PD124736 – Final report

My project's aim was to gather information on various aspects of how temperature affects female reproduction in oviparous Lacertid lizards, through experimental tests and in part, using an integrative climate model. However, regarding the latter method, I was facing serious difficulties in accomplishing this part of the project, that I am going to elaborate below. I decided to section the results of this research based on topics rather than on timing because in some cases, publications gather results partially from other work periods.

Thermal preference and temperature-dependent physiological performance in alternative reproductive strategies

During the first work period I focused on patterns of temperature preference in different reproductive states and temperature dependent locomotor performance of a single-clutch and a multiple-clutch species, *Lacerta viridis* and *Podarcis tauricus* before and after oviposition. The collected data from the reproductive season in 2018 and results gathered partially in 2020 were presented in national and international conferences, all stating the source of funding from National Research, Development and Innovation Office:

Bajer Katalin, Molnár Orsolya, Mészáros Boglárka, Jordán Lilla, Török János: Gyíkok szaporodási befektetése és termális környezet, VI. Herpetológiai Előadóülés, Budapest, 2019. február 26. pp. 4, 2019

Jordán Lilla, Mészáros Boglárka, Molnár Orsolya, Bajer Katalin: A környezeti hőmérséklet és a szaporodási befektetés kapcsolata gyíkoknál, XX. Eötvös Konferencia, Budapest, 2019. április 12-13. pp. 90-91., 2019

Jordán Lilla, Mészáros Boglárka, Molnár Orsolya, Bajer Katalin: A környezeti hőmérséklet és a szaporodási befektetés kapcsolata gyíkoknál, XXIV. Bolyai Konferencia, Budapest, 2019. április 6-7., 2019

Lilla Jordán, Orsolya Molnár, Boglárka Mészáros, János Török, Katalin Bajer: Females' reproductive state influences temperature-dependent locomotor performance differently in two Lacertid lizards (Lacerta viridis, Podarcis taurica), Reproductive strategies from genes to societies – Frontiers in animal and plant reproduction research, Debrecen, november 7-9., 2019

My findings from the first and partially from the last work period (relative reproductive effort described as RCM) formulated a manuscript with the co-autorship of Lilla Jordán, an MSc student who participated in the research and also presented its results, Boglárka Mészáros, a PhD student who since had defended her thesis and currently works at Balaton Limnological Research Institute Tihany, Orsolya Molnár and János Török. The manuscript is currently under review at Frontiers of Ecology and Evolution, so I present it unchanged:

Are female reproductive strategies of oviparous lizards plastic enough to escape climate warming?

Lilla Jordán, Boglárka Mészáros, Orsolya Molnár, János Török, Katalin Bajer

Abstract

Gravidity in ectotherms involves various costs to survival and future reproduction, however, how changing temperature affects these costs is yet to be uncovered. Climate models predict a dramatic increase of global atmospheric temperature by the end of this century, while rapid heat events impose substantial thermal stress to oviparous lizards. Phenotypically plastic reproductive traits can provide a short-term response to increasing environmental temperature and thus compensate for fitness costs of climate warming. We measured the reproductive output and tested the effect of gravidity on the temperature-dependent physiological performance of two sympatric oviparous Lacertid lizards, Lacerta viridis and Podarcis tauricus. These two species show distinct reproductive strategies in our study populations, notably, laying a single clutch vs. multiple clutches per year, respectively. Locomotor performance of gravid, post-lay and 2. post-lay (in P.tauricus) females was measured on 5 different temperatures throughout their species-specific activity temperature range. Our results show that both strategies had similar reproductive output per reproductive season in terms of relative clutch mass, however, this resulted in more eggs for the single-clutch strategy. Gravid females in both species kept their body temperature at suboptimal values compared to post-lay state, that in turn decreased their physiological performance. Multiple-clutch females showed close to peak performance in a wider temperature range after first oviposition, however, their performance was again limited to a narrow range following second oviposition. Laying multiple clutches can spread the costs of investment in one reproductive bout and therefore compensate for deleterious effects of egg-bearing. However, this strategy seems to be more sensitive to high temperatures during subsequent gravid periods and further increasing clutch frequency to compensate for high temperatures' limiting effects on offspring survival is unlikely. Considering the negative connection between heat stress on reproductive females' physiological performance raises concern regarding lizard conservation in the face of climate warming.

Introduction

Anthropogenic climate change imposes diverse detrimental effects on species across a wide range of taxa around the globe (e.g. Seddon et al. 2016, Arias et al. 2021) causing distributional and phenological shifts, often leading to population- and species-level extinctions (McLaughlin et al. 2002, Poloczanska et al. 2013, Scranton 2017). Climate models predict that global atmospheric temperature will increase at least by 1.5°C by 2100. (IPCC 2018). However, empirical studies suggest that even short-term temperature changes, "extreme heat events" with temperatures above the animals' critical thermal maxima can cause substantial mortality and reduce population fitness below replacement rate, resulting in population declines (McKechnie & Wolf 2009, Huey et al. 2012).

Hence, it is important to uncover the effects of short term thermal shifts on populations in order to assess their vulnerability to rapid thermal changes such as extreme heat events (Sinervo et al. 2010, Hoffman & Sgró 2018, Garcia-Porta 2019).

A population's responses to thermal stress occur on various different time scales. While genetic adaptation is a key factor in long term survival (Angilletta et al. 2002, Valladares 2014), it is slow and constrained by genetic covariation (Chevin et al. 2010). Therefore, a population's capacity to survive extreme heat events by short term phenotypic changes (i.e. phenotypic plasticity) within an animal's lifespan is crucial to provide sufficient time for evolutionary rescue (Gienapp 2008, Morley et al. 2019, Visser 2008). Without the buffering effect of temperature-sensitive plastic traits, populations can drift towards extinction when faced with suboptimal environmental conditions (Sinervo et al. 2010, Dupoué et al. 2017). In a thermally heterogenous environment a population's short-term survival may depend on diverse strategies the individuals use to cope with rapid thermal events (Morley et al. 2019, Pottier et al. 2022), including changes in geographic range (Valladares et al 2014), seasonal timing (Van Asch et al. 2007), habitat use (Ashton et al. 2009) and reproduction (Pankhurst 2011).

Ectotherms are especially sensitive to temperature changes, as ambient temperature directly affects multiple aspects of their physiology, fitness and behaviour (Huey and Berrigan 2001, Clusella-Trullas et al. 2011, Clusella-Trullas & Chown 2014), therefore, ectothermic organisms typically limit their activity to a specific temperature range (Pough & Gans 1982) through physiological changes or behavioural thermoregulation (Angilletta 2009). The thermal requirements and constraints of ectothermic species can be described by a number of well-established and widely accepted indices. Thermal tolerance ranges can be characterized by the critical thermal minima and maxima (CTmin and CTmax), the lowest and highest thermal limits of activity (Spellerberg 1972). Optimal body temperature is most closely related to preferred body temperature (Gilbert & Miles 2017, Clusella-Trullas & Chown 2014), which is the selected body temperature (Tpref) in an environment without any ecological constraint. Thermal parameters vary greatly spatially and temporally and in both inter- and intraspecific context (Gunderson & Stillman 2015, Gilbert & Miles 2017, Telemeco et al. 2017). Temperature-dependent behaviour in the activity range can be described by thermal performance curves (TPCs, Angilletta 2006). TPCs basically depict how a certain aspect of performance changes with body temperature (Huey & Stevenson 1979, Vasseur et al. 2014), most frequently the temperature dependence of locomotor activity. Locomotor performance reflects survival probability (Feder & Lauder 1986), through its effects on e. g. foraging (Diaz 1994), predator avoidance (Ekner-Grzyb et al. 2013) or thermoregulation (Gilbert & Miles 2017). Better performance signals better quality via its relations to food availability (Gilbert & Miles 2016), muscle mass (Vanhooydonck et al. 2006), metabolism (Garland et al. 1984) and immune activity (Zamora-Camacho et al. 2015).

Effects of heat stress on terrestrial ectotherms is more thoroughly studied in adult males or non-reproductive females (Angilletta & Angilletta 2009) and even in early ontogenic stages (De-Lima et al. 2022, Pottier et al. 2022). However, the costs and plasticity of gravid females' reproductive effort in different thermal regimes is poorly understood (but see Charnov & Ernest 2006, Clarke 2012). A population's persistence depends not only on survival of adult individuals but also on recruitment rates (Huey and Berrigan 2001). Heat stress may inflict a substantially negative effect on maternal reproductive effort if females fail to compensate for thermal changes either by physiological or behavioural plasticity (Telemeco et al. 2017), leading to reproductive failure and catastrophic population decline or even virtual extinction of the population (i.e recruitment rate is continuously lower then mortality rate) even with an existing adult population. Females' strategies to cope with stress factors include a wide variety of phenotypical traits, such as changes in life-history strategy through differential reproductive allocation (Massey 2022), number or size of clutches, offspring size, timing of reproduction (Ma et al. 2018) or nesting behaviour (Levy et al 2015). Relative clutch mass (RCM) represents the ratio of clutch mass with respect to female body mass (Gerald and Miskell 2007), commonly used as an indicator of female reproductive effort in reptiles (Seigel & Fitch 1984). In lizards, laying multiple clutches in a reproductive season seems to be widespread (Meiri et al. 2020). Moreover, clutch size shows the most variation of all life history traits used to estimate reproductive effort in squamates, from clutches consisting of a fixed, small number of eggs (Vitt and Caldwell 2013), up to 90 eggs per clutch in some chameleon species (Meiri 2018). If patterns of reproductive allocation show high variability within and across taxa, they might provide an opportunity to plastic physiological responses to increase fitness in spite of changing thermal environments, especially because gravid females are less mobile and their foraging capability is also compromised by the physiological effects of gravidity (Andrews and Rand 1974, Ashton 2005, Johnson 2010, Schwarzkopf 2010). Consequently, they are less likely to change their habitat use and are more forced to cope with local environmental temperature changes.

