Title

Climate change and a little brown lizard: the impact of climate on maternal thermoregulation, offspring phenotype, and host-parasite interactions in the viviparous European common lizard (Zootoca vivipara)

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CLIMATE CHANGE AND A LITTLE BROWN LIZARD: THE IMPACT OF CLIMATE ON MATERNAL THERMOREGULATION, OFFSPRING PHENOTYPE AND HOST-PARASITE INTERACTIONS IN THE VIVIPAROUS EUROPEAN COMMON LIZARD (*ZOOTOCA VIVIPARA*)

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by

Pauline R. Blaimont

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Abstract

Climate change and a little brown lizard: the impact of climate on maternal thermoregulation, offspring phenotype, and host-parasite interactions in the viviparous European common lizard (*Zootoca vivipara*)

by

Pauline R. Blaimont

Climate change is predicted to severely impact species distributions and extinction risk in the coming decades. Ectotherms, such as lizards, are of particular concern due to their dependence on environmental temperatures to survive and reproduce. The predictions of extreme weather events and increases in global mean temperatures will affect the ability of these organisms to carry out important functions such as feeding or breeding. While we predict that these organisms will face challenges from climate change, examining whether they show evidence of coping with these changes is critical for determining extinction risk and making conservation decisions. In this dissertation, I use treatments of 3-hour (short), 6-hour (mid/control), and 9-hour (long) access to basking heat to investigate how different climate extremes, predicted to increase and worsen under climate change, would impact various aspects of pregnant viviparous European common lizards (*Zootoca vivipara*). The 3-hour treatments reflect an unusually long series of cool days limiting thermoregulation, 6-hours reflects the "normal" period of contemporary climate and 9-hours reflects periods of long-duration heat spells.

In **Chapter 1,** I look at the flexibility of females to modify their behavior to different basking treatments and how intraspecific variation between populations

plays a role. My results demonstrate these lizards exhibit plasticity in basking behavior in response to varying thermal opportunity. However, the magnitude to which they modify their behavior is significantly tempered by environmental characteristics of their population of origin. In **Chapter 2,** I investigate how basking treatments influence gestation and offspring phenotype, as the offspring life history stage is one of great importance. We found that offspring phenotype was significantly affected by mother basking treatments with local population differences and sex-dependent outcomes on size and viability in juveniles. My findings indicate that cooler and warmer temperatures have different effects on progeny phenotype and may have cascading impacts under climate change in the next generation. In **Chapter 3,** I examine how parasite load varies between populations and whether basking treatments influence a lizard's ability to fight infection. The basking treatments influenced changes in parasite load in three of the five populations, where we observed a relatively small increase in parasite abundance in lizards in the short and mid/control basking treatment compared to the long treatment. This difference between populations suggests a context-dependent impact of basking opportunities on the capability of lizards to clear parasite infections, under the warm environment treatment (9-h) and provides further evidence that ectothermic host-blood parasite relationships are likely to be impacted by future and contemporary climate change.

The results of this dissertation ultimately highlight the complex impacts climate change can have on these organisms and will hopefully encourage further research while raising awareness of this pressing issue.

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Introduction

Broad context

Investigating if and how organisms adapt to novel environments is not a new topic in science. Organisms, like *Escherichia coli,* that have shown their ability to adapt over thousands of generations in the lab (Lenski et al. 1991) and the rare minnows that manage to live in hot springs and thrive (Vinyard 1996), are just a few of the extraordinary examples from natural systems. As one moves onto larger organisms with longer generation times, the opportunity and ability to quickly move through the process of natural selection diminishes greatly. Now, we as scientists have a relatively new global scientific experiment in asking whether and/or how animals will (or will not) adjust to novel environments: climate change. Climate change is arguably one of the greatest threats to our planet (if you don't count the humans causing it) and it is happening faster than we previously thought (IPCC 2018).

Climate change is predicted to severely impact organisms across the globe in the coming decades, with ectotherms being of particular concern due to their dependence on environmental temperatures to survive and reproduce (Kearney et al. 2009). For example, actual extirpations of present-day lizard populations suggest levels of extinction will reach 30% by 2080 due to the joint impacts of ecophysiological limits being exceeded (20%) and impacts from competition (~10%) (Sinervo et al. 2010, 2018). This is due to the expectation of extreme weather events and global mean temperatures increasing which will modify species ranges and increase hours of restriction (already observed during a severe warm spell in Mexico in 1998; Sinervo et al. 2011), essentially modifying when these organisms can carry out important functions such as feeding or breeding (Kearney 2013; Sinervo et al. 2018). While at a broad scale we predict organisms will face challenges as a result of climate change, delving deeper into whether they show evidence of being able to cope with ongoing and future changes is critical in determining their extinction risk and making decisions around their conservation.

As an ectotherm, the ability and opportunity to properly thermoregulate has important fitness consequences. These consequences range from maximizing energy demands (Glanville and Seebacher 2006), to improving performance (Clusella-Trullas et al. 2011), maintaining temperatures in a range that does not harm embryos in viviparous taxa (Beuchat 1988; Rodríguez-Díaz and Brañ 2011) and even influencing host-parasite interactions (Oppliger et al. 1996; Paranjpe et al. 2014). Climate change has the capacity to alter thermoregulatory opportunity and, as such, is likely to have cascading impact on the aforementioned traits.

In this dissertation I address three sub-topics that are expected to be impacted as a result of climate change in the viviparous European common lizards (*Zootoca vivipara*). Climate change is expected to raise mean global temperatures, while additionally increasing the occurrence and intensity of extreme weather events (Buckley and Huey 2016a). Therefore, I implemented thermal treatments in the lab that were designed to represent the ecological variation in thermal extremes predicted under climate change, using a 3-hour treatment, 6-hour treatment (used as control) and a 9-hour access to heat treatment as the set-up for the questions I ask in all three of my chapters. Short treatments reflect cold spells or cloud cover and the long treatment reflects more consistently warm environments (Easterling et al. 2000; Meehl and Tebaldi 2004; Hare and Cree 2010; Sunday et al. 2014). The control of six hours is the average daily time this species spends thermoregulating in the field and was allotted in prior laboratory studies on this species (Rodríguez-Díaz and Brañ 2011; Rutschmann et al. 2016b). Using this experimental design, I investigated: if females are able to respond to these treatments through modification of their thermoregulatory behavior (**Chapter 1**), if gestation time and offspring phenotype are affected (**Chapter 2**), and if host-parasite interactions change over time in response to these basking treatments (**Chapter 3**), all while considering variation between populations.

All of these topics are critical to address because anthropogenic climate change continues to threaten species persistence. Using a cross-sectional approach, I was able to experimentally probe how differences in basking opportunity influenced important aspects relevant to common lizard survival and reproductive success in a rapidly changing climate.

Dissertation Outline

In **Chapter 1,** “Interpopulation variation in thermoregulatory response to basking opportunity of common lizards (*Zootoca vivipara*)”, I specifically investigate the ability of pregnant females to modify their behavior in response to these basking opportunities and how their responses vary as a result of the climate variability that differs across the populations. I found time of emergence varied across populations, with populations at higher elevations emerging significantly earlier than those at lower elevations, supporting previous work showing these lizards have to bask more at higher elevations (Gvoždík 2002). In addition, thermal range of a population (the breadth between the minimum temperature and maximum environmental temperature recorded at a population) was strongly correlated with the amount of time spent basking between treatments. Specifically, populations that experienced the largest breadths in maximum and minimum daily air temperature showed the most dramatic differences in time spent basking between the extreme treatments in support of the climate variability hypothesis (Gutiérrez-Pesquera et al. 2016). This suggests those populations that experience more extreme fluctuations in temperature are better able to respond to changes in their environment and showcases the degree of variation that exists among populations in the capacity to modify their thermoregulatory behavior.

In **Chapter 2,** "Effects of basking opportunity during gestation on offspring phenotype of the common lizard *(Zootoca vivipara*)", I investigated if and how basking opportunity affected gestation time and ultimately offspring phenotype and viability. I found that increased thermal opportunity resulted in smaller offspring overall, but sex-dependent differences remained, with males being smaller than females across treatments. In addition, female offspring had differential survival (during gestation) between treatments, having lower viability in the short and higher viability in the long basking treatment, while male viability was equal amongst treatments. These results showcase that even a short period of time in these treatments has the capacity to significantly influence offspring phenotype. As such, the extreme weather events predicted under future climate change may severely impact this species.

In **Chapter 3,** "An experimental study of host-parasite interactions of the common lizard (*Zootoca vivipara*) in the wake of climate change", I investigated if the relationship between this species and their blood parasite, *Karyloysus,* is modified in response to these basking opportunities. Specifically, I asked if thermal environment affects their ability to fight off an infection. In three out of five populations sampled, parasite abundance was significantly higher at the end of exposure to the long treatment (9-h) as opposed to the short (3-h) or mid/control (6-h) treatment. This matched the findings that warmer populations in general had higher parasite loads. As such, the global increase in mean temperature has the potential to significantly impact these host-parasite interactions.

The results of this dissertation highlight the complexity of the impacts of a changing climate on organisms and how nuanced it can be within a single species when also taking into consideration interpopulation variation.

Chapter 1: Interpopulation variation in thermoregulatory response to basking opportunity of common lizards (*Zootoca vivipara)*

Abstract

Rising temperatures due to climate change pose new challenges to ectotherms that use behavioral thermoregulation. These rising temperatures are of particular concern to viviparous (live-bearing) organisms where females need to thermoregulate within a precise range of body temperatures during embryogenesis. Modification of basking behavior in response to prolonged exposure to extreme environmental temperatures is one potential avenue females can use to avoid overheating. We used the European common lizard (*Zootoca vivipara*), a cold-adapted specialist, to investigate behavioral plasticity in thermoregulation. We captured pregnant females (N = 207) from nine populations across an elevational gradient and split them amongst three experimental basking conditions until parturition. These treatments were designed to represent the observed ecological variation in climate extremes in studied populations: a 3-hour treatment (low basking opportunity), 6-hour treatment (used as control) and a 9-hour treatment (prolonged basking). We performed behavioral assays to determine whether the time available for basking affected the time of emergence and subsequent basking behavior. We also investigated if the patterns were influenced by the population of origin. We found that female basking responses to treatments correlated to the natural thermal characteristics of their

population. Specifically, females from populations that experienced wider breadths of thermal range showed greater basking responses to treatments, in support of the climatic variability hypothesis. In addition, time of emergence appeared to be a conserved trait as it was not affected by treatments but rather highly linked to the elevation of a population. This work adds to the growing body of knowledge on how organisms may respond to climate change and warrants a need for further studies that consider a variety of populations that may differ in selection pressures to draw species-level conclusions.

Introduction

Climate change is happening at rates faster than previously predicted, with widespread local population extinctions of many organisms as a direct result (Hof et al. 2011; Urban 2015; IPCC 2018; Resplandy et al. 2018). The persistence of ectotherms is of particular concern due to their dependence on environmental temperatures to regulate their own body temperature and perform all necessary functions (Kearney et al. 2009) (Fig 1.1). Lizards are an ideal model system for studying the physiological and behavioral costs and constraints imposed by climate change. Warmer mean environmental temperatures and increased extreme weather events are predicted to limit their hours of activity and increase energy costs as a result (Sinervo et al. 2010). Actual measurements of extirpations of contemporary lizard populations suggest levels of extinction will reach 30% by 2080 from ecophysiological limits being exceeded (20% extinctions, worldwide, but higher in the tropics, 40%) or from invasion of a competitor with a superior thermal niche

(~10%) (Sinervo et al. 2010, 2018). Such predictions warrant the need to investigate the capabilities of species to mitigate these ecophysiological costs through modification of thermoregulatory behavior (Huey et al. 2012).

In heliothermic reptiles, thermoregulatory behavior refers to the decision made by an individual to bask versus remain in thermal refugia or carry out other activities based on environmental temperatures (Huey 1982) and has the potential to be critically impacted by climate change. If the normal range of temperatures for behavioral thermoregulation is surpassed, animals risk death from their upper critical thermal limits being exceeded (Sinervo et al. 2010; Sunday et al. 2014).

In addition, thermoregulation during reproduction imposes even greater thermal constraints as temperature during embryogenesis can critically impact offspring viability and other phenotypic traits such as size or performance (Lourdais et al. 2004; Yan et al. 2011). Oviparous females retain eggs for a shorter time; hence the effects of maternal thermoregulation may have a limited effect on embryological development compared to nest site placement. In contrast, viviparous (live-bearing) lizards have a higher risk of exposure to temperatures deleterious to embryonic development. The prevailing hypothesis of the origin of viviparity is that this mode of reproduction evolved in cold climates such that females could compensate for cold conditions by maximizing time basking and optimize thermal conditions for embryo development (Elphick and Shine 2002). Viviparous mothers are able to regulate their body temperatures during development, which is not possible when eggs are oviposited in the environment. Several studies have shown that pregnant female lizards have lower preferred temperatures (T_{pref}), which is defined as the average temperature an individual chooses when allowed to freely thermoregulate, and field active (T_b) the temperatures recorded upon capture in the field (Le Galliard et al. 2003). Thus, recent analyses predict viviparous species are expected to be especially vulnerable to hotter environments based on current global warming trends (Ji et al. 2007; Pincheira-Donoso et al. 2013). For example, viviparous *Sceloporus* lizards in Mexico have a rate of climate forced extirpation that is 5 fold-higher at the low elevation limit of their range compared to oviparous lizards (Sinervo et al. 2010). This increased risk is in part due to the conserved nature of thermal physiology traits and thus investigating how behavioral modifications may assist ectotherms in getting around these restrictions is critical (Van Damme et al. 1990).

The viviparous European common lizard (*Zootoca vivipara*) is a model system for investigating the capacity of ectotherms to mediate predicted future climatic changes via modifications of thermoregulatory behavior. Females of this species have already been shown to select for lower body temperature during gestation, suggesting increasing global mean temperature will impose constraints their ability to properly thermoregulate (Le Galliard et al. 2003).

For this study we addressed the following two questions:

1. Do *Zootoca vivipara* exhibit behavioral responses to new environmental conditions via behavioral modifications?
2. Does population of origin play a role in that response?

We combined a cross-sectional study and experimental manipulations to answer these questions and determine factors shaping thermoregulatory behavior. We studied how intraspecific variation in thermal behavior of pregnant females (N = 207) captured from 9 populations co-varied with climatic gradients and whether these behaviors were exacerbated or mitigated by rapid changes in their thermal environment. That is, we randomly assigned pregnant females to one of three treatments that differed in the duration of available time for the lizard to bask (access to basking light): 3-hours (short), 6-hours (control) and 9-hours (long). Females were maintained in these treatments until parturition. Long and short treatments were chosen to represent ecologically relevant extremes that are predicted to increase as a result of climate change. The short treatment is an analog for prolonged cold spells or increasing cloud cover, which lowers thermal opportunities whereas the long treatment portrays anticipated warmer environments which can restrict activity if it is too warm and force females to retreat to shelter (Easterling et al. 2000; Meehl and Tebaldi 2004; Hare and Cree 2010; Sunday et al. 2014). The control treatment of six hours is the average daily time spent thermoregulating in the field. Moreover, the six hour treatment matches the time females were provided basking opportunities in the lab during previous experiments (Rodríguez-Díaz and Brañ 2011; Rutschmann et al. 2016b).

We additionally characterized the thermal microclimatic conditions at each population. We calculated an index of “thermal range” as the difference between the minimum (T_{min}) and maximum daily air temperatures (T_{max}) experienced by the lizards because use of only the mean daily temperature (T_{mean}) masks the extreme temperatures organisms face in their respective environments (Camacho et al. 2015). Impacts of thermal range *per se* is still widely unexplored but it is hypothesized organisms in more fluctuating environments should have increased acclimation capacity and/or more genetic variation due to exposure to different selection pressures invoked by their local environments, a concept known as the climatic variability hypothesis (Hoffmann and Sgrò 2011; Clusella-Trullas et al. 2011; Bozinovic et al. 2011; Gutiérrez-Pesquera et al. 2016; Sheldon and Dillon 2016; Sears et al. 2016).

