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The role of metabolism in understanding the altitudinal segregation pattern of two potentially interacting lizards

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ABSTRACT

Sympatric species from the same ecological guild, that exhibit partial altitudinal segregation, can potentially interact in areas of syntopic occurrence. Besides general species' ecology, physiology can provide important answers about species interactions reflected in altitudinal patterns. Lizards *Podarcis muralis* and *Iberolacerta horvathi* exhibit partial altitudinal segregation, while they strongly resemble in overall morphology and ecology (diet, daily and seasonal activity pattern), but show certain degree of physiological dissimilarity. They have similar mean preferred body temperatures and patterns of seasonal and daily variations but differ in the magnitude of seasonal variation. Since an ectotherm metabolism is highly dependent on body temperature, thermoregulation is expected to directly affect their metabolism. We compared metabolic rates of adult males from an area of sympatry, measured under two temperature regimes (20 °C and 28 °C). Both species increased metabolic rates with temperature in a similar pattern. We also compared electron transport activity from tail tissues which provide values of species' potential metabolic activity (enzymatic capacity). Species clearly differed in potential metabolic activity; *I. horvathi* attained higher values than *P. muralis*. No difference was detected in how species exploited this potential (calculated from the ratio of electron transport activity and metabolic rates). However, we observed higher potential metabolic activity *I. horvathi* which together with the ability to thermoregulate more precisely could represent a higher competitive advantage over *P. muralis* in thermally more restrictive environments such as higher altitudes. Understanding of metabolism seems to provide valuable information for understanding recent distributional patterns as well as species interactions.

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1. Introduction

Sympatric species from the same ecological guild are likely to be in interaction when in areas of syntopic occurrence (Begon et al., 2006). One of the common distributional patterns of interacting species is partial altitudinal segregation, where one species is occupying higher altitudes, the other lowlands and there is a zone of spatial overlap at intermediate altitudes (e.g. Vrezec and Tome 2004, McHugh and Budy 2005, Pasch et al. 2013, Žagar et al. 2013). Such sympatric zones of overlap provide ideal opportunity to study species interactions.

The first step of species interaction studies is usually a comparison of species' general ecological traits, and only rarely physiology is included although it can provide elusive answers. Especially metabolism could be an important physiological trait providing a better understanding of

species distribution patterns and interactions (e.g. Bennet et al., 1984; Nagy et al., 1984). From a number of factors that may influence the metabolic rate (MR) of reptiles, the most dramatic effects are probably those produced by changes in the activity level mediated by body temperature (Bennett and Dawson, 1976; Huey, 1982). However, within a species, body size, sex, reproductive and nutritional conditions, time of day and seasonal acclimatization also affect the metabolic rate (Bennett, 1982; Patterson and Davies, 1984; Brown et al., 1992; Zari, 1996, 1999; Dorcas et al., 2004; Steffen and Appel, 2012). Also, different species that share similar habitats may display divergent metabolic rates even as a result of separate evolutionary history (Thompson and Withers, 1994; Randriamahazo and Aime, 1998; Hare et al., 2006).

The sympatric lacertid lizards from the Northern Dinaric Mountains, Common Wall Lizard (*Podarcis muralis* Laurenti, 1768) and Horvath's Rock Lizard (*Iberolacerta horvathi* Méhely, 1904), exhibit partial altitudinal segregation pattern (Žagar et al., 2013). *I. horvathi* is most abundant at higher altitudes while *P. muralis* in lowlands and a broad zone of overlap exists at mid-altitudes. A specific characteristic of the overlapping area at mid-altitudes in SE Europe is high forest cover (Perko and Orožen Adamič, 1998) where open areas, suitable for reptile occurrence

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and basking, are limited (Žagar et al., 2013). Males of many species of the family Lacertidae are known to exhibit aggressiveness against other males in spatial context, namely for the best basking places and shelters (e.g. Olsson, 1994; López and Martín, 2002). Thus, while such favourable parts of the habitat are limited in the area of sympatric occurrence of the studied species, there is a high potential for interactions.

Both species strongly resemble in morphology (Žagar et al., 2012) and ecology (diet, daily and seasonal activity pattern and reproduction, De Luca, 1989; Lapini et al., 1993; Richard and Lapini, 1993; Cabela et al., 2007), but show some degree of physiological dissimilarity. Osojnik et al. (2013) recently described that both species have similar mean preferred body temperatures (T_p) and the pattern of daily and seasonal variations of T_p . The only interspecific difference detected was the magnitude of seasonal variation of T_p ; the “high altitude” species, *I. horvathi*, selected similar temperatures throughout the year, while the “low altitude” species, *P. muralis*, displayed seasonal variation. This suggests that *I. horvathi* is potentially a more precise thermoregulator than *P. muralis*. Alongside, *I. horvathi* was found to lose less water than *P. muralis* when exposed to dry conditions, suggesting additional physiological trait enabling it to spend more time in sun basking without risking water stress (Osojnik et al., 2013).

Since acclimatization of preferred body temperature (T_p) in response to environmental seasonality has been demonstrated to vary between lizard species (e.g. Angilletta, 2001; Osojnik et al., 2013) and temperature is the key determinant of metabolic rate in ectotherms (Bennett, 1982), difference in metabolism between species is expected. As a general rule in ectotherms, species from cold climates tend to have a higher metabolic rate at low temperatures than those from warm climates. For example, the cold-climate lizard *Zootoca vivipara* attained higher metabolic rates than the warmer-climate lizard *Podarcis hispanica* (Patterson and Davies, 1989). With altitude, the most obvious changing environmental trait is the temperature (dropping with increase of altitudes) which has a pronounce direct effect on ectotherms, such as lizards (Huey et al., 2012).

