.001	0.0003
(W18-W19)/W8	8	0.001	0.0007	0.003	0.001	0.0003
(W19-W20)/W8	8	0.002	0.0008	0.003	0.001	0.0003

Appendix 2. ANOVA cumulated WI of each time intervals between species and sexes using the covariates SVL and W_0. $(W_0\text{-}W_1)/W_0$.

		$(W_0\text{-}W_1)/W_0$		**$(W_0\text{-}W_2)/W_0$**		**$(W_0\text{-}W_3)/W_0$**		**$(W_0\text{-}W_4)/W_0$**		**$(W_0\text{-}W_5)/W_0$**		**$(W_0\text{-}W_6)/W_0$**	
	df	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Species	1, 30	1.54	0.22	3.16	0.09	0.36	0.55	3.38	0.08	2.29	0.14	3.00	0.09
Sex	1, 30	1.9	0.18	0.09	0.77	0.3	0.59	0.49	0.49	0.57	0.46	1.02	0.32
Species*Sex	1, 30	0.1	0.75	0.75	0.39	0.19	0.67	0.70	0.41	0.43	0.52	1.12	0.30
SVL (covar.)	1, 29	0.07	0.79	0.04	0.85	0.11	0.76	0.10	0.75	0.08	0.78	0.05	0.83
Species	1, 29	1.52	0.23	3.08	0.09	0.37	0.55	3.33	0.08	2.26	0.14	2.94	0.10
Sex	1, 29	1.85	0.18	0.09	0.77	0.29	0.59	0.47	0.50	0.55	0.46	0.99	0.33
Species*Sex	1, 29	0.17	0.69	0.66	0.42	0.28	0.60	0.73	0.40	0.48	0.49	0.97	0.33
W_0 (covar.)	1, 29	5.99	0.02*	7.70	0.01	2.36	0.14	6.47	0.02	7.30	0.01*	11.7	$<10^{-3}$*
Species	1, 29	0.61	0.44	0.33	0.57	0.42	0.52	0.15	0.70	0.54	0.47	0.97	0.33
Sex	1, 29	3.35	0.08	0.01	0.94	0.61	0.44	1.26	0.27	1.47	0.23	2.75	0.11
Species*Sex	1, 29	1.10	0.30	0.51	0.48	0.22	0.65	0.40	0.53	0.76	0.39	0.70	0.41
SVL (covar.)	1, 28	1.77	0.19	1.93	0.18	0.95	0.34	2.08	0.16	2.16	0.15	2.99	0.09
W_0 (covar.)	1, 28	7.83	0.01*	9.83	$<10^{-3}$*	3.20	0.08	8.66	0.01	9.65	$<10^{-3}$*	15.4	$<10^{-3}$*
Species	1, 28	1.2	0.28	0.81	0.37	0.75	0.39	0.54	0.47	1.18	0.29	1.99	0.17
Sex	1, 28	3.74	0.06	0.03	0.87	0.7	0.41	1.51	0.23	1.76	0.20	3.31	0.08
Species*Sex	1, 28	0.25	0.62	0.03	0.87	0.01	0.94	$<10^{-3}$*	0.95	0.08	0.78	0.03	0.87

		$(W_0\text{-}W_7)/W_0$		**$(W_0\text{-}W_8)/W_0$**		**$(W_0\text{-}W_9)/W_0$**		**$(W_0\text{-}W_{10})/W_0$**		**$(W_0\text{-}W_{11})/W_0$**		**$(W_0\text{-}W_{12})/W_0$**	
	df	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Species	1, 30	3.79	0.06	3.84	0.06	3.31	0.08	2.69	0.11	2.32	0.14	2.52	0.12
Sex	1, 30	0.60	0.44	0.52	0.48	0.39	0.54	0.65	0.43	0.69	0.41	0.63	0.43
Species*Sex	1, 30	0.84	0.37	1.53	0.23	1.6	0.22	1.97	0.17	2.05	0.16	2.29	0.14
SVL (covar.)	1, 29	0.15	0.70	0.10	0.75	0.14	0.71	0.29	0.60	0.32	0.57	0.06	0.80
Species	1, 29	3.75	0.06	3.77	0.06	3.27	0.08	2.70	0.11	2.35	0.14	2.47	0.13
Sex	1, 29	0.59	0.45	0.50	0.49	0.38	0.54	0.63	0.43	0.68	0.42	0.61	0.44
Species*Sex	1, 29	0.94	0.34	1.41	0.24	1.55	0.22	2.09	0.19	2.22	0.15	1.89	0.18
W_0 (covar.)	1, 29	11.6	$<10^{-3}$*	17.2	$<10^{-4}$*	18	$<10^{-4}$*	18.1	$<10^{-4}$*	16.9	$<10^{-4}$*	24.4	$<10^{-5}$*
Species	1, 29	0.65	0.43	1.48	0.23	1.93	0.18	2.42	0.13	2.47	0.13	3.99	0.06
Sex	1, 29	1.94	0.17	2.21	0.15	1.93	0.18	2.61	0.12	2.61	0.12	3.12	0.09
Species*Sex	1, 29	0.93	0.34	0.96	0.33	0.98	0.33	0.73	0.40	0.58	0.45	1.01	0.32
SVL (covar.)	1, 28	3.93	0.06	5.21	0.03*	5.93	0.02 *	7.48	0.01*	7.28	0.01*	6.86	0.01 *
W_0 (covar.)	1, 28	16.4	$<10^{-4}$*	24.8	$<10^{-5}$*	26.7	$<10^{-5}$*	29.1	$<10^{-5}$*	27.20	$<10^{-5}$*	36.10	$<10^{-6}$*
Species	1, 28	1.70	0.20	3.31	0.08	4.21	0.05	5.47	0.03*	5.49	0.03 *	7.64	0.01 *
Sex	1, 28	2.50	0.13	2.98	0.10	2.72	0.11	3.81	0.06	3.78	0.06	4.39	0.05
Species*Sex	1, 28	0.05	0.83	0.02	0.89	0.01	0.92	0.02	0.90	0.04	0.83	0	0.95

Appendix 3. ANOVAR results from the Tp interaction experiment data with and without SVL and W0 as covariates. Significant effects are marked with an asterisk (*). Data are log-transformed.

	Tp experiment results			**Wl experiment results**		
ANOVAR	**df**	**F**	***p***	**df**	**F**	***p***
Species	1,30	0.0	0.89	1,21	1.82	0.19
Sex	1,30	2.5	0.12	1,21	1.41	0.25
Species*Sex	1,30	0.0	0.90	1,21	0.32	0.58
Time	9,270	2.3	0.02*	11,231	2.54	0.01*
Time*Species	9,270	1.1	0.33	11,231	1.89	0.04*
Time*Sex	9,270	1.6	0.12	11,231	0.89	0.55
Time*Species*Sex	9,270	1.0	0.42	11,231	1.36	0.19
ANCOVARs						
Covar. (SVL)	1,29	0.22	0.64	1,20	10^{-4}	0.99
Species	1,29	0.01	0.91	1,20	1.69	0.21
Sex	1,29	2.43	0.13	1,20	1.33	0.26
Species*Sex	1,29	0.03	0.87	1,20	0.21	0.65
Time	9,261	0.33	0.96	11,220	0.38	0.96
Time*(SVL)	9,261	0.35	0.96	11,220	0.38	0.96
Time*Species	9,261	1.13	0.34	11,220	1.94	0.04*
Time*Sex	9,261	1.55	0.13	11,220	0.87	0.58
Time*Species*Sex	9,261	0.55	0.84	11,220	1.00	0.45
Covar. (W_0)	1,29	0.18	0.68	1,20	32.48	0.00*
Species	1,29	0.04	0.84	1,20	6.49	0.02*
Sex	1,29	2.19	0.15	1,20	6.76	0.02*
Species*Sex	1,29	0.02	0.90	1,20	4.80	0.04*
Time	9,261	0.81	0.60	11,220	0.48	0.92
Time*(W_0)	9,261	1.00	0.43	11,220	0.66	0.78
Time*Species	9,261	1.43	0.18	11,220	1.26	0.25
Time*Sex	9,261	1.57	0.12	11,220	0.93	0.51
Time*Species*Sex	9,261	1.10	0.36	11,220	1.26	0.25
Covar. (SVL)	1,28	0.11	0.74	1,19	3.99	0.06
Covar. (W_0)	1,28	0.07	0.80	1,19	41.32	$<10^{-6}$*
Species	1,28	0.01	0.91	1,19	8.56	0.01*
Sex	1,28	2.17	0.15	1,19	6.99	0.02*
Species*Sex	1,28	0.06	0.81	1,19	1.61	0.22
Time	9,252	0.41	0.93	11,209	0.49	0.91
Time*(SVL)	9,252	0.47	0.89	11,209	0.54	0.87
Time*(W_0)	9,252	1.11	0.36	11,209	0.81	0.63
Time*Species	9,252	1.44	0.17	11,209	1.20	0.29
Time*Sex	9,252	1.52	0.14	11,209	0.90	0.54
Time*Species*Sex	9,252	0.71	0.70	11,209	0.96	0.49

Manuscript II

Field body temperatures of two *Podarcis* species (Reptilia: Lacertidae) in sympatry in north-eastern Iberian Peninsula

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Abstract

Many reptilian species are able to regulate their body temperature by using the environmental conditions of their ecological niche. Some are even able to keep it within a narrow range near the preferred body temperatures (Tp) attained in the absence of thermoregulatory constraints. In order to understand the thermoregulatory strategy and thermal requirements of species, field body temperatures (Tb) must be compared with Tp.

Tb of *P. liolepis* and *P. muralis* individuals was assessed during a field survey in a sympatric area in northern Catalonia, Spain, and compared with known Tp. Abiotic factors (air and substrate temperature and relative humidity of the air) were recorded on each capture.

Tb in the field was lower than Tp for both species. Male *P. liolepis* and *P. muralis* tend to select higher Tb and Tp than females. Males and females of *P. liolepis* and *P. muralis* selected similar Tb in the field. No differences on the relevance of each abiotic factor for each species or sex were detected. The Tb of both species and sexes was positively correlated with air and substrate temperature and negatively correlated with humidity.

The importance of a bigger sample regarding *P. liolepis* individuals is stressed because it would allow unequivocal comparison of Tb among species in the sympatry area.

Keywords: Field body temperature, preferred temperature, sympatry, *Podarcis*

Introduction

Variations of environmental conditions over space and time directly influence physicochemical processes of ectotherms (Schmidt-Nielsen, 1997). Some ectotherms are not able to keep a body temperature (Tb) different from the ambient temperature and thus are called thermoconformers. On the other hand, perfect thermorregulaters are able to keep a constant Tb over a range of air or soil temperatures (Hertz, Huey, & Stevenson, 1993). As an example, lacertids in temperate climates are accurate and efficient thermorregulaters (Van Damme, Bauwens, & Verheyen, 1990) while several tropical and nocturnal lizards do not termorregulate carefully while active (Huey, 1982; Gil, Guerrero, & Pérez-Mellado, 1994).

For thermorregulating lizards, keeping an adequate Tb, i.e. close to preferred body temperature (Tp) (which is attained in the absence of thermoregulatory constraints; Van Damme 1989), is vital because it allows keeping the internal physicochemical processes at high performance rates (Huey, 1976; Gvoždík & Castilla, 2001; Veríssimo & Carretero, 2008; Angilletta Jr., 2009).

However, the Tb selected in the field by lizards is constrained by the thermal environment (Christian, Weavers, Green, & Bedford, 1996; Tosini & Avery, 1996). In consequence, Tb in the field may vary according to external factors as the time of day and season but also to internal factors as reproductive condition and feeding status (Licht, Dawson, & Shoemaker, 1966; Huey & Slatkin, 1976; Huey, 1982). These factors influence the precision of Tb selection, i.e. the difference between Tb and Tp (Sievert & Hutchinson, 1988, but see Hertz et al. 1993).

In order to comprehend the thermoregulatory strategy of a species it is necessary to determine (i) its preferred body temperature (Tp) which is reached when there are no thermoregulatory constraints (Huey & Stevenson, 1979; Van Damme, Bauwens, & Verheyen, 1987; Bauwens et al., 1995) (ii) body temperature attained in the field (Tb), and (iii) the distribution of operative body temperatures (Te) which is obtained by placing copper lizard models in all available microhabitats at all times (Hertz et al., 1993). Certainly, even when different species share the same habitat, they may display different thermoregulatory strategies (Avery, 1978; Pollo & Pérez-Mellado, 1989; Van Damme et al., 1990; Castilla, Van Damme, & Bawens, 1999; Grbac & Bawens, 2001).

Podarcis liolepis and *P. muralis* are two lacertid species separated by more than 8my (Kaliontzopoulou, Pinho, Harris, & Carretero, 2011) found in sympatry in mountains of northeast Catalonia, NE Iberia (Llorente, Montori, Santos, & Carretero, 1995; Diego-Rasilla, 2009). *P. muralis* has the widest range among species of the genus but in

Iberia it is restricted to the mountain ranges of the north and centre (“IUCN,” 2012) while *P. liolepis* occupies Mediterranean areas but reaches mountain areas up to 1700 metres in NE Iberia (Llorente et al., 1995). Although *P. muralis* displays saxicolous habits across its range, in Iberian Peninsula it tends to be more ground dweller in sympatry areas while the other species occupy the rocks with more intensity (Diego-Rasilla, 2009).

In experiments conducted under controlled conditions, individuals from both species selected for similar Tp when alone. However, *P. liolepis* tended to select for higher temperatures when in the presence of either conspecifics or *P. mularis* (Carneiro et al., *unpub.*). This suggests that an asymmetric interaction between both species could affect the thermal ecology of *P. liolepis*.