We hypothesized that females would optimize their time basking as a result of these treatments, with females basking significantly more in the short treatment during our behavioral surveys when they only have 3-hours per day to bask vs the 9-hour treatment where females have opportunities to shuttle in and out from the basking light. Similar results have been shown in a previous study in other cool-adapted lizards in the genus *Sceloporus*, which do not use the full range of time during the day, compared to hot-adapted populations that use all the time provided (Sinervo and Adolph 1989; Sinervo 1990a). In addition, we hypothesized that initial variation and changes in thermal behavior would coincide with environmental conditions. We predicted a response in support of local adaptation in conjunction with the countergradient theory, which indicates populations at lower elevations should bask less overall and select lower temperatures, because their habitats are generally warmer and the opposite result for higher elevations (Laugen et al. 2003). This expectation is supported by previous work showing that at higher elevations *Z.*

vivipara bask significantly longer (Gvoždík 2002). Lastly, we posited field temperatures experienced by females would influence their response to treatments, with individuals from more variable environments being more responsive to local thermal opportunities for basking (Paaijmans et al. 2013). We quantified "basking response", which represents the mean difference in time spent basking between individuals in long and short treatments by population, essentially representing the difference in the proportion of time spent basking by the two extreme treatments per population. Because we have sampled multiple populations along an elevational gradient, we have the opportunity to use intraspecific variation to create a comprehensive profile of how a species may respond to rapidly changing thermal environments (Herrando-Pérez et al. 2019).

Materials & Methods

Study species and populations

The common lizard *Zootoca vivipara (Lacertidae)* occurs across Eurasia and has two reproductive modes. Most populations are viviparous in the northern part of the range, but oviparous in the Pyrenees, the Southern Alps and the Mountain ranges in the Balkan Peninsula (Surget-Groba et al. 2006). In the viviparous populations of the Massif Central of France we studied, males and females emerge from hibernation in April-May. Courtship and mating occur directly after emergence from hibernation. Gestation occurs from mid-late May through mid-late July. Parturition historically occurred late July through early August. However, recent changes in breeding phenology as a result of climate change has significantly advanced the dates of parturition with some populations having an onset of birth as early as late June in warmer years and all populations finishing before August (Rutschmann et al. 2016b).

Field data

We spent approximately one – two days capturing females at each site mid-late June of 2018. We used three iButton ® temperature data loggers (Maxim Integrated Products, Sunnyvale, CA, USA, ±0.0625°C) per population to record ambient temperatures. Loggers were placed in shaded vegetation and have been successfully used in previous studies to record daily temperatures experienced at a finer scale (Rutschmann et al. 2016c, b; Dupoué et al. 2017). We measured microclimatic temperatures at each site from date of capture until we released females post parturition. We used a standardized sampling period from June 29th to July 21st in 2018 to calculate temperature variables such as T_{mean} (mean temperature averaged over the population) and thermal range (difference between T_{min} and T_{max} of a population) (Table 1.2).

Experimental Design

We captured female lizards from nine different field locations. We obtained approximately 24 females per population (N=207 in total) distributed along an elevational gradient. These populations have been sampled every other year as part of an ongoing long-term monitoring study in the Massif Central (Table 1.2). Each female was given a unique toe clip for individual identification, brought into the lab and measured for the following: SVL, mass, external parasites, and ventral coloration

(Vercken et al. 2006). External parasites were removed upon entry into the lab and females were treated with 0.29% fipronil (Frontline®) to ensure mites were eliminated per methods described in (Wiechert 2007). Females were individually housed in a 11 x 18 x 11 cm plastic terrarium with a layer of sterilized soil and a heat lamp (25 W) on one side and a cardboard shelter on the other (Massot and Clobert 2000). Females were fed every 3 days and watered three times daily.

After a 48-hour acclimation period in standard conditions of six hours basking time, we measured T_{pref} of post-absorptive females using methods described in (Artacho et al. 2017). In brief, females were placed in a thermal gradient made of long planter boxes (125 cm in length) consisting of a lightbulb at one end and nothing at the other with a temperature gradient of approximately 22-43°C from the cool to warm end. Females were matched up by size and two females were run in each box at a time (in order to measure all females due to space and time limitations) and allowed to freely move along the thermal gradient. Previous work has shown this species have similar body temperatures whether tested alone or in groups (Patterson and Davies 1978). We recorded the temperature of each female using an infrared thermometer (Raytek RAYST61 ST Pro Plus Infrared Thermometer, Raytek Corporation, Santa Cruz, USA) and took a body temperature measurement every 15 minutes for 3 hours yielding 24 records per female. Previous work has confirmed cloacal temperatures measured highly correlated to surface skin temperatures supporting the less invasive use of an IR-gun to infer temperature preference (Artacho et al. 2017). This T_{pref} data represents acclimatization and /or evolved differences among individuals either due to local environments that they have experienced during their life (acclimatization) and/or remote past (evolved).

After the initial phase where we assessed female T_{pref}, females were randomly assigned to a treatment of either 3-hours (short), 6-hours (control) or 9-hours (long) of basking time. Females did not differ in body mass (short: N=70, mass = 4.73 ± 0.15 g; mid/control: N=67, 4.76 ± 0.16 g; long: N=70, 4.64 ± .15 g) among treatments ($F_{2,205}$=0.17; p =0.84). Lights were on timers from 9 AM (all treatments) to 12 PM for the first treatment, 4 PM for the next, and 7 PM for the last. We kept the lights off for a period of one hour (12-1 pm) for all treatments, this coincides with the peak of the heat of the day and at a time when lizards are generally inactive in the field.

Basking behavior

After 12 – 14 days in the treatment the behaviors of females were sampled to assess impacts of their time in treatments. We recorded observations between 8:30 AM and 12 PM at fifteen-minute intervals. We began focal sampling at 8:30, 30 minutes before the basking lights turned on to determine the time of first emergence. Behavior was then sampled (at a single point) by scanning the terrariums every 15 minutes by an naive observer who recorded the behavior of each female using the following code: 1-Basking, the female was entirely outside or on top of the cardboard shelter; 2-Half-Basking, female was half exposed out of the shelter; 3-Active, female was actively moving around the enclosure, and 4-Hiding, female was within the shelter or buried in the soil. These behavioral categories were previously confirmed to capture the range of behaviors exhibited by females (Cote et al. 2006).

Statistical Analysis

All data were analyzed in the R statistical environment v. 3.5.3 (R Core Team 2019) and JMP version 14. T_{pref} was calculated as an average of all the temperatures recorded for an individual during the trial (N = 14 recorded temperatures per individual) as per methods described in (Gilbert and Miles 2017). A generalized regression in JMP was used to evaluate how T_{pref} (the response variable) differed among populations. An adaptive lasso with AIC validation was applied to determine the best fit model for explaining population variation while including environmental characteristics including elevation, T_{mean} and thermal range as variables. For time of first emergence for each observation between 8:30 AM-12:00 PM we translated times into minutes, with -30 representing 8:30 AM before lights came on to +180 at 12 PM when observations finished. Data were analyzed with a generalized linear model using elevation, treatment and their interaction as fixed independent variables and used the Anova function in the R package car to determine to interpret the model results. Individuals that never emerged by the time the focal sampling ended were excluded (N=8 out of 207 obs.). We next analyzed the amount of time spent basking within the 9-12 pm window when lights were on for all treatments by population using a generalized regression with zero inflated Poisson distribution to account for the number of zeros (implying an individual never performed said behavior in the 3-hour period) and determined a posteriori the best model fit using AIC validation. We then used the difference in basking time between the short and long treatment as a metric to measure "basking response" within population and compared this to the thermal range of a population. In addition, because pregnant females show changes in their thermoregulatory behavior throughout gestation, we verified whether the pattern of basking behavior was related to a female's gestational state (e.g., days before parturition) or body size (Le Galliard et al. 2003). Values are reported throughout as the mean ± standard error (SE).

Results

Thermal Preference

Thermal preference (T_{pref}) differed among populations ($F_{8,194}$4.82, $p <.0001$) and was strongly influenced by an interaction between elevation, thermal range and mean temperature ($F_{7,195}$=5.42, $p<.0001$) with individuals from lower elevations and warmer environments generally exhibiting lower thermal preferences. Female size ($F_{1,203}$=21.44, $p<.0001$) was significant as well, with larger females exhibiting lower temperatures. Proximity to parturition had no influence on T_{pref} ($F_{20,169}$=1.19, $p=0.27$).

Time of first emergence

Time of first emergence was significantly influenced by elevation ($\chi^2_1 = 8.53$, $p=0.0035$), while effects of treatment were non-significant ($\chi^2_2 = 1.13$ $p =0.57$) and there was no significant interaction between the two ($\chi^2_2 = 1.48$, $p =0.48$) (Figure 1.3). The populations at the highest elevations, BEL and BOB emerged before lights came on. The time of emergence before the onset of warm conditions was approximately 10 minutes. In contrast, the population at the lowest elevation (MON) emerged 24 minutes on average after lights came on (Table 1.4).

Basking behavior

We found T_{mean} (χ^2_1 = 4.47, p=0.03) and the quadratic term for T_{pref}^2 (χ^2_1 = 6.30, p=0.012) best explained the amount time spent basking by individuals within a population regardless of treatment (Figure 1.5). In addition, the thermal range at the population location significantly influenced the basking response between short and long treatments (R^2 =0.52, $F_{1,25}$=26.58, p <.0001) (Figure 1.6). However, there was no influence of gestational state on basking behavior ($F_{21,184}$=0.92, p=0.56) nor was there influence of female size ($F_{1,203}$=.06, p=0.8234).

Discussion

There are both costs and benefits for the flexibility that viviparity brings to females for maintaining body temperature under a range of conditions. While it allows females flexibility in temperature selection during gestation, it also means they need to be able to respond to both short and long-term climatic events that may occur in their environment. Our results show these lizards exhibit plasticity in basking behavior in response to varying thermal opportunity. However, the magnitude to which they modify their behavior is significantly tempered by environmental characteristics of their population of origin.

Population variation in thermal preference was influenced by an interaction between elevation, T_{mean} and thermal range. This finding supports the countergradient effect whereby populations at lower elevations (generally warmer conditions) have lower T_{pref}. This has been similarly shown in other lizard species like *Liolaemus pictus* (Artacho et al. 2017) and in frog larvae of *Rana sylvatica*, with tadpoles from closed-canopy ponds (cooler and more stable temperatures) having higher preferred temperatures than tadpoles in open-canopy ponds (warmer and more variable temperatures) (Kealoha Freidenburg and Skelly 2004). Another study showed that exposure to temperature fluctuations reduced the optimum and critical maximum temperature of mosquitos (Paaijmans et al. 2013). We find a similar effect is occurring in these lizards. This conflict of selecting for cooler temperatures in warmer environments has alarming ecophysiological implications if global mean temperatures continue to increase as suggested from predicted trends in climate change.

Daily time of emergence was negatively correlated to elevation, which we would expect considering common lizards at higher elevations require more basking time (Gvoždík 2002). The populations at the highest elevations, BEL and BOB emerged before lights came on approximately 10 minutes prior to the onset of warm conditions. In contrast, the population at the lowest elevation (MON) emerged 24 minutes on average after lights came on (Table 1.4). These responses suggest individuals from higher elevations benefit from emerging earlier since they are known to require more basking time. Furthermore, treatment had no influence on time of emergence, indicating it is likely a conserved trait controlled by a circadian rhythm (See Fig. 1.3). This is further supported by studies showing circadian rhythms in lizards are deeply entrained and can remain even in complete darkness (Ellis et al.

2008; Clusella-Trullas and Chown 2014).

Amount of time spent basking varied between populations as a result of T_{mean} and T_{pref}^2, regardless of treatments. Organisms from warmer environments basked less overall and exhibited lower T_{pref}. This additional evidence supports the countergradient pattern; individuals originating from warmer environments have less necessity to emerge early. A striking finding of this study was the strong and positive correlation between treatment induced basking response and thermal range experienced by individuals of a population. Individuals originating from more thermally variable environments showed greater differences between treatments in the amount of time spent basking (Fig. 1.6). This pattern is particularly evident when comparing the two populations that show the most drastically opposing basking responses, MON and PAR. PAR is the most thermally varied environment and had the greatest distinction between the short and long treatment, with individuals in the short treatment basking nearly twice as much as those in the long treatment during the 3-hour survey period. MON, the population with the most stable and least varied environment, in contrast, had no discernible treatment effects. MON, in addition, is classified as endangered according to IUCN guidelines (Dupoué et al. 2017) and continues to support predictions that it is vulnerable to collapse in other studies, from having high pronounced stress responses and shorter telomeres in comparison to other populations (Dupoué et al. 2017, 2018). While these results support the hypothesis that organisms exposed to more fluctuating environments are likely to have higher acclimation potential, thermal range is shown to have a complex and interactive effect on fitness (Bozinovic et al. 2011). Thus, further studies will be required to better determine how adaptive these behavioral responses are in the context of climate change.

Patterns of selection are expected to change as novel thermal challenges arise as a result of climate change and determine the critical outcome of phenotypic plasticity and/or adaptive thermoregulatory behavior in assessing a species persistence (Hoffmann and Sgrò 2011). A critical next step is to initiate genetic studies for examining the potential for adaptive evolution in response to shifting thermal environments. Estimates of heritability and genetic variation of a population would provide insights into the relative roles of phenotypic plasticity, adaptive evolution or a combination There is a dearth of studies linking climate change to direct genetic changes and thus more work looking at the molecular underpinnings (e.g., analysis of transcriptomes) of our findings on plasticity (Corl et al. 2018) would move the field in an important direction (Merilä and Hendry 2014). In addition, based on the link we observed between thermoregulatory behavior and thermal range of an environment, a similar study comparing the effects of fluctuating versus stable thermal treatments rather than standard consistent timed treatments would be of further interest. Finally, we only performed this experiment on pregnant females but looking at variation between sexes could potentially provide more answers to just how plastic (or conserved) these behaviors are between populations.

We have shown the ability of these populations to mitigate temperature extremes predicted by climate change via behavioral adjustment is largely dependent on their environmental context and supports our hypothesis that local environmental conditions play a significant role in shaping thermoregulatory behavior plasticity in this species. One major insight from this work is the importance of considering variation among populations. Our study incorporated nine populations along an elevational gradient that differed in thermal profiles as well as vegetation and humidity allowing us to capture a broad range of habitat types. If we had only chosen two populations, we would have certainly missed the incredible levels of variation that exists. Our research indicates that these lizards have the capacity for adjusting thermoregulatory behavior in the short-term to experimentally induced variation in thermal opportunity. However, our results revealed that shifts in regulatory behavior to laboratory treatments in the laboratory are also contingent on prevailing local population environmental conditions. Current climate projections suggest more extreme weather patterns and unpredictable seasons between years in the future. Thus, subsequent investigations into how ectotherms can mitigate the ecophysiological costs at both the intra- and interspecific level is crucial.