Organisms' metabolism is constraint by the enzymatic capacity of tissues. This can be measured in several ways, most often by assessing citrate synthase activity or cytochrome *c* oxidase (e.g. Simon and Robin, 1971; Lannig et al., 2003; Seebacher et al., 2003), whereas electron transport system (ETS) activity was less frequently used (e.g. Lannig et al., 2003) but provides similar information. Measurements of ETS activity indicate the amount of oxygen consumption that would occur if multi-enzyme complexes, located in the inner membrane of mitochondria, functioned maximally (Muskó et al., 1995). The ratio between ETS activity and observed oxygen consumption (ETS/MR ratio) is an important index of an organism's metabolism (Muskó et al., 1995) because it provides a measure of the fraction of total metabolic potential that is actually exploited for respiration. Several studies also calculated the ETS/MR ratio (but only in invertebrates) and reported that it differs between species having different ecological demands and consequently inhabiting different habitats (Muskó et al., 1995; Simčič and Brancelj, 1997; Simčič et al., 2005). Measuring metabolic potential provides information on species' fundamental physiology and is therefore crucial for a comprehensive understanding of an organisms' metabolism.

Our study was set to explore the metabolic respiration, the response of metabolism to temperature change and to determine metabolic potential, which was compared between studied species to elucidate the underlying distributional pattern (partial altitudinal segregation) and the potential for interaction in syntopic populations. We measured metabolic rate (oxygen consumption) and determined metabolic potential (ETS activity) under two realistic temperature regimes: at 20 °C, which is a less favourable temperature for lizard activity, and at 28 °C, the temperature close to their T_p (Osojnik et al., 2013). If both species were adjusted to their current altitudinal ranges, the metabolic rates and potential should be higher in the mountain species (*I. horvathi*) than in the lowland one (*P. muralis*), while the use of metabolic

potential (calculated from the ratio of metabolic rate and metabolic potential at the same temperature) should be lower for the mountain species ensuring a reserve for thermally critical periods. Furthermore, metabolic response to temperature was expected to differ; species with a lower precision of thermoregulation (*P. muralis*) was expected to have higher metabolic rate at lower temperatures as a consequence of compensation for attaining lower T_p in spring, when the thermal environment is more restrictive (Osojnik et al., 2013). Obtained results were linked to species ecology to provide a better understanding of potential interspecific interaction.

2. Material and methods

2.1. Field study sites and lizard collection

Lizards were collected in the area of species' sympatric occurrence (Kočevje, SE Slovenia: lat. 45°38'N, long. 14°51'E, datum = WGS8410). To discard effects of ontogeny or egg production only adult male lizards were collected. Collecting sites were similar in habitat structure and altitude (*P. muralis* site: 723 m a.s.l., and *I. horvathi* site: 986 m a.s.l.), and were located at a distance of 9.4 km one from another. Previously seasonal differences in enzymatic activity have been detected in several terrestrial species of lizards (e.g. Garland and Else, 1987; Zari, 1996, 1999), thus to avoid possible effect of season, all collections were conducted in the summer in three sampling campaigns between 26th June and 15th August 2013. Live lizards were quickly transported to the laboratory in the dark in order to reduce stress effects. Before the experiments, lizards were kept in individual housing terraria for three to five days with food (*Tenebrio molitor* larvae) and water provided ad libitum, and under a natural regime of light and temperature maintained by switching infra-red bulbs on for the period of 8 am to 18 pm (see also Osojnik et al., 2013). Food supply was interrupted two days previous to the experiment.

2.2. Collection permit and ethical procedures

All individuals used in the experiments were collected under the special licences 35601-14/2013-5 issued by the Slovenian Environment Agency. All animal handling procedures were done following the EU and Slovenian Government-established norms and procedures.

2.3. MR and ETS activity

Metabolic rate (MR, as a measure of oxygen consumption) was measured consecutively at two temperature regimes (20 °C and 28 °C). The experiment was performed in randomised trials from 08.30 to 16.30 h Central European Time (CET), the period of daily activity of both species observed in the field (Brana, 1991; De Luca, 1992). Lizards were put in 200 mL individual glass flasks with an impermeable lid and MR was measured with a fibre-optic oxygen meter (PreSens, Regensburg, Germany), each lizard individually in one flask. In the first part of the experiment, the flasks with lizards were first kept for 20 min at 20 °C to acclimatize. Then we measured the oxygen concentration at hourly intervals for four consecutive times, each time in the same order starting from flask number one, continuing until the last number. Each measuring session was done in less than three minutes to minimise disturbance of lizards during the experiment. After this, flasks were opened, with lizards remained kept inside, and moved to 28 °C where lizards were left for 20 min to heat-up. After that we closed the flasks and left it for an additional 20 min after starting the first measurement. Again we measured the oxygen concentration at hourly intervals for four consecutive times. In each series we used 18–28 flasks, depending on the number of individuals used per session. In all cases two of the flasks were left empty and represented controls. Oxygen consumption by each lizard was determined as the difference between oxygen consumption in the flask with the animal and mean oxygen consumption in control flasks. Oxygen consumption in each flask was calculated as the