In this study, we tested the temperature dependent effects of gravidity on the physiological performance of lizards with alternative reproductive strategies. We studied two sympatric, temperate species (*Podarcis tauricus* and *Lacerta viridis*), each population showing distinct reproductive patterns in terms of clutch laying frequency (i.e., single clutch per year and multiple clutches per year).

To estimate temperature dependent cost of reproduction, we conducted locomotor performance tests before and after egg laying, each time throughout 5 temperatures between CT_{min} and CT_{max}, including Tpref. We assumed that if laying eggs in separate batches over a period of time versus laying all eggs at once have different costs on reproduction, then the two strategies should result in distinct physiological performances that are affected differently by temperature.

To describe the two distinct reproductive strategies' respective costs, we formulated the following questions: does peak locomotor performance of *L. viridis* or *P. tauricus* change with reproductive state? Do thermal preference and tolerance of *L. viridis* and *P. tauricus* change with reproductive state? Do the differences in reproductive strategy between *L. viridis* and *P. tauricus* appear as differences in reproductive output (RCM)? Does reproductive strategy affect the patterns of changes in temperature dependent locomotor performance before and after egg laying?

Material and Methods

Study species

Our study species are both diurnal, actively foraging heliothermic Lacertid lizards that maintain their body temperature by active behavioural thermoregulation. (Rismiller & Heldmaier 1988, Castilla et al. 1999, Václav 2007).

The Balkan wall lizard (*P. tauricus* Pallas 1814) is a small (adult snout-vent length [SVL]: 6-8 cm) lizard that occupies open, dry habitats with low elevation. It can be found throughout the Balkan Peninsula from Greece to Crimea, with the northernmost edge of its geographic distribution being Hungary. According to our observations during the last decade, females in our study population usually lay 2, occasionally 3 clutches (2-6 eggs) per reproductive season (Bajer, personal observation).

The European green lizard (*L. viridis* Laurenti 1768) is a medium sized (adult SVL: 8-20 cm) lizard that is widely distributed throughout Europe and occupies diverse habitats, from open, grassy areas to forest edges and even forests with less dense foliage. In our study population females lay a single clutch of 5-20 eggs per reproductive season (Bajer, personal observation).

Capturing and laboratory conditions

We collected females of *P. tauricus* (N=36) and *L. viridis* (N=27) from two populations near Tápiószentmárton, Hungary (47°20'25" N, 19°47'11" E, 05/2014) in the spring of 2018 and 2019. Females were captured by noose and checked for mating scars. Gravid females were taken to the laboratory of Eötvös Loránd University in Budapest, Hungary. Lizards were housed individually in plastic terraria (80 x 40 x 30 cm; length x width x height, respectively) with 20 cm deep soil substrate and a shelter. Food (*Tenebrio molitor* larvae) and water was provided *ad libitum* throughout the experiment. Each terraria was illuminated with Repti Glo 2.0 Full Spectrum Terrarium Lamps (Exo Terra, Rolf C. Hagen Inc., Holm, Germany). We used heat cables (50W, Exo Terra, Rolf C. Hagen Inc., Holm, Germany) to maintain adequate ambient temperature (28 ± 2 °C day time and 21 ± 2 °C night time). Photoperiod was held natural (14L:10D).

Experimental layout

After capture females had an acclimatization period of 10 days and then were assigned for the performance tests. Body size (SVL; snout-vent length) and gravid body weight was measured to the nearest 0.001g at the end of the acclimatization period.

We measured the locomotor performance of females before and after oviposition, at 5 different temperatures (for experimental design, see below). In order to minimise stress, we introduced a resting day between each performance test, so a full set of tests took 9 days. After the first set of tests (i.e. gravid state) females were resting until oviposition. We checked their substrate for eggs twice a day, we then measured the eggs and the females to the nearest 0.001 g with a digital scale. Eggs were placed in an incubator (GQF HOVA BATOR 1602N, GQF, UK) at 27-29°C until hatching. Hatchlings were released at the site of their respective parents' capture.

After oviposition, females rested for a day and then the second set of locomotor performance tests were carried out. Following the second set of performance tests, we shortly released *L. viridis* females. We then continued monitoring the oviposition twice a day for *P. tauricus*. For those females that laid a second clutch, we measured female body weight, then placed the eggs into an incubator and hatched them as described above. Performance tests were repeated for *P. tauricus* females after their second clutch following the protocol described below. Females had a week for recovery with *ad libitum* food and water and then released at the site of capture.

We characterised reproductive output with clutch size (CS; number of eggs per clutch), clutch mass (CM; total mass of eggs) and RCM (relative clutch mass; clutch mass divided by the female's body mass after oviposition).

Locomotor performance trial

We used thermal performance curves (TPCs) to describe individual temperature dependent physiological performance. First, we measured the species' CT_{min} and CT_{max} by cooling down or heating up 6-6 gravid females of each species (using spot lamps for heating and icepacks for cooling) gradually until the loss of righting reflex, which determined the thermal limits of performance (Voituron 2002). These individuals were excluded from further experiments. The rest of the females were placed separately into a rectangular track where we provided a thermal gradient (-4°C – +40°C) using icepacks and heating lamps on the opposite ends of the track. Lizards were able to thermoregulate freely for a 60 min period, during which we measured body temperatures every 2 min on the back of each individual with a laser thermometer (Raytek Rayngger ST, Raytek GmbH, Berlin, Germany). Measurements were made by the same person who the lizards could not see during the experiment. T_{pref} was defined as the average of the 30 body temperature measurements. We then defined 4 more temperatures on the thermal activity range evenly (2 between CT_{min} and T_{pref} and 2 between T_{pref} and CT_{max}). During a 9-day set of tests, performance of each individual was measured at all 5 temperatures (by definition, performance at CT_{min} and CT_{max} is zero). After reaching the desired body temperatures by gradual heating or cooling, lizards were run in a circular arena until the loss of righting reflex, signalling the limit of their physiological performance. Running distance was registered in centimetres. Temperature values defining the TPCs were 10C, 18C, 24C, 30.2C, 34C, 38C, 41.5 for gravid, 11C, 17C, 22C, 27C, 32C 37C, 42C after first clutch and 11.3C, 18C, 23C, 28C, 33C, 38C, 42.9C after second clutch for *P. tauricus* and 10C, 14C, 20C, 26C, 32C, 36C, 40C for gravid and 9.9C, 16C, 22C, 28C, 34.5C, 38C, 41C after single clutch for *L. viridis*.

Thermal performance curves and performance variables

TPCs were acquired by plotting performances (distance run in cm) against temperatures (°C) for each individual and fitting a curve with the Kumaraswamy function designed by Cordeiro & de Castro (2011, for a schematic depiction of a TPC see Fig.1). After the experiments we had 2 TPCs for *L. viridis* and *P. tauricus* females that laid a single clutch (describing gravid and post-lay performance) and 3 TPCs for *P. tauricus* females that laid 2 clutches (describing gravid, 1. post-lay and 2. post-lay performance).

We defined 3 variables to describe temperature dependent locomotor performance: T_{pref} (preferred body temperature, see above), MaxPerf (performance measured at T_{pref}) and Breadth (i.e. effective performance range, a temperature range in which performance is at least 80% of the maximum).

We used R (R Core Team 2013) for curve fitting and TableCurve 2D (SYSTAT Software Inc.) for calculating variables.

Statistical analyses

Variables of the different reproductive states (CS, CM, RCM, T_{pref}, Breadth, MaxPerf) were compared using paired t-tests and Wilcoxon-tests (depending on normality, tested with q-q plot analysis and Shapiro-Wilks test). We designed general linear models (GLM) to explore the relationships of the variables describing reproductive output (CS, CM, RCM) and temperature dependent locomotor performance (T_{pref}, MaxPerf, Breadth). Dependent variables were the CS, CM and RCM. Independent variables were the T_{pref}, MaxPerf and Breadth. We applied backward stepwise model simplification based on P = 0.05 significance. Statistical analyses were performed using R (R Core Team 2013).

Results

CS of *L. viridis* was 6.67±0.49 (mean ± SE, N=15), and CM was 4.31±0.32 g (mean ± SE, N=15). 46% of the gravid *P. tauricus* females layed two clutches. The first clutches consisted of 3.17±0.19 eggs (mean ± SE, N=30) and the second clutches of 2.47±0.37 (mean ± SE, N=15), while CM of P. tauricus was 1.07±0.07 g (mean ± SE, N=30) for the first clutch and 0.82±0.09 g (mean ± SE, N=15) for the second clutch. In *P. tauricus* females, CS and CM did not differ between the first and second clutches (CS: t = 1.7938, df = 24, P = 0.09).

We examined the relationship of body size and clutch parameters in both species with General Linear Models. We found that SVL positively correlated with CS in both species (*P. tauricus*: SVL and CS of first clutch: B = 0.109, SE = 0.039, df = 28, t = 2.826, P = 0.009, Fig. 2/a; SVL and total egg number: B = 0.2293, SE = 0.0892, df = 28, t = 2.571, P = 0.0158, Fig. 2/b; *L. viridis*: B = 0.234, SE = 0.107, df = 13, t = 2.179, P = 0.048, Fig. 2/c). We also found a positive correlation between first CM and condition (regression of body mass on SVL) of *P. tauricus* (B = 0.338, SE = 0.094, df = 28, t = 3.706, P < 0.001, Fig. 3/a), however, condition did not correlate with mean egg mass (B = 0.011, SE = 0.015, df = 28, t = 0.757, P = 0.46), so larger females did not lay larger eggs (Fig. 3/b).

RCM of *L. viridis* females did not differ from that of *P. tauricus* females' first clutch (paired t-test; t = -1.336, df = 28.07, P = 0.19, Fig.4/c). For *L. viridis*, however, this reproductive output resulted in significantly more eggs (t = 6.571, df = 18.63, P < 0.0001; Fig.4/a). We found that the RCMs of P. tauricus females' first clutches were marginally higher than those of the second clutches (t = 2.033, df = 26.48, P = 0.052, Fig.4/c).