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Tables and Figures

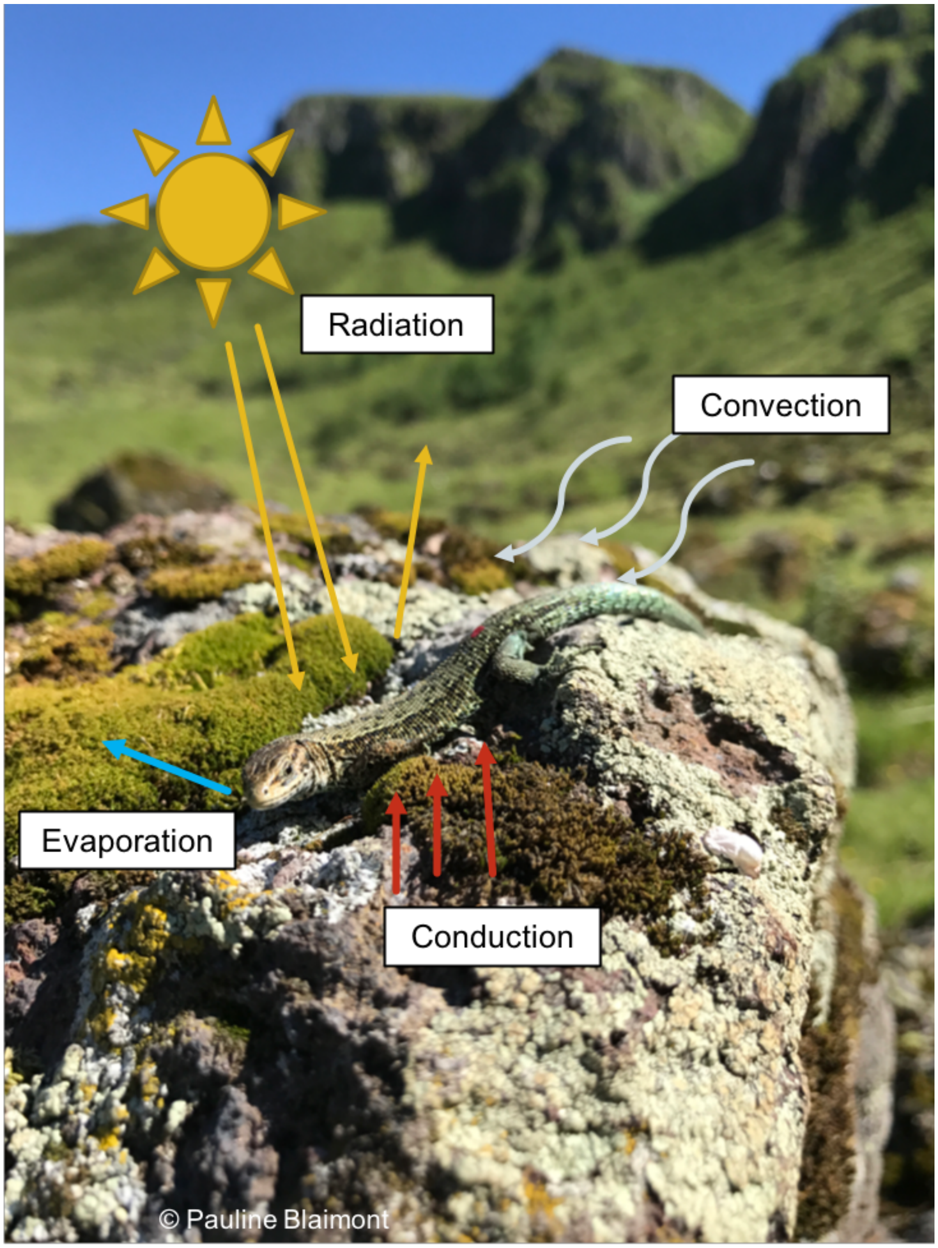

Figure 1.1. Example of ectotherm thermoregulation in the Common lizard (*Zootoca vivipara*). The text boxes represent the different components of heat gain and loss which all factor into regulating body temperature in ectotherms. Photo Credit: Pauline Blaimont.

Table 1.2. Summary of populations used in the study including codes, coordinates, elevation, maximum, minimum, mean temperature and thermal range. Thermal range is given by (Δ T_{min} and T_{max}) experienced in the interval between capture and release of individuals in 2018. We deployed iButton® data loggers in the field between June 29th and July 21st, 2018 to obtain thermal measurements.

Population	Pop Code	Coordinates	Elevation (m)	Tmin (°C)	Tmax (°C)	Tmean (°C)	Thermal Range (°C)
Montselgues	MON	N44° 30.613', E4° 00.485'	1049	10.4	28.45	17.62	18.05
Carmantran	CARM	N45° 09.474', E2° 50.273'	1212	6.81	32.11	17.32	25.3
Lajo	LAJO	N44° 50.660', E3° 25.864'	1330	8.71	32.35	16.94	23.64
Barraque Bouvier	BOU	N44° 45.225', E3° 30.923'	1390	8.33	29.49	16.64	21.16
Lou Paradis	PAR	N44° 36.063', E3° 33.583'	1396	7	34.16	18.91	27.16
Barrandon	BAR	N44° 26.534', E3° 37.108'	1403	8.96	29.43	16.06	20.47
Col du Cheval Mort	COM	N44° 40.041', E3° 31.916'	1405	8.8	27.44	16.1	18.64
Belair	BEL	N44° 40.360', E4° 01.492'	1418	8.15	34.49	16.52	26.34
Bout de la Barre	BOB	N44° 49.437', E4° 13.694'	1432	8.44	28.86	15.66	20.42

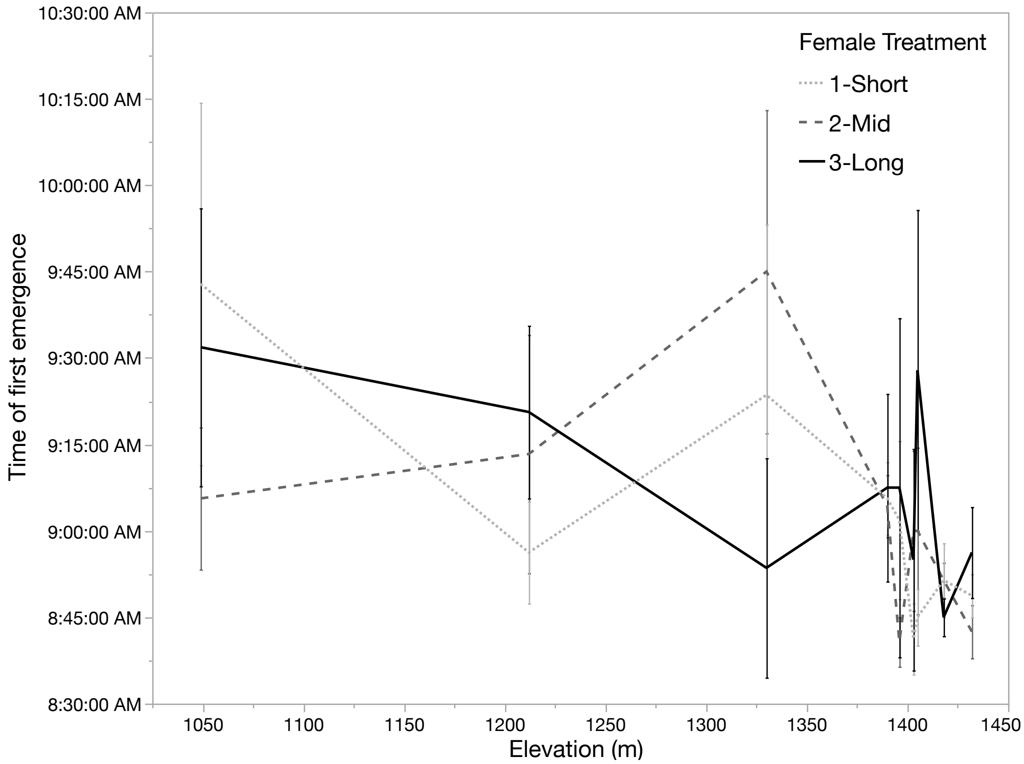

Figure 1.3. Population time of first emergence by treatment (ordered from lowest elevation to highest). Time of first emergence was significantly influenced by elevation (χ^2_1 = 8.53, p=0.0035), while effects of treatment were non-significant (χ^2_2 = 1.13 p =0.57) and there was no significant interaction between the two (χ^2_2 = 1.48, p =0.48).

Table 1.4. Average emergence times for each population in relation to elevation. A positive value means that individuals of a population emerged came out *n* minutes on average after lights came on, whereas a negative value means individuals of a population emerged *n* minutes on average before the lights came on. Values are reported as the mean ± SE.

Population	Elevation (m)	Emergence Time Average (Minutes)
MON	1049	24.55 ± 13.69
CARM	1212	10.2 ± 9.15
LAJ	1330	18.158 ± 14.89
BOU	1390	11.25 ± 7.88
PAR	1396	-3.57 ± 9.76
BAR	1403	-7.5 ± 7.66
COM	1405	8.75 ± 11.2
BEL	1418	-10.5 ± 2.69
BOB	1432	-10.23 ± 3.47

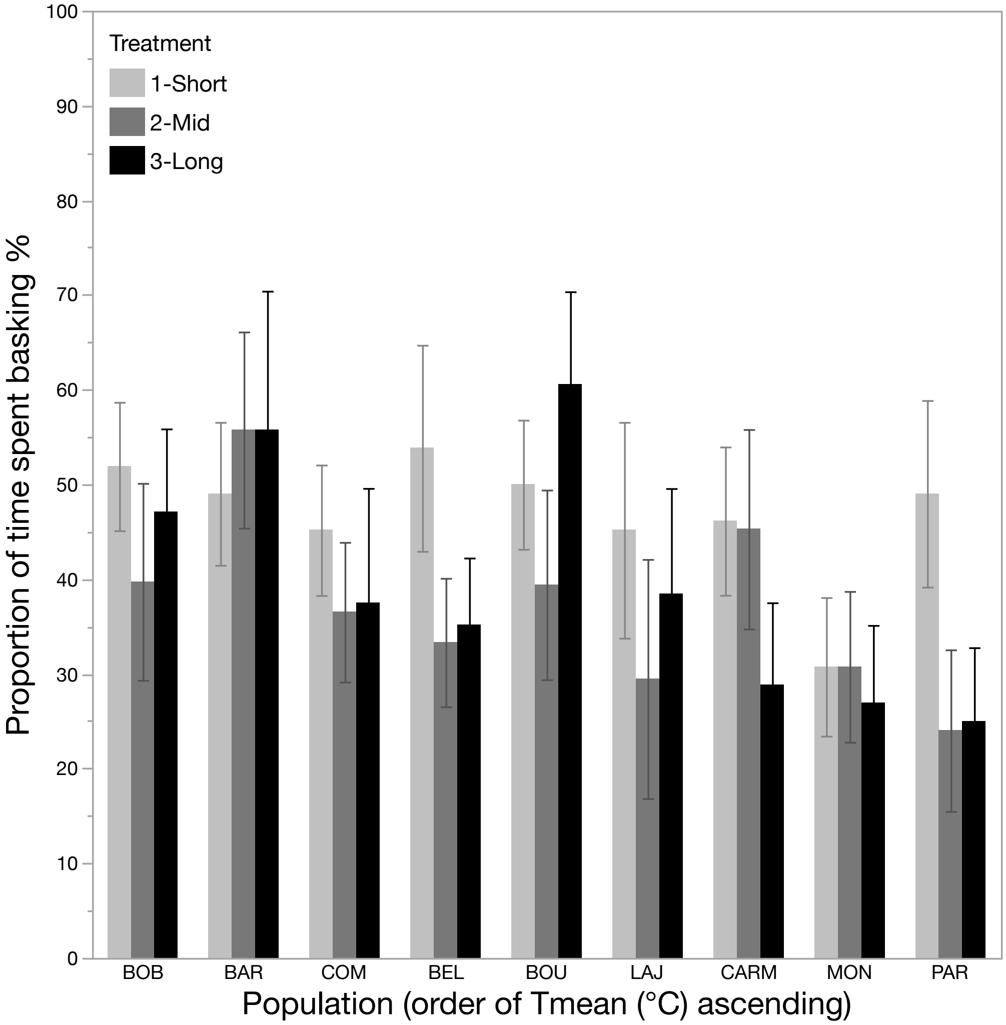

Figure 1.5. Mean proportion of time lizards spent fully out basking by population and treatment (± S.E.) is shown.

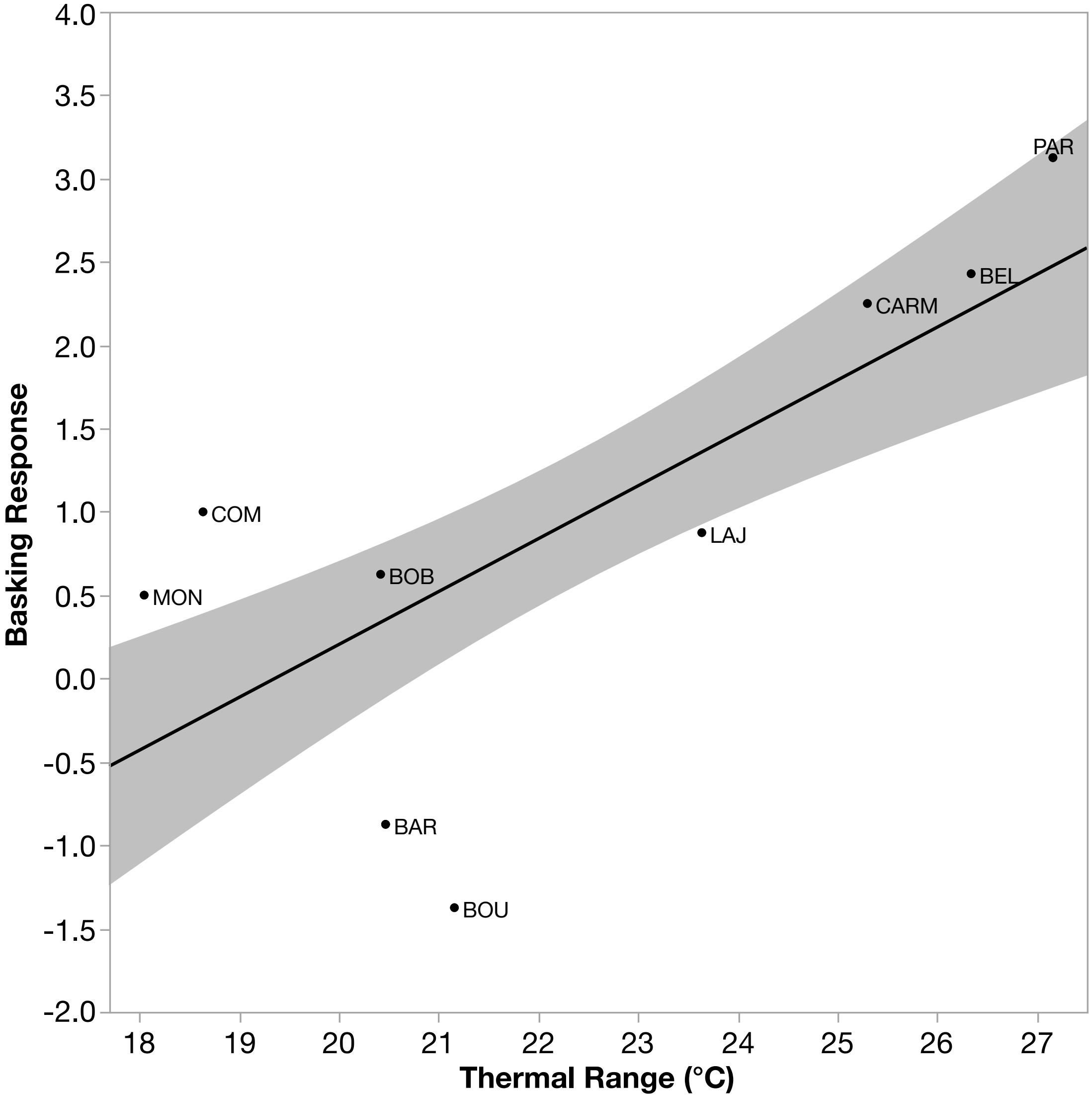

Figure 1.6. Correlation of thermal range (△ T_{min} and T_{max}) at each site and differences in basking response (proportion of time spent basking) between short and long treatments among populations ([mean short basking time- mean long basking time] for each population). Populations that experienced the widest range of temperatures at their local population exhibited greater differences in basking time response between extreme treatments.