In this study we aim at determining the field Tb of *P. liolepis* and *P. muralis* in a sympatry area during activity period in order to assess whether the pattern of Tp recorded in the lab (Carneiro et al. *unpub.*) remains under natural conditions.

Material and Methods

During the beginning of May 2012, active adult specimens of two *Podarcis* species were captured in a sympatry area in the Montseny massif, a mountain range within the pre-Pyrenean region of Catalonia, NE Spain. In the collection site, Turó del Home (41°46′26″N 2°27′43″E; 1700m altitude), the highest peak of this mountain range, *P. liolepis* and *P. muralis* are found in syntopy (personal observations). The study area is dominated by boreoalpine vegetation community mainly composed of shrubs and herbs and scattered presence of pine trees (Roijals et al., 2002).

Adult individuals of *P. liolepis* (larger than 38mm in snout-vent length (SVL), Carretero, & Llorente, 1993; Kaliontzopoulou, *et al*., 2006) and *P. muralis* (larger than 46mm, Diego-Rasilla, 2009) were captured by noose or by hand. The reproductive status of females was not determined but considering the phenology of both species, it is likely that they would be in the beginning of the reproductive period (Diego-Rasilla, 2009) since no external evidences of pregnancy (i.e. copulation scars, deformed abdomen) were observed.

Tb of each individual was taken immediately after capture by inserting a probe of a digital-termocouple thermometer HIBOK®18 (precision ±0.1ºC) 2 mm in the cloaca within ten seconds after capture. Subsequently, substrate temperature (Ts) in the place of first observation was taken using the same thermometer while air temperature (T_{air})

and the relative humidity (RH) were measured using a Fluke®-971 Hygrothermometer 50 cm placed above ground level. All measurements were taken in the shade. The solar time was registered along with the sex and species of the lizard. A team of two or three people was used in order to perform all measurements promptly.

The specimens collected were marked with a number on the belly and were taken to the facilities of the Faculty of Biology, University of Barcelona in order to measure their SVL using digital callipers (to the nearest 0.01mm) and their weight with a digital balance (precision ±0.0001g). Afterwards they were released in the collection site.

Normality of the data was determined through Kolmogorov-Smirnov and Lilliefors tests and since this assumption was not fully met, data were log-transformed, maintaining the criterion of the work on preferred temperatures in the laboratory (Carneiro et al., *unpub.*). Homoscedasticity was confirmed through univariate Levene's tests ($P>0.05$ in all cases).

Correlation matrixes were built to determine potential correlation between SVL, W_0, Tb, T_{air}, Ts and HR. We first analysed partial correlations of Tb of both species and sexes together, with each environmental variable (T_{air}-Tb; Ts-Tb; RH-Tb) in order to determine which variable influences most the Tb of *Podarcis* in the Montseny. Then, partial correlations of Tb of each species separately were done; and finally, partial correlations of Tb of each sex and species. In order to test whether all individuals were captured in similar weather conditions, ANOVAs were performed using T_{air}, Ts or RH as within-subject factors and Species and Sex as between-subject factors.

To test for possible significant variation of Tb in the field between the studied species and sexes, we performed ANOVA with Species and Sex as factors and Tb as dependent variable. In addition, to ascertain possible size-mass effect on the selection of Tb we performed ANCOVAs using W_0, SVL, or both W_0 and SVL as covariates. The effects of the covariates on Tb were assessed through ANOVAs for each covariate alone using Tb as within-subject factor.

All analyses were performed using Statistica 7.1 (StatSoft, 2005).

Results

Eight adult individuals of *P. liolepis* (four males and four females) and 42 of *P. muralis* (22 males and 20 females) were captured.

ANOVAs on the covariates revealed W_0 as statistically different between species and sexes while for SVL no statistical differences between species and sexes were found

(Appendix 1). ANCOVA on W_0 with SVL as covariate revealed statistical differences between species ($F_{1,45}$= 12.94; $p=<10^{-3}$), *P. muralis* being more robust than *P. liolepis*, and between sexes ($F_{1,45}$=14.12; $p=<10^{-3}$), males being more robust than females.

The Tb of both species and sexes was positively correlated with T_{air} and Ts and negatively correlated with RH (Table 1). When analysing species separately, Tb of *P. muralis* follows the same trend while Tb of *P. liolepis* is only negatively correlated with HR. *P. muralis* revealed the same pattern even when both sexes were analysed separately while Tb of male and female *P. liolepis* was not correlated with any environmental variable. In addition, it seems that the weight and length do not influence the thermoregulatory processes of these species because there are no significant results except for W_0 when accounting for species and sexes together (Table 1).

Table 1. Regressions of Tb with W_0, SVL and with each environmental variable log-transformed (T_{air}, Ts, RH; see abbreviations in Material and Methods). * indicates p<0.05.

Type of correlation	Sex	Species	Variables	r^2	p	Regression equation
All data together	Both sexes	Both species	W_0 : Tb	0.10	0.02*	y = 0.2084 + 0.3439*x
			SVL: Tb	0.02	0.30	y = 1.0868 + 0.5298*x
			T_{air} : Tb	0.22	$<10^{-3}$*	y = 2.1601 + 0.3869*x
			Ts : Tb	0.34	$<10^{-5}$*	y = 1.6549 + 0.5488*x
			HR : Tb	0.33	$<10^{-4}$*	y = 4.7782 - 0.4122*x
Between species (sexes together)	Both sexes	*P. muralis*	W_0 : Tb	0.04	0.18	y = 2.9365 + 0.2277*x
		P. liolepis		0.35	0.12	y = 2.6326 + 0.466*x
		P. muralis	SVL: Tb	0.01	0.55	y = 1.8769 + 0.3384*x
		P. liolepis		0.04	0.65	y = -0.2276 + 0.8384*x
		P. muralis	T_{air} : Tb	0.22	$<10^{-3}$*	y = 2.1793 + 0.3872*x
		P. liolepis		0.36	0.12	y = 1.9746 + 0.4158*x
		P. muralis	Ts : Tb	0.35	$<10^{-5}$*	y = 1.7063 + 0.5384*x
		P. liolepis		0.50	0.05	y = 0.8388 + 0.7916*x
		P. muralis	HR : Tb	0.28	$<10^{-4}$*	y = 4.7071 - 0.3887*x
		P. liolepis		0.71	0.01*	y = 5.0717 - 0.5142*x

Table 1. ***Continued***

Between species and sexes	M	*P. muralis*	W_0 : Tb	0.06	0.26	y = 2.8258 + 0.3023*x
	F	*P. muralis*		0.02	0.55	y = 3.0323 + 0.1562*x
	M	*P. liolepis*		$<10^{-4}$*	0.99	y = 3.2432 + 0.0077*x
	F	*P. liolepis*		0.21	0.55	y = 2.562 + 0.5093*x
	M	*P. muralis*	SVL: Tb	0.12	0.12	y = -1.788 + 1.2439*x
	F	*P. muralis*		0.07	0.25	y = 7.1077 - 0.9587*x
	M	*P. liolepis*		0.26	0.49	y = 9.9285 - 1.6571*x
	F	*P. liolepis*		0.12	0.65	y = -4.8559 + 1.9746*x
	M	*P. muralis*	T_{air} : Tb	0.22	0.03*	y = 2.0969 + 0.4164*x
	F	*P. muralis*		0.21	0.04*	y = 2.2648 + 0.3561*x
	M	*P. liolepis*		0.35	0.41	y = 2.4871 + 0.2701*x
	F	*P. liolepis*		0.57	0.27	y = 1.531 + 0.542*x
	M	*P. muralis*	Ts : Tb	0.37	$<10^{-3}$*	y = 1.4917 + 0.6143*x
	F	*P. muralis*		0.33	0.01*	y = 1.9078 + 0.4661*x
	M	*P. liolepis*		0.65	0.19	y = 1.5594 + 0.5719*x
	F	*P. liolepis*		0.21	0.54	y = 0.2956 + 0.9587*x
	M	*P. muralis*	HR : Tb	0.31	0.01*	y = 4.9362 - 0.4511*x
	F	*P. muralis*		0.26	0.02*	y = 4.4968 - 0.3325*x
	M	*P. liolepis*		0.73	0.14	y = 4.6488 - 0.3816*x
	F	*P. liolepis*		0.75	0.13	y = 5.0646 - 0.5285*x

Males and females of *P. liolepis* and *P. muralis* selected similar Tb in the field (Table 2). Both SVL and W_0 showed no effect on the Tb selected by species and sexes in the field. The same was verified for the environmental variables (Table 2).

Table 2. ANOVA and ANCOVAs for Tb between species and sexes, with and without the covariates SVL and/or W_0, T_{air}, Ts and RH.

	df	**F**	***p***
ANOVA			
Species	1,46	1.79	0.19
Sex	1,46	2.44	0.12
Species*Sex	1,46	1.56	0.22
ANCOVA			
Covar Log (SVL)			
Log SVL	1,45	0.21	0.65
Species	1,45	1.34	0.25
Sex	1,45	2.06	0.16
Species*Sex	1,45	1.42	0.24
ANCOVA			
Covar. Log (W_0)			
Log W_0	1,45	1.91	0.17
Species	1,45	0.24	0.63
Sex	1,45	0.58	0.45
Species*Sex	1,45	0.86	0.36

Table 2. *Continued*

ANCOVA			
Covar. Log (SVL) and Log (W_0)			
Log SVL	1,44	0.77	0.38
Log W_0	1,44	2.47	0.12
Species	1,44	0.09	0.77
Sex	1,44	0.26	0.62
Species*Sex	1,44	0.65	0.42
ANCOVA			
Covar. Log (T_{air})	1,45	13.14	$<10^{-3}$*
Species	1,45	2.63	0.11
Sex	1,45	1.68	0.20
Species*Sex	1,45	1.75	0.19
ANCOVA			
Covar. Log (Ts)	1,45	24.66	$<10^{-5}$*
Species	1,45	3.63	0.06
Sex	1,45	1.62	0.21
Species*Sex	1,45	1.36	0.25
ANCOVA			
Covar. Log (HR)	1,45	20.81	$<10^{-5}$*
Species	1,45	2.28	0.14
Sex	1,45	0.97	0.33
Species*Sex	1,45	1.19	0.28

Table 3 shows the mean values of each variable and covariate used on all statistical analyses. Note that *P. liolepis* females recorded the lowest values for all variables included in the study.

Table 3. Descriptive statistics of W_0, SVL and environmental variables for each sex of *P. liolepis* and *P. muralis*. Numbers indicate mean ± SE and range. See Material and Methods section for variables' abbreviations.

	P. liolepis		***P. muralis***	
	Males	**Females**	**Males**	**Females**
	N=4	N=4	N=22	N=20
W_0	3.64 ± 0.26	2.43 ± 0.20	4.29 ± 0.17	3.69 ± 0.16
	2.69-4.16	2.12-3.01	2.37-6.07	2.45-5.13
SVL	56.48 ± 1.21	53.71 ± 0.41	58.14 ± 0.78	57.01 ± 0.77
	53.15-60.16	51.99-55.87	47.41-64.32	50.56-63.47
Tb	25.26 ± 1.89	20.50 ± 1.74	26.73 ± 1.21	25.90 ± 1.14
	20.30-30.40	16.70-24.10	14.00-34-60	16.70-32.80
T_{air}	17.12 ± 2.78	15.63 ± 1.95	16.73 ± 0.94	15.73 ± 0.96
	11.50-26.60	12.40-21.30	10.20-23.00	10.60-23.00

Table 3. ***Continued***

Ts	19.22 ± 2.05	17.00 ± 0.69	18.35 ± 1.07	17.75 ± 1.00
	15.90-26.70	15.30-18.30	12.10-32.70	9.60-24.90
			N=22	
HR	45.28 ± 7.36	50.23 ± 6.31	44.17 ± 2.96	46.96 ± 3.22
	27.10-62.20	32.70-60.70	28.40-72.30	27.60-69.30

Significant differences in terms of T_{air}, Ts and RH were not detected for each species and sex supporting the fact that they were captured in similar microenvironments in terms of T_{air}, Ts and HR conditions (Table 4).

Table 4. ANOVAs for T_{air}, Ts and HR (data log-transformed).

	df	**F**	***p***
T_{air}			
Species	1,47	0.08	0.78
Sex	1,47	0.61	0.44
Species*sex	1,47	0.04	0.84
Ts			
Species	1,46	0.13	0.72
Sex	1,46	0.76	0.39
Species*Sex	1,46	0.25	0.62
HR			
Species	1,47	0.001	0.97
Sex	1,47	1.36	0.25
Species*Sex	1,47	0.46	0.50

Comparisons of Tp (Carneiro et al, *unpub*) revealed *P. muralis*' Tb was approximately 5ºC below Tp while for *P. liolepis* higher differences in mean and standard error between Tb and Tp were registered (Table 5). Males of both species tend to select higher Tb and Tp than females.

Discussion

Both species selected similar Tb in the sympatry area possibly due to the environmental constraints. The study area is located on a mountain range and characterized by higher precipitation and relative humidity of the air, and lower air temperatures. Such abiotic conditions are known to constrain species' ranges and

Table 5. Comparison between mean Tp±SE (ºC) selected in the laboratory and Tb±SE (ºC) in the field of *P. liolepis* and *P. muralis*.