In *L. viridis* T_{pref} and MaxPerf changed significantly with reproductive state, both variables being higher after oviposition (T_{pref}: t = -9.146, df = 14, P < 0.0001; MaxPerf: t = -7.121, df = 14, P < 0.0001; Fig.5/a,b). However, Breadth did not differ significantly between the gravid and post-lay females (t = -0.817, df = 14, P = 0.43; Fig.5/c).

In *P. tauricus*, in addition to preferred body temperature (t = -6.481, df = 29, P < 0.0001, Fig.6/a) and MaxPerf (t = -8.319, df = 29, P < 0.0001, Fig.6/b), Breadth also showed significant increase at post-lay state (t = -3.45, df = 29, P = 0.0017, Fig.6/c). RCM of the first clutch showed negative correlation with the increase of peak locomotor performance following oviposition (GLM; B = -0.0011, SE = 0.0005, df = 27, t = -2.104, P = 0.045, Fig.7). In individuals that layed two clutches, we found that T_{pref} did not show further change (t = -1.039, df = 14.14, P = 0.31, Fig.6/a), whereas MaxPerf further increased after laying the second clutch (t = -4.176, df = 14, P < 0.0001, Fig.6/b). Interestingly, after second oviposition, Breadth decreased significantly (t = 2.156, df = 25.83, P = 0.04), resulting in Breadth values being similar to those at gravid state (t = -0.539, df = 32.4, P = 0.59, Fig.6/c).

Discussion

According to life-history theory, when energy expenditure of producing offspring constrains future reproduction and/or survival, animals should spread energetical costs between current and future reproduction (Nilsson & Svensson 1996, Roff 2002). While short-lived species tend to favor current reproduction to increase their fitness, long-lived species show variance in their reproductive investment to balance current adjacent costs and future benefits (Stearns 1992, Roff 2002). Thermal environment can change life-history traits of ectotherms through its substantial effect on their feeding, metabolism, behaviour and reproduction (Adolph & Porter 1993, Sorci & Clobert 1997). Ectothermic populations might be more sensitive to shifting temperature than others, and understanding to what extent they might be capable of coping with rapid temperature changes through phenotypic plastic traits is of particular importance regarding their conservation.

Reproductive investment of the two studied species with alternative reproductive strategies showed a number of distinct traits, however, some general patterns seem to be apparent independently of the number of reproductive bouts. Bigger females had more eggs in a single bout (Fig.2/a,c) in both species, and this remained true when considering the total number of laid eggs during multiple ovipositions for *P. tauricus* (Fig.2/b). Maternal body volume, fat bodies and energy stores are of substantial importance in lizards' vitellogenesis and optimizing clutch size (Bauwens & Díaz-Uriarte 1997). However, egg size seems to be optimized in a number of Lacertid species (e.g. Du 2006, Amat 2008) and also other squamates along the constraints of a minimum viable egg size and a maximum egg size compared to maternal body size (Shine et al. 1998, Thompson & Pianka 2001). Second clutches of *P. taurica* consisted of fewer eggs, probably because of the depleted maternal energy and nutrient storages. While the total reproductive effort relative to their post-lay body mass (cumulative RCM) did not differ between *L. viridis* and *P. taurica* (Fig.4/c), in a single clutch the former could create a greater number of eggs (Fig.4/a).

Temperature-dependent fitness costs of reproduction in oviparous ectotherms originate from various sources, including the mismatch between optimal maternal and offspring temperature (Huey & Kingsolver 1989). In our study, females of both species preferred lower temperatures in gravid state than they did after oviposition, irrespective of their reproductive strategy (Fig.5/a, 6/a). This suggests general constraints like embryonic heat sensitivity (Van der Have 2002) and an effort of females to protect embryos from dangerously high maternal body temperature, because embryos have a narrow heat tolerance compared to adult lizards and basically no opportunity to cope by behavioural thermoregulation (Cordero et al. 2018). A number of studies reported on deleterious effects of long-term high incubation temperatures (e.g. Shou et al. 2003, Andrews & Schwarzkopf 2012), but how rapid, extreme temperature elevation (like extreme heat events) affects embryonic development is still unclear. Recent works showed that even acute heat stress applied on hatching eggs caused suppressed embryonic development and survival (Hall & Warner 2020), while embryos from heat-stressed females showed even higher sensitivity to elevated temperatures (Telemeco et al. 2017). Embryonic heat tolerance seems to be narrow and rigid across species, which provides an explanation why gravid females compromise their own metabolic activity by maintaining lower body temperature, as seen in other taxa, even in live-bearing lizards, where the opposite is more general (e.g. Mathies & Andrews 1997, Gainsbury 2020). Interestingly, *P. tauricus* females that laid two clutches did not select for higher body temperatures after second oviposition compared to their first post-lay state (Fig.6/a), suggesting that the thermal constraints were lower for the second clutch. In many lizards, females are able to arrest embryonic development through oviductal egg retention (Bauwens 1999, Shanbhag 2003, Rafferty & Reina 2012). If embryos of the second clutch go through early stages of ontogenesis before first oviposition, low maternal body temperature provides optimal thermal environment for their development, as lizard embryos are found to be less robust to heat stress in early stages (Sanger et al. 2018, Hall & Warner 2019). After the first oviposition, females can maintain higher body temperature and compensate for the delay in embryogenesis via higher metabolic activity (Angilletta & Sears 2000), indeed, in our experiment, the time interval between *P. taurica* females' first and second oviposition was similar to that of females in a separate experiment where individuals were housed from the time of copulation to first oviposition (Bajer et al. unpublished data).

Peak locomotor performance and preferred body temperature had similar patterns in both *L. viridis* and *P. tauricus*, gravid females showing lower performance than that of post-lay females (Fig.5/b, 6/b). Both species are active foragers and gravidity imposes not only a physical burden on females by the clutch itself (Shine 1980, Le Galliard et al. 2003), locomotor impairment can occur also because of physiological constraints. Such constraint is the above mentioned shifting thermal preference that forces females to be more settle, which in turn decreases foraging success (Shine 1980). However, locomotor activity affects predator avoidance as well, and more cryptic individuals might not be burdened by predators as much as more active females, increasing the probability of survival and mitigating the costs of reproduction (e.g. Cooper et al. 1990, Weiss 2001). This is further supported by the fact that energy needed for reproduction is stored in form of fat bodies over winter and predation implies a greater risk than starvation during egg development. On the contrary, habitat use of females will contribute to thermoregulatory precision, which in turn increases the reproductive success (Mathies & Andrews 1997, Woolrich-Piña et al. 2015, Juri et al. 2018), thus restricted locomotor performance that hinders optimal habitat use of females may impose higher costs on reproduction than one could assume (Miles & Sinervo. 2000). *P. tauricus* females further increased their locomotor performance after their second oviposition, showing that the second clutch imposes physical and physiological constraints to the mother similar to the first clutch, a cost of reproduction females would be unlikely to bear when laying eggs in a single reproductive bout.

While peak performance (T_{pref}) of *P. tauricus* females increased after the first oviposition, the degree to which they were able to perform better depended on their relative reproductive investment into the first clutch (Fig.6). Females investing more heavily into their first oviposition showed a lower increase in locomotor performance on preferred body temperature, a clear sign of energetical constraints of reproductive allocation (e.g. Cox et al. 2010), however such connection was not present in the case of *L. viridis*. It is possible that accommodating optimal egg size while maximizing their reproductive output is more costly for small species that are forced to limit their clutch size and compensate with other phenotypic plastic traits like, for example, elevated nutrient allocation to increase offspring quality (Warne et al. 2012), multiple egg laying to eventually increase number of offspring, as in the case of *P. tauricus* (this study, Chondropoulos & Lykakis 1983).

Maintaining effective physiological performance across a variety of thermal environment is crucial for ectotherms. Lizards, especially active foraging species, often change their thermoregulatory behaviour depending on their habitat, for example, actively thermoregulating when in open and passively when in closed habitats (Huey 1974, Gillis 1991). Increased thermoregulatory precision in gravid lizards is reported in several species (e.g. Mathies & Andrews 1997, Woolrich-Piña 2015), likely due to thermal sensitivity of developing embryos (Sanger et al. 2018). In lizards, locomotor performance reaches close to maximum levels at a relatively narrow temperature range that is species-specific (effective performance or Breadth, Huey & Kingsolver 1989), and this usually does not coincide with thermal requirements of optimal embryonic development (Telemeco et al. 2017). However, when reproductive females are only able to maintain effective locomotor activity in a suboptimal temperature range, it may limit their foraging ability and predator avoidance (Huey & Slatkin 1976), imposing additional costs to reproduction (Galliard 1993). We found that these costs manifest differently in alternative reproductive strategies. *L. viridis* females could perform close to maximum values in a similarly wide temperature range before and after oviposition (Fig.5/c). Contrarily, gravid P. tauricus females' effective locomotor performance was confined to a narrow temperature range that expanded after oviposition (Fig.6/c), showing that in this species gravidity imposes even greater costs on suboptimal temperatures that are more likely to grow with increasing heat stress.

Even if laying multiple clutches distributes the costs of investing into the current reproductive bout, clutch frequency is limited by the available energy that a female is able to allocate into further clutches (Warne et al. 2012). Such depletion of energy stores manifests in the decreased effective locomotor performance of *P. tauricus* females with two clutches. Notably, females were again able to attain close to peak locomotor performance only in a narrow temperature range (Fig.6/c), similar to gravid state, this time probably because of the need for a suitably high metabolic rate that enables recovery from repeated reproductive effort. Females were again forced to thermoregulate according to the requirements of rapid energy restoration, rather than their individual optimum. Lizards with both reproductive strategies realized similar reproductive output (similar RCMs), however, *P. tauricus* was only able to do so in two reproductive bouts, suggesting that further increasing the number of clutches to compensate for the detrimental effect of high temperature on offspring survival is unlikely. Because of the higher energetic cost of prolonged gravidity, the phenotypic plastic trait of clutch frequency in all probability would not provide an opportunity to escape climate warming because producing more clutches would inflict unbearable energetic limitations and result in lower survival.