Chapter 2: Effects of basking opportunity during gestation on offspring phenotype of the common lizard (*Zootoca vivipara)*

Abstract

Viviparous species in particular are presumed to have a higher extinction risk under climate change as viviparity is thought to be an evolved response to cold climates, allowing females to better control temperatures during embryogenesis compared to oviparous species. Here, we study how various climate forecasts impact gestation and offspring phenotype in a viviparous ectotherm, the European common lizard (*Zootoca vivipara*). We captured pregnant female lizards from 17 locations in the Massif Central, France between mid-late June in 2017 and 2018. Females were randomly assigned to a treatment of either 3-hours (short), 6-hours (control) or 9-hours (long) of basking time. We found a joint effect of population of origin and maternal basking treatment on the phenotype of neonates. However, the expression of phenotype in response to the basking treatments differed depending on the sex of the offspring. Females with longer access to heat produced smaller offspring. We detected treatment effects on the viability of female neonates, but not in males. In addition, we did not detect evidence for maternal effects on offspring thermal preference. Our findings suggest that shorter and longer basking opportunity have dissimilar effects on fitness-related traits of the progeny and altered phenotypes in

response to different basking opportunity and may have cascading impacts under climate change.

Introduction

Ectotherms are of particular concern in the wake of climate change as they rely on external sources of heat to maintain their body temperature within a range that facilitates optimal performance to promote survival and reproduction (Huey 1982; Deutsch et al. 2008; Sinervo et al. 2010, 2018; Paaijmans et al. 2013). Predictions from the recent IPCC suggest that irreversible aspects of climate change are a mere decade away (IPCC 2018) and organisms are projected to experience dramatic changes (Wapstra et al. 2009; Santidrián Tomillo et al. 2015; Refsnider and Janzen 2016), with many, such as shifts in breeding phenology (Rutschmann et al. 2016b), already occurring. The stakes are increased during the breeding season for females of viviparous (live-bearing) species as proper embryonic development relies heavily on the female's ability to behaviorally mediate environmental temperatures (Huey 1982) and as such, it is likely to increase extinction risk in the face of climate change (Pincheira-Donoso et al. 2013). This is because environmental basking opportunity for viviparous females, particularly during gestation, is critical for reproductive success and climate change is expected to impact that ability through increased extreme weather events and overall global mean temperatures rising rapidly (Buckley and Huey 2016b). Indeed, the current extirpation risk is 5 times higher at the warmest parts of viviparous species ranges, compared to oviparous species ranges in Mexican

Sceloporus lizards (Sinervo et al. 2010) and viviparous females are likely to face similar challenges globally (Wang et al. 2017a).

An underappreciated aspect of species responses to rising temperatures are the shifts in offspring phenotypic traits as a consequence of temperature during embryogenesis. Elevated temperatures during development, predicted to occur as a result of climate change, regardless of reproductive mode, can have drastic and potentially long lasting consequences (Sinervo 1990b; Shine and Harlow 1993; Elphick and Shine 2002; Reedy et al. 2013; Noble et al. 2018). For example, in species with temperature-dependent sex determination the sensitivity to changes of even a few degrees Celsius can result in imbalanced sex ratios of offspring (Refsnider and Janzen 2016), even in viviparous species (Zhang et al. 2010; Tang et al. 2012). In some species warmer temperatures result in smaller offspring (Booth et al. 2004) whereas other species have larger offspring at warmer temperatures (Wapstra 2000; Elphick and Shine 2002), thus emphasizing the importance of maternal basking opportunity in determining offspring phenotype and ultimately fitness in a given environment. The European common lizard (*Zootoca vivipara*) is already shown to have complicated relationships with warming temperatures. Individuals gain a fitness advantage as offspring from earlier parturition in warmer years, allowing more time to grow before hibernation. Consequently individuals grow larger and faster increasing the fecundity of females the following year (Chamaille-Jammes et al. 2006). However, recent studies have shown this accelerated growth leads to a “live fast, die young” strategy, resulting in higher adult mortality (Bestion et al. 2015).

These examples showcase the variability in responses by species to climate change and one notable and underexplored pattern is the potential for ontogenetic conflict, or differential fitness impacts, between sexes or life-history stages.

Beyond attempting to control temperature, there is evidence females can influence developing offspring through maternal effects as well (Shine and Downes 1999; Swain and Jones 2006). In common lizards (*Z. vivipara*), corticosterone levels of pregnant females influence offspring locomotion (Meylan and Clobert 2004), maternal feeding rate affects offspring sprint speed (Sorci and Clobert 1997) and water restriction of pregnant females influences offspring behavior (Rozen-Rechels et al. 2018). However, the effects of altered basking opportunities for pregnant females on the phenotypes of offspring are unknown.

In this study we addressed the following questions. First, how is parturition date in females altered by differences in basking opportunity? Second, how is the viability and phenotype of neonates affected by female basking behavior? Third, does the thermal opportunity experienced by females during pregnancy affect the thermal preference of their neonates? Finally, how does local variation in the natal environment moderate responses to temperature variation?

To answer these questions, we captured female common lizards from 17 populations across the Massif Central region of France in mid to late June of 2017 and 2018 (See Figure 2.1). Females were randomly assigned to one of three basking opportunity treatments of 3-hour (short), 6-hour (control) and 9-hour (long) access to heat. These treatments allow us to test the sensitivity of gestation to temperature variation (Easterling et al. 2000; Meehl and Tebaldi 2004; Hare and Cree 2010; Sunday et al. 2014). Previous studies on this species have shown that temperatures experienced by females at mid-gestation influence the tradeoff between offspring number and size. The form of the tradeoff varies significantly among populations (Rutschmann et al. 2016a). We leveraged the results from recent studies to further investigate additional potential trade-offs that may arise in offspring size and survival in response to experimental manipulation of basking opportunity in pregnant females.

Previous work has shown how the duration of basking opportunity can influence reproductive performance in oviparous lizards. Here, we predicted that females with reduced basking opportunities should delay parturition, due to the lower availability of optimal developmental temperatures slowing development (Wapstra 2000; Wapstra et al. 2010; Tang et al. 2012). We also predicted that cooler developmental temperatures would result in larger offspring. In addition, we captured females from sites that represent a gradient in ambient temperatures. Thus, we predicted that the local ambient conditions of each population would modulate the relationship between developmental time and offspring phenotype. Furthermore, the thermal preference of neonates has yet to be assessed. In the oviparous lizard *Uta stansburiana* there is evidence that thermal preference of the offspring (T_{pref}) is influenced by conditions experienced by the dam (Paranjpe et al. 2013). Thus, we have a unique opportunity evaluate whether variation in thermal preference is influenced by maternal effects, local adaptation to environmental conditions, or evidence of phenotypic plasticity in this species.

In this study we use a cross-sectional approach to experimentally probe how differences in basking opportunities available to pregnant females influence offspring phenotype. We quantified sex ratio of the litter, neonate body size, and viability. We also measured thermal preferences and thermoregulatory behavior of offspring immediately after parturition.

Methods

Field collection

We captured gravid female lizards from 17 different sites along an elevational gradient in the Massif Central region of France (Figure 2.1). We captured females from nine sites in 2017 and nine in 2018. We sampled one population, Montselgues, in both 2017 and 2018. We placed three iButton ® temperature data loggers (Maxim Integrated Products, Sunnyvale, CA, USA, ±0.0625°C) per population. Loggers were placed in shaded vegetation as described in previous studies to record daily temperatures (Dupoué et al. 2017). We used a standardized sampling period from June 29th to July 21st in 2018 to calculate temperature variables. These included mean temperature (T_{mean}; mean temperature averaged over the population), minimum temperature (T_{min}), maximum temperature (T_{max}), and used it to additionally calculate thermal range (difference between T_{min} and T_{max} of a population) (Table 2.2).

Experimental Design

Females were given a unique toe clip identification (Perry et al. 2011) in the field before being brought into the lab and measured for the following: SVL (to the nearest .5 mm), mass (to the nearest .1 g), and ventral color. We placed females in individual plastic terrarium (11 x 18 x 11 cm) with a layer of sterilized soil and a heat lamp (25 W) on one side and a cardboard shelter on the other (Massot and Clobert 2000). Females were fed 2-3 times per week and watered three times daily. Females were given one day to acclimate and on day two, the post-absorptive females had their thermal preference (T_{pref}) experimentally measured using an IR thermometer (Raytek RAYST61 ST Pro Plus Infrared Thermometer, Raytek Corporation, Santa Cruz, USA) every 15 minutes for 3 hours with a thermal gradient per methods described in Artacho et al. (2017). Cloacal temperatures are highly correlated to surface skin temperatures in this species supporting the less invasive use of an IR-gun to infer temperature preference (Artacho et al. 2017). Females were matched up by size and two individuals were run per box at a time (in order to measure all females due to space and time limitations). Females were allowed to freely move along the thermal gradient and previous work has shown this species have similar body temperatures whether tested alone or in groups (Patterson and Davies 1978).

Females were then randomly assigned to one of three treatments that provided differing durations of basking opportunity. Females were exposed to either 3-hours (1-short), 6-hours (2-control) or 9-hours (3-long) of heat per day. These times were chosen based on 6 hours being the average time they spend thermoregulating in the field. In addition, 6 hours of basking has been the standard treatment for previous studies (Rodríguez-Díaz and Brañ 2011; Rutschmann et al. 2016a). We also ensured that the average female size among treatments was similar. Lights were set on timers to go on at 9 am and went off at 12 pm for the first treatment, 4 pm for the next, and 7 pm for the last with a lights-off period from 12-1 pm for all treatments.

Offspring Phenotype

We measured the body length (SVL to the nearest 0.5) and body mass (to the nearest 0.01g) of each neonate. We then determined the sex of each individual using scale counts (Lecomte et al. 1992). We scored the litter of each female by assigning offspring as viable, stillborn, or aborted. Unfertilized eggs were also recorded.

We measured T_{pref} of the offspring within 48 hours of birth. T_{pref} measurements were conducted in only 2018. We used the same methods as we did for the females but reduced the time in the gradient to 1 hour and sampled body temperature every 10 minutes. Behavior during T_{pref} was also recorded with "0" for out and actively thermoregulating and a "1" for hiding (buried) and an average was taken across the measurements to estimate the proportion of time each neonate spent basking or took refuge.

Statistical Analysis

All data were analyzed in R software (v 3.5.2, R Core Team) and JMP v. 14. Because females may have been at different stages of gestation when captured, we quantified "gestation time", which was the difference between the date of initiation into the treatments and the date of parturition. We used an ANOVA to assess whether there were treatment and/or population effects on the timing of parturition and a least square means contrast approach. We used elevation, T_{min} and T_{mean} as our independent factors looking at population effects on gestation time. We used T_{mean} as a proxy for T_{max} and thermal elevation due to a loss in degrees of freedom and T_{mean} was a covariate with T_{max} and thermal range. Juvenile analyses were performed using individual females as a random factor to account for litter effects, because siblings are non-independent (Massot et al. 1994). In addition, we considered the potential that male and female offspring may exhibit dissimilar responses in phenotype to the basking treatments and ran each analysis by sex. Viability for each offspring was designated as a binary variable: 0 (not viable) and 1 (viable). Viability data to discern treatments effects were analyzed using an ordinal logistic fit model for viable and non-viable progeny at birth in association with sex, treatment and population. Offspring size analysis by treatment was performed with a restricted maximum likelihood analysis. Offspring size analysis by population was performed using a mixed model using T_{min}, T_{max}, T_{mean} and elevation as population predictors. Female and hatchling T_{pref} was calculated by averaging their recorded temperatures (N=13 observations averaged per female/3 hours; N=6 observations per juvenile/1 hour) per standard methods as described in (Gilbert and Miles 2017). Generalized linear mixed models were used to examine the treatment effects of neonate T_{pref} and behavior. We included population as a random effect. We used a one-way ANOVA to determine those populations that were significantly different in juvenile behavior. Values are reported as the mean ± standard error (SE) unless otherwise noted.

Results

We captured 404 females during the 2-year study (N= 209 in 2017 and N=195 in 2018). Of these, 389 females gave birth (N=209 in 2017, N=184 in 2018). We obtained a total of 2,475 offspring [N=1,232 (Male N=642, Female N=413) in 2017; N=1,253 (Male N=706, Female N=426) in 2018], which were used in the following analyses (Table 2.3).

Gestation time

Gestation time differed among the populations ($F_{16,338} = 19.99$, $p < 0.0001$) and treatments ($F_{2,338} = 4.31$, $p = 0.014$), however their interaction was not significant ($F_{32,338} = 0.69$, $p = 0.90$), suggesting the that the effect of treatment was unaffected by population. Females in the 9-hour treatment gave birth earlier than those from the short treatments ($F_{1,338} = 8.28$, $p < 0.004$) (Table 2.3). A distribution of female parturition events by treatment is shown in Figure 2.4. T_{mean} was the only significant population factor influencing parturition date ($F_{3,385} = 10.57$, $p < 0.0001$).

Offspring viability

Offspring viability was significantly influenced by an interaction between offspring sex and treatment ($\chi^2_6 = 19.17$, $p = .0039$), with female offspring in the long treatment having significantly higher viability than female offspring in the short treatment and males having consistent viability across treatments (Figure 2.5).

Populations significantly differed in offspring viability as well [χ^2_{16} = 93.67, p < 0.0001 (Figure 2.6). Specifically, in T_{min} ($F_{1,1347}$= 19.24, p <.0001) and elevation ($F_{1,875.8}$= 4.87, p = 0.028), with greater offspring survival as T_{min} decreased and elevation increased.

Offspring morphology

Male offspring were significantly smaller than female offspring across all treatments (Male = 19.69 ± 0.03 mm; Female = 20.4 ± 0.037 mm; $F_{1,2181}$= 229.85, p < 0.0001). Exposure of females to different basking treatments significantly influenced offspring size. Females gave birth to smaller offspring regardless of sex in the 9-hour treatment than either the control or 3-hour treatments (Females – $F_{2,835}$= 4.34, p = 0.0136]; Males – $F_{2,1342}$= 9.09, p <.0001 (Figure 2.7). In addition, offspring size was also influenced by environmental variables of the source population. Specifically, T_{min} (Females – $F_{1,640.9}$= 24.78, p<.0001; Males – $F_{1,916.9}$= 27.8, p <.0001) and T_{max} (Females – $F_{1,424.7}$= 8.29, p=.0042; Males – $F_{1,501.3}$= 7.48, p =.0065) affected offspring size, with larger offspring as T_{min} decreased and smaller offspring as T_{max} increased.

Offspring thermal preference

There was no correlation between mother T_{pref} and hatchling T_{pref} ($F_{1,988}$= 0.19, p = 0.66). Additionally, there was no overall effect of female treatment ($F_{2,152}$= 0.0891, p = 0.91) or population ($F_{8,152}$= 0.75, p = 0.65) on offspring T_{pref} (Figure 2.8). There was also no effect of female treatment on offspring behavior ($F_{2,166}$= 0.77, p = 0.46) and it was marginally insignificant for population ($F_{8,160}$= 1.74, p = 0.09). However, a least square means contrasts analysis revealed an outlier population with offspring from MON exhibiting more time spent hidden during T_{pref} trials than other populations ($F_{1,187}$=5.93, p=0.016) (Figure 2.9).