	P. liolepis		***P. muralis***	
	Males	**Females**	**Males**	**Females**
Tp	31.97 ± 0.51 n=9	31.22 ± 0.32 n=10	31.76 ± 0.17 n=7	31.28 ± 0.26 n=8
Tb	25.26 ± 1.89 n=5	20.50 ± 1.74 n=4	26.73 ± 1.21 n=22	25.90 ± 1.14 n=20
Difference	6.71 ± 1.38	10.72 ± 1.42	5.03 ± 1.04	5.38 ± 0.88

distributions along with their physiological performance due to more severe conditions than in lowland. For instance, anoles lizards living in high altitudes spend more time basking than lowland populations to compensate for the lower air temperatures and cloudy weather (Huey, 1976). Temperate species maintain lower Tb during activity in high altitude because T_{air} is lower than at sea level (Gvoždík, 2002; Macnab, 2002). In addition, in altitude the reproductive peak may occur later, when the environmental conditions are more adequate (Carretero, 2006).

Comparing the Tb of *P. liolepis* and *P. muralis* when active in the field with data on Tp obtained in the previous reproductive season (Carneiro et al., *unpub.*), Tb attained by the lizards fell quite lower than those preferred in the photo-thermal gradient regardless the species and sex. Similar results have been observed in other works on lacertid lizards (Van Damme et al., 1987; Grbac & Bauwens, 2001).

This may relate to seasonal variation since this study was conducted in the beginning of the activity season when thermal constraints are expected to be high. This may also occur during autumn when environmental conditions are similarly likely to impose constraints to thermoregulation. This has been confirmed with *P. muralis* in other areas of its European distribution and with other *Podarcis* species. For instance, the Tb attained by *P. muralis* males and females on an island in Croatia during autumn was 30.4± 0.4 ºC (n = 46) and *P. muralis* was shown to maintain Tb closer to Tp than *P. melisellensis* (Grbac & Bawens, 2001).

On the other hand, the Tb attained during the reproductive season is higher suggesting few thermoregulatory constrains. For instance, *P. muralis* males in northern Spain during the reproductive season registered Tb around 33.58 ± 1.79ºC (n=48) and did not differ from Tp in males and non-pregnant females (Braña, 1993). Tp of *P. liolepis* males from NE Iberia (Bellaterra, Cerdanyola del Valles, UTM 31TDF2395, 150 m altitude) collected during the peak of oviposition was around 33.75 ± 0.24ºC (n=9) (Carretero, Marcos, & de Prado, 2006). The discrepancy of Tp values with our results (Carneiro et al., *unpub*.; Table 5) may be related to the month or the location where each study was conducted (Carretero, 2006).

In northern Spain during summer the Tp of *P. muralis* males was around 33.77 ± 1.79ºC (n =135) and pregnant females 32.62 ± 1.58ºC (Braña, 1993) while in Central Spain, also during summer, it was 34.15ºC (n=15; males and females) (Bawens et al., 1995). The results by Bauwens et al. (1995) cannot be used in comparison because they pooled males and females together. However, the discrepancy of Tp between the northern Spain population of *P. muralis* (Braña, 1993) and the population from the Montseny Park (Table 5) suggest seasonal variation on the Tp attained. A lower Tp in spring than in the summer has also been reported for another population of *P. muralis* in Slovenia (Osojnik, Žagar, Vrezec, & Carretero, 2010).

Nonetheless, the small sample size of *P. liolepis* does not allow a full comprehension of the thermoregulatory behaviour of this species and the influence of environmental conditions on the selection of Tb and its impacts on lizard’s performance. Such low number of captures is likely due to lower density of this population in comparison to *P. muralis*’ in this specific sympatry area (unpublished data). *P. muralis* was found in high populational density in Turó del Home while *P. liolepis* was scarce. High population densities of *Podarcis* are known to affect the thermoregulatory patterns and time budgets along the day when thermal constraints are weak. For instance, *P. hispanica* type Ib spends more time basking when population density is higher and thus, the time available for feeding decreases (Avery, 1982; Diego-Rasilla & Pérez-Mellado, 2000). In addition, in higher densities, individuals spend more time interacting which may reduce the efficiency of basking (Diego-Rasilla & Pérez-Mellado, 2000).

The high disparity in species densities (empirically estimated by the number of captures divided by effort of capture (the time between capturing one individual from a species until capturing a conspecific) may reflect the biogeographic origin of each species studied and their characteristic habitat. *P. muralis*, being a central-European element, would be more capable of occupying and thriving under high humidity and low insolation conditions typical of Atlantic/mountain climate, as observed, while *P. liolepis* predominates in Mediterranean climate regions and is very scarce in mountain areas (MAC, personal observation in the Cantabric region). Hence, in the Montseny massif *P. liolepis* was likely at its ecological limits (Llorente et al., 1995) because it occurred in areas with increased RH, decreased air and substrate temperature (Table 3).

In contrast, it is hypothesized that the confined presence of *P. muralis* to mountains in Iberian Peninsula may be due to interaction with other lacertid species ecologically similar rather than its phylogeographic origin. In fact, in other Mediterranean regions *P. muralis* is found in lowland areas as observed in the Balkan or Italian Peninsulas where it reaches the southernmost limits (Llorente et al., 1995; Gasc, 1997).

The standard error of Tb was more than the double of Tp's indicating that temperatures selected in the field vary more than in controlled conditions (Table 5). Nonetheless, the low sample sizes should not be disregarded. The lower precision of thermoregulation and the lower Tb may be a consequence of restrictions imposed by the thermal environment (Huey, 1982; Sears & Angilletta Jr., 2003) but also may be due to shifts in the thermal setpoints as a temporary response to unsuitable conditions.

The negative difference between Tb and Tp reveals that lizards in the field were active at suboptimal body temperatures otherwise they would only be found active in places and times with suitable conditions (Grbac & Bauwens, 2001). However, during this field work, we captured active animals at low T_{air} and Ts and with dense cloud cover, conditions less suitable for thermoregulating lizards.

P. muralis is an active thermoregulator because it is able to reach a Tb different from external conditions (Table 1). This is shown through the low slope of the regression equations of T_{air}:Tb for *P. muralis* (Hertz et al., 1993). The Ts also looks relevant for maintaining a suitable Tb indicating that *P. muralis* termorregulates not only through irradiative heat but also by conduction with the substrate. In fact, this thermoregulatory ability is underestimated because the T_{air} and Ts were in fact results of a previous "selection" by the lizard (i.e. it was not active in other microhabitats and conditions). Similar conclusions could be taken for *P. liolepis* but the sample size requires caution in such assessments.

Nonetheless, in order to properly assess if the lizards termorregulate precisely, i.e., reach a Tb within the range of Tp, at least when the environmental temperatures allow them, it is further necessary to use copper models to determine the operative temperatures (Te). Copper models mimic lizards features (size, shape, irradiative properties) and biophysical factors capable of affecting heat balance (Castilla et al., 1999; Hertz et al., 1993). Determining Te will clarify if lizards are inhibited of reaching optimal Tbs by environmental conditions throughout the year in mountain areas and whether they can only reach higher Tbs in microhabitats which may constrain foraging or reproductive behaviours (Grbac & Bauwens, 2001). Also, comparing Te with Tb selected in the field allows understanding in what extent these species are able to termorregulate precisely.

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Supplementary material

Appendix 1. ANOVA for W_0 and SVL (data log-transformed).

ANOVA for W_0	**df**	**F**	***p***
Species	1,47	15.27	0.0003 *
Sex	1,47	12.70	0.0009 *
Species*Sex	1,47	2.40	0.13

ANOVA for SVL	**df**	**F**	***P***
Species	1,47	0.33	0.57
Sex	1,47	0.27	0.61
Species*Sex	1,47	0.12	0.73

Manuscript III

Predicting current and future distribution patterns of two *Podarcis* Wall lizards in north-eastern Iberia

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Abstract:

Environmental factors constrain the distribution of species and interactions among them. Determining the most influencing factors is important to understand their range limitations. Here, environmental niche-based models (ENMs) are produced using Maxent software in order to determine the current suitable areas of two locally sympatric species in north-eastern Iberia: *P. muralis* and *P. liolepis*. The relevance of the climatic variables used is assessed and ENMs for future climate change scenarios are produced.

The most contributing variables for the models are water-related (precipitation of wettest month and precipitation seasonality). Models predicted an extensive area of similarly suitable conditions for both species in the north of the study area.

The models for the future climate conditions reveal that *P. muralis* is simply expected to retract from the peripheral areas in the south and east while *P. liolepis* will became the only species in such places and will progress high up in the Pyrenees. In the harshest scenario (A2a; warmer and drier), *P. liolepis* will disappear from central west due to aridification. *P. muralis* range will decrease but will be little fragmented while *P. liolepis* range will shift northwards and, hence, depend on dispersal. Therefore, *P. liolepis* could have the worst part of climate change effects.

A higher resolution of bioclimatic variables and presence records are needed in order to unravel the type of interaction occurring in the predicted sympatry areas.

Keywords: Environmental Niche-based models, bioclimatic variables, *Podarcis*, sympatry, future scenarios

Introduction

The geographic distribution of species is constrained by their ecology and evolutionary history which work at different scales and intensity (Soberón & Peterson, 2005). Abiotic and biotic factors, dispersal conditions and evolutionary capacity to adapt are factors that constrain and shape species' distribution.

Ectotherms are directly dependent on the external ecological conditions to keep their physiological processes (Huey 1976; Angilletta et al. 2002). Among the abiotic factors, temperature is known to impose severe constraints for thermorregulating lizards (Huey & Slatkin, 1976). However, being important, temperature may not be the most constraining abiotic factor for many species. This is the case of some lacertid lizards which have been recently demonstrated to depend more on humidity (García-Muñoz & Carretero, *submitted*; Osojnik et al. *submitted*.; Carneiro et al. *unpub*). Abiotic factors are likely to change in the future due to environmental climatic changes eventually leading to a decrease or increase of suitable environmental conditions either by causing habitat fragmentation or expanding suitable conditions, respectively (Gaston, 2009).

The suitability of habitat can be assessed by Ecological Niche-based Models (ENM) which estimate the ecological niche under a spatial perspective. By estimating the habitat suitability of different species, ENMs may give further insights on interspecific interactions and on the environmental characteristics limiting and shaping species distributions. ENMs relate presence records of species with ecological or geographical characteristics of the study sites (Elith, Burgman, & Regan, 2002).

In the Iberian Peninsula, species of the *Podarcis* genus are mainly found in parapatry while fewer are found in sympatry (Carretero 2008). *P. muralis* and *P. liolepis* partly overlap in North-eastern Iberian Peninsula at geographic scale while they are locally sympatric (syntopic) only in a few localities (Llorente et al. 1995). Their ecology and morphology are fairly similar but they have different biogeographic origins (Oliverio, Bologna, & Mariottini, 2000; Kaliontzopoulou et al., 2011) and geographic ranges (Gasc et al., 1997). *P. muralis* is the member of the genus with the broadest distribution range as it is widely spread from North Iberia to Western Turkey and from Southern Italy and Balkans to Central Europe (Gasc et al., 1997). In the Iberian Peninsula, it is restricted to the north and mountain regions with low temperatures and medium/high humidity levels, contrasting with the continuous pattern also extended to the Mediterranean areas observed in the Italian peninsula and the Balkans (Pleguezuelos et al. 2002; Loureiro et al. 2010). More specifically, the Iberian range is quite continuous in the north from Pyrenees to the Cantabric region, but it splits in isolated nuclei in the centre, namely in the east Central System above 1230m (in the Massif of Guadarrama) and in North Iberic System from 1000m to 2200m of altitude in Urbión, Cebollera and Moncayo Mountains as well as the southern isolates in the South Iberian System of Sierra de Gúdar, and Penyagolosa (Diego-Rasilla, 2009). In Catalonia, north-eastern Iberia, *P. muralis* occupies 26.8% of the total area and is mostly distributed from 30m asl to 2300m asl. This is the most abundant lizard species in the areas with no Mediterranean climate and the western limit of distribution is drawn by the 800mm of annual rainfall and it is usually not found in places with medium annual temperature above 11ºC (Llorente et al. 1995). In contrast, *P. liolepis* is widely and continuously distributed in the Mediterranean areas of NE Iberia, where it occupies 76.8% of the Catalonia area, but it is restricted to the plains in SE France (Llorente et al. 1995; Renoult et al. 2010; Kaliontzopoulou et al. 2011).

In this study we use ENMs aiming at determining i) the main environmental factors defining the presence of both *P. muralis* and *P. liolepis* and whether they differ between them; ii) the current predicted distribution for each species; iii) indirect evidence of interaction at geographic level between both species; and iv) the future putative areas of distribution, and eventual interaction, under different available scenarios of climate change.

Material and Methods

Study area and species datasets

The partial distributions of *P. muralis* and *P. liolepis* were modelled in Catalonia, north-eastern Spain (Fig. 1). This is a relatively large area that provides a good representation of different environments and where no other *Podarcis* species are present. A total of 439 historic records of *Podarcis* (321 *P. liolepis* and 118 *P. muralis*) in the study area were combined with bioclimatic variables to develop ENMs of habitat suitability for both species. Historical records were obtained from the Spanish Atlas updated until 2005 (Montori et al., 2005) represented on the WGS84 grid in a georeferenced database and displayed using ArcMap 9.3 GIS (ESRI, Redlands, California, USA).