Acknowledgements

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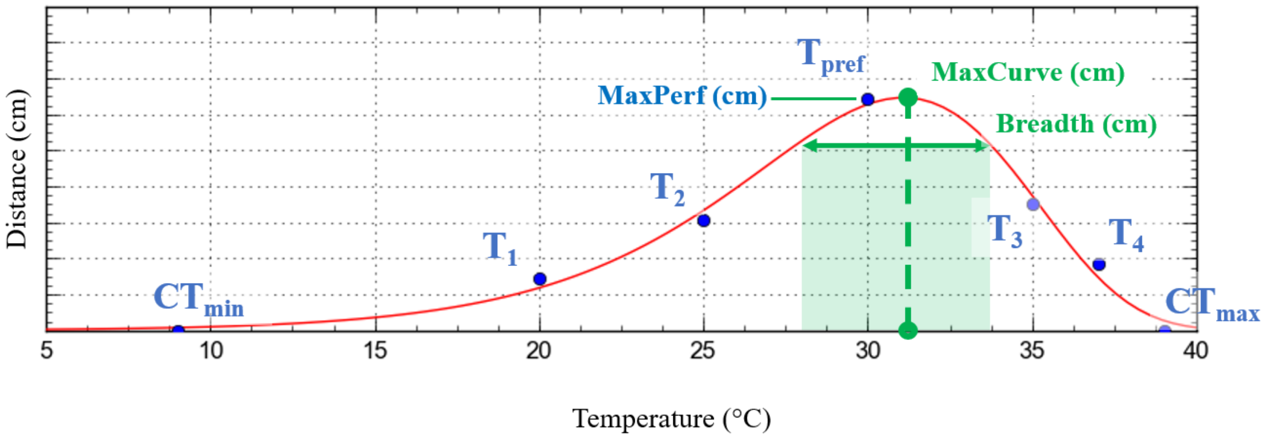

Figure.1. Schematic depiction of a thermal preference curve (TPC). Full dots represent performance measured on particular temperatures; red line shows curve fitted by Kumaraswamy function. Green horizontal arrow marks 80% of maximum calculated performance (MaxCurve) while green shaded area covers thermal range at which at least 80% of maximum performance is shown (Breadth).

Figure 2

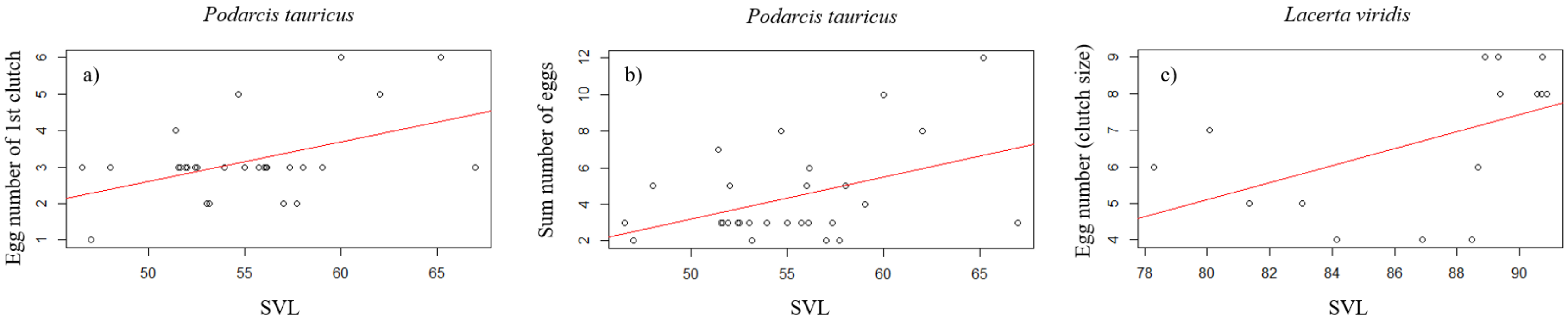

Figure.2. General Linear Models (GLMs) describing the relationship between SVL and (a) number of eggs laid is the first clutch and (b) total number of eggs in the two clutches for P. tauricus, and (c) the total number of eggs in the single clutch for L. viridis. Hollow dots are model residuals, red lines show a liner fitted curve.

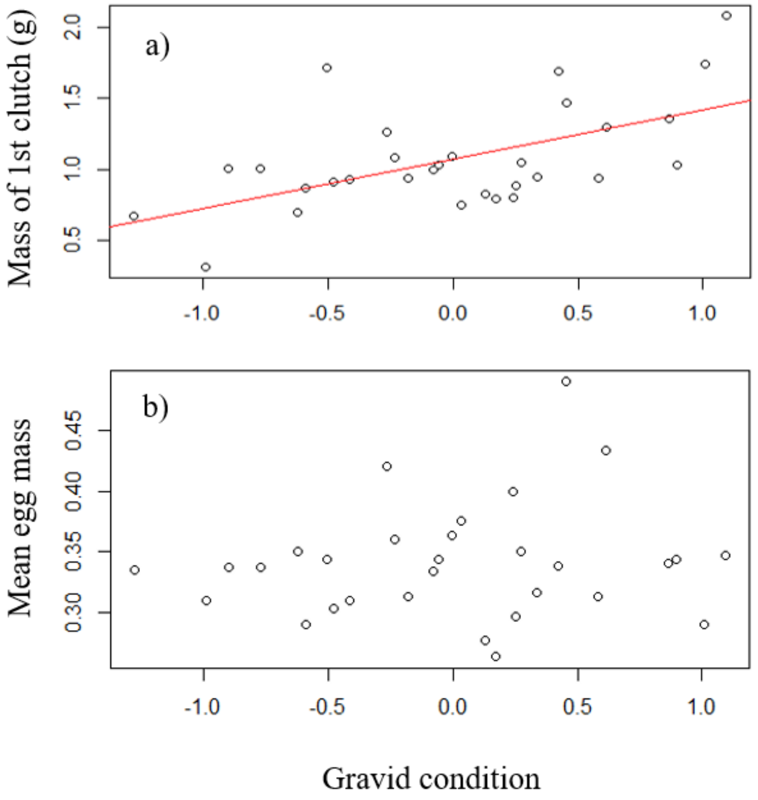

Figure 3. General Linear Models (GLMs) showing (a) the positive connection between female gravid body condition and clutch mass of the first clutch in P. tauricus. Contrary to expectations, body condition did not show any correlation with mean egg mass in the same species (b). Body condition was defined as a function of body mass and SVL. Hollow dots are model residuals, red lines show a liner fitted curve.

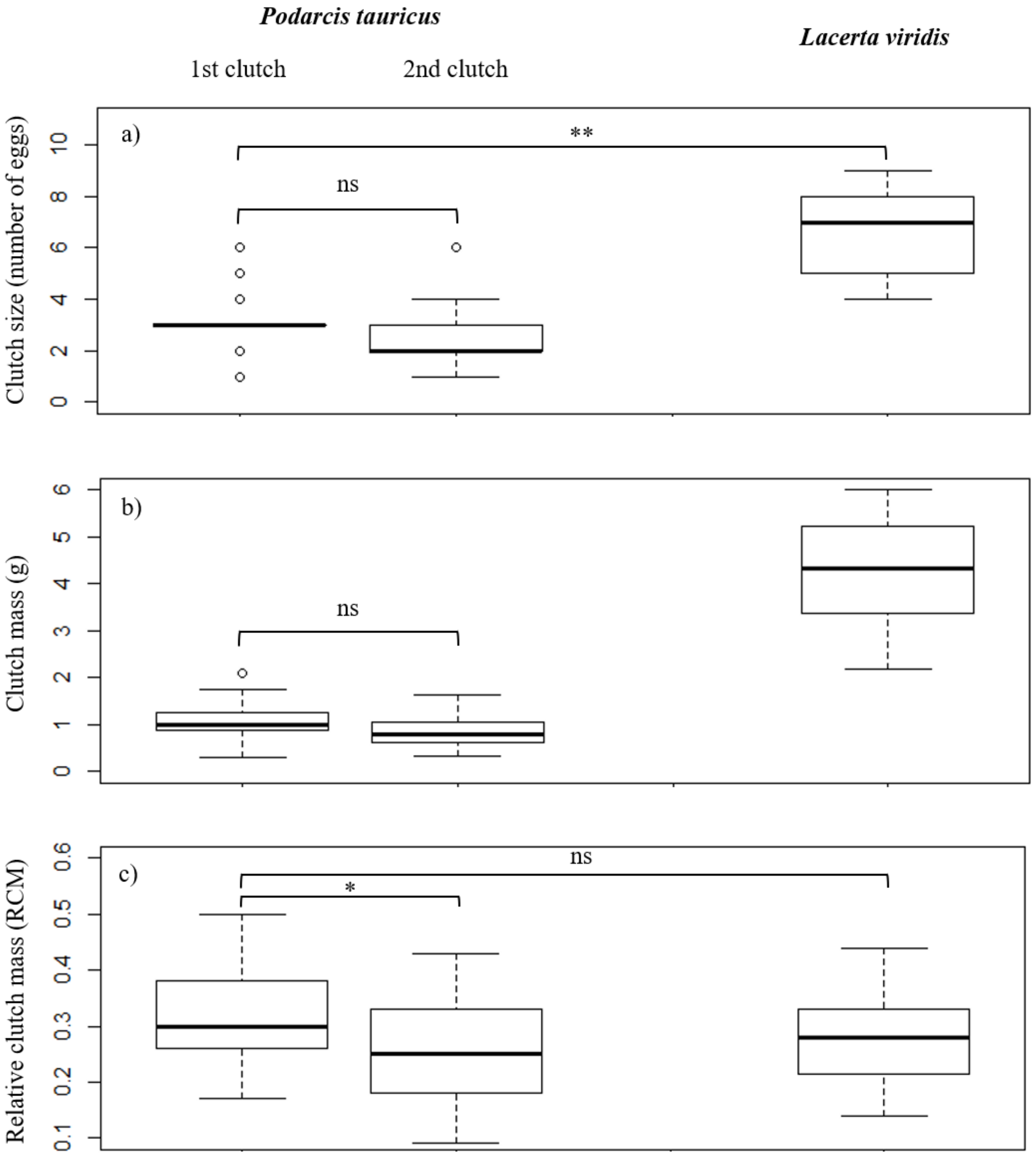

Figure 4. Reproductive variables compared within multiple clutches of P. tauricus and between the two study species. (a) Clutch size did not vary between first and second clutch of P. tauricus, but was significantly lower than that of L. viridis. (b) Clutch mass also showed no intraspecific difference, but was significantly higher in L. viridis. (c) Relative clutch mass (RCM) showed no interspecific difference but was significantly higher for the first clutch in P. tauricus. Solid lines represent median, boxes represent lower and upper quartiles while whiskers represent 95% confidence interval.