Discussion

We found that female parturition dates and offspring phenotypes varied among populations and with basking treatments. Extreme weather events such as those mimicked by our treatments, including heat waves or long stretches of rain have been observed in the past and are expected to increase and worsen with climate change (Easterling et al. 2000; Buckley and Huey 2016a). Our study showcases that extreme weather occurring during a female's gestation period can significantly impact reproduction in these lizards, considering a few weeks in our basking treatments significantly influenced parturition date and offspring phenotype. Females with experimentally shortened basking opportunities exhibited longer gestation times. The prolonged duration of gestation is consistent with our predictions, because it is well established that less access to heat slows down embryogenesis (Wapstra 2000; Lourdais et al. 2002; Tang et al. 2012). These gestation times and the conditions under which they occur have ecological significance on when neonates enter the environment which in turn affects their growth trajectory before hibernation and is known to have cascading impacts on their survival and fecundity the following season (Chamaille-Jammes et al. 2006).

In respect to offspring phenotype and treatment effects, we found that female viability was influenced by mother basking treatment, whereas male offspring viability remained consistent across treatments. Female offspring born from the short treatment had significantly lower viability than those born from the long treatment. This is not the first case of sex-dependent effects in response to incubation temperature, as it was found in seed beetles only male offspring benefitted from warming temperatures after multiple generations of acclimating to warming temperatures (Rogell et al. 2014). In the case of these lizards, however, we observed that females are significantly larger than males at birth across treatments as well. This makes sense considering females are larger than males as adults and must reach a minimum size before reaching reproductive maturity, thus being born small would be a greater fitness consequence to a female than male (Holmes and Cree 2006).

Interestingly, while females in the long treatment were smaller than females in the short or control treatments (See Figure 2.7) they also had the highest viability (See Figure 2.5). This suggests the benefits gained from being born earlier may counteract potential fitness consequences of smaller size at birth in females. Offspring size overall was influenced by treatments equally across the sexes with smaller offspring in both sexes coming from the long treatment. This pattern confirms many studies on a variety of taxa showing larger offspring at lower temperatures either in SVL or other morphometric traits such as head size or limb length (Perrin 2006; Yan et al. 2011). In addition, in *Z. vivipara* while offspring size is observed as becoming smaller as access to heat increases in this study, there is a trend of body sizes increasing as mean global temperature increases in sexually mature *Z. vivipara* (Chamaille-Jammes et al. 2006, personal observations) contrary to global trends of shrinking body size (Sheridan and Bickford 2011). This confirms females are able to produce smaller offspring earlier in warmer environments while still having offspring developing into larger individuals.

These findings further suggest the potential for ontogenetic conflict, which is generally defined as the expression of the same allele moving sexes differentially (or life-history stages) towards or away from phenotypic optimum (Rice and Chippindale 2008). Although viability is equal between the sexes in the control treatment, females had higher viability in the long treatment and lower viability in the short treatment (Figure 2.5). Evidence of ontogenetic conflict occurring between the sexes has been shown in other lizard species such as *U. stansburiana* (Sinervo and Calsbeek 2003; Sinervo and McAdam 2008). In addition, this conflict is evident between life history stages in *Z. vivipara* because while offspring size is observed as becoming smaller as access to heat increases in this study, there is a trend of body sizes increasing as mean global temperature increases in sexually mature *Z. vivipara* (Chamaille-Jammes et al. 2006, personal observations) contrary to global trends of shrinking body size (Sheridan and Bickford 2011). Thus confirming females are able to produce smaller offspring earlier in warmer environments while still having offspring developing into larger individuals.

In addition, our results detected population level differences in offspring traits and viability. T_{min} and T_{max} of a female's population of origin was a strong predictor of offspring size, with offspring from populations with lower T_{min} being larger at birth, and higher T_{max} being smaller at birth. This is consistent with our treatment findings as the SVL of neonates from treatments where females had limited access to heat were also larger. In addition, these findings are supported with evidence that variation in female parturition date reaction norms are correlated to local thermal conditions (Rutschmann et al. 2016a). These population effects in conjunction with our treatments support early work indicating variation in offspring phenotype would be a result of female selection for the optimal offspring phenotype in their given environment (Shine and Downes 1999). Thus raising further concerns on just how far these females can physiologically go in terms of size-matching offspring to the environment should climate change continue to push limits on extreme temperatures.

Despite our evidence that females influence their parturition date and offspring traits in response to their environment, there are surprisingly only a handful of studies looking at the potential for "maternal-effect rescue". Maternal-effect rescue refers to the ability of females to alter progeny to essentially buffer them from climate warming effects, as has been suggested in previous studies (Paranjpe et al. 2013; Sinervo et al. 2018). However, there is a lack of studies investigating these ideas, likely because it is poorly understood, despite being a potentially viable way for organisms to respond to climate change (Meylan et al. 2012; Sinervo et al. 2018). Our study showed no evidence of maternal effects in response to population or mother treatment on offspring thermal preference. However, thermoregulatory behavior of offspring was only marginally insignificant, with MON offspring exhibiting increased thermoregulatory avoidance behavior compared to other populations (See Fig. 2.9). MON is a population considered on the verge of collapse as a result of climate change impacts (Dupoué et al. 2017). The fact that this population stood out amongst the others in amount of time offspring spent hiding suggests there is reason to further delve into potential heritability of thermoregulatory behavior. In addition, a previous study that used the same basking treatments revealed this population also spent the least time basking when given the opportunity, suggesting offspring may be exhibiting similar basking-avoidance behaviors (Blaimont et al. in prep). Extreme populations like MON are able to provide important information on the warning signs of population extirpation that can be applied when looking at other populations.

Ultimately, we found that offspring phenotype was significantly affected by basking treatments with evidence of local population differences and sex-dependent outcomes in juveniles. However, we did not find significant evidence for strong maternal effects of thermal preference on progeny thermal preference. Observing how gestation is impacted by basking opportunity and its cascading effects on offspring phenotype and viability offers an interesting avenue for further work investigating the heritability and long-term fitness impacts of such effects. Our results demonstrate considering gestation and the offspring life-history stage is critical in drawing conclusions on how robust a population and ultimately, a species, may be in response to climate change.

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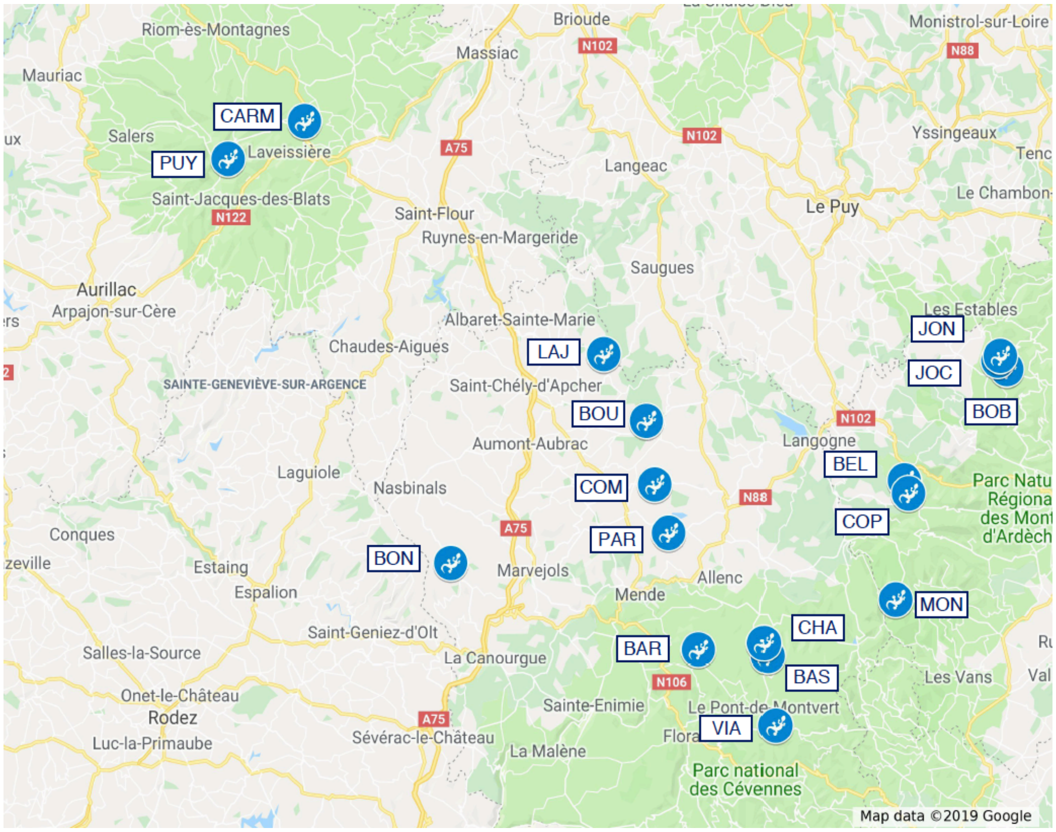

Figure 2.1. A map depicting the locations of the 17 populations used in this study in 2017 and 2018.

Table 2.2. Names, GPS coordinates, elevation, and temperature data collected from the populations in 2017 and 2018.

Population	**Pop Code**	**Year**	**Coordinates**	**Elevation (m)**	**Tmin (°C)**	**Tmax (°C)**	**Tmean (°C)**	**Thermal Range (°C)**
Montselgues	MON	2017	N44°30.613', E4° 00.485'	1049	9.88	26.38	16.74	16.5
Vialas	VIA	2017	N44°20.288' E3°46.045'	1141	8.45	28.04	15.67	19.59
Source Loire	JOC	2017	N44°50.113' E4°12.650'	1296	7.71	23.59	14.75	15.88
Col de Bonnecombe	BON	2017	N44°33.610' E3°07.674'	1344	9.73	24.46	15.4	14.73
Col du Pendu	COP	2017	N44°39.290' E4°01.855'	1375	8.48	25.93	15.16	17.45
Gerbier de Jonc	JON	2017	N44°50.512' E4°12.913'	1390	10.48	22.87	15.01	12.39
Chalet	CHA	2017	N44°26.934' E3°44.883'	1429	8.93	23.25	14.43	14.32
Puy Mary	PUY	2017	N45°06.410' E2°41.188'	1450	11.39	25.26	15.98	13.87
Barnesac	BAS	2017	N44°25.997' E3°45.333'	1515	7.74	26.69	15.06	18.95
Montselgues	MON	2018	N44°30.613', E4 00.485'	1049	10.4	28.45	17.62	18.05
Carmantran	CARM	2018	N45°09.474', E2° 50.273'	1212	6.81	32.11	17.32	25.3
Lajo	LAJO	2018	N44°50.660', E3° 25.864'	1330	8.71	32.35	16.94	23.64
Barraque de Bouvier	BOU	2018	N44°45.225', E3° 30.923'	1390	8.33	29.49	16.64	21.16
Lou Paradis	PAR	2018	N44°36.063', E3° 33.583'	1396	7	34.16	17.63	27.16
Barrandon	BAR	2018	N44°26.534', E3° 37.108'	1403	8.96	29.43	16.06	20.47
Col du Cheval Mort	COM	2018	N44°40.041', E3° 31.916'	1405	8.8	27.44	16.1	18.64
Belair	BEL	2018	N44°40.360', E4° 01.492'	1418	8.15	34.49	16.52	26.34
Bout de la Barre	BOB	2018	N44°49.437', E4° 13.694'	1432	8.44	28.86	15.66	20.42

Table 2.3. Summary treatment response data including female gestation time (days), juvenile SVL (mm), juvenile body mass (g), and juvenile survival (0= non-viable, 1=viable). Data from 2017 and 2018 were pooled. Values are mean ± SE.

	1- Short		**2- Control**		**3- Long**	
Gestation time (days)	18.46 ± 0.39 days		18.17 ± 0.39 days		17.17 ± 0.37 days	
Juvenile SVL (mm)	M= 19.75 ± 0.052	F= 20.46 ± 0.061	M= 19.78 ± 0.054	F= 20.47 ± 0.057	M= 19.53 ± 0.052	F= 20.27 ± 0.057
Juvenile BM (g)	M= 0.163 ± 0.0011	F= 0.164 ± 0.0014	M= 0.166 ± 0.0011	F= 0.169 ± 0.0013	M= 0.159 ± 0.0011	F= 0.162 ± 0.013
Juvenile Survival (0-1)	M= 0.948 ± 0.010	F= 0.922 ± 0.014	M= 0.953 ± 0.011	F= 0.952 ± 0.013	M= 0.95 ± 0.010	F= 0.973 ± 0.013

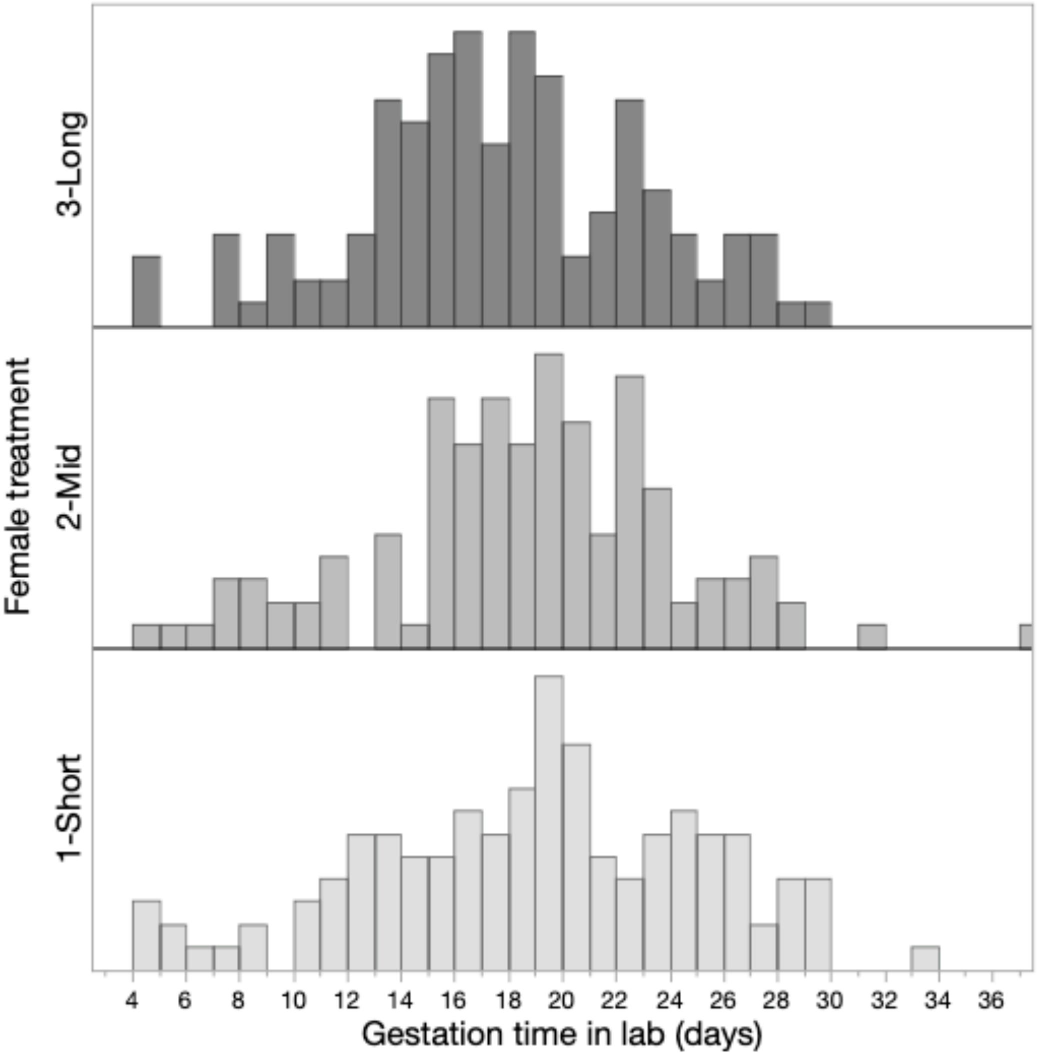

Figure 2.4. Temporal distribution of parturition events in 2017 and 2018 in relation to treatment. (See Table 2.3 for exact values).