difference between the oxygen concentration during incubation, multiplied by the volume of the flask which was subtracted by the animal volume, and divided by the incubation time. We used values calculated after each hourly interval and after the two last hours of the experiments when the rate has stabilized more; the latter is denoted as MR-last-2-hr. The animals were weighed to the nearest ± 0.1 mg (Sartorius, Goettingen, Germany) and measured for snout to vent length (SVL) to the nearest 0.1 mm using a digital calliper. We then removed the last third of the tail that was used for non-lethal ETS measurements. Tail tip removal has already been demonstrated to produce minimal disturbance in the subsequent lizard behaviour in the field (García-Muñoz et al., 2011). All lizards were released back at capture sites after experiments.

In a previous study it was shown that ETS activity of a whole animal can be estimated on the basis of the measured ETS activity of a part of an animal body which can be regenerated afterwards (Simčič et al., 2012). ETS activity was measured using the method originally proposed by Packard (1971) and improved by G.-Tóth (1999). A 10 mm long segment of the tail, taken from the posterior third of the whole tail was homogenized in liquid nitrogen using a mortar. A weighed amount (100–400 mg wet mass) was sonicated in 4 mL of ice-cold homogenization buffer (0.1 M sodium phosphate buffer pH = 8.4; 75 μ M MgSO₄; 0.15% (w/v) polyvinyl pyrrolidone; 0.2% (v/v) Triton-X-100) for 20 s (4710; Cole-Parmer) and centrifuged at 8500 \times g for 4 min at 0 °C (Centrifuge Sigma). Three 0.5 mL samples from each homogenate were incubated for 40 min at 20 °C (standard temperature) in 1.5 mL substrate solution (0.1 M sodium phosphate buffer pH = 8.4; 1.7 mM NADH; 0.25 mM NADPH; 0.2% (v/v) Triton-X-100) with 0.5 mL 2.5 mM 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) solution. The reaction was ended by addition of 0.5 mL of stopping solution (formalin: H₃PO₄ concentration = 1:1 v/v). Blanks (1.5 mL substrate solution and 0.5 mL INT solution) were incubated and treated as for the samples, followed by addition of 0.5 mL of homogenate. Formazan production was determined spectrophotometrically from the absorbance of the sample at 490 nm against the control blank within 10 min of stopping the reaction (WTW PhotoLabSpektral). ETS activity was calculated according to (Kenner and Ahmed, 1975). All data on ETS activity at 20 °C (ETS₂₀) were obtained directly from the measurement and for ETS activity values at 28 °C (ETS₂₈) we used a transformation formula $ETS_{28} = ETS_{20} \cdot \exp^{E_a(1/T_{20} - 1/T_{28})/R}$, where E_a is the activation energy (a value of 15 kcal mol⁻¹ was used; Bamstedt, 1980; Packard et al., 1975), T₂₀ and T₂₈ are the absolute temperatures at 20 °C and 28 °C, respectively, and R the gas constant. We also calculated the ratio between ETS activity and MR (using the formula: ETS/MR-last-2-hr) to estimate the fraction of the metabolic potential that was used by the individual at two temperature regimes. Finally, the mean Q₁₀ value, was calculated according to equation (Lampert, 1984): $Q_{10} = (k_2/k_1)^{(10/(T_2 - T_1))}$, where k_1 and k_2 are the reaction rates (using MR-last-2-hr at 20 °C and 28 °C) at temperatures T₁ (20 °C) and T₂ (28 °C), respectively.

2.4. Statistics

Log-transformed values of SVL and weight of both species were compared by means of one-way ANOVA once normality and homoscedasticity were ensured. For data sets of MR and ETS measurements, we used Shapiro–Wilk's test and Levene's test to examine the normality of the data distribution and to assess homoscedasticity. Consequently all values (MR, ETS and ETS/MR-last 2-hr-values) were log-transformed to ensure normality and homoscedasticity (which was checked again) prior to analysis to meet the analysis criteria. To examine the effect of two temperature regimes, species and time on MR and ETS, we used a repeated measures ANOVA design (rm ANOVA) with species and temperature (Temp) as between-subject factor and time interval (Time) of the measurement as a within-subject factor. Next, we performed an analysis of MR-last-2-hr values first without (one-way ANOVA) and second including (two-tailed ANCOVA) average log₁₀weight as a continuous

predictor to account for size differences (Carretero et al., 2005) and species and Temp as between-subject factor. For analysis of ratios, factorial ANOVA with species and Temp as between-subject was used (ETS values were already size corrected). For pairwise post-hoc comparisons we used Duncan's significant difference test. All statistical analyses were performed with Statistica (Statsoft 2013).

3. Results

In total 32 *P. muralis* and 26 *I. horvathi* adult males were collected and used in the experiments. Individuals of both species were similar in size (logSVL: $F_{1, 56} = 2.80$, $P = 0.10$) but not in weight; *P. muralis* males were heavier (mean \pm SE: 4.3 ± 0.1 g) than *I. horvathi* males (mean \pm SE: 3.7 ± 0.1 g; log₁₀W: $F_{1, 55} = 6.03$, $P < 0.05$). Table 1 displays descriptive statistics with sample sizes used in analysis for MR considering species, temperature regimes and measurement time interval.