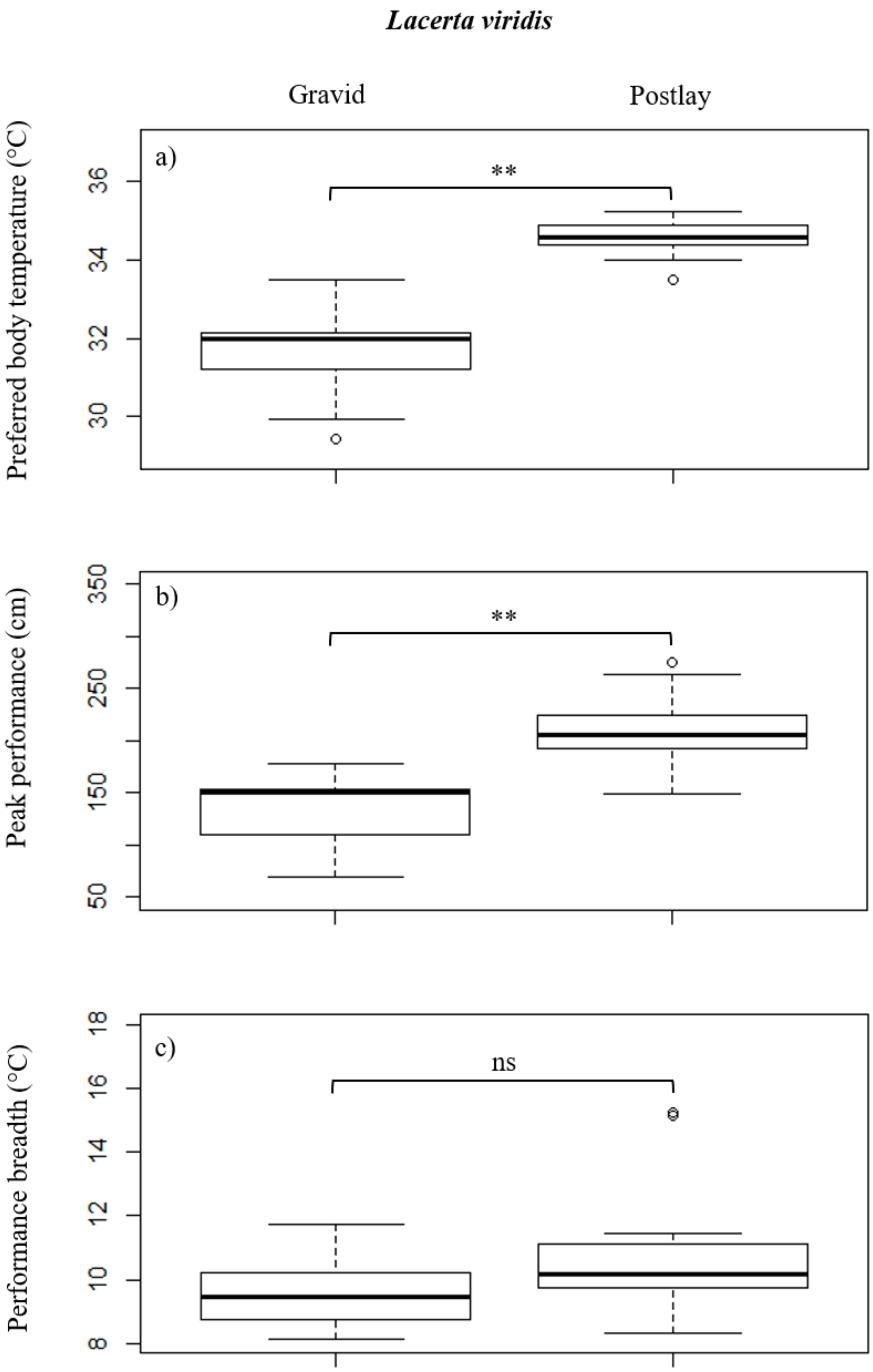

Figure 5. Difference in performance variable between gravid and after depositing single clutch state of L. viridis females. (a) Preferred body temperature was significantly lower in gravid state. (b) Peak performance also significantly increased after oviposition. (c) Performance breadth showed no change before and after oviposition. Solid lines represent median, boxes represent lower and upper quartiles while whiskers represent 95% confidence interval.

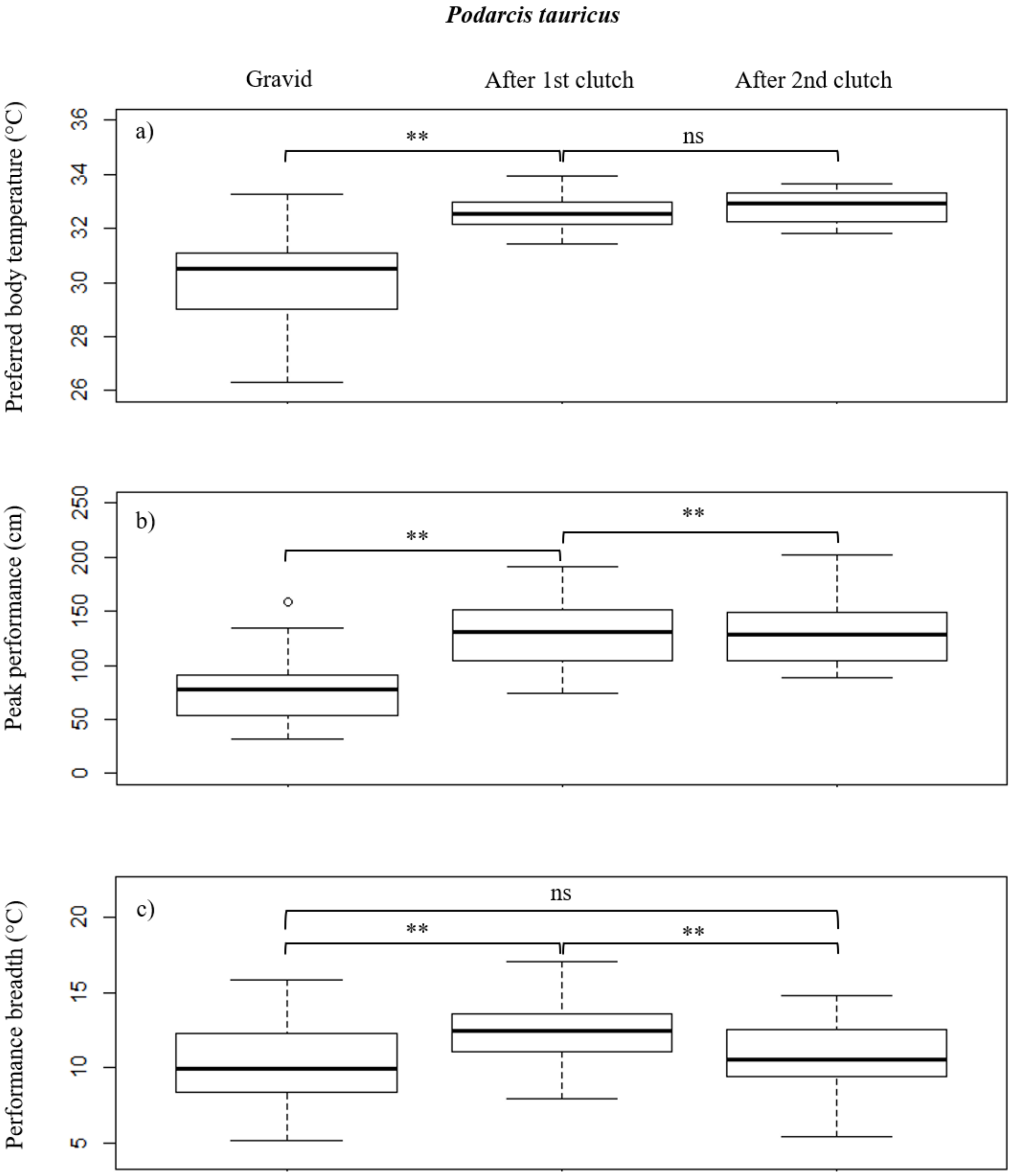

Figure 6. Difference in performance variables between gravid, after depositing first clutch and after depositing second clutch in P. tauricus females. (a) Preferred body temperature was significantly lower in gravid state than after first oviposition but did not change after second oviposition. Peak performance also significantly increased after first oviposition, and continued to significantly increase after the second clutch. (c) Performance breadth significantly increased after first oviposition, and significantly decreased after second clutch. Solid lines represent median, boxes represent lower and upper quartiles while whiskers represent 95% confidence interval.