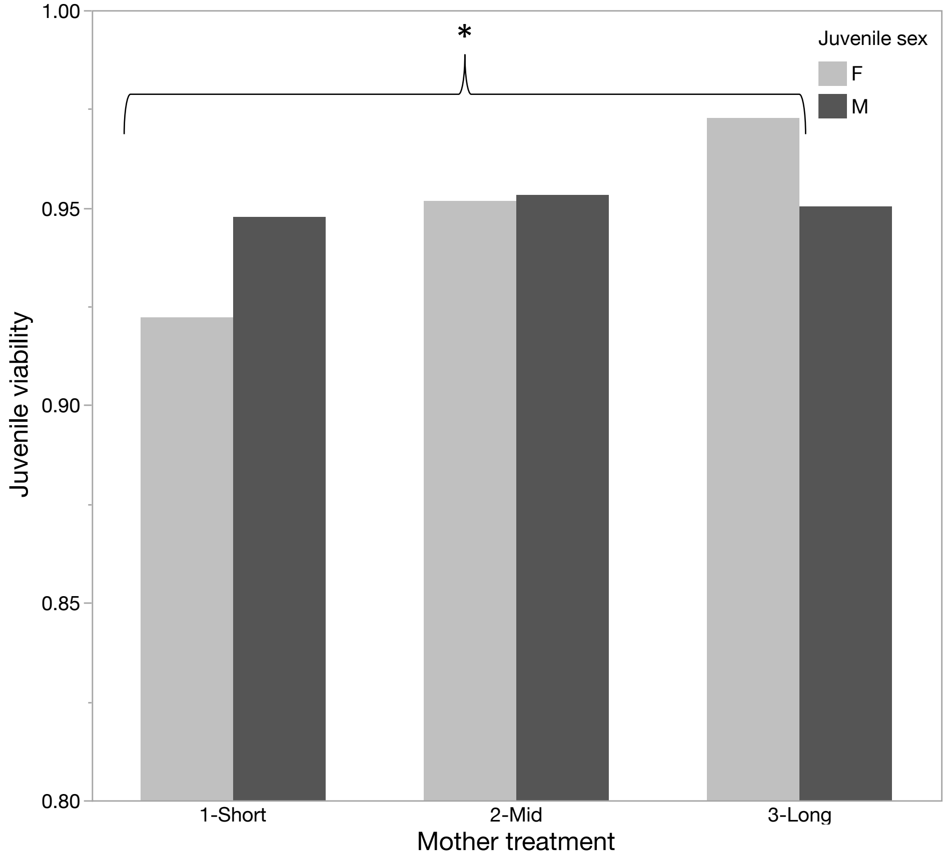

Figure 2.5. Mean juvenile viability by sex and treatment (0= not viable, 1= viable).

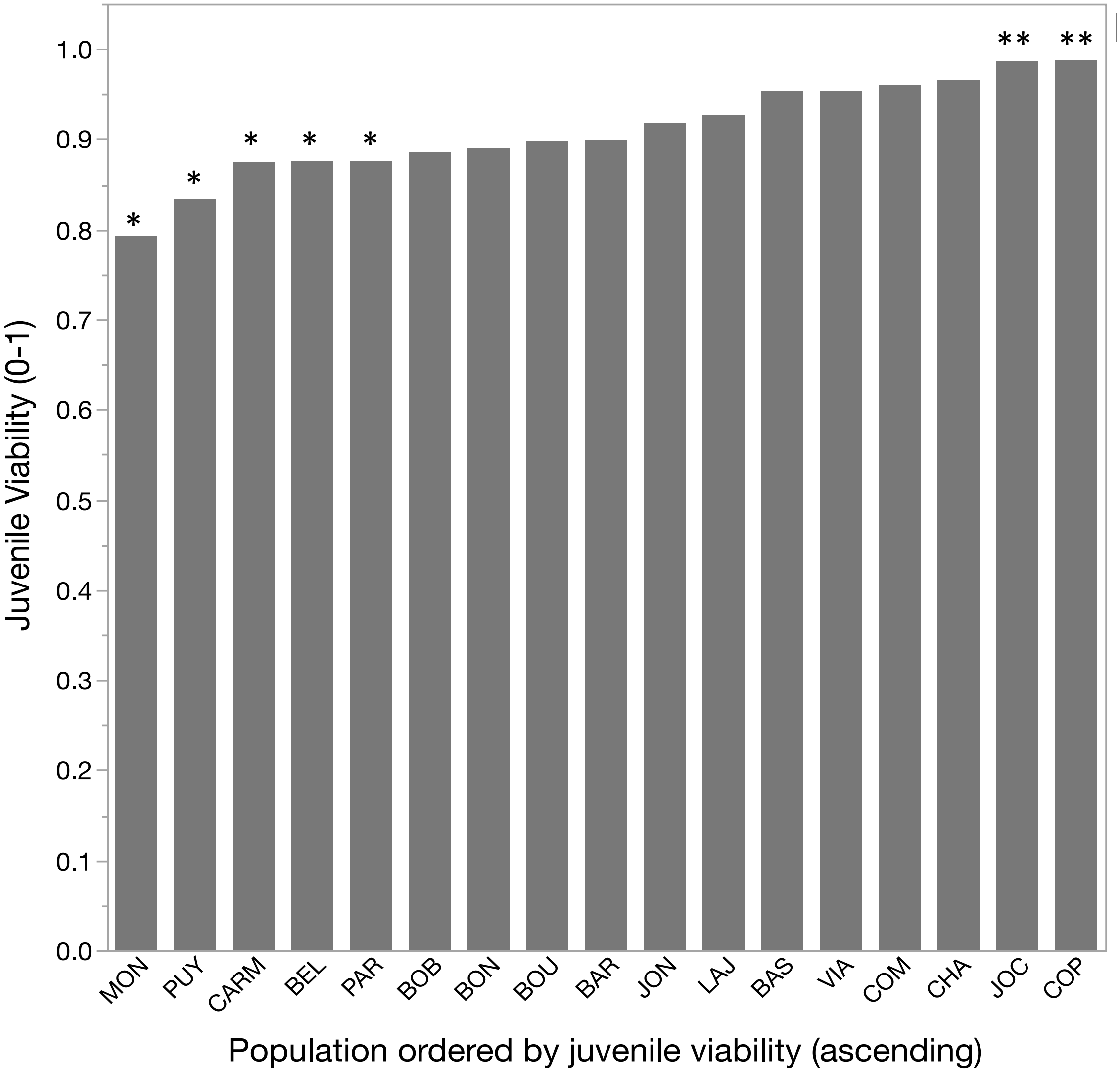

Figure 2.6. Mean juvenile viability by population (0=not viable, 1=viable).

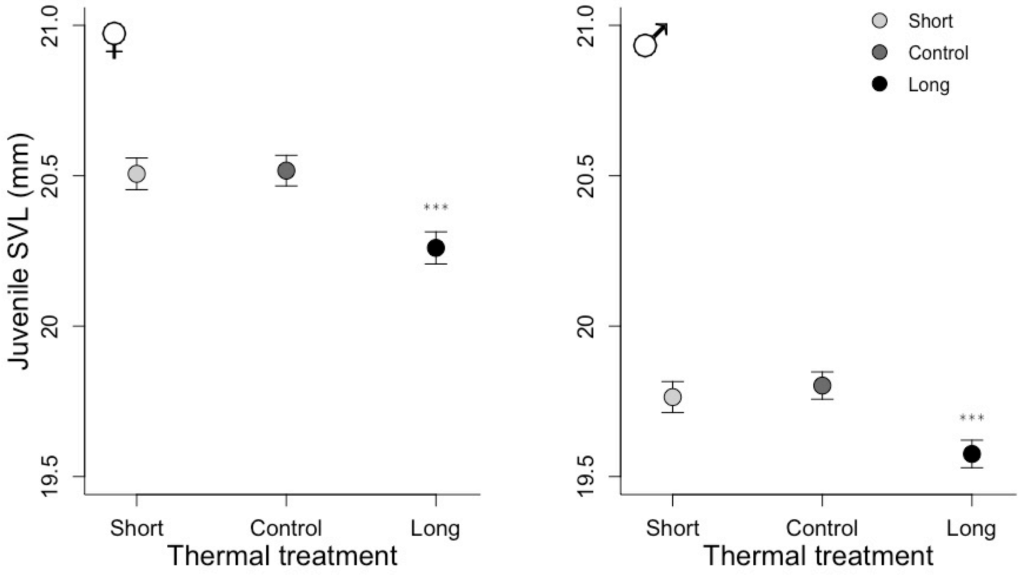

Figure 2.7. Mean offspring size by sex in response to mother basking treatment. Offspring in long treatments were significantly smaller than those in either the short or mid treatments.

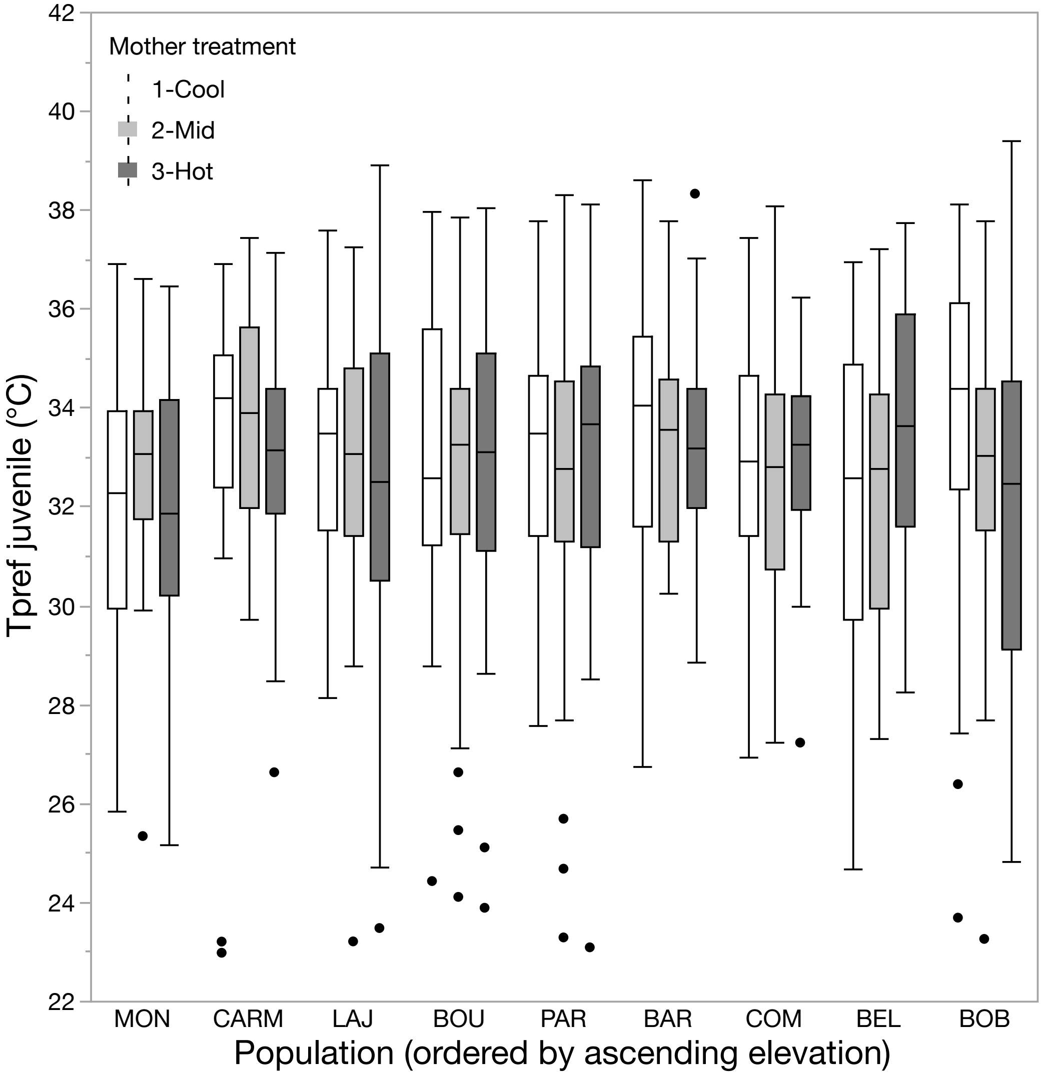

Figure 2.8. Figure showing mean juvenile T_{pref} by population and mother treatment. There was no influence of female treatment ($F_{2,1007}$=.0385, p=0.9623) or population ($F_{8,1007}$=.6139, p=0.7650) on offspring T_{pref} overall.

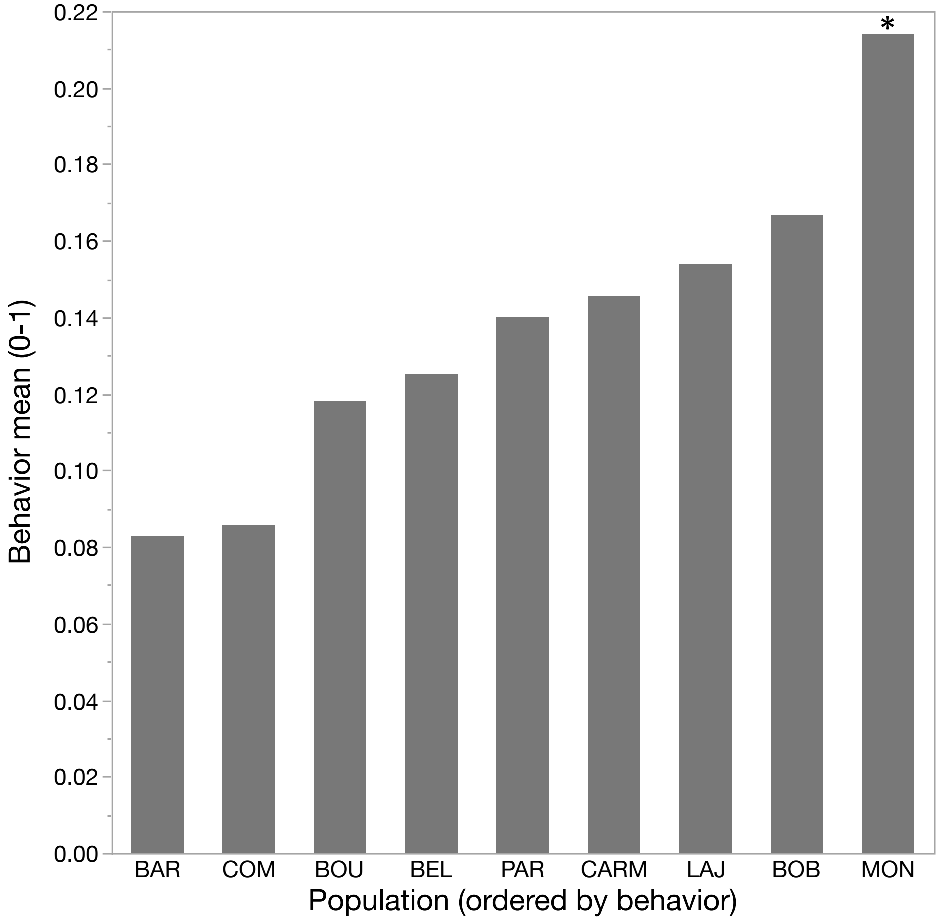

Figure 2.9. Juvenile behavior during T_{pref} trials by population, representing proportion of time spent active (0) and hidden (1). Population was marginally insignificant overall ($F_{8,1007}$= 1.74, p=0.09), however, a least square means contrast revealed MON was hidden significantly more than other populations ($F_{1,1007}$= 5.93, p=0.02).