First we examined the effects of species and temperature regime on MR in time (in three consecutive hourly intervals). MR of both species was higher at higher temperature with no interspecific difference (Table 2, Fig. 1). There were significant differences between consecutive measurements under the same temperature regime, but interaction between temperature and time was also significant (Table 2, Fig. 1).

Results of the second analysis using MR-last-2-hr values re-confirmed a similarity between the species at both temperatures (at 20 °C: $F_{1, 52} = 0.87$, $P = 0.35$ and at 28 °C: $F_{1, 39} = 3.20$, $P = 0.08$), also when we introduced the log₁₀weight (log₁₀W) as a continuous predictor to account for eventual mass-related allometry in MR (at 20 °C: species * log₁₀W, $F_{2, 50} = 0.06$, $P = 0.94$ and at 28 °C: $F_{2, 37} = 2.80$, $P = 0.08$).

LogETS values were significantly higher at 28 °C than at 20 °C in both species (Temp, $F_{1, 45} = 2.91 \cdot 10^{14}$, $P < 0.001$), but were also lower in *P. muralis* than in *I. horvathi* at both temperatures (Temp * Species, $F_{1, 45} = 4.97$, $P < 0.05$, Fig. 2). On the other hand, we did not detect interspecific differences in the ratios (ETS/MR-last-2-hr, Temp * Species, $F_{1, 28} = 0.09$, $P = 0.76$). Nevertheless, the ratios were higher at 20 °C than at 28 °C in both species (Temp, $F_{1, 28} = 4.08$, $P = 0.05$, Fig. 2).

The mean Q₁₀ value at 20 °C and 28 °C also did not differ between species either uncorrected (One-way ANOVA, Species, $F_{1, 35} = 1.62$, $P = 0.21$) or with mass-corrected analysis (One-way ANOVA, Species * log₁₀W, $F_{2, 33} = 0.84$, $P = 0.44$). The mean and standard error of Q₁₀ were 2.986 ± 0.221 for *I. horvathi* and 2.628 ± 0.160 for *P. muralis*.

4. Discussion

Results of our study provide evidence for physiological dissimilarity in metabolic potentials (measured as ETS activity) of two sympatric lizard species, *I. horvathi* and *P. muralis*. The pattern of partial altitudinal segregation of the studied species that has been found across their overlapping distribution (De Luca, 1989; Lapini et al., 1993; Cabela et al., 2007; Žagar et al., 2013) was reflected in their metabolism, namely in the difference of ETS activity. *I. horvathi* had higher ETS activity determined from the tail tip tissue than *P. muralis*. However, contrarily to the initial expectation, interspecific differences were not found in measured metabolic rates (MR). Our results showed that MR was similar between species at the ranges of variation of both species at tested temperature regimes.

Weight was the morphological trait showing some variation between species (our results here and Žagar et al., 2012). Therefore weight was included as the covariate into analysis of MR to account for size differences, but similar results persisted, showing size independent similarity in MR between species. ETS activity values were already size corrected (see formula used in Section 2), thus obtained results reflect intrinsic difference between species in metabolic potentials.

Table 1
Mean metabolic rates of three consecutive hourly measurements and the mean of the last two measurements of *Podarcis muralis* and *Iberolacerta horvathi* males measured under two different temperature regimes (20 °C and 28 °C). MR-1 denotes the first, MR-2 the second and MR-3 the third of the three consecutive hourly measurements of metabolic rate. MR-last-2-hr denotes the mean of the last two consecutive hourly measurements of metabolic rate. n denotes sample size.

Species	Temp. regime	MR-1 (mg O ₂ /g/h ± SE) n	MR-2 (mg O ₂ /g/h ± SE) n	MR-3 (mg O ₂ /g/h ± SE) n	MR-last 2-hr (mg O ₂ /g/h ± SE) n
<i>P. muralis</i>	20 °C	0.23 ± 0.030 31	0.241 ± 0.018 31	0.226 ± 0.016 30	0.229 ± 0.013 30
	28 °C	0.724 ± 0.057 22	0.543 ± 0.034 20	0.531 ± 0.035 17	0.529 ± 0.029 19
<i>I. horvathi</i>	20 °C	0.307 ± 0.048 25	0.291 ± 0.028 25	0.228 ± 0.024 24	0.251 ± 0.020 24
	28 °C	0.769 ± 0.047 25	0.617 ± 0.047 23	0.592 ± 0.034 22	0.603 ± 0.030 22

Metabolism of organisms is particularly in ectotherms highly dependent on body temperatures due to the temperature dependence of involved biochemical process (Gillooly et al., 2001), thus we tested also for the response of MR to temperature change. Results of MR measured at two temperatures (20 °C and 28 °C) showed that MR values were higher at 28 °C than at 20 °C with no interspecific difference found. Our results confirm that biochemical reactions rather than ecophysiological traits govern the temperature dependence of metabolism (Randriamahazo and Aime, 1998; Angilletta, 2001; Gillooly et al., 2001).

While we found that both species possess different metabolic potentials (ETS activity) that could be exploited for energy production, we were interested in how the potential is exploited following the change in temperature. Significantly, the effect of temperature (all measures were recorded in the same season) was positive and parallel in both species. ETS activity was calculated higher for 28 °C than for 20 °C for both. Nevertheless, the interspecific difference persisted at both temperature regimes; *I. horvathi* ETS activity values were higher than *P. muralis* at 20 °C and at 28 °C. Detected higher ETS activity in *I. horvathi* than *P. muralis* at the same temperature means that former species maintains higher level of enzymes which can assure adequate production of energy when demands are increased.