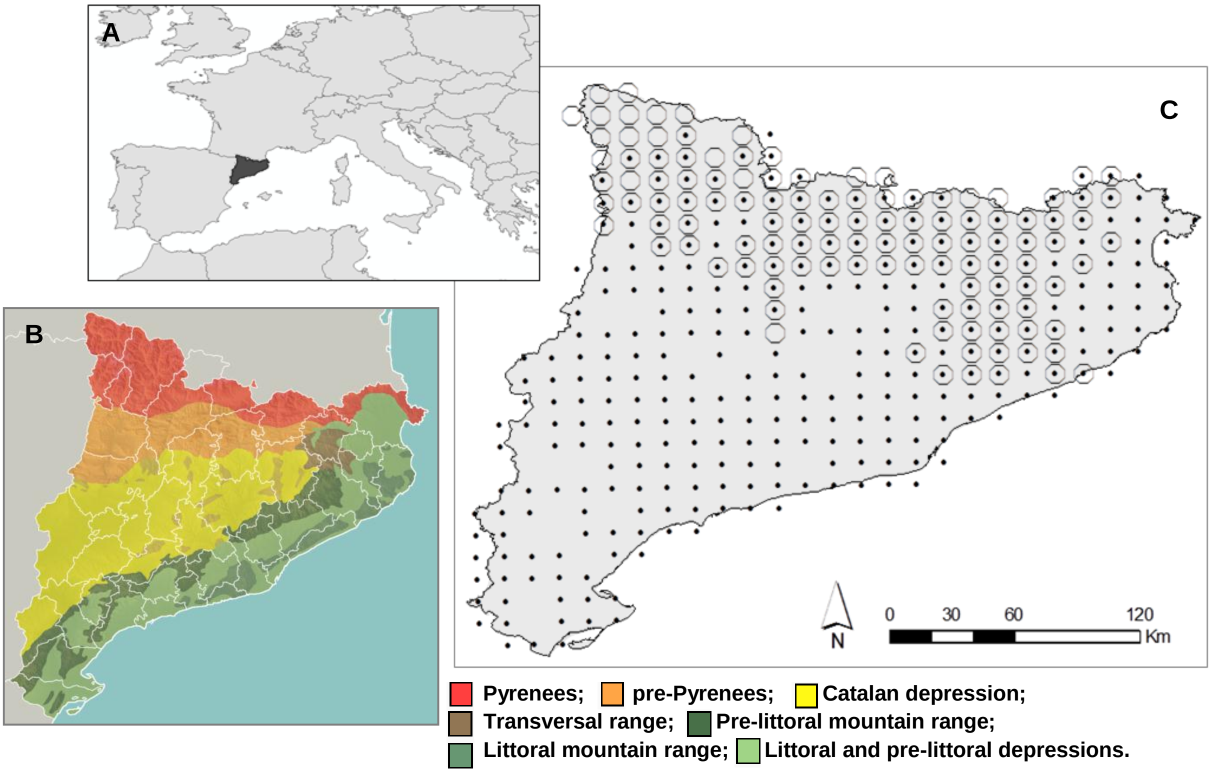


Fig. 1. Location (A) and geography (B) of Catalonia; presence points of *P. muralis* (open circles) and *P. liolepis* (closed circles) (C). Areas occupied by both species are marked with both circles.

The study area was divided in 10 x 10 km squares to match the resolution of the points of species which is an acceptable scale for ENMS (Llorente et al. 1995; Ribeiro et al. 2009).

Environmental data for current and future scenarios

Climatic variables (CVs) with a resolution of 5 arc-minutes (~10 km spatial resolution) were obtained from the WorldClim data base in the datum WGS84 (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005; http://www.worldclim.org/). Of the 19 CVs available, six with a Pearson correlation lower than 0.75 were selected (Table 1; Sillero, 2009).

Table 1. Climatic variables used on modelling the distribution of *Podarcis liolepis* and *P. muralis* in Catalonia, northeastern Spain. BIO2 = Mean Diurnal Range (Mean of monthly (max temp - min temp)); BIO7 = Temperature Annual Range. Source: Hijmans et al. 2005.

Climatic variables (*r*<0.75 in all cases)
Bio3 = Isothermality (Bio2/Bio7) (* 100)
Bio4 = Temperature Seasonality (standard deviation *100)
Bio8 = Mean Temperature of Wettest Quarter
Bio9 = Mean Temperature of Driest Quarter
Bio13 = Precipitation of Wettest Month
Bio15 = Precipitation Seasonality (Coefficient of Variation)

The two predictive models of each species for the current environment were intersected in order to determine the areas with equally suitable conditions for both species.

For the future climate we used three coupled atmosphere-ocean models (CCCMA, HadCM3 and CSIRO) each with three emission scenarios (A1b, A2a and B2a) for three time periods (2020, 2050, 2080). The A1b scenario predicts rapid human population expansion and more environmental consciousness; the A2a predicts the highest human population expansion, changes in land-use, high increase in CO^2 emissions along with high air temperatures; the B2a predicts low human population growth and slower land-use changes (IPCC, 2003).

The mean models of the three coupled atmosphere-ocean models for each time period were joined. The operation was repeated for each emission scenario giving a total of 18 projections (3 scenarios x 3 years x 2 species).

Ecological Niche-based Models

The ENMs for current conditions of the two species were generated using Maxent software version 3.3.3k available at http://www.cs.princeton.edu/~schapire/maxent/. This software calculates the realized niche of species (Sillero, 2010) by using the environmental data from the only-presence records and the background sample (finite number of points from the landscape to which values of CVs are associated) to estimate the ratio “probability density of covariates across locations within landscape where the species is present”/”probability density of covariates across landscape” (Elith et al., 2011). This is done by choosing the statistical model with the maximum entropy

(Phillips et al. 2004; Phillips et al. 2006; Phillips 2012). Pixels with no presence records are not treated as absences. Maxent makes a post-transformation of the raw output providing a logistic output that makes assumptions about prevalence and sampling effort to achieve a better estimate of the suitability of the environment for a species' presence rather than the probability of occurrence (Phillips 2012).

It has been shown that the predictions of Maxent coincide more with those of mechanistic models even if considering predictions for the future (Hijmans & Graham, 2006).

The final current models and the 18 projections were the average of 20 replicates which were run with random seed (a different random sample is used each time Maxent is run). A random test percentage of 30% was chosen so that the program sets aside 30% of the sample records for testing (33 *P. muralis* and 84 *P. liolepis*) and uses 70% as training data set (78 *P. muralis* and 196 *P. liolepis*) on each run. The test data set is used to evaluate the performance of the model. Bootstrap was the sampling technique employed since it allows sampling with replacement. Models were run with auto-features and recommended default values and options were used (Phillips et al. 2006). The output was in logistic format which gives an estimate between 0 and 1 of suitability.

Models were tested with the area under the curve (AUC) of the receiver operating characteristics (ROC) plot as it is a measure of individual model fit (Fielding & Bell, 1997). A ROC plot is produced by relating the proportion of presences correctly predicted (sensitivity) with the proportion of pseudo-absences incorrectly predicted (1-specificity). Higher values of AUC may translate the proportion between the total size of the distribution area of the species and the size of the study area: the larger the proportion the larger the value of AUC (Lobo, Jiménez-Valverde, & Real, 2008).

The relevance of each variable to the Maxent prediction was determined by Jackknife analyses of the training and test gain and of AUC. In Jackknife analysis each CV is excluded in turn and a model is created with the remaining variables; then another model is created using each variable in isolation; and finally, a model is created using all variables. The value of the gain for each CV indicates if the variable contributes more in predicting the suitability than a uniform distribution (which would have zero gain).

Response curves of each CV were performed to also assess its importance in predicting the suitability of habitat. The values of the 20 replicates of each CV were averaged and represented by a tendency curve adjusted by a polynomial equation of sixth degree. These curves give the probability of contribution of CV for the raw prediction of the models which indicate the values of the CVs suitable for each species (following Martínez-Freiría et al. 2008).

Results

Models for both species were significantly better than random predictions and test and training data sets are not correlated (Table 2). Training AUC is higher for *P. muralis* while the value for *P. liolepis* is closer to 0.5 (Table 2).

The CV more explaining the variation in the model of *P. muralis* is Precipitation of Wettest Month (Bio13) while Mean Temperature of Wettest Quarter (Bio8) contributes the least to the model (Table 2**Table**). The CV that more contributes to fitting the model of *P. liolepis* is Precipitation Seasonality (Bio15) while Temperature Seasonality (Bio4) has less impact on the prediction (Table 2).

Table 2. Percentage contribution of each climatic variable and mean training and test AUC for the 20 Maxent models of *P. liolepis* and *P. muralis* in Catalonia, Spain. Minimum and maximum values within brackets. See abbreviations in Table 1.

	P. muralis	***P. liolepis***
Training AUC	0.85 (0.84 – 0.88)	0.69 (0.65 – 0.72)
Test AUC	0.82 (0.78 – 0.87)	0.57 (0.52 – 0.67)
Test gain	0.80 (0.64 – 1.02)	0.01 (0.009 – 0.18)
Bio3	2.80 (0.00 – 11.26)	13.56 (2.44 – 28.05)
Bio4	2.90 (1.21 – 7.58)	11.13 (4.22 – 28.26)
Bio8	2.00 (0.22 – 6.15)	12.17 (1.22 – 27.35)
Bio9	8.60 (0.28 – 22.38)	14.15 (2.61 – 26.80)
Bio13	76.9 (65.39 – 85.79)	13.41 (2.15 – 31.16)
Bio15	6.90 (0.95 – 25.00)	35.57 (17.40 – 62.65)

The results of Jackknife analyses corroborate these findings (Fig.). The CVs Bio13 and Bio15 alone contribute most for the prediction of distribution of *P. muralis* while Isothermality (Bio3) is the less contributing variable (Fig2). Excluding Bio13 will decrease the training and test gain (Fig. 2A and 2B). Excluding Bio 4 will improve the predictive performance of the model (Fig. 2B). The results of the three Jackknife analyses are concordant.

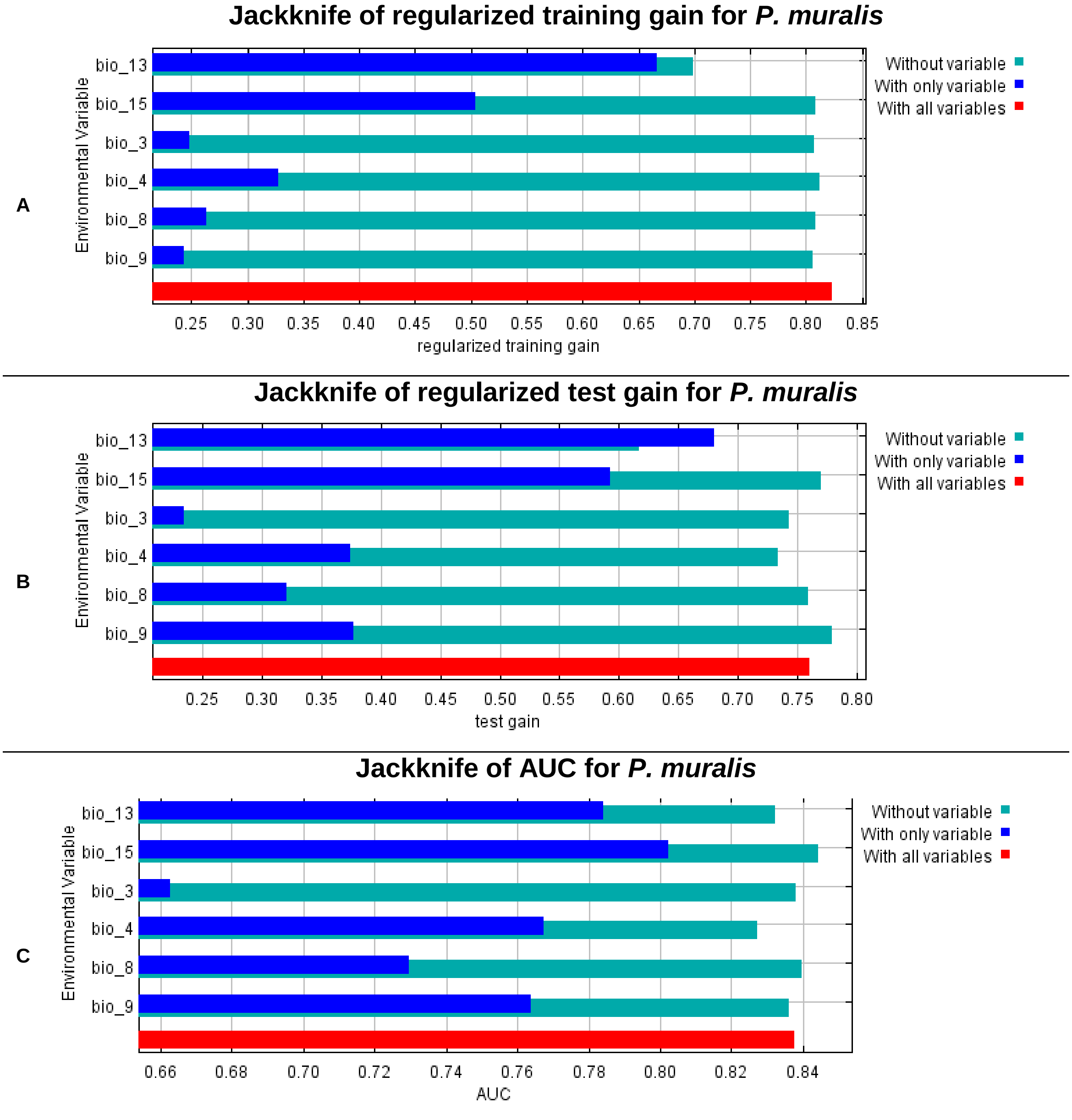


Fig. 2. Jackknife of Regularized Training (A) and Test Gain (B) and of AUC (C) of *P. muralis* model distribution.

The variable that contributes most individually on predicting the distribution of *P. liolepis* is Bio15 followed by Bio13 and Mean Temperature of Wettest Quarter (Bio8) (Fig. 3A and 3B). The model performs better if using only Bio15. If using only Mean Temperature of Driest Quarter (Bio9) to predict suitability for *P. liolepis* the model would perform worse than random prediction (Fig. 3B). When variables Bio8 and Bio9 are not used, the predictive performance of the models improves (Fig. 3C).