Chapter 3: Interpopulation variation of endoparasite clearance in response to basking opportunity in the common lizard (*Zootoca vivipara*)

Abstract

Host-parasite interactions are common and widespread across taxa. In the case of many ectotherms, host-parasite interactions are thermally mediated, often resulting in changes to the host behavior in how they thermoregulate. Investigating how these interactions may be impacted by climate change can provide insight into these complicated interactions and better project how organisms may fare under future climate forecasts. Here, we use pregnant female common lizards *Zootoca vivipara*, collected from five populations in the Massif Central of France, to investigate how infection by the blood parasite *Karyolysus* is affected by experimental differences in thermal environment. Treatments were designed to portray the potential thermal environments predicted by climate change models. We focused on pregnant females, where either their behavioral or immune responses against parasites might compromise the development of embryos. We quantified thermal preference (T_{pref}) and sampled blood from each female before placing them into basking treatments. We provided either 3-hours (short), 6-hours (mid/control) or 9-hours (long) access to basking heat. After parturition and before release we resampled for blood parasites. We used the before and after blood samples to investigate whether differences in basking treatments affected parasite abundance. Females from three out of five

populations exhibited a smaller increase in parasite abundance when exposed to the mid/control heat access compared to the short and long treatment. In contrast, the remaining two populations showed no treatment effect on parasite abundance. The difference between populations suggests a context-dependent impact of thermal environment on the capability of lizards to combat parasitic infections. Our study provides further evidence that ectothermic host-blood parasite relationships are sensitive to host's basking opportunities and are likely to be impacted by climate change.

Introduction

Current climate forecasts have projected a 1.5 °C increase in mean global temperature by 2030 under the 4.5 RCP emission scenario (IPCC 2018). Although this increase in temperature may seem small for ectotherms, whose body temperatures depend on external sources of heat to thermoregulate, this 1.5 °C is likely to result in a variety of deleterious ecophysiological consequences. Besides changes in mean temperature, prolonged heat spells (1998) have shut down reproduction in female *Sceloporus* lizards of Mexico (Sinervo et al. 2011), precipitating local extirpations in the ensuing decade (Sinervo et al. 2010). A physiological metric of the potential cause of these effects has been termed hours of restriction, which is a description of limits to the time available for foraging, growth and reproductive opportunities (Sinervo et al. 2018). This limit is due to the normal range of temperatures for behavioral thermoregulation being surpassed, as animals risk death from upper critical thermal limits being exceeded (Sinervo et al. 2010; Sunday et al. 2014).

The consequences of limited hours of activity have broad impacts on food sources, habitats and even other ecological relationships, such increased susceptibility to parasite infections (Kearney 2013). Host-parasite interactions impact diverse traits in the host including coloration (Megía-Palma et al. 2016a; Côte et al. 2018), body condition (Sánchez et al. 2018), and behavior (Hart 1992). For example, the trematode, *Leucochloridium paradoxom*, infects the snail *Succinea putris* by entry through the eyes, and causes disturbing green pulsating patterns designed to attract predators. This trematode ultimately manipulates the snail's brain, overriding their natural aversion to daylight to aid discovery by a bird, the final avian host of this parasite (Wesołowska and Wesołowski 2014). Despite the critical importance of these host-parasite interactions, there have been few empirical studies investigating how they are likely to be disrupted by climate change (Brooks and Hoberg 2007). However, the few studies that have been published suggest they will be critically impacted (Levi et al. 2015; Gehman et al. 2018).

In lizards, a critical trait in the context of climate change is thermoregulatory behavior, which acts to maintain their body temperature within a rather narrow range that maximizes performance (Huey and Kingsolver 1989). However, parasite infections are known to impact thermoregulatory behavioral changes in lizards (Deen and Hutchison 2001; Zamora-Camacho et al. 2016). Experiments and modeling have shown that host and parasites often respond differently to temperature and as such may exhibit dissimilar responses to climate change (Gehman et al. 2018). In addition, infection can induce multiple thermoregulatory responses in hosts. For example, two common responses reported in a variety of ectotherms have included heat-seeking "fever" (Chatfield and Richards-Zawacki 2011; Rakus et al. 2017) and cool-seeking "hypothermia" thermoregulatory behaviors (Müller and Schmid-Hempel 1993; Moore and Freehling 2002; Paranjpe et al. 2014, Megía-Palma et al. in review). These behaviors are further complicated by the fact that they may affect the host and parasite in different ways and in some cases it is unclear whether the parasite is manipulating the behavior of the host or if the host is attempting to affect the parasite (Schmid-Hempel 2011; Paranjpe et al. 2014). However, the potential negative fitness impact of the parasite on the host should favor thermoregulatory behaviors that reduce infections (Ouedraogo et al. 2004). Ultimately, the capacity of hosts to tolerate an increase or decrease in the body temperature as a departure from their preferred temperature depends on how physiological performance is compromised. Furthermore, the magnitude of the response may depend on intensity of the infection (Żbikowska 2011).

In addition, parasite loads have been shown to correlate with a variety of environmental factors including elevation (Álvarez-Ruiz et al. 2018), temperature (including mean, variance etc.) (Rohr and Raffel 2010), seasonality (Daversa et al. 2018) and precipitation (Megía-Palma et al., in prep) as well as interactions between all of the above. Warmer temperatures are predicted to aid in the spread of parasites and their vectors as well (Rogers and Randolph 2000). As a result, parasite load has been used as a metric to determine ecosystem stress, making it an ideal candidate to study climate change impacts (Marcogliese and Cone 1997; Marcogliese 2005).

The potential of host-parasite relationships to affect thermoregulatory behavior has interesting and expectedly complex implications in the context of climate change. For example, the relationship between amphibian declines, climate change and *Batrachochytrium dendrobatidis (Bd)* (a harmful fungus to amphibians) is a topic still under debate. Many studies suggest that amphibian declines were due to stress from increased temperatures as a result of climate change and thus increased disease risk to *Bd* (Pounds et al. 2006; Bosch et al. 2007). However, other studies have shown that elevated temperatures were actually an effective treatment against *Bd* as this fungus prefers cooler temperatures (Chatfield and Richards-Zawacki 2011). In addition, previous studies were shown to be temporally confounded in determining amphibian declines in response to climate change and susceptibility to disease and certain environmental variables such as thermal variability were not accounted for (Rohr and Raffel 2010). This example goes to showcase the complicated history of the topic and the fact that these host-parasite interactions and outcomes to climate change generally have to be established on a species by species basis.

In this study, we investigate the host-parasite relationship between the Eurasian common lizard, *Zootoca vivipara* (Squamata: Lacertidae), and the protozoan heamogregarine blood parasite *Karyolysus spp*. (Apicomplexa: Adeleorina: Karyolysidae). Earlier studies on *Z. vivipara* focused on the effects of maternal parasite load on reproduction (Sorci and Clobert 1995; Sorci et al. 1996). However, recent research on lacertids have been focused on male lizards in the context of tests of sexual selection theory (on male signals) (Megía-Palma et al. 2016b). Fewer studies have evaluated the impacts of parasites on adult females during the breeding season. Females face high energetic demands during vitellogenesis and carrying embryos. As a result, gravid females may have a higher risk of infection by parasites, because of low body condition and compromised immune system. While a previous longitudinal study on *Z. vivipara* found a limited ability for individuals to clear blood parasites (Sorci 1995), we were specifically interested in whether basking opportunity would influence these clearance rates.

We used a cross-sectional study to assess how abiotic factors affect host-parasite dynamics in *Z. vivipara*. We sampled pregnant females from five populations in the Massif Central region of France and at each site measured microclimatic variables to investigate any environmental factors contributing to parasite load. We brought females back to the lab and measured their thermal preference (T_{pref}), took blood samples for parasite analysis and exposed the lizards to different basking treatments during gestation. Treatments were designed to represent the potential variation in basking opportunity predicted by climate change. We randomly allocated females in to one of three thermal treatments: allowing them access to basking heat for 3-hours (short) treatment, 6-hours (mid/control) treatment and 9-hours (long). The former treatment represents cool conditions expected from higher levels of cloud cover and increased frequency/amounts of precipitation. The latter treatment represents warmer environments, as global mean temperatures are expected to rise. The 6-hour treatment is a control treatment designed to mimic the daily hours currently available for basking and is the standard in laboratory experiments previously done with this species (Rodríguez-Díaz and Brañ 2011; Rutschmann et al. 2016b). Because lizards have been shown to change thermoregulatory behavior when infected as previously discussed, it is possible certain climatic events such as a particularly warm-season or cold-spell as mimicked by our treatments would give lizards thermal opportunities or rather, impose constraints, on their ability to clear parasites. Thus, we hypothesized both the short treatment and long treatment would impose stressors to either the lizard and/or parasite and we would expect to see either higher or lower rates of parasite clearance depending. We measured T_{pref} within 48 hours of capture to determine whether there was evidence of cool-seeking (hypothermia) or heat-seeking behaviors (behavioral fever) as a function of infection status. We hypothesized parasite abundance over time would be affected by access to heat as thermoregulatory opportunity has been shown to influence the ability to fight infection in other ectotherms like locusts (Ouedraogo et al. 2004). Therefore, we also took a second blood sample before releasing females back into the wild to determine whether a change in parasite load occurred while in basking treatments. Overall, our study adds to the growing body of knowledge on the intricacies of host-parasite relationships and works to further develop how these relationships may be impacted by climate change.

Methods

Study species

The European common lizard *Z. vivipara* is a small-bodied species in the family Lacertidae. The species is found across Eurasia with most populations being viviparous in the northern part of the range, but oviparous in the Pyrenees, the Southern Alps and the Mountain ranges in the Balkan Peninsula (Surget-Groba et al. 2006). Maximum female lifespan is 11 years and 7 years for males (Richard et al. 2005). The breeding season from copulation to parturition occurs in May – July. Litter size varies from 1-12 neonates. Litter size is positively correlated with female body size (Richard et al. 2005; Rutschmann et al. 2016b).

Individuals of *Z. vivipara* are commonly infected by the blood parasite *Karyolysus* spp. The a genus includes 10 species and has been primarily reported to occur in European lizards (Haklová-Kočíková et al. 2014)(See Fig. 3.2). They have an indirect life cycle with merogeny (a form of asexual reproduction of protozoans) occurring in intermediate lizard hosts while gamogony (gamete formation by sexual reproduction) and sporogony (spore formation by sexual reproduction) occur in the gut of a final invertebrate host (Telford 2008). The main invertebrate known to be vector of *Karyolysus* is the mite genus *Ophionyssus* (Arachnida: Acari: Macronyssidae) (Haklová-Kočíková et al. 2014)

Field collection

Gravid female *Z. vivipara* were captured at five sites throughout the Massif Central region, southern France in early June 2018 as part of a long-term monitoring study already in place. Populations were chosen based on parasite prevalence, which was previously assessed in a pilot study in 2016. These populations had over 50% in prevalence of blood parasites (unpublished data). We placed three iButton ® temperature data loggers (Maxim Integrated Products, Sunnyvale, CA, USA,

±0.0625°C) per population. Loggers were placed in shaded vegetation as described in previous studies to record daily temperatures experienced at a finer scale (Dupoué et al. 2017). Microclimatic temperatures such as mean temperature (T_{mean}), minimum temperature (T_{min}) and maximum temperature (T_{max}) and water density (a measure of humidity; g H_2O/m^3) at the capture site were measured from date of capture to date of release. We standardized the sampling period from June 29th to July 21st in 2018 to calculate temperature variables (See Table 3.1)

Experimental Design

Females were brought in (N=121 lizards; [CARM, N=24; BOU, N=24; BAR, N=24; PAR, N=24; TIO, N=25]), given a unique toe clip identification (see Perry et al. 2011) and measured for traits including mass (to the nearest 0.01 g) and length (SVL, to the nearest .5 mm). Ectoparasites (mites and ticks) were counted upon arrival into the lab, removed with tweezers and then treated with 0.29% fipronil Frontline® to ensure any mites potentially missed were eliminated per methods described in Wiechert (2007). This removal of mites was designed to avoid re-infection during the treatments. Females were individually housed in 11 x 18 x 11 cm plastic terraria with a layer of sterilized soil and a heat lamp (25 W) on one side and an cardboard shelter at the other (Massot and Clobert 2000). Females were fed 2-3 times per week and watered three times daily. After 48-hours of acclimation in the lab they then had their T_{pref} measured per methods described in Artacho et al. (2017). Females were placed in a 125 cm long track with a thermal gradient of 23° – 42°C.

This method allows females to thermoregulate freely for 3-hours with body temperature recordings taken every 15 minutes for 3 hours via an infrared thermometer (Raytek RAYST61 ST Pro Plus Infrared Thermometer, Raytek Corporation, Santa Cruz, USA).

Females were randomly placed in treatments with access to basking light for 3-hours, 6-hours, and 9-hours. We endeavored to size match females in each of the treatments: 3-hour/short: N=40, mass = 5.21 ± 0.21 g; 6-hour/mid: N=40, 5.22 ± 0.19 g; long: N=41, 5.20 ± .21 g ($F_{2,118}$=.0009; p-value=.9991). After T_{pref} and before the start of treatments females had a drop of blood taken from the post-orbital sinus with a capillary tube per methods described in (Dupoué et al. 2017). Gestation time/length of time in treatment was calculated as the difference between the date placed in treatment and the date a female gave birth. After giving birth and before release back to the site of capture, females had another blood sample taken to determine any treatment effects on parasite abundance (approximately 18.72 ± .57 days spent in treatment). The blood drop was smeared on a slide, stained with Giemsa per methods described in Paranjpe et al. (2014) and analyzed under a Leica Microsystems DMLB microscope at 1000x (10x ocular x 100x objective) magnification under oil-immersion for 6 minutes per methods described by J. Schall (http://www.uvm.edu/~jschall/pdfs/techniques/scanningsmears.pdf) (See Fig 3.2).

Statistical Analysis

All analyses were performed in JMP-SAS version 14 and R-software v.3.4.3 (R core team, 2017). We excluded females from the analysis who were not pregnant

(N=9). We had a final sample size of 35, 38, and 39 gravid females in the short, mid, and long treatments, respectively. Females were classified as “not infected” if we did not detect any parasites or “infected” if they had any quantity of parasites. Body condition was calculated by regressing log body mass against log SVL and calculating the residuals Dunlap and Mathies (1993). We analyzed whether body condition differed between infected and uninfected females using an ANOVA. We used the same method in order to determine if any relationship existed between tick and mite load as well.

We used a general linear model (LM) in R to analyze factors contributing to T_{pref}, which would provide additional evidence of interpopulation variation. T_{pref} was calculated as an average of all the temperatures recorded for an individual during the trial (N = 14 recorded temperatures per individual). Population, body condition, infection status, SVL, and number of mites and ticks (separately) were included as independent variables and we used VIF scores to screen for collinearity.

To investigate factors contributing to infection status (0=absent, 1=present) upon entry into the lab, we used a GLM in R fitted with a binomial distribution linked to a logit function in R. Population, body condition, SVL, and number of mites and ticks were included as independent variables. We used the corrected Akaike information criterion for small sample sizes (AICc) to estimate the relative importance of each predictor using the R-package MuMIn (Barton, 2013). We considered models with $\Delta AICc \leq 4$ sufficiently informative (Burnham and Anderson, 2004). Then, we summed the weights of all the models where the predictor appears to calculate the relative importance of each variable in the final model. We used the dredge function in R, which calculates the relative importance of equiprobable models, to further confirm population was a suitable variable to represent the suite of environmental variables (See Table 3.3). We used population to represent the physical structure that accounts for differences between populations that we don't have all the data to explain seeing as temperature and humidity variables were highly collinear and did not significantly explain as much of the variation seen as population as a whole. In addition, we calculated the significance of the coefficients for each predictor using model averaging that included the effect (i.e., conditional average). We calculated the maximum likelihood estimate and its standard error for all the predictors.