By calculating the ratio between the actual metabolic rate and the ETS activity we detected that both species, with no interspecific difference, use less of the potential at 20 °C than at 28 °C. The implications of calculated ratios from other studies show that they varied with temperature (since ETS activity is a direct enzymatic process) and depended upon enzyme concentration (Bamstedt, 1980) and their characteristics (Packard, 1971). Previous studies on aquatic invertebrates also revealed that in species with high ETS/MR ratio, the capacity to increase metabolism is maintained (Fanslow et al., 2001; Simčič et al., 2005). Despite the fact that we have not detected any direct interspecific difference in ETS/MR ratio, results of higher ETS activity in *I. horvathi* than in *P. muralis*, provide evidence that *I. horvathi* has a higher capacity of metabolism.

There are evidences that thermoregulatory behaviour and thermal physiology are co-adapted in lizards (Angilletta et al., 2002), as well as both are reciprocally in connection with metabolism and performance (Angilletta, 2001). Higher thermoregulatory precision of *I. horvathi* (Osojnik et al., 2013), integrated with higher capacity of metabolism

(ETS activity) as found here, could mean that *I. horvathi* can actually better exploit its greater metabolic potential due to a better ability of obtaining higher body temperatures than *P. muralis* when environmental temperatures are more restrictive, i.e. in spring. We suspect that these attributes of *I. horvathi* could pose competitive advantage against more generalist but thermoregulatory less efficient *P. muralis*, at least in colder environments, i.e. at high altitudes.

Moreover, metabolism is highly positively dependent on temperature (Bennett, 1982), reflecting in the increase of energy requirements (Angilletta, 2001) as well as in overall metabolic production of proteins. This in turn affects food intake (feeding time, feeding mode, e.g. Nagy et al., 1984; Angilletta, 2001) and other life-important activities such as reproduction, territorial defence, anti-predatory behaviour (Bennett, 1982; Patterson and Davies, 1984; Martín and López, 2001). Results from this study suggests that *I. horvathi* should in syntopic sites be able to outcompete *P. muralis*, since metabolic potential combined with more precise thermoregulation should be reflected in higher metabolic rates that are in turn reflected in other, above mentioned, life-important activities. Nevertheless, *I. horvathi* is more abundant than *P. muralis* only at higher altitudes (De Luca, 1989; Lapini et al., 1993; Cabela et al., 2007; Žagar et al., 2012), thus in the light of species interactions, the question remains why this physiological advantage of *I. horvathi* seems to only provide an advantage over *P. muralis* at higher altitudes and does not reflect also at lower altitudes. These results corroborate to suggest that interactions likely still persist in syntopic populations with *I. horvathi* as competitive subordinate species, when higher

Table 2
Results of the rm ANCOVA analysis of metabolic rates of *Podarcis muralis* (n = 17) and *Iberolacerta horvathi* (n = 19) males using temperature (Temp) and species as between-subject factors, and time interval (Time) as a within-subject factor.

Effects	F	MS	df	P
Temp	331.50	7.36	34	<0.001
Species	2.11	0.10	34	0.16
Temp * Species	0.20	<0.01	34	0.66
Time	7.34	0.23	68	<0.01
Temp * Time	4.98	0.14	68	<0.01
Time * Species	0.39	0.01	68	0.68
Temp * Time * Species	0.26	0.01	68	0.77

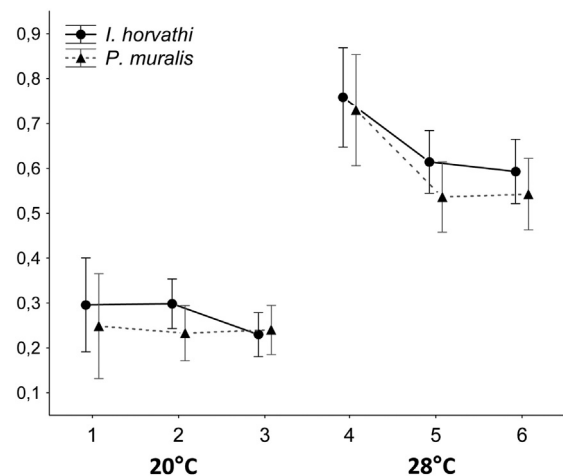


Fig. 1. Hourly metabolic rates (MR) of *Podarcis muralis* and *Iberolacerta horvathi* males measured at 20 °C and 28 °C. Metabolic rates were measured each hour as a measure of oxygen consumption with a fibre-optic oxygen meter. Lizards were kept at two temperature regimes: first at 20 °C (1, 2 and 3 on the X axis) and next at 28 °C (4, 5 and 6 on the X axis). Error bars represent 95% confidence intervals. Sample sizes used are given in Table 1.