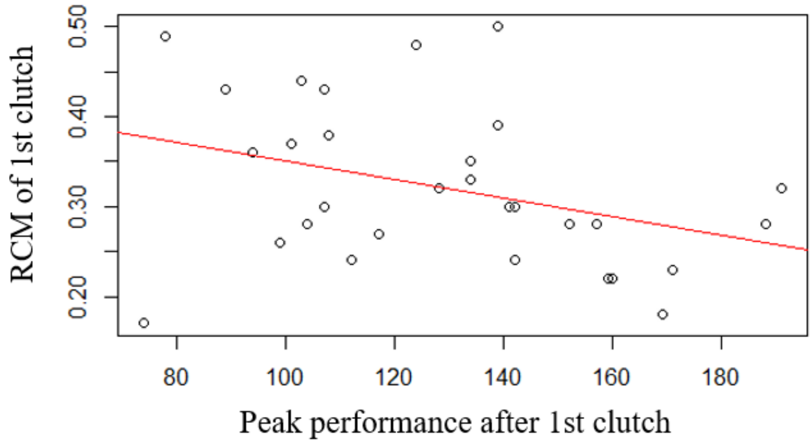

Figure 7. General Linear Model showing negative correlation between RCM of the first clutch and Peak performance after laying the first clutch in P. tauricus. Hollow dots are model residuals, red lines show a liner fitted curve.

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Thermal preference and temperature-dependent physiological performance in alternative thermal environments

In the second work period I investigated the temperature dependent locomotor performance of *Iberolacerta cyreni* and *Psammodromus algirus* in gravid and post-lay state. The two populations live in two distinct thermal environments, notably, in a stochastically changing mountain habitat (no buffering effect) and a lower elevation, „constant" habitat (buffering effect of vegetation), respectively. The study was carried out near Madrid, in collaboration with Prof. José Martín (MNCN-CSIC, Spain). Our fieldwork and experiments involved 1 PhD and 2 graduate students from the research group led by Dr. Martín. This research resulted in a manuscript presented below and has since contributed to a successful PhD proposal of Dr. Gonzalo Rodríguez Ruiz and is currently under review at the research group of MNCN.

Climate warming triggers higher reproductive costs and lower reproductive investment according to the selective thermal environment in lizards

Lilla Jordán, Boglárka Mészáros, Gonzalo Rodríguez Ruiz, Orsolya Molnár, Katalin Bajer

Introduction

Thermal regimes induced by a changing climate threaten to affect species across all taxa, but are predicted to have a pronounced effect on terrestrial ectotherms with limited mobility, since their physiological performance is strongly dependent on environmental temperature (Buckley et al., 2015; Logan et al., 2014; Sinervo et al., 2010). Studies investigating lizard populations have gathered substantial amount of evidence regarding temperature-dependent locomotor performance and its potential effects on individual survival (Cabezas-Cartes et al., 2019; Clusella-Trullas et al., 2011; Gilbert & Miles, 2016), and models predicting extinctions rates are largely based on survival rate estimates (Sinervo et al., 2010). Nevertheless, survival is not the only contributor to total fitness: population dynamics are significantly influenced by reproductive output as well as survival (Huey & Berrigan, 2001), but studies investigating the plasticity and thermal constraints of females' reproductive investment are scarce (but see Charnov & Ernest, 2006). These fitness components become especially important considering that heat stress may alter nesting behaviour and embryonic development (Hall & Warner, 2021; Telemeco et al., 2017), as well as increase physiological costs of females such as elevated levels of stress hormones (Ensminger et al., 2018) and oxidative damage (Dupoué et al., 2020). If thermal regimes restrict both survival and reproduction rates, that can lead to reproductive failure when recruitment rate remains below mortality rates and the populations start declining.

Estimating the overall effects of reproductive investment is commonly done by measuring locomotor performance plotted on a thermal performance curve (TPC; Angilletta, 2006) before and after oviposition. As TPCs are not only affected by environmental temperature, but also increased reproductive investments (Domínguez-López et al., 2018; Olsson et al., 2000), they are highly suitable to measure overall physiological burden on females expressed as a combination of environmental temperature and reproductive allocation. Decreasing performance will affect fitness components such as foraging efficiency (Pagan et al., 2012), thermoregulation (Gilbert & Miles, 2017) and avoiding predators (Gilbert & Miles, 2016).

However, females have been demonstrated to exhibit various levels of plasticity in their reproductive traits such as timing of reproduction (Ma et al., 2018), differential allocation (Massey et al., 2022) and nesting behaviour (Levy et al., 2015). When estimating individual reproductive investment, relative clutch mass (RCM) is a widely used index of female reproductive effort, calculated as a function of clutch mass and female body mass (Gerald & Miskell, 2007). RCM often shows seasonal changes within populations (Du & Shou, 2008), environmental temperature (Jin & Liu, 2007), and resource availability (Doughty & Shine, 1998). Therefore, it can be assumed that various thermal environments result in different constraints related to reproduction and females will be able to alter their reproductive investment in light of the physiological burden the environment represents. It is therefore possible that harsher environmental regimes will lead to decreased reproductive investment on the females' part.

For our study, we chose to investigate to sympatric lizard populations (*Iberolacerta cyreni* and *Psammodromus algirus*) inhabiting highly different thermal environments. The two areas are differentially exposed to effects of climate warming, which allows for testing the effects of climate change in female reproductive physiology. Both populations have been described to lay only a single clutch of eggs, reproductive investment is therefore not distributed across multiple clutches and can only be manipulated through relative clutch mass or clutch size. To estimate female performance, we recorded TPC for all females before and after oviposition. We hypothesized that strict thermal regimes will result in decreased reproductive investment and/or a decrease in various aspects of physiological performance. We were also expecting to see various physiological variables affected by gravidity due to different selective pressures expressed by different environments. Our goal was to compare the physiological costs or reproduction in different thermal environments.

Material and Methods

Study species

We chose two sympatric lizard species inhabiting two microhabitats representing two different thermal regimes and differentially affected by climate change. As we had previously shown that males of these same populations show different physiological adaptations to the two thermal environments (Mészáros et al., 2019), we investigated whether females would also demonstrate alternate reproductive strategies.

The Carpetian rock lizard (*Iberolacerta cyreni*) is a mountain lacertid native to Spain, distributed across the Sierra de Bejar, Sierra de Gredos, La Serrota and Sierra del Guadarrama 1800–2500 m above sea level (Pérez-Mellado et al., 2009). Populations inhabit exposed, rocky, open areas with low or no vegetation, and are therefore characterized by inordinate microclimatic conditions. Females lay 6 eggs of average with a mean egg mass around 0.5 g (Monasterio et al., 2016).

We chose the Algerian Psammodromus (*Psammodromus algirus)* as our second study species, which is native to pine and oak forests of France and Spain, ranging from 0 to 2600 m altitudes (Mateo et al., 2009). Contrary to *I. cyreni*, *P. algirus* inhabits an environment with a much more heterogenous and stable thermal regime, where the thick vegetation buffers the effects of extreme temperature shifts. Between 22-64% of females of this species lay a second clutch, with an average egg mass being approximately 0.44 for both clutches, and an average clutch size of 6.5 and 5.5 for the first and second clutch respectively (Díaz et al., 2007).

Collection and morphological measurements

In the reproductive season on 2019, we captured 15 *I. cyreni* and 16 *P. algirus* gravid females in the mating season of 2018in their native distribution areas in the Madrid area of Spain. *I. cyreni* was collected from 'Puerto de Navacerrada' (40° 47' 04.1" N, 4° 00' 44.8" W; datum = WGS84) and *P. algirus* from 'La Golondrina' (40° 45' 06.0" N4°02' 03.6"W; datum=WGS84). Animals were caught by noosing, placed individually in fabric bags and transported back to the facilities of the 'El Ventorrillo' field station (Museo Nacional de Ciencias Naturales, CSIC). Animals were then measured for their snout-vent-length (SVL) and with a digital calliper (Mitutoyo, Kawasaki, Japan) and body weight (BW) with an analytical balance (Ohaus Scout Pro SPU-2001, Pine Brook, USA), and then housed individually in plastic tanks measuring 60x60x40 cms. Tanks were equipped with a uniform shelter and placed in a temperature control chamber providing optimal operative temperature (T_{day} = 28± 2 °C, T_{night} = 21 ± 2 °C). We used full spectrum lights for lighting 10 hours a day and additional spotlights to create basking spots. Females were fed *Tenebrio molitor* larvae and water *ad libitum*. Mealworms were kept on a high-carotenoid diet.

Locomotor performance

Once captured, measured and housed, females had a 3-day acclimatization period, after which we began to establish and record performance curves. On the first day after acclimatization, females were offered a temperature gradient in a rectangular arena, where they were able to move around freely and their body temperature was measured every 2 min with a laser thermometer (Raytek Raynger ST, Raytek GmbH, Berlin, Germany) for 60 min, resulting in 30 measurements per individual. We then averaged these 30 measurements to define preferred body temperature (T_{pref}) for all individuals, and also determined minimum (CT_{min}) and maximum (CT_{max}) temperatures as those at which animals no longer showed the righting reflex (Spellerberg, 1972). Since T_{pref} and CT_{max} values were much closer to each other than T_{pref} and CT_{min}, we defined one further temperature value between T_{pref} and CT_{max}, and another three between

T_{pref} and CT_{min}, thereby creating a range of five evenly distributed temperature values between the physiological tolerance marked by CT_{min} and CT_{max} (Table 1). Following another day of rest, we started measuring physiological performance of each of the five temperatures for all individuals. We randomized the sequence of temperatures for each female and ran them once a day on the temperature assigned for that day. Between each performance trial, we allowed for a day of rest. Before each trial, we set animals to the desired body temperature using spotlights and/or icepacks, and placed them into a circular arena. We encouraged them through gentle chasing to run until they no longer gave the writing reflex, and recorded the distance ran for each female at each temperature.

We conducted the above-described protocol for all females during their gravid state and after oviposition. No animals had laid a second clutch. After concluding all experiments, females were released at the site of capture. No lizards died or suffered any injury as a result of housing, handling or sampling, all animals gained weight and were observed to be active and showing normal feeding and basking behaviour on the date of release.