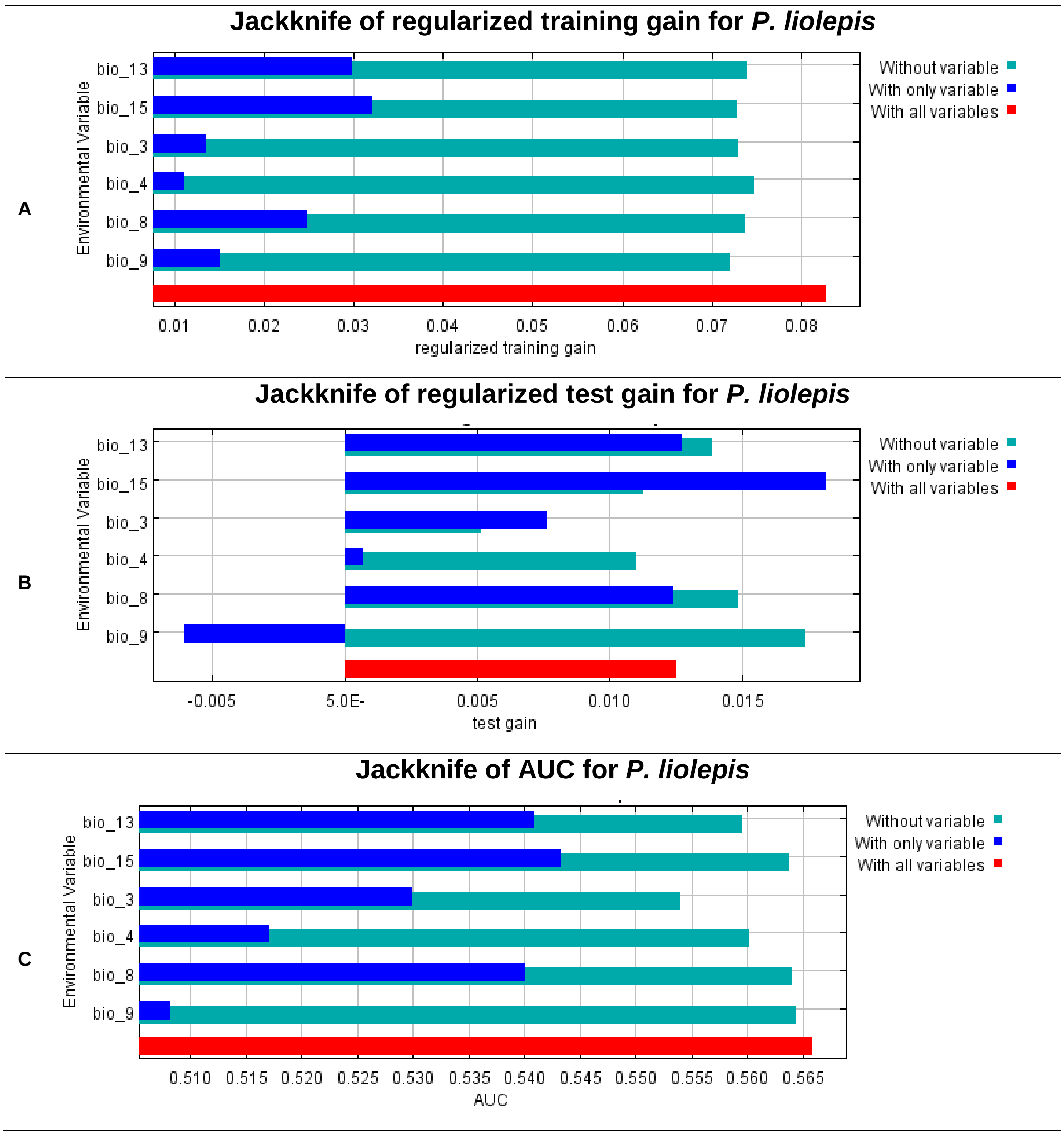


Fig. 3. Jackknife of Regularized Training (A) and Test Gain (B) and of AUC (C) of *P. liolepis* model distribution.

Low isothermality (bio3) increases the probability of occurrence of both species while higher values are suitable only for *P. liolepis* (Fig. 4). *P. muralis* is more likely to occur in areas with middle values of temperature seasonality (bio4) while *P. liolepis* is likely to occur in areas with the entire range of seasonality values with a probability of occurrence of around 0.5. Regarding the mean temperature of the wettest quarter (bio8) prediction of the model for *P. muralis* is better for lower values and slowly decreases towards the higher values while for *P. liolepis* it is lower for lower temperatures during the wettest quarter and is constant for higher temperatures. During the driest quarter (bio9), *P. muralis* is likely to be found in colder areas and its probability of occurrence rapidly decreases for medium to high temperatures. *P. liolepis* is equally likely to occur in the whole range of temperatures during the driest quarter.

Low precipitation levels (bio13) are suitable for *P. liolepis* while higher values of humidity likely increase the suitability for the presence of *P. muralis.* However, medium values of humidity are somewhat similarly adequate for both species. Low values of precipitation seasonality (bio15) are similarly appropriate for both species but higher seasonality in only suitable for *P. liolepis* since the probability of occurrence of *P. muralis* for those values is very small (Fig. 4).

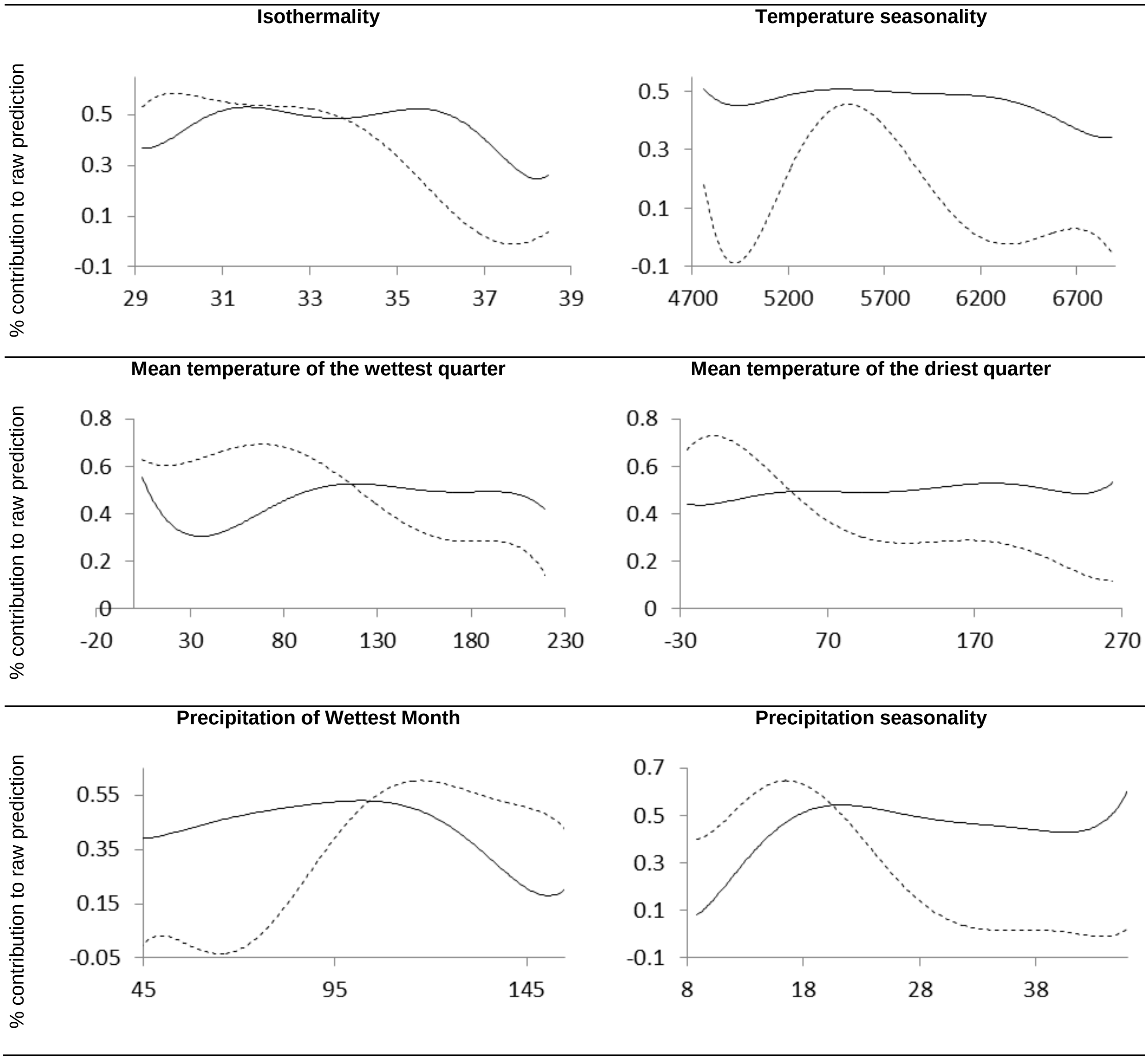


Fig. 4. Response curves of *P. muralis* (dotted line) and *P. liolepis* (full line) to the selected climatic variables.

Highly suitable current conditions for *P. muralis* are detected in the northern part of Iberian Peninsula following the Pyrenees line until near the upper eastern shore line (Fig. 5). The high suitability area also includes northern pre-Pyrenees and the Transversal mountain range (see Fig. 1 for the geography of Catalonia). To the south of this area decreasing suitability areas are predicted. In the south-eastern part of the Central Depression the lowest suitability values for *P. muralis* are found.

The mean model of the predicted distribution of *P. liolepis* reveals mildly suitable conditions across the entire study area except for the north-western Pyrenees where it is fairly low. Also, few pixels of low habitat suitability occur in the eastern part of Catalonia in the area of the Littoral mountain range and the southernmost less suitable pixels are located in the pre-Littoral mountain range.

Low to mildly suitability values are detected simultaneously for both *P.* muralis and *P. liolepis* in the areas of the Pyrenees, pre-Pyrenees, Transversal range and north-eastern areas of the Littoral and pre-Littoral depressions (Fig. 5; Intersection). They closely follow the predicted distribution for *P. muralis* with few exceptions in the north-western Pyrenean areas. Simultaneous high habitat suitability conditions for both species were not detected in the study area. From the pre-Pyrenean region southwards no common suitable conditions for both species are found.

Predictive models for the three future climate scenarios reveal different trends of putative distributional ranges between the two species (Table 3) as expected from the current distribution.

According to the A1b climatic scenario, suitable conditions for *P. muralis* are likely to decrease in the north-eastern coastal areas along the years. In addition, the A1b scenario predicts a range contraction towards the Pyrenees with suitable conditions no longer available in the pre-Pyrenees. In the Transversal mountain range, habitat suitability for *P. muralis* decreases along the years. The A2a scenario also predicts a severe range contraction from 2020 to 2050 in the pre-Pyrenees and north-eastern coastal areas whereas by 2050 and 2080 medium suitability conditions will appear in north-western Pyrenees. By 2080 the entire Central Depression becomes completely unsuitable for *P. muralis*. According to the B2a predictions for *P. muralis*, suitability conditions will remain rather constant and favourable for the species in the north of the study area although by 2080 they slightly decrease in the northern extreme (Table 3).

The A1b scenario for *P. liolepis* predicts medium habitat suitability across Catalonia which will slightly decrease in the Catalan depression along the years. The areas with higher suitability values area located in some pixels in the pre-Pyrenees and the lowest values of suitable conditions are located in the north-western Pyrenees (Table 3).

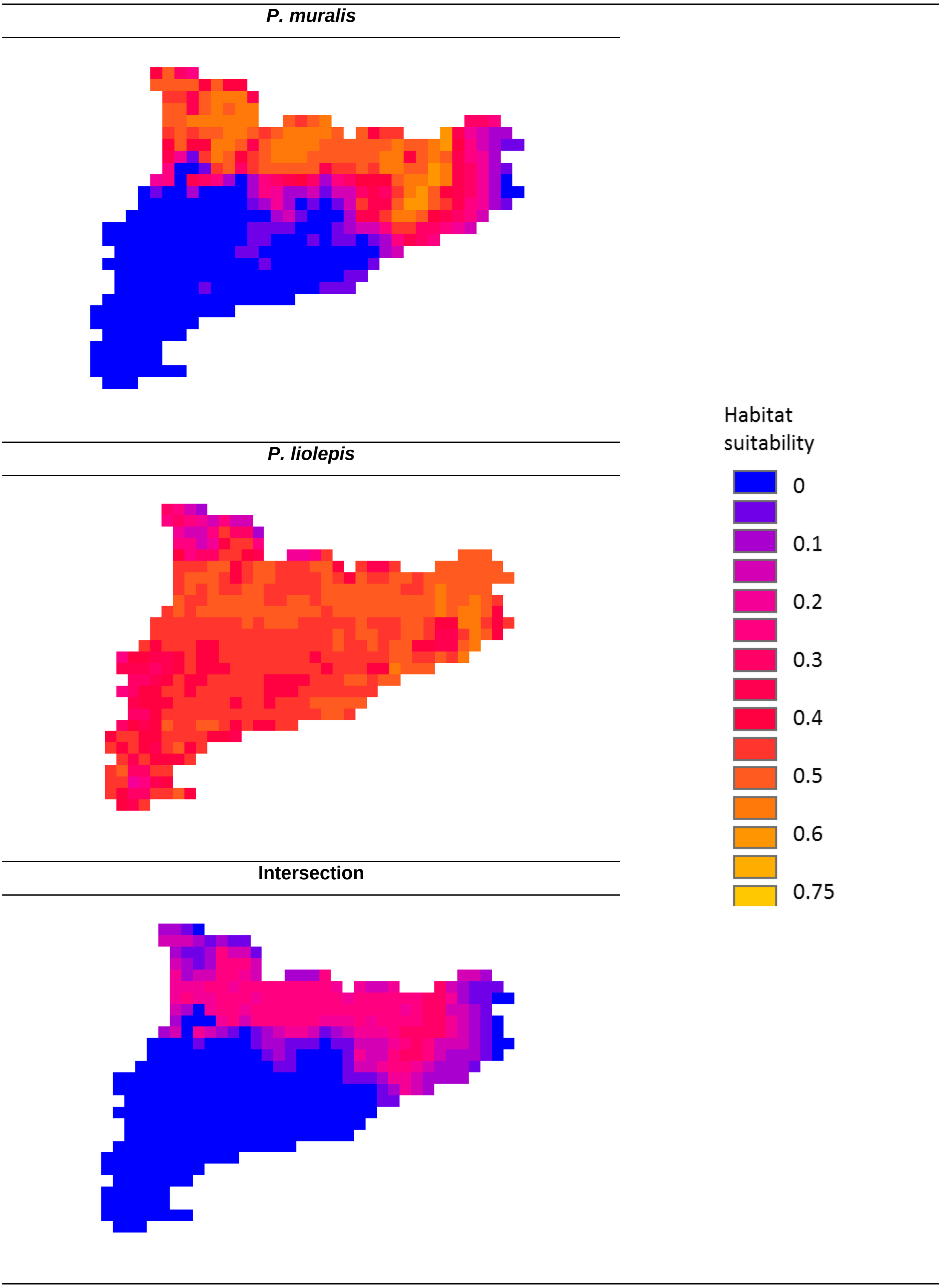


Fig. 5. Mean predicted distribution of *P. liolepis* and P. *muralis* in Catalonia based on current bioclimatic variables (see Table 1). The two maps of current predictions for each species were intersected ("Intersection" map). One pixel=10km^2. Legend gives the suitability probabilities.