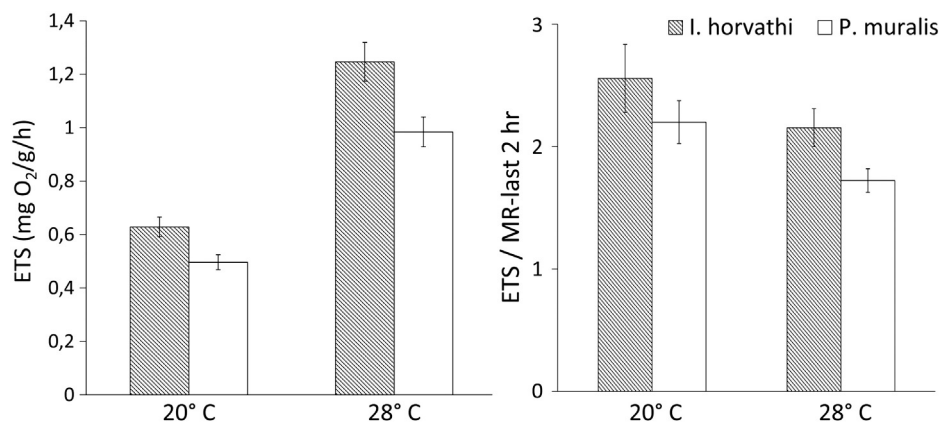


Fig. 2. The metabolic potential (left plot) and the use of metabolic potential (right plot) of *Podarcis muralis* and *Iberolacerta horvathi* males determined for 20 °C and 28 °C. We obtained the values of electron transport system (ETS) activity (metabolic potential) under two realistic temperature regimes; at 20 °C which is a less favourable temperature for lizard activity and at 28 °C, the temperature close to their T_p for *Podarcis muralis* ($n = 26$) and *Iberolacerta horvathi* ($n = 21$) males, represented on figure A. On figure B are ratios calculated from electron transport system (ETS) activity divided by the mean metabolic rate of the last two hours of the experiment (MR-last-2-hr) for *Podarcis muralis* ($n = 17$) and *Iberolacerta horvathi* ($n = 17$). Bars represent the mean and whiskers standard error values. Measurements were done using tissue from lizards' tail tips.

environmental temperatures do not promote *I. horvathi* any metabolic advantage.

We found out yet another specialisation of *I. horvathi* that suggests its better adaptation to colder environments which to a certain level explains its distributional pattern with higher abundances at higher altitudes. On the opposite, different physiology of *P. muralis* explains its higher abundances at lower altitudes and is probably not likely to try to occupy higher altitudes. Jointly, dissimilarities in metabolic potential between studied sympatric species seem to contribute to understanding their altitudinal segregation pattern. However, we emphasize that in studies of species interactions, metabolism cannot be viewed solely and other factors (independent or connected to metabolism) will certainly play a role and provide additional explanation of the distribution pattern of both species.

In conclusion, we have shown that metabolic studies can be of prominent importance for understanding species interactions and to some extent for explaining altitudinal patterns, but need to be integrated with other results of species ecology, physiology and behaviour. Further work needs to focus on ascertaining the significance of different physiological traits in the field, especially in energy economics, which can at last reflect in different reproduction outputs which would give further explanation of the output of studied species interaction. Finally, the competitive advantage due to differences in physiological traits which suggest for a spatially limited specialisation to certain climatic conditions in the environment, i.e. to higher altitudes, raises the question of threats under future climate changes for this specialized species. The future work should be in the direction of hypothesizing what could be the effects of global warming (Sinervo et al., 2010, Huey et al., 2012) on such ectothermic reptile communities.