	I. cyreni					***P. algirus***				
	T1	**T2**	**T3**	**Tpref**	**T4**	**T1**	**T2**	**T3**	**Tpref**	**T4**
Gravid	12	18	24	29.9	34	14	21	28	34.5	38
Postlay 13	20	27	35.2	36	14	21	28	36.1	39	

Table 1. Temperature values calculated for physiological performance curves for each species before (Gravid) and after oviposition (Postlay)

Oviposition and clutch measurements

After finishing all performance trials in their gravid state, females were housed in their tanks until showing signs of oviposition. Once they had laid their eggs, we recorded their BW and carefully collected the eggs from the tank. Clutch size (number of eggs) and egg size (g) were recorded for each female, and used to calculate clutch mass (total mass of egg) and Relative Clutch Mass (RCM; clutch mass/maternal mass), to characterize relative reproductive energy allocation of each female. Eggs were placed into boxes filled with wet perlite, covered with a thin layer of the same material and placed in incubators until hatching. Hatchlings were released on the females' site of capture.

The day after oviposition, females underwent the same performance measurement protocol described above.

Statistical analyses

We used a statistical method developed for performance curves to extract our variables for physiological performance (Cordeiro & de Castro, 2011). We plotted the distance measured at each temperature for each physiological state of each female against the body temperature of the trial and fitted the curve to the Kumaraswamy function. We determined the variable characterizing maximum locomotor performance (MaxPerf; the performance measured at T_{pref}) and the effective locomotor performance (Breadth; the temperature interval where the performance is at least 80% of the maximum locomotor performance = the difference of the two x-values where the y-value is 80% of the maximum y-value).

Normal distribution was tested by analysing q-q plots and running Shapiro-Wilk tests. Depending on the results, we used either paired t-tests or Wilcoxon matched-paired tests for normal and non-normal distribution, respectively. For each species, we compared T_{pref}, MaxPerf and Breadth values before and after oviposition. Furthermore, we ran Generalized Linear Models (GLZ) separately for each species to test which performance variables are connected to RCM. Dependent variable was RCM, whereas independent variables were MaxPerf before and after oviposition, MaxPerf before and after oviposition, T_{pref} before and after oviposition and SVL. We applied backward stepwise model simplification in all models, nonsignificant explanatory variables were deleted one by one in decreasing order of P, and final models included only the significant main effects. Statistical analyses were performed using R (R Core Team, 2016), performance curves were created with TableCurve (SYSTAT Software Inc., 2002).

Results

Locomotor performance before and after oviposition

When measuring T_{pref} before and after oviposition, females of both species showed a significant increase in their preferred body temperature after having laid their eggs (*I. cyreni*: V=14, P=0.0067; *P. algirus*: V=3, P>0.0005; Figure 1.).

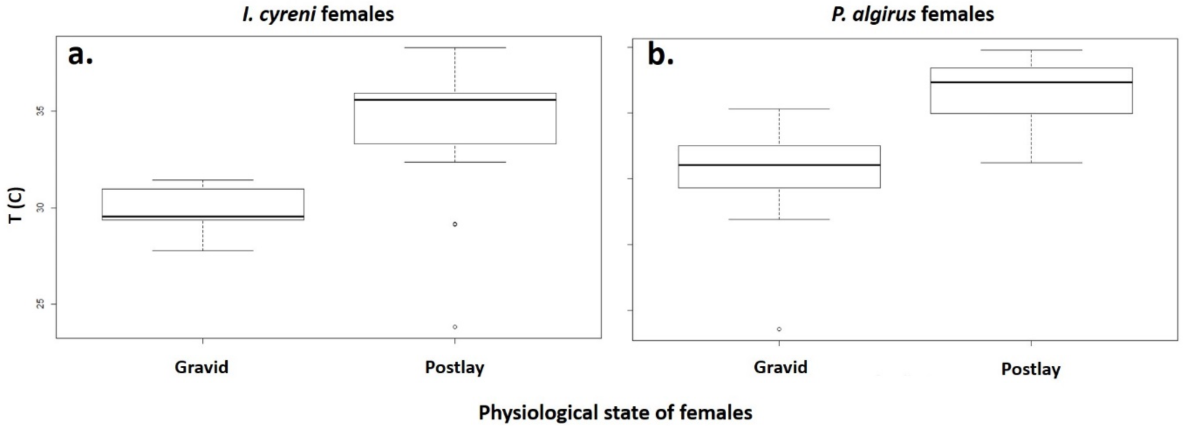

Figure 1. Difference in T_{pref} before (Gravid) and after oviposition (Postlay) in (a) *I. cyreni* and (b) *P. algirus* females.

When comparing MaxPerf before and after oviposition, *P. algirus* females showed a significant increase in their maximum locomotor performance (t=-3.22, P=0.005), but we found no difference in MaxPerf between the two physiological states for *I. cyreni* females (t=-1.68, P=0.12; Figure 2).

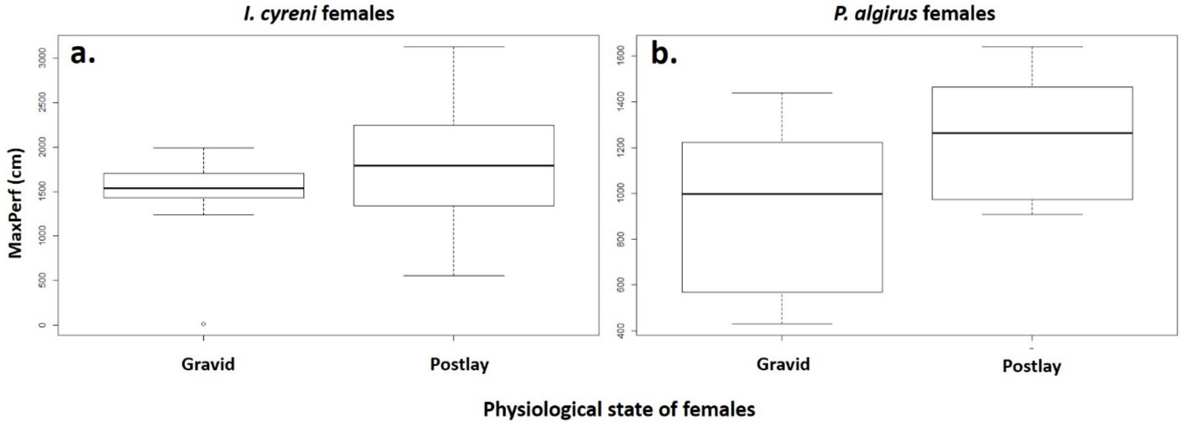

Figure 2. Difference in MaxPerf before (Gravid) and after oviposition (Postlay) in (a) *I. cyreni* and (b) *P. algirus* females.

At the same time, *I. cyreni* showed a significant increase in their effective locomotor performance after oviposition (t=-6.50, P>0.0001), while *P. algirus* showed no difference in Breadth before and after oviposition (t=-0.06, P=0.96).

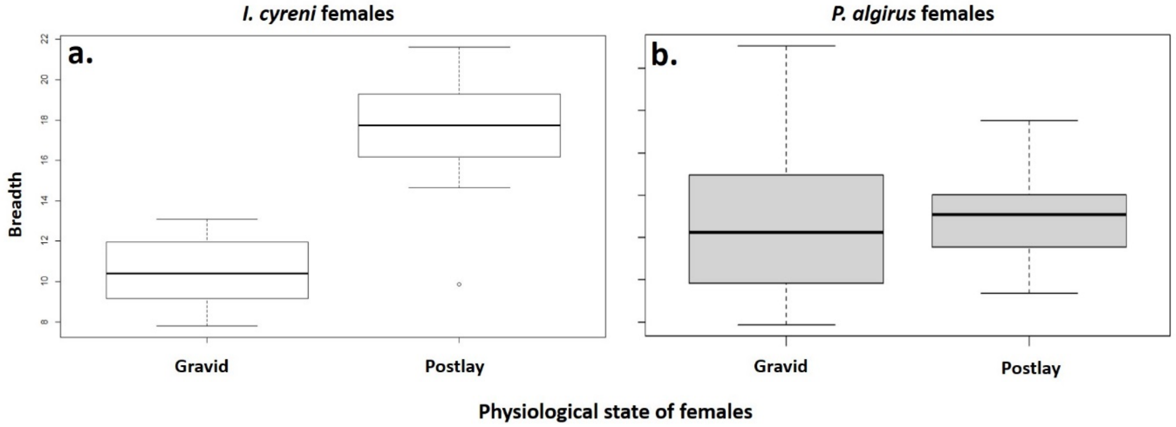

Figure 3. Difference in Effective locomotor performance (Breadth) before (Gravid) and after oviposition (Postlay) in (a) *I. cyreni* and (b) *P. algirus* females.

When investigating reproductive allocation of females, while RCM was not associated with any of the examined performance variables in *I. cyreni* females, in *P. algirus* it showed a significant negative connection with maximum locomotor performance measured before oviposition and a significant positive connection with preferred body temperature measured after oviposition, meaning animals with higher RCM had a lower MaxPerf in a gravid state and a higher Tpref after laying their eggs (Figure 3.).

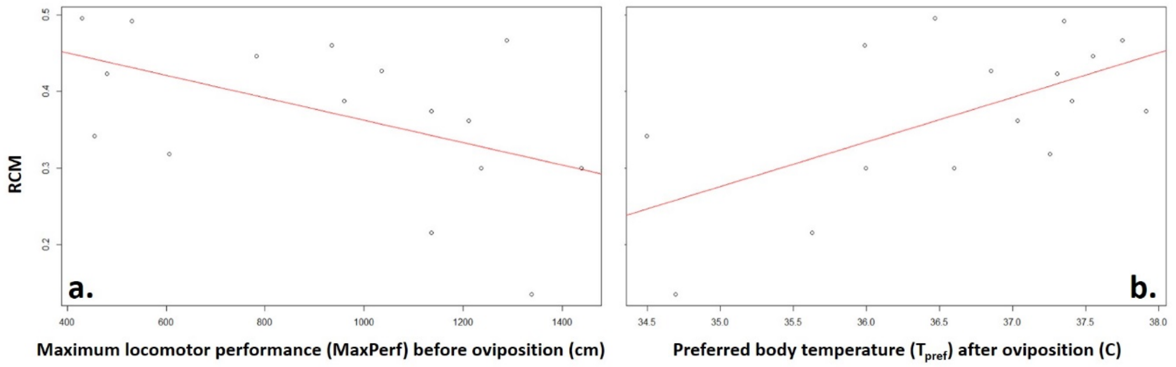

Figure 4. Connection between relative clutch mass (RCM) and (a) maximum locomotor performance (MaxPerf) measured before and (b) preferred body temperature (T_{pref}) measured after oviposition in *P. algirus* females.