However, in the north-western extreme in the lower altitude areas in that region, medium suitable conditions will appear by 2080. The A2a predicts medium to high suitability conditions for *P. liolepis* in the north-western region along the years. To the south of the Pyrenees the suitability is low except for some pixels in the delta of the Ebro River. The B2a scenario for *P. liolepis* also predicts values of high habitat suitability in north-western Pyrenees by 2020 and 2050 although by 2080 those values decrease but progressively shift towards the south. In the remaining study area, medium habitat suitability is predicted except for some pixels in the western Catalan depression and in eastern Littoral depression with low values.

Similar suitable conditions for both species were predicted in north-eastern Catalonia by the A2a scenario in 2020 and in north-western Pyrenees and Transversal range by the B2a scenario in 2020 and 2050.

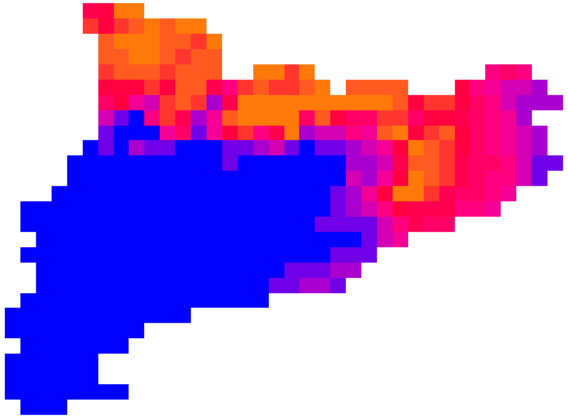
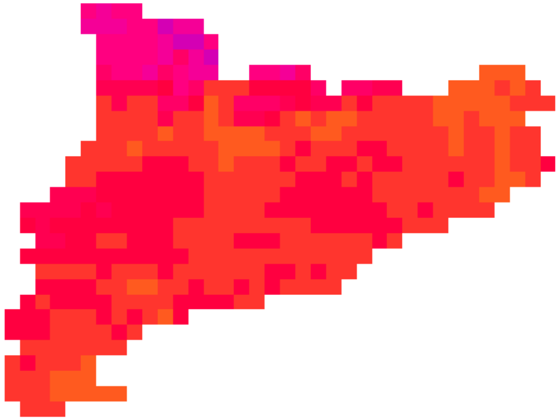
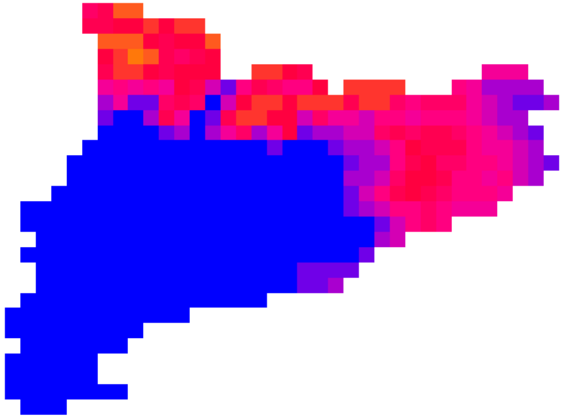
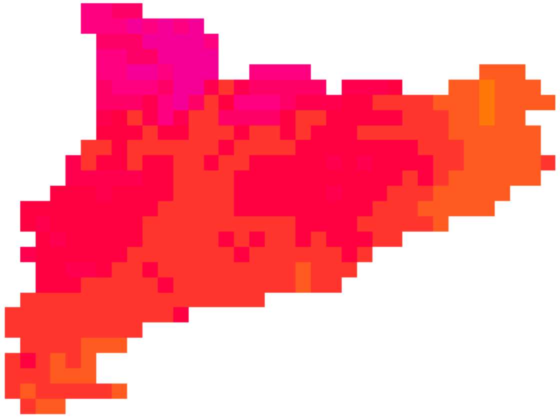
Discussion

The ENMs of both *P. muralis* and *P. liolepis* revealed that their distribution patterns are mostly influenced by water-related CVs (Table 2). Such finding is somewhat unexpected since *Podarcis* lizards are thermorregulating species so in principle environmental temperature could be expected to have the most preponderant role in predicting their distribution. Similar patterns are reported for *P. vaucheri* in North Africa (Kaliontzopoulou et al., 2008).

The distribution models of *P. muralis* and *P. liolepis* predict current suitable conditions in the areas where they are currently found (Llorente et al. 1995; Pleguezuelos et al. 2002) supporting a good model performance under this working scale. In fact, the low value of AUC for *P. liolepis* may only reflect the low proportion between size of distribution and size of the study area (Lobo et al., 2008).

P. muralis was predicted to occur in areas with low seasonal variation of temperature and humidity, high precipitation and low temperatures, that is, Eurosiberian/Atlantic climate. *P. liolepis* was predicted to occur in areas with high seasonal variation of humidity, low precipitation and high temperatures in addition to indifferent conditions of seasonal variation of temperature, that is, Mediterranean climate.

Table 3. Mean models (ccma, csiro and hadcm3 together) of each scenario (A1b, A2a, B2a) for each year. One pixel=10Km^2. Legend gives the probabilities of habitat suitability.

	P. muralis	***P. liolepis***
	A1b	
2020		
2050		

Habitat suitability

0

0.1

0.2

0.3

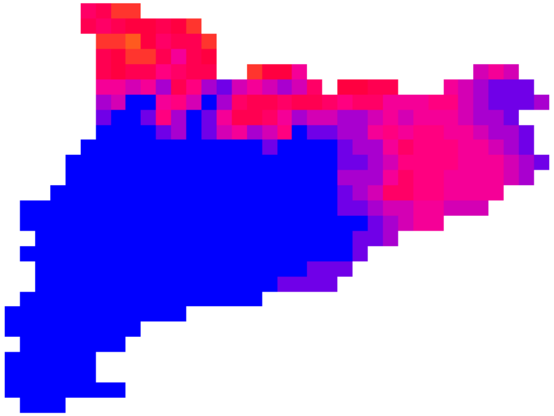
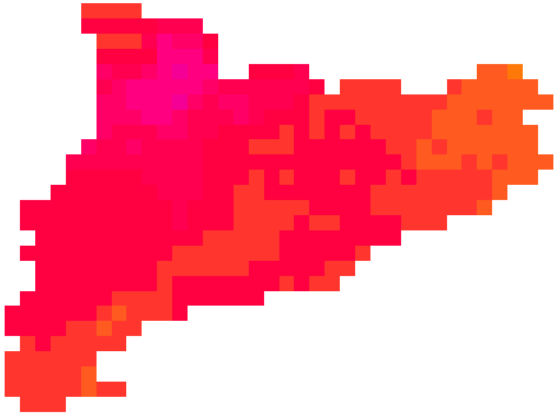
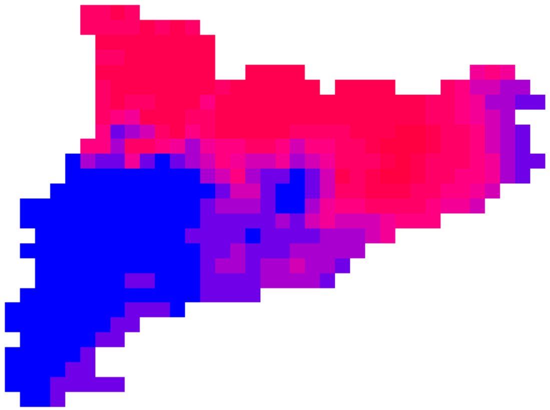
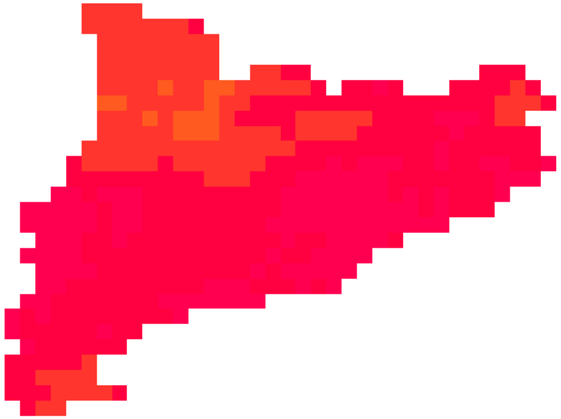
0.4

0.5

0.6

0.75

Table 3. ***Continued***

	P. muralis	***P. liolepis***
	A1b	
2080		
	A2a	
2020		

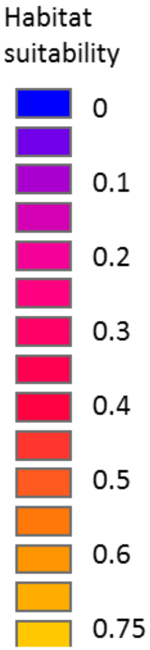
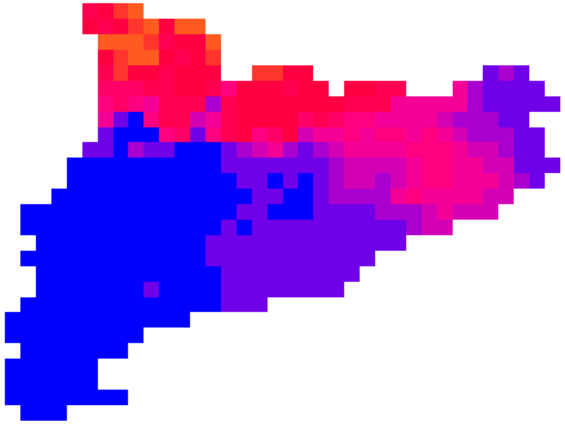
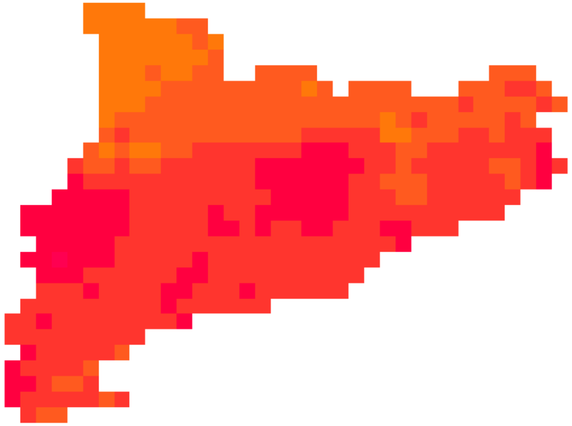
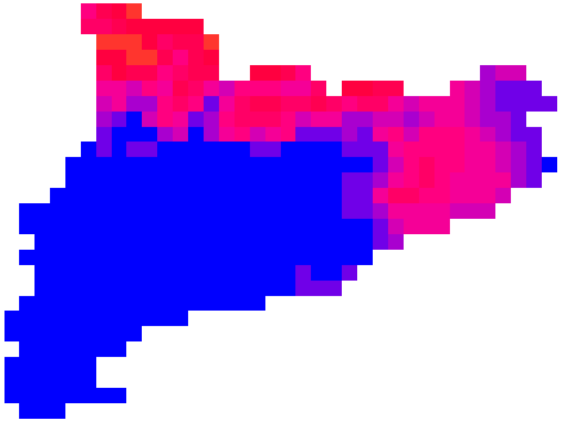
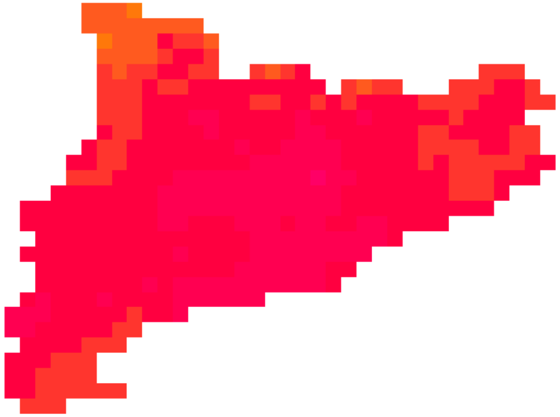