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References

- Angilletta Jr., M.J., 2001. Thermal and physiological constraints on energy assimilation in a widespread lizard (*Sceloporus undulatus*). *Ecology* 82, 3044–3056.
- Angilletta Jr., M.J., Niewiarowski, P.H., Navas, C.A., 2002. The evolution of thermal physiology in ectotherms. *J. Therm. Biol.* 27, 249–268.
- Bamstedt, U., 1980. ETS activity as an estimator of respiratory rate of zooplankton populations. The significance of variations in environmental factors. *J. Exp. Mar. Biol. Ecol.* 42, 267–283.
- Begon, M., Townsend, C.R., Harper, J.L., 2006. *Ecology: From Individuals to Ecosystems*, 4th edition. Blackwell Publishing, (738 pp.).
- Bennett, A.F., 1982. The energetics of reptilian activity. In: Gans, C., Pough, H.F. (Eds.), *Biology of the Reptilia*. vol. 13. Academic Press, London, pp. 155–199.
- Bennett, A.F., Dawson, W.R., 1976. Physiology A. In: Gans, C., Dawson, W.R. (Eds.), *Biology of the Reptilia*. vol. 5. Academic Press, New York.
- Bennett, A.F., et al., 1984. Physiological correlates of natural activity and locomotor capacity in two species of lacertid lizards. *J. Comp. Physiol. B* 154, 113–118.
- Brana, F., 1991. Summer activity patterns and thermoregulation in the wall lizard, *Podarcis muralis*. *Herpetol. J.* 1, 544–549.
- Brown, R.P., Perez-Mellado, V., Diego-Rasilla, J., Garcia, J., Naranjo, A., Speakman, J., 1992. Individual and population energetics of a lizard on a Mediterranean islet. *Oecologia* 91, 500–504.
- Cabela, A., Grillitsch, H., Tiedemann, F., 2007. Habitatpräferenzen von *Podarcis muralis* (Laurenti, 1768) und *Iberolacerta horvathi* (Mehely, 1904) bei gemeinsamem Vorkommen. *Herpetozoa* 19, 149–160.
- Carretero, M.A., Roig, J.M., Llorente, G.A., 2005. Variation in preferred body temperature in an oviparous population of *Lacerta (Zootoca) vivipara*. *Herpetol. J.* 15, 51–55.
- De Luca, N., 1989. Taxonomic and biogeographic characteristics of Horvath's rock lizard (*Lacerta horvathi* Méhely, 1904, Lacertidae, Reptilia) in Yugoslavia. *Scopelia* 18, 1–48.
- De Luca, N., 1992. Notes on biology and ecology of the Horvath's rock lizard (*Lacerta horvathi* Méhely, 1904, Reptilia: Lacertidae). *Proc. Sixth Ord. Gen. Meet. S. E. H., Budapest 1991*, pp. 129–135.
- Dorcas, M.E., Hopkins, W.A., Roe, J.H., 2004. Effects of body mass and temperature on standard metabolic rate in the eastern diamondback rattlesnake (*Crotalus adamanteus*). *Copeia* 2004, 145–151.
- Fanslow, D.L., Nalepa, T.F., Johengen, T.H., 2001. Seasonal changes in the respiratory electron transport system (ETS) and respiration rate of the zebra mussel, *Dreissena polymorpha* in Saginaw Bay, Lake Huron. *Hydrobiologia* 448, 61–70.
- G.-Tóth, L., 1999. Aktivität des Elektronentransportsystems. In: von Tümping, V., Friedrich, G. (Eds.), *Biologische Gewässeruntersuchung. Methoden der Biologischen Wasseruntersuchung 2*. Gustav Fischer Verl., Jena, Stuttgart, pp. 465–473.
- García-Muñoz, E., Ceacero, F., Pedrajas, L., Kaliontzopoulou, A., Carretero, M.A., 2011. Tail tip removal for tissue sampling has no short-term effects on microhabitat selection by *Podarcis bocagei*, but induced autotomy does. *Acta Herpetol.* 6, 223–227.
- Garland Jr., T., Else, P.L., 1987. Seasonal, sexual, and individual variation in endurance and activity metabolism in lizards. *Am. J. Physiol.* 252, R439–R449.
- Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M., Charnov, E.L., 2001. Effects of size and temperature on metabolic rate. *Science* 293, 2248–2251.
- Hare, K.M., Pledger, S., Thompson, M.B., Miller, J.H., Daugherty, C.H., 2006. Daily patterns of metabolic rate among New Zealand lizards (Reptilia: Lacertilia: Diplodactylidae and Scincidae). *Physiol. Biochem. Zool.* 79, 745–753.