Finally, we compered the RCM of the two species and found that of *P. algirus* to be significantly larger than that of *I. cyreni*, confirming that the two species differ in their reproductive allocation strategy (F=19.74, P<0.0001)

Discussion

Our results show that although some physiological traits such as preferred body temperature are under similar constraints in species living in different thermal environments, other physiological performance variable show altered adaptation to the respective thermal regimes.

Females preferred a higher body temperature after oviposition in both species. Egg production has been experimentally shown to be in trade-off with survival in lizards (Landwer, 1994), and has a negative effect on embryonic development and survival in oviparous lizards (Hall & Warner, 2021). Females may therefore prefer lower temperatures during egg development to maintain an optimal thermal environment for embryos and thus increase hatching rate. Furthermore, climate change is expected to decrease thermal heterogeneity in many habitats and thereby manifest as a limiting factor on offspring fitness (Hall & Warner, 2020).

Alternatively, the increase in preferred body temperature can be the result of physiological constraints on the female. Egg development requires substantial physiological investment, which often compromises regenerative capacities and survival (Bleu et al., 2013; Cox & Calsbeek, 2010; Frýdlová et al., 2013; Miles et al., 2000; Weiss et al., 2011). As ectotherm species require an optimal body temperature to maximize their physiological performance (Barr et al., 2021), and metabolic rates also increase with increasing environmental and body temperature of the female (Yue et al., 2012; Zari, 1991), it is therefore possible that females facilitate their physiological regenerative processes by choosing higher body temperature.

I. cyreni females' peak locomotor performance did not change between their gravid and post-lay state, but their effective locomotor performance significantly increased. This means that although they were able to realize a similar thermal performance curve stayed in the same position, it changed it's shape allowing females to exhibit at least 80% of their maximum performance on a larger temperature range after oviposition. One explanation is that there is strong selection for maintaining maximum locomotor performance, and females cannot afford to reallocate energy and compromise their maximum speed even during egg development. Microhabitat has a significant effect on escape strategies shown by lizard populations (Batabyal et al., 2017) and escaping predators generally represent a strong selective pressure (Wagner & Zani, 2017). As *I. cyreni* occupies open, rocky mountain areas, basking animals are exposed to predators and are forced to quickly initiate flight and seek refuge between boulders. Therefore, decreasing their maximum locomotor performance may also decrease their chances of survival to an extent that compromises their fitness despite increased maternal investment (C. P. Qualls & Shine, 1998).

Nevertheless, females showed a significant increase in their effective locomotor performance after laying eggs, which suggests that decreased performance breadth may appear as a cost of egg development. In an effort to maximize their fitness through both their own and their offspring's survival, females have been shown to optimize their escape behaviour during reproductive season (Cabezas-Cartes et al., 2018; Domínguez-López et al., 2018). Lowered performance on suboptimal temperatures can however be compensated for by remaining closer to the refuge (Domínguez-López et al., 2018; Iraeta et al., 2010), which is further in line with our previous result on gravid females preferring lower body temperature and thus requiring less basking time.

Contrary to *I. cyreni*, *P. algirus* females showed no difference in their locomotor performance breadth before and after oviposition, but showed a significant increase in their maximum locomotor performance after having laid their eggs. This means that the shape of their performance curve didn't change, but it had moved to a higher position than during gravid state, suggesting that decreased maximum locomotor performance appears as a cost of egg development. It has been shown in this species that gravid females escape predators through a larger number of shorter runs than non-egg bearing individuals (Iraeta et al., 2010), which could be a compensatory strategy for their decreased maximum locomotor performance. Microhabitat structure also has a significant effect on escape behaviour, with refuge availability being a key factor (Domínguez-López et al., 2018; Iraeta et al., 2010). When interpreting the results on effective locomotor performance, it is possible that there is strong selection for lizards to perform on a wider range of temperature even if that results in lower amount of energy allocated into their offspring. However, considering the environment of our sample population, refuges are available in high density and effects of thermal shifts are buffered by dense vegetation. Therefore, and alternative explanation is that broad availability of resources such as optimal thermal habitats and refuges decreases selective pressure and allows females to maintain their locomotor performance breadth without having to compromise maternal investment. Similar differences between populations inhabiting diverse types of habitats have been shown to affect sprint speed (Itonaga et al., 2012; F. J. Qualls & Shine, 1997) and alter intrinsic rate of energy allocation into offspring (Hall et al., 2020).

Additional to the differences in performance, gravid maximum locomotor performance decreased with relative clutch mass in *P. algirus*, but not in *I. cyreni*, meaning females proportionately allocating larger amount of energy into their offspring suffered a larger decrease in their maximum performance. These results demonstrate the elevated costs associated with larger broods, representing both a physical and a physiological constraint (Olsson et al., 2000). Females allocating larger amount of energy into their offspring my increase their fitness through higher offspring survival even by decreasing their own survival. Although it has been suggested that older females with larger body masses are able to afford larger energy investments into their clutches (Roitberg et al., 2013; Sciences, 2008; Telemeco & Baird, 2011), our analyses corrected RCM for female SVL, it therefore appears to be strategy conducted by females of all age. This could also suggest a higher availability of resources and/or higher plasticity in the reproductive allocation strategy followed by individuals (Olsson et al., 2002; Wang, Zeng, et al., 2017).

P. algirus females also showed a significant positive connection between RCM and preferred body temperature after oviposition, meaning animals exhibiting higher investment into their clutch preferred higher body temperatures postpartum. As described above for difference in preferred body temperature before and after egg deployment, females are expected to require physiological regeneration after egg development. For females producing larger relatively larger clutches and thereby investing more energy into their reproductive success, regeneration might be more intense and thus require higher temperatures. Available environmental temperature determines key physiological variables such as digestive energetics (Plasman et al., 2019), oxygen consumption (Steffen & Appel, 2012) and superoxide levels (Ballen et al., 2012), it can therefore be assumed that more intense regeneration will also require higher environmental and body temperatures.

Finally, when comparing the RCM between the two study species, that of *P. algirus* was significantly higher than that of *I. cyreni*. Taken together with the former showing more correlations between reproductive allocation and physiological performance variables, results suggest that *P. algirus* can generally afford a larger investment into offspring and bears mor physiological costs than *I. cyreni*. One explanation is the higher abundance of resources such as diverse thermal microhabitats, refuges and/or prey availability in the environment inhabited by *P. algirus*. Inhabiting a more stable environment allows females to invest more energy into reproduction since the chances of being able to regenerate are higher. At the same time, *I. cyreni* occupying an area more exposed to climate shifts with large scale changes in available temperature may select for a decreased maternal investment and aim to maximize fitness more through maternal survival, supporting the environmental matching hypothesis (Wang, Li, et al., 2017).

In summary, our results demonstrate that different thermal environments have significant influence of reproductive allocation strategies. On the one hand, different physiological performance variables come under different selective pressure in stable and stochastic environments, and species are able to adapt to their environment through differential investment into maximum locomotor performance and locomotor performance breadth. On the other hand, the number of physiological variable affected by reproductive energy allocation also changes between species inhabiting different environments, which suggests that animals in stable environments can afford higher investment and bear larger costs when investing into their clutches. These results highlight the importance of considering the multitude of factors that can affect maternal investment, and provide contextual interpretation for various hypotheses discussing strategies for reproductive allocation.

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Reproductive investment and thermal constraints on a species-scale

By the third work period I gathered data on short-term plastic changes in reproductive strategy of female lizards that might act as tools for compensate for climate warming. Specifically, the plastic changes in clutch parameters (CS, CM and RCM). I also conducted locomotor performance tests on *Timon lepida and Podarcis guadarrama*, however, the former species formulated a group with only a small sample, not enough for analysis. The latter species has the smallest body size among our sampled Lacertids, which might cause a higher risk of stress and exhaustion during the tests. For reasons uncovered, *P. guadarrama* females showed extremely poor feeding behaviour and rapidly declining condition during the tests, so I eventually excluded this group of the analysis. These species have to be replaced or retested in the future. The previously collected reproductive effort data was included the above presented manuscripts, and also in the Master's Thesis of the graduate student I worked with in Hungary, all stating funding from National Research, Development and Innovation Office:

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Besides analyzing reproductive costs in current thermal environment, I planned to make predictions for future climate change as well. However, this has been postponed due to changes in personal circumstances (I was diagnosed with high-risk pregnancy and therefore asked for a maternity leave [4. "inactive" work period] in May 2020, I resumed work in July 2021, last work period). In the meantime, the publication of SUNBEAR was still delayed in 2020 and I did not have the full program package to use it with my own data. According to our original plans, historical and current thermal data should have been provided and possibly collected at the study population's habitat by the help of the Sinervo lab in order to incorporate sex-specific data as a new dataset. However, due to the untimely passing of Prof. Barry Sinervo, the creator and co-developer of the SUNBEAR package, it is a question if I can find a solution for this lack of advancement.

We therefore redesigned the study and aimed to collect long-term temperature data from our sites of collection to identify possible trends in environmental temperatures. Although I had acquired some of the previously recorded temperature data from the area, it turned out that only daily average temperature had been recorded on a very limited number of sites. This raises the serious issue of not being able to estimate daily temperature shifts, which is one of the crucial defining factors of stable and stochastic thermal environments. Furthermore, the low number of recording sites does not provide a high enough resolution to compensate for movement due to behavioural thermoregulation, animals could therefore have been exposed to thermal habitats highly different from the ones recording were conducted in. Nevertheless, with the two manuscripts currently under review, we are in the process of designing a new collaboration with our partners at MNCN to make up for this lacking thermal database.