Table 3. ***Continued***

	P. muralis	***P. liolepis***
	A2a	
2050		
2080		

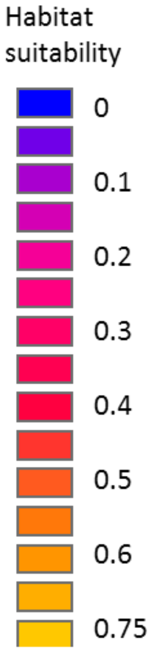
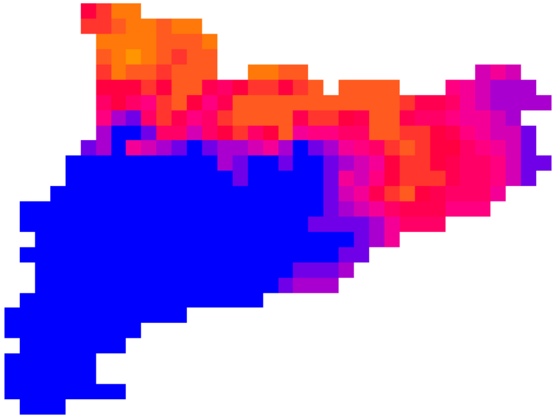
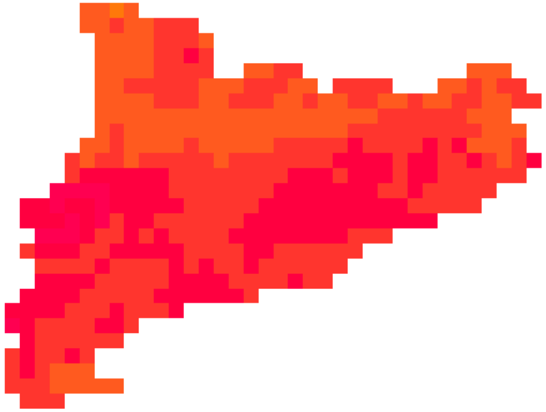
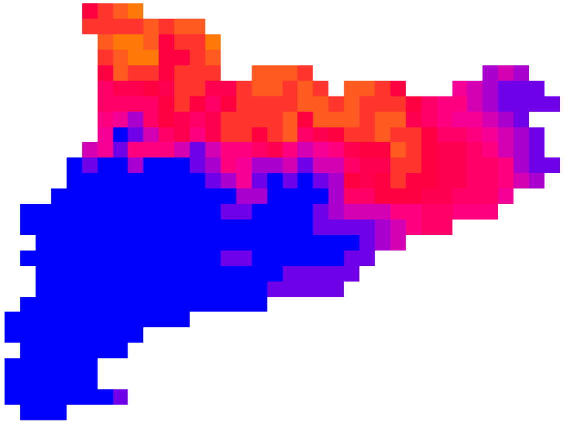
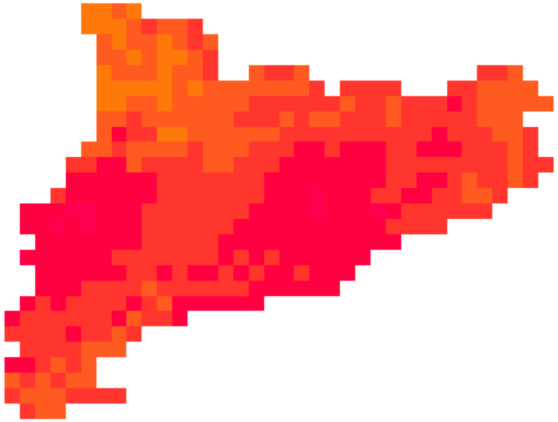


Table 3.*Continued*

	P. muralis	*P. liolepis*
	B2a	
2020		
2050		

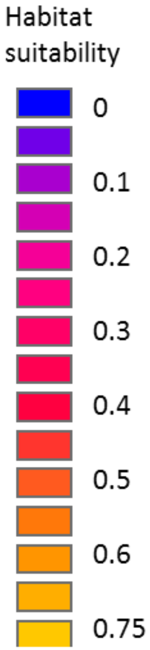
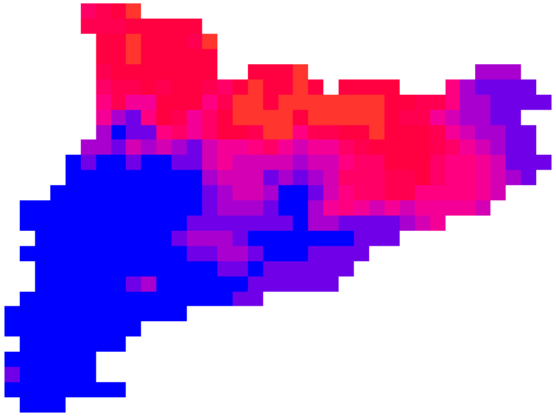
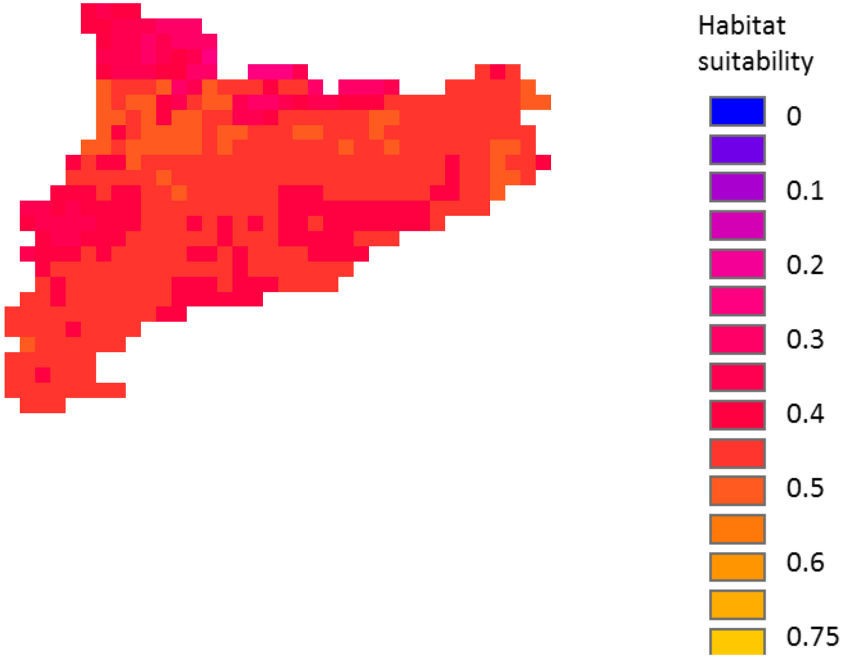


Table 3.*Continued*

	P. muralis	***P. liolepis***
	B2a	
2080		

The bioclimatic requirements shown (Fig. 4) suggest that *P. muralis* is prone to thrive in mountain areas because of their lower air temperatures, more humid climate and low precipitation seasonality. This is in accordance with its presence in areas of Atlantic climate in the Iberian Peninsula since the predicted suitable areas for *P. muralis* overlap with the Atlantic climate in the north-east of the study area (Sillero et al., 2009). The same occurs for the other mountain areas in Iberian Peninsula where *P. muralis* is found (the Central and Iberian mountain ranges). The explanation for this repeated pattern could simply be the presence of suitable areas for *P. muralis* in high altitudes. However, in mountain ranges of the pre-Pyrenees with altitudes higher than that of Montseny or Central System, this species does not occur while *P. liolepis* is present. Conversely, *P. muralis* can be found in western Catalonia in areas with low altitude (300m) near the coast also with Atlantic climate (Sillero et al., 2009) corroborating the assumption that this type of climate is the one influencing the distribution pattern of *P. muralis*.

Conversely, *P. liolepis* has more suitable areas in regions of Mediterranean climate characterized by higher temperatures but also high thermal and hydric seasonality (Fig. 4). However, *P. liolepis* can be found in the Pyrenees but only on the south-facing slopes (Rica 1983; Pleguezuelos et al. 2002) while *P. muralis* is found on the north-facing slopes.

The smooth snake, *Coronella austriaca*, displays a similar pattern to that of *P. muralis* at a different geographic scale. It has a wide European distribution and in Iberian Peninsula it continuously inhabits the northern regions. However, isolated patches occur in the centre and south of the Peninsula (Santos et al., 2009). Its distribution is correlated with Atlantic areas and the isolated patches are probably due to decrease of habitat suitability that may have led to local extinctions. It is not unreasonable that the same has happened for *P. muralis* populations in the Central and Iberian mountain ranges: the occurrence of isolated nuclei in those areas is likely due to climatic fluctuations during the Pleistocene that lead to local extinctions (Santos et al., 2009). The progressively reduced areas of suitable habitat for *P. muralis* are likely due to temperature increase and precipitation reduction predicted by the models for future climate scenarios.

The predicted overlap area of highly suitable conditions (Fig. 5; Intersection) does not necessarily imply that both species live in sympatry. In fact, field observations (Llorente et al. 1995) revealed that in most areas they are not in the same location which may indicate competitive exclusion: environmental conditions are suitable for both but only one is found in the area (Costa, Wolfe, Shepard, Caldwell, & Vitt, 2008). However, a better resolution of presence records and bioclimatic variables is needed to more precisely determine putative sympatry areas and determine if species are competitively interacting rather than occupying different microclimates. Nonetheless, this somewhat coarse resolution does not fail in predicting the general patterns.

Regarding the predictions of the A1b future climate scenario, the possible expansion of *P. liolepis* towards the north-east may be caused by an increased global air temperature. This may also explain the contraction of *P. muralis* away from coastal areas which are predicted to get warmer. In fact, the current models predict that *P. liolepis* founds suitable conditions in warmer and drier areas than *P. muralis* (Fig. 5).

The A2a is the most rigorous scenario and predicts an overall range contraction for both species although new suitable areas in the north-west Pyrenean region are detected for *P. muralis* and for *P. liolepis*, which is currently an area of Atlantic climate. This pattern is likely to be related with the increase of air temperature as previously discussed. In addition, non-suitable conditions in coastal areas are possibly caused land-use changes and by the high expansion of human population since these areas are mostly covered by cities which are likely to expand.

The B2a predictions are the least changing for both species along the years. This may be related with low land-use changes and higher environmental protection. In fact, natural parks located in the pre-Pyrenean areas (such as the Montseny Natural Park) may be favoured.

In conclusion, *P. muralis* and *P. liolepis* have adequate environmental conditions to remain in mountain areas in the north-eastern part of Catalonia. *P. muralis* is likely to remain in the same areas where it is currently found although it may suffer a slight contraction in its range, disappearing from the most coastal mountain areas. *P. liolepis* may expand towards the northernmost limit of Catalonia where it is not currently found if the climate temperature rises to considerable values.

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3 General discussion

The results obtained following three independent methodological approaches (laboratory tests, field records and ecological modelling) corroborate each other revealing that environmental humidity rather than temperature is the factor most constraining the distribution patterns of *P. liolepis* and *P. muralis*. However, some of the conclusions, particularly in the field, are based on a low sample size, namely in terms of number of *P. liolepis* individuals in the sympatry area (Carneiro, García-Muñoz, & Carretero, *unpub*).

Thermal ecology has been the main focus on reptile ecophysiological studies (Huey, 1976). Nonetheless, some studies focus on the physiology of some taxa regarding skin permeability, scales or other mechanisms by which water can be lost (Lillywhite, 2006; Calsbeek et al., 2006). However, the importance of humidity is still left aside in most studies on reptile physiology. Obviously, water is of paramount importance for all living organisms. At global level, reptiles are mostly associated either to tropical areas characterized by high, stable temperatures and humidity levels, or to desert areas, where high air temperatures and low environmental humidity are the main constraints for their activity. However, in temperate areas the relative influence of these two factors is more dubious and must be adequately addressed (Schmidt-Nielsen, 1997a).

In the Iberian Peninsula, located in temperate-climate latitude, two main climatic areas are found: Atlantic and Mediterranean. While the Atlantic climate ranges from the western and northern coastal areas along the north-eastern areas of the peninsula, the Mediterranean climate predominates in the remaining area with few exceptions on the higher mountain ranges (Sillero et al., 2009). Both regions differ mainly in (summer) precipitation regimes while temperature is more variable according to the altitude and degree of continentality, although it is somewhat lower in the Atlantic climate.

Climate models for future climate change scenarios reveal that the Mediterranean climate will expand northwards (IPCC, 2007). The ENM predicting more severe changes in environmental conditions, the A2a, (Carneiro & Carretero, *unpub*) forecasts that highly suitable areas for *P. liolepis* will appear near the Pyrenees, the highest mountain range in the peninsula, which is concordant with its preference for hotter and drier conditions according to the model's predictions.

Also, the ENMs predicted areas with highly suitable conditions available for both species. The most relevant variables detected by the models are mainly related to hydric ecology. In parallel with this information provided by the models, preferred temperatures of *P. liolepis* and *P. muralis* are not statistically different suggesting similar thermal requisites. This is quite concordant with the ENMs which do not attribute high relevance to the temperature-related variables for explaining the presence of both species at geographical scale.