- Huey, R.B., 1982. Temperature, physiology, and the ecology of reptiles. In: Gans, C., Pough, F.H. (Eds.), *Biology of the Reptilia* Vol. 12, Physiology C. Academic Press, London, pp. 25–91.
- Huey, R.B., Kearney, M., Krokenberger, A., Holtum, J.A.M., Jess, M., Williams, S.E., 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Philos. Trans. R. Soc. Lond. B* 367, 1165–1679.
- Kenner, R., Ahmed, S., 1975. Measurements of electron transport activities in marine phytoplankton. *Mar. Biol.* 33, 119–127.
- Lampert, W., 1984. The measurement of respiration. In: Downing, J.A., Rigler, F.H. (Eds.), *A Manual on Methods for the Assessment of Secondary Productivity in Fresh Water*, IPB Handbook 17, 2nd ed. Blackwell Scientific Publications, Oxford, pp. 413–468.
- Lannig, G., Eckerle, L., Serendero, I., Sartoris, F.-J., Fischer, T., Knust, R., 2003. Temperature adaptation in eurythermal cod (*Gadus morhua*): a comparison of mitochondrial enzyme capacities in boreal and Arctic populations. *Mar. Biol.* 142, 589–599.
- Lapini, L., Richard, R., Dall'Asta, A., 1993. Distribution and ecology of *Lacerta horvathi* Mehely, 1904 (Reptilia, Lacertidae) in North-Eastern Italy. *Atti Museo Civ. Stor. Nat. Trieste* 14, 213–230.
- López, P., Martín, J., 2002. Effects of female presence on intrasexual aggression in male lizards, *Podarcis hispanicus*. *Aggress. Behav.* 28, 491–496.
- Martín, J., López, P., 2001. Hindlimb asymmetry reduces escape performance in the lizard *Psammotromus algirus*. *Physiol. Biochem. Zool.* 74, 619–624.
- McHugh, P., Budy, P., 2005. An experimental evaluation of competitive and thermal effects on brown trout (*Salmo trutta*) and Bonneville cutthroat trout (*Oncorhynchus clarkia utah*) performance along an altitudinal gradient. *Can. J. Fish. Aquat. Sci.* 62, 2784–2795.
- Muskó, I.B., G.-Tóth, L., Szábo, E., 1995. Respiration and respiratory electron transport system (ETS) activity of two amphipods: *Corophium curvispinum* G. O. Sars and *Gammarus fossarum* Koch. *Pol. Arch. Hydrobiol.* 42, 547–558.
- Nagy, K.A., Huey, R.B., Bennett, A.F., 1984. Field energetics and foraging mode of Kalahari lacertid lizards. *Ecology* 65, 588–596.
- Olsson, M., 1994. Rival recognition affects male contest behavior in sand lizards (*Lacerta agilis*). *Behav. Ecol. Sociobiol.* 35, 249–253.
- Osojnik, N., Žagar, A., Carretero, M.A., García-Muñoz, E., Vrezec, A., 2013. Ecophysiological dissimilarities of two sympatric lizards. *Herpetologica* 69, 445–454.
- Packard, T., 1971. The measurement of respiratory electron-transport activity in marine phytoplankton. *J. Mar. Res.* 29, 235–244.
- Packard, T.T., Devol, A.H., King, F.D., 1975. The effect of temperature on the respiratory electron transport system in marine plankton. *Deep Sea Res.* 22, 237–249.
- Pasch, B., Bloker, B.M., Phelps, S.M., 2013. Interspecific dominance via vocal interactions mediates altitudinal zonation in neotropical singing mice. *Am. Nat.* 182, E161–E173.
- Patterson, J., Davies, P., 1984. The influence of temperature, sexual condition, and season on the metabolic rate of the lizard *Psammotromus hispanicus*. *J. Comp. Physiol. B* 154, 311–316.
- Patterson, J., Davies, P., 1989. A possible effect of climate on resting metabolic rate in lizards. *Copeia* 1989, 719–723.
- Perko, D., Orožen Adamič, M., 1998. Slovenia: Landscape and People. Mladinska knjiga, Ljubljana.
- Randriamahazo, R., Aime, H.J., 1998. Activity temperatures in *Oplurus cyclurus*, *Oplurus cuvieri* and *Zonosaurus laticaudatus* and resting metabolic rates in the latter two species. *Amphibia–Reptilia* 19, 215–220.
- Richard, J., Lapini, L., 1993. Trophic niche overlap in syntopic populations of *Lacerta horvathi* and *Podarcis muralis* (Reptilia, Lacertidae). *Atti Museo Civ. Stor. Nat. Trieste* 45, 151–157.
- Seebacher, F., Guderley, H., Elsey, R.M., Trosclair, P.L., 2003. Seasonal acclimatisation of muscle metabolic enzymes in a reptile (*Alligator mississippiensis*). *J. Exp. Biol.* 206, 1193–1200.
- Simčič, T., Brancelj, A., 1997. Electron transport system (ETS) activity and respiration rate in five *Daphnia* species at different temperatures. *Hydrobiologia* 360, 117–125.
- Simčič, T., Lukančič, S., Brancelj, A., 2005. Comparative study of electron transport system activity and oxygen consumption of amphipods from caves and surface habitats. *Freshw. Biol.* 50, 494–501.
- Simčič, T., Pajk, F., Vrezec, A., Brancelj, A., 2012. Size scaling of whole-body metabolic activity in the noble crayfish (*Astacus astacus*) estimated from measurements on a single leg. *Freshw. Biol.* 57, 39–48.
- Simon, L.M., Robin, E.D., 1971. Relationship of cytochrome oxidase activity to vertebrate total and organ oxygen consumption. *Int. J. Biochem.* 2, 569–573.
- Sinervo, B., Méndez-de-la-Cruz, F.R., Miles, D.B., Heulin, B., Bastiaans, E., Villagrán-Santa Cruz, M., Lara-Reendiz, R., Martínez-Méndez, N., Calderón-Espinosa, M.L., Meza-Lázaro, R.N., Gadsden, H., Avila, L.J., Morando, M., de la Riva, I., Sepúlveda, P.V., Rocha, C.F.D., Ibarguengoytia, N.R., Aguilar Puntriano, C., Massot, M., Lepetz, V., Oksanen, T.A., Chapple, D.G., Bauer, A.M., Branch, W.R., Clobert, J., Sites Jr., J.W., 2010. Erosion of lizard diversity by climate change and altered thermal niches. *Science* 328, 894–899.
- Steffen, J.E., Appel, A.G., 2012. The effect of temperature on standard metabolic rate of Brown Anoles. *Amphibia–Reptilia* 33, 297–302.
- Thompson, G.G., Withers, P.C., 1994. Standard metabolic rates of two small Australian varanid lizards (*Varanus caudolineatus* and *V. acanthurus*). *Herpetologica* 50, 494–502.
- Vrezec, A., Tome, D., 2004. Altitudinal segregation between Ural Owl *Strix uralensis* and Tawny Owl *S. aluco*: evidence of competitive exclusion in raptorial birds. *Bird Study* 51, 264–269.
- Žagar, A., Osojnik, N., Carretero, M.A., Vrezec, A., 2012. Quantifying the intersexual and interspecific morphometric variation in two resembling sympatric lacertids: *Iberolacerta horvathi* and *Podarcis muralis*. *Acta Herpetol.* 7, 29–39.
- Žagar, A., Kos, I., Vrezec, A., 2013. Habitat segregation patterns of reptiles in Northern Dinaric Mountains (Slovenia). *Amphibia–Reptilia* 34, 263–268.
- Zari, T.A., 1996. Seasonal metabolic compensation in the fringe-toed lizard, *Acanthodactylus boskianus* (Reptilia: Lacertidae). *J. Therm. Biol.* 21, 145–150.
- Zari, T., 1999. Seasonal acclimatization in metabolic rate of the fan-fingered gecko, *Ptyodactylus hasselquistii* (Reptilia: Gekkonidae). *J. Therm. Biol.* 24, 137–142.