Both species are usually rock-dwelling but *P. muralis* is displaced to the ground when in sympatry with *P. liolepis* (Diego-Rasilla, 2009). However, such displacement may not occur if both species are not syntopic but segregate at geographic level (Carneiro, et al, *unpub*). The upwards shift of Tp of *P. liolepis* due to the presence of *P. muralis* in the interaction tests may not constitute a disadvantage for the former if climate in sympatry areas warms, providing sufficient thermal resources. This contrasts with the stability of thermal selection by *P. muralis* either in the presence of conspecifics or heterospecifics. Certainly, *P. muralis* may face some thermal constrains if climate is warming but likely heterogeneous habitats will still provide adequate thermal environments. However, the subsequent aridification may be more problematic because lizards would have problems in dealing with abrupt humidity variations. These will be especially constraining for *P. liolepis* occurring in the driest areas but also for *P. muralis* currently inhabiting peripheral areas of the range. While the first will simply derive from the suitability of abiotic conditions for the physiology of *P. liolepis*, the second may result from a combination of the climate suitability from both species and their (asymmetric) interactions. According to our lab tests, we may expect that *P. liolepis* would outperform *P. muralis* in syntopy in terms of thermal/hydric environment if climate gets warmer and drier as future scenarios forecast.

Further environmental data is necessary to complete the view of possible sympatry and the inter-specific impacts on the physiology of *P. liolepis* and *P. muralis*. Detailed data on temperature of air and substrate, relative humidity along with wind speed, direction of solar rays and insolation should be determined in order to fully comprehend the ecological requisites of these species. Temperature and humidity data loggers would allow more reliable information on the operative temperatures and water loss rates of lizards (Hertz et al., 1993; Castilla et al., 1999). This methodology should be applied in the termogradient and in the water loss experiments, in mesocosms (semi-natural conditions where presence and abundance of heterospecifics can be manipulated) and *in situ*, in putative sympatry areas (Scheers and Van Damme, 2002).

Additional experimental methodology integrating simultaneously thermal and hydric gradients should be developed. Some attempts have been made (Appendix 1) but proved unfruitful due to physical constraints (water evaporation increase leads to an increase of temperature impeding constant gradients of both factors). In addition, the same ecophysiological characters determined with these studies should be assessed for the other *Podarcis* species (García-Muñoz et al., 2011; Carretero et al., 2012) in order to obtain further insight on the influence of phylogenetic relations on the physiology of species within this genus.

Information on other species (Bauwens et al., 1995; Castilla et al., 1999; Grbac and Bauwens, 2001; Carretero et al., 2006; Veríssimo and Carretero, 2008) would also give further insights on the ecophysiology and patterns of distribution of *P. liolepis* and *P. muralis*. The ecological requirements of both putative prey and predator species should be studied namely by interaction field experiments in order to unravel their preponderance on the distribution of these *Podarcis* species. In addition, conditions for egg incubation and hatchlings should be assessed since a species may be absent because of unsuitable conditions during incubation and hatching even if conditions for the adults are suitable (Van Damme, Bauwens, Braña, & Verheyen, 1992; Ji & Braña, 1999).

Assessing ecophysiological information and producing ENMs with correlative distribution modelling tools proved to be useful to understand the ecological requirements of *P. liolepis* and *P. muralis*. In the future, directly integrating such information to produce models of distribution would increase their reliability. This can be achieved with mechanistic models which are based on functional traits and physiological constraints (Hijmans and Graham, 2006) thus being completely independent of species records (Kearney et al., 2010, 2009). These models are able to determine the fundamental niche (Kearney and Porter, 2004; Kearney et al., 2009) by explicitly integrating limiting factors of species distributions and abundances (Kearney et al., 2009; Fei et al., 2012) such as preferred body temperature (Carneiro et al., unpub), lethal temperature (Monahan, 2009), core temperature, evaporative heat exchange in ectotherms, metabolic heat in endotherms or energy transport processes in plants (Kearney et al., 2009).

Since mechanistic models use information intrinsic to each species and base predictions on real cause–effect relationships they allow assessing possible distributions independently of the current range (Kearney and Porter, 2004). Mechanistic models provide better and more correct prediction for instance in range-shifting species in comparison with correlative models (Kearney et al., 2009). Also, these models lead to a more reliable prediction of the impacts of climate change (Kearney et al., 2010) and of species' future distribution because they are dynamic, stochastic and based on non-equilibrium which makes more sense in ecological terms (Pickett et al., 1994). The mechanistic approach does not directly account for interspecific interactions or for evolutionary affects (Soberón and Peterson, 2005) and they can only model the part that exists in the environment (Sillero, 2011). Regardless of the numerous advantages that mechanistic models offer, they are, in practical terms, difficult to implement because assessing physiological data for several parameters through field and/or laboratory experiments raises logistic problems.

In conclusion, data used to produce correlative models are easier to obtain and apply because environmental variables such as bioclimatic, topographic, habitat-related and biological are easily measured in the field and they are fairly good correlated with species patterns observed, while determining physiological aspects of species' biology may require specialized and expensive material. The outputs of the models are easier to compare with most studies on changing environments (Guisan and Zimmermann, 2000), are valuable to present-day scenarios and can be used to obtain a first assessment over large areas. Nonetheless, physiological data should also be integrated to improve parameterization in correlative models for the future since these two approaches are complementary.

4 General conclusions

- Studies as this bring new insights on the impacts of humidity on physiology and distribution of Lacertid species and highlights that this abiotic factor should be more stressed in future studies on reptilian species.
- Convergence of thermal physiology seems to occur among two *Podarcis* species non-directly related as they select similar preferred body temperatures.
- Water ecophysiology varied interspecifically among *Podarcis* species throughout time in one normal activity day although the final amount of water lost was similar.
- Asymmetric interaction between species regarding body temperature attained when two heterospecific individuals were together caused an increase in the temperature preference of *P. liolepis* while *P. muralis* remained invariant. When two conspecifics were together no differences in preferred temperature were detected for either species.
- Preliminary field sampling indicated that body temperatures in the field are more affected by air temperature although soil temperature and humidity also affect body temperatures. More field effort is needed to discriminate eventual differences between both species.
- Environmental niche-based models indicate that variables related with water availability are more important than those related with temperature for predicting the presence of both species.
- Models differ between both species but are inclusive regarding their competitive interaction due to limitations of data scale and fitting of modelling methods.
- Projection of the models for the climate change scenarios predicts a contraction of the range of the species occupying the Atlantic climate (*P. muralis*) with increasing temperature and decreasing relative humidity; a geographic shift involving occupation of current peripheral Atlantic zones and disappearing from the most arid Mediterranean areas is observed for species with higher Mediterranean affinities (*P. liolepis*).

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Glossary

Basking - behaviour that consists in orienting the body perpendicularly to the solar rays direction and spreading the legs and flattening the body (Scheers and Van Damme, 2002; Schmidt-Nielsen, 1997b).

Parietal eye - photoreceptive structure located on the top of the head; it responds to the presence of light and to different light wavelengths. Shielding the parietal eye with filters to different wavelengths causes a decrease in the upper and lower set point temperatures in 1-2ºC (Tosini and R. Avery, 1996). It is possible that this photoreceptive structure is responsible for detecting the beginning and the end of the daily photophase but it is not the only involved in the regulation of thermoregulatory processes (no effects on mean heating rates, and bask and forage durations were detected; Tosini & Avery (1996)).

The effects of the parietal eye on thermoregulation are probably mediated by the hormone melatonin (Rismiller and Heldmaier, 1987). This hormone secreted from the pineal and the parietal eye affects the thermoregulatory processes because its secretion is inhibited by light (Rismiller and Heldmaier, 1982).

Q_{10} - rate of a physiological process at one temperature divided by the rate of that process at a temperature lower by 10ºC. This parameter is used to describe the sensitivity of an organism to a change in Tb (Macnab, 2002).

Supplementary material

Appendix 1

An attempt for a simultaneous gradient of temperature and humidity

After determining the thermal and hydric requirements separately for each species, we considered interesting to determine the exact thermal and hydric requirements of species at a given time. We tried to develop a methodology that would simultaneously combine thermal gradients and variable hydric regimes. To do so, the thermal gradient would have to be maintained under different hydric regimes.

To conduct this trial we collected *P. bocagei* individuals from Moledo, northern Portugal (41º51'N 8º51'E; 6m altitude) in October 2011, in a transitional habitat between beach and abandoned agriculture fields.

This trial was conducted by placing five terraria with different relative humidity side by side on a laboratory with constant air temperature (±26ºC). Each had a fine plastic net on the bottom as the substrate with which the animal would be in direct contact with.

Two of the terraria had highly humid conditions created by a towel saturated with 0.4L of water ("Humid"); two had 450g of silica gel creating dry conditions ("Dry"), and the "Control" terrarium only had the net on the bottom and had the same temperature and humidity conditions as the laboratory.

In each terrarium, a thermal gradient was created by placing an infra-red bulb of 150V, 15 cm above the substrate as in the standard experiment (see Manuscript I). Each hour, for six consecutive hours (simulating the morning part of the lizard activity), a Fluke®-971 Hygrometer was used to register the temperature and relative humidity inside the terrarium by placing it in the middle.

The first day of experiments was conducted with no lizards and, in the subsequent five, one individual was placed in each terrarium.

The results obtained revealed several difficulties with the methodology. The main problem was the inability of maintaining humidity conditions constant (Figure 1). It was observed that in the "Dry" terraria the silica gel was saturated after three hours in the first day ("Control" had 30.5% and "Dry" had 26.4%) and after the third hour the "Control" and the "Dry" revealed the same trend. This was also observed in the subsequent days. This probably translates shifts in the humidity of the room caused by the presence of the other terraria, in particular the "Humid", since water was continuously evaporating from them.

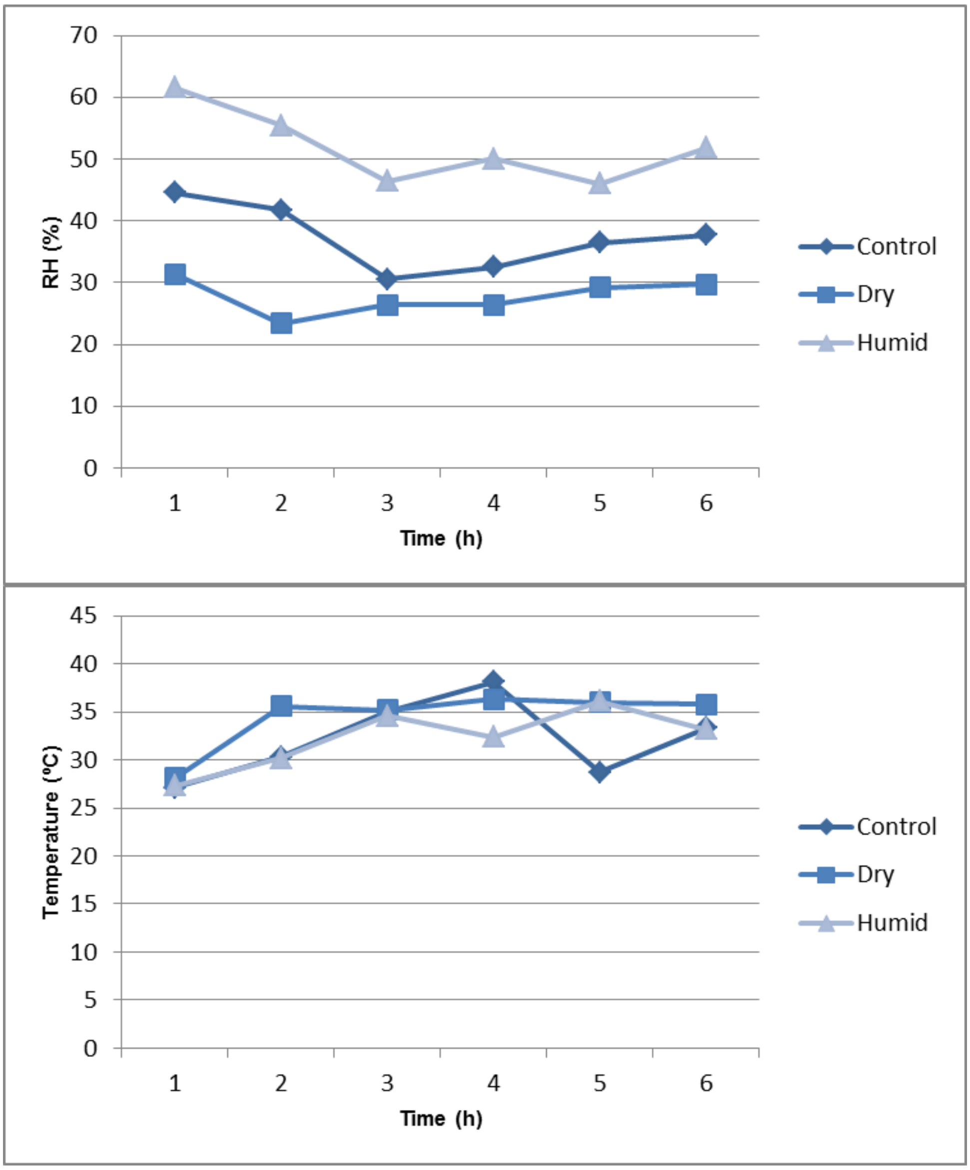

Figure 1. Variation of humidity (A) and temperature (B) conditions inside each terrarium during one-day experiment (6 hours). "Control" – terrarium with the same conditions of temperature and humidity as the laboratory it was in; "Dry" - terraria with silica gel; "Humid" – terraria with water-saturated towel.

In fact, RH ranged from 61.6 to 51.8%, decreasing throughout time. At a given point in time, each terrarium no longer had the initial conditions (Figure 1 A).

The temperature in the same place of the terraria was not constant (Figure 1 B) (varied between 27.1 and 38.1ºC) probably due to interaction with physical properties of water. Another bias potentially introduced by this the design is the inability of weighing the excrements of the animals leading to a loss of statistical power since the weigh is always taken with high accuracy (0.0001g).

Also, the results of each day were not consistent with each other (not displayed) so this methodology was not replicable. This proved to be a non-viable methodology and alternative procedures should be implemented in future assessments of Tp and WI simultaneous.

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