Next, to determine predictors explaining the initial parasite load observed in the lizards, we used a GLM model in R fitted with a negative binomial distribution and linked to a log function that accounts for the typically over dispersed distribution shown by parasite counts (Pennycuick 1971). Population, body condition, SVL, T_{pref}, and number of mites and ticks (separately) were included as independent variables and we used VIF scores to screen for collinearity.

Finally, to analyze the effect of the experimental basking treatments on parasite load we calculated the log-transformed initial parasite load and final parasite load and took the difference as a metric to determine change in load over time in treatments. Then, we used a generalized linear model with gaussian distribution and identity link function where log parasite load difference was set as dependent variable and treatment was included as a factor. Mite load, tick load, body condition, SVL, number of days in treatment and T_{pref} were included as continuous predictors. In both cases we evaluated the parametric assumption of our models checking the normality (Shapiro-Wilk test), kurtosis (Anscombe test), and skewness (Agostino test) of the residuals (D'Agostino 1970; Anscombe and Glynn 1983). The heteroscedasticity of the models was visually evaluated. When we observed deviations from homoscedasticity, we reported robust covariance matrix estimators *a la* White (den Haan and Levin 1997). We performed omnibus tests and confirmed our models were significantly better than a random null model using a likelihood ratio test.

Results

Infection status upon entry to lab

In our study, 77 pregnant females of 112 (68.75%) were infected with endoparasites of the genus *Karyolysus* (See Figure 3.4). Infected females had significantly worse body conditions than non-infected females ($F_{1,110}$=4.19, p=0.043) (See Fig 3.5). T_{pref} significantly differed by population ($F_{4,100} = 3.36$, $P = 0.0126$; CARM=31.31 ± .28 °C, TIO=31.6 ± .25 °C, BOU=32.31 ± .34 °C, PAR=31.07 ± .28 °C, BAR=31.55 ± .29 °C), however, other variables had no effect. Additionally, there was no significant relationship between tick and mite load ($F_{1,110} = 0.21$, $P = 0.65$) (See Fig. 3.6 for mite and tick numbers by population). Infection status (0 = absent, 1 = present) was significantly explained by population ($\chi^2_4 = 31.55$, $P < 0.001$) and tick load ($\chi^2_1 = 5.00$, P =0.25).

In regard to initial parasite abundance upon entry into the lab, our glm revealed that despite mites being the presumed vector for *Karyolysus*, mite load and *Karyolysus* infection at capture was not significant (z= 0.93, p=.93). However, infection by *Karyolysus* was negatively correlated with tick number (z = -3.02, p = 0.0025) (See Fig. 3.7) and T_{pref} had no significant relationship to initial parasite load upon entry into the lab (z = 1.59, p = 0.11).

Treatment effects

There was no significant difference among basking treatments in number of infected vs. not infected individuals at the beginning of the experiment (χ^2_2= .64, p=.73) (See Fig. 3.8). Our initial model included all five populations used to analyze the effect of the three basking treatments did not meet the assumptions of the statistical test. In particular, the residuals were non-normally distributed and showed evidence of heteroscedasticity, skewness, and kurtosis. However, we graphically explored the interaction plot population*treatment and found 3 populations (PAR, BOU and CARM) that showed a similar effect of treatment on parasites while BAR and TIO showed no effect. We ran the same model only using the three populations where we graphically observed an effect of the basking treatments and the model properly fit the expected parametric assumptions of normality and homoscedasticity. Thus, we found a significant difference between parasite abundance over time in PAR, BOU and CARM and they followed the same general pattern with the control/mid treatment having the lowest increase in number of parasites throughout time in treatments compared to the long and short ($F_{2,24}$= 5.55, p = 0.0105) (See Fig. 3.9).

Discussion

Parasites can negatively affect host fitness through lowered survival and reproductive success (Sorci and Clobert 1995; Martínez-de la Puente et al. 2010). Optimal performance and other critical physiological processes, including immune response, are known to be temperature dependent (Wright and Cooper 1981; Mondal and Rai 2001) and, thus, altered thermal niches predicted by current climate models are likely to exacerbate the effect of parasites on their host. In support of this prediction, we found evidence of a thermally-mediated host-parasite relationship between *Z. vivipara* and its endoparasite *Karyolysus* because the change in parasite load was significantly different depending on the basking treatment. However, this relationship was only evident in three out of five of the populations sampled.

We found evidence of infection in all populations we surveyed, and body condition of infected individuals was significantly lower than non-infected individuals. Body condition is shown to be a predictor of important traits in lizards such as hatchling size and survival in lizards (Weiss 2006; Weiss et al. 2009). Thus our findings support the general understanding that parasites have a negative influence on the health of their host and/or that individuals in poor body condition are more susceptible to infections (Martínez-de la Puente et al. 2010; Sarasa et al. 2011).

Our model for parasite intensity revealed significant differences between populations with highest infection rates in the two warmest, most thermally varied environments regardless of elevation. This is partially congruent with the experimental portion of our study because lizards maintained in the longest basking treatment showed large increases in parasite abundance over time compared to the control treatment in the three populations (PAR, CARM and BOU) included in our final model. However, we also found that individuals in the short treatment had significantly greater parasite abundances at the end of treatments compared to the control. This suggests that both extremes impose stressors on the host enabling them to be more susceptible to endoparasite infection. However, we only found this pattern in three of the five populations and there are a variety of factors that might explain the lack of influence of the treatments in TIO and BAR. In 2018, TIO was collected last and also had earlier parturition dates than the remaining populations, influencing their time in our treatments. As a result, females from TIO may have shown no influence of treatment because their average exposure to the treatment was significantly less than other populations, suggesting there may be a minimum amount of time necessary in treatments to influence parasite abundance. BAR, on the other hand, was in the lab on average the same as the three populations used in analysis but has the lowest values for the environmental factors included in our analysis (T_{mean}, temperature variation, and humidity) and as such may not follow the same pattern as the other populations. This difference between populations suggests a context-dependent impact of basking opportunities on the capability of lizards to clear parasite infections that might be linked to particularities in the environmental conditions at each sampling site and local phenomena of host-parasite co-adaptation (Oppliger et al.

1999). In support of this idea, the significant differences found in thermal preferences between populations provide a signal of local adaptation to the dissimilar environmental conditions observed (e.g. Sinervo 1990). However, further research would be necessary to determine the exact cause of the lack of response in TIO and BAR.

Ultimately, our data suggests greater exposure to warm environments has a negative influence on the lizards' ability to fight off an infection. This supports previous work suggesting increasing mean global temperature will expand vector-borne infections (Patz and Reisen 2001) as the abundance of the majority of vectors are positively related to temperature and transmission may be increased (Lindsay and Birley 1996). That said, it is a generalized view and species-by-species host-parasite interactions and results are likely to be far more complex. For example, lizards of the genus *Gallotia* in the island of Tenerife were found to have parasite loads best explained by summer precipitation (Megía-Palma in prep). Our results are limited by the fact that we only sampled at one time point, however, they do point towards the idea that global warming will negatively affect these lizards' ability to fight off parasites. Future studies could consider long-term sampling of environmental parameters during the breeding season and looking at the change over time in environmental variables to better determine the most influential factors in the host-parasite relationship of *Z. vivipara* and *Karyolysus*.

We additionally did not find evidence for an influence of parasite load on thermal preference in this study. However, one explanation is that females of this species change thermal preference when gravid, tending to select lower temperatures (Le Galliard et al. 2003). This suggests they may be prioritizing temperatures best for embryogenesis and not actively thermoregulating in response to parasite infection. Thus, future work using both gravid and non-gravid females and/or males in a side by side comparison looking at thermal preference would be necessary to determine if there is evidence of thermoregulatory behavior changes in this species in response to parasite infection.

In addition, while there was no apparent correlation between tick and mite load, nor a relationship of mite load (the vector of *Karyloysus*) with initial *Karyloysus* abundance, we found tick load had a significant negative relationship to *Karyolysus* abundance upon entry to the lab. One study has similar findings between ticks and blood parasites in the European male green lizards (*Lacerta viridis*) and suggest tick bites may induce an immune reaction, that in turn, reduces blood parasite count (Wikel et al. 1997 in Molnár et al. 2013). Ticks and mites have also been shown to be inversely correlated to different environmental variables (mite infestation has been shown to increase with elevation and vegetation cover, but decrease with human disturbance and grazing by livestock, ticks shown the inverse relationship) in this species (Wu et al. submitted). Another explanation may simply be that an individual lizard can only handle so many parasites at a time and survive. Along those same lines we are limited by the lizards that are active and available for us to capture and lizards with higher parasite loads may either be deceased or inactive as they work to fight off a large infection. However, more research would be required to solidify any of these hypotheses, as confirming these interspecific microparasite interactions is known to be complex (Fenton et al. 2010) and looking at single parasites in isolation may result in overlooking important patterns of infection risk (Telfer et al. 2010).

The findings from this study open the door to many ideas and opportunities for future research. Identifying those environmental variables that best predict changes in host-parasite interactions is critical for determining how predicted climate change may alter the interaction between species in the future (Gehman et al. 2018). As a result, future studies should work to obtain more detailed data of the physical landscape to better decipher the factors affecting parasite abundance. While we mentioned in this study the importance of this work as it applies to pregnant females for which there is a dearth of knowledge, future work should consider a mix of males and females as sex-dependent differences in parasite abundances within species are common (Duneau and Ebert 2012). These differences are often driven by sexual selection in males (Mills et al. 2008b), versus costs of reproduction such as offspring size and number in females (Sinervo and Svensson 1998; Sinervo and McAdam 2008) and the potential for sexually antagonistic selection over immune function has been shown in previous studies in *Uta stansburiana* (Svensson et al. 2009). Further delving into these potential trade-offs between the sexes and their effects on host-parasite interactions will be important in determining how the species as a whole may be impacted by climate change.

The prevalence of host-parasite interactions across taxa in conjunction with the knowledge that climate change is happening at rates faster than previously predicted makes it more important than ever to better understand the influence of environmental perturbations on these interactions. For example, the incidence of dengue-fever in humans is up 30-fold from 50 years ago and climate change has been an important driver, increasing the geographic range of the *Aedes* mosquito vector (Ebi and Nealon 2016). Thus, at a broad-level, a better understanding of host-parasite relationships across taxa, including these lizards, may help scientists draw patterns in parasite, disease, vector and host relationships to better prepare for and anticipate the potential for new disease-spread in the wake of climate change.

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Table 3.1. Table of all populations used in this study and environmental variables associated including: coordinates, elevation (m), T_{min} (°C), T_{max} (°C), T_{mean} (°C), thermal range (°C), and water density (gH_2O/m^3).

Population	Pop Code	Coordinates	Elevation (m)	T_{min} (°C)	T_{max} (°C)	T_{mean} (°C)	Thermal Range (°C)	Water Density (gH_2O/m^3)
Carmantran	CARM	N45°09.474', E2°50.273'	1212	6.81	32.11	17.32	25.3	13.25
Tioule	TIO	N44°35.315', E3°06.421'	1275	6.66	32.39	17.37	25.73	13.51
Barraque Bouvier	BOU	N44°45.225', E3 30.923'	1390	8.33	29.49	16.64	21.16	13.22
Lou Paradis	PAR	N44°36.063', E3°33.583'	1396	7	34.16	17.63	27.16	13.54
Barrandon	BAR	N44°26.534', E3°37.108'	1403	8.96	29.43	16.06	20.47	12.38

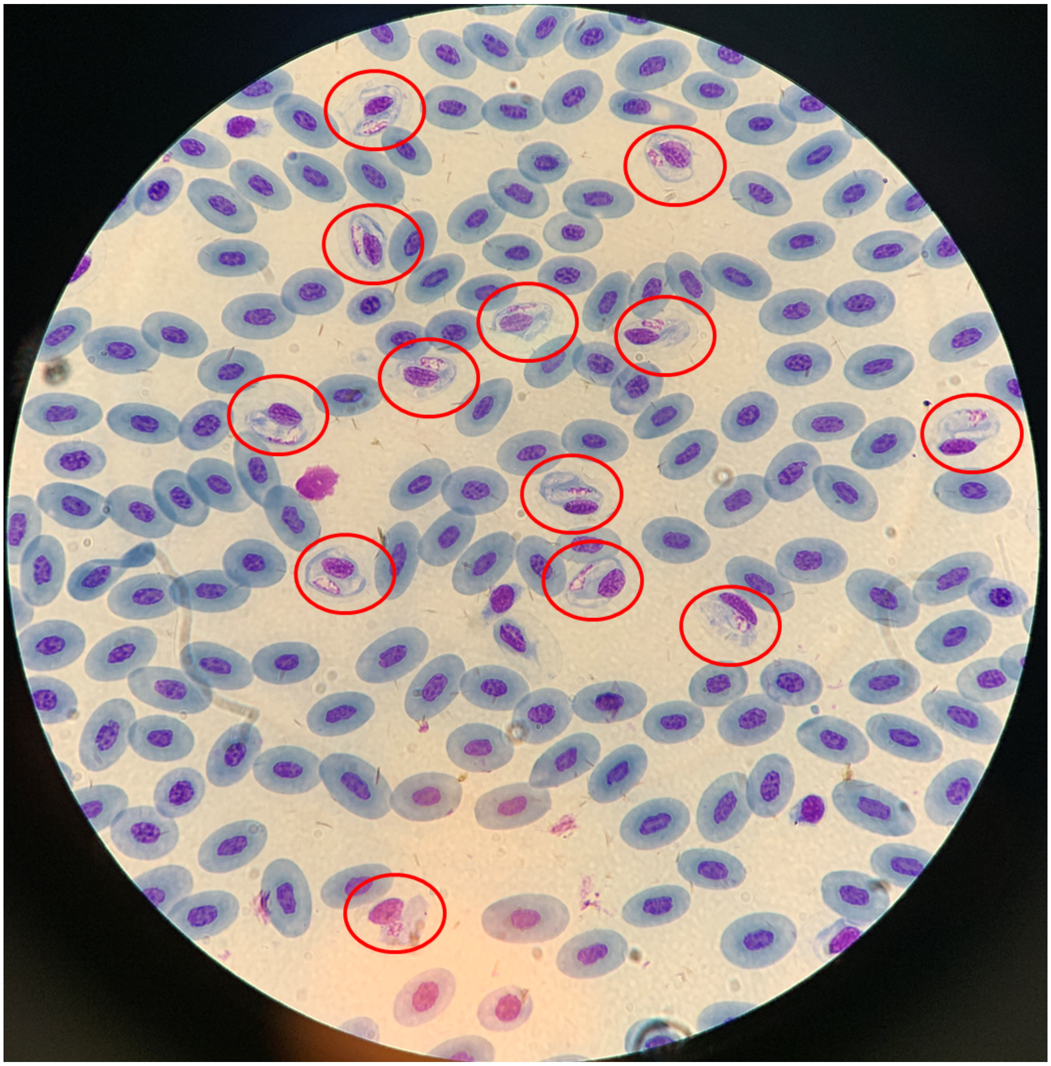

Figure 3.2. Photo of a *Z. vivipara* blood smear used in this study under oil-immersion 1000x (10x ocular x 100x objective) magnification showing blood cells infected with endoparasites of the genus *Karyolysus*.

Table 3.3. Results of automated model selection analysis in R using the dredge function on our model investigating variables that affect infection status. This indicates relative variable importance over 14 equiprobable models, supporting our use of population as a proxy in our analysis.

	Population	**Ticks (N)**	**T_{mean} (°C)**	**SVL (mm)**	**Body Condition**	**Mites (N)**
Importance	1.00	0.88	.50	.28	.18	.10
N containing models	14	12	7	6	4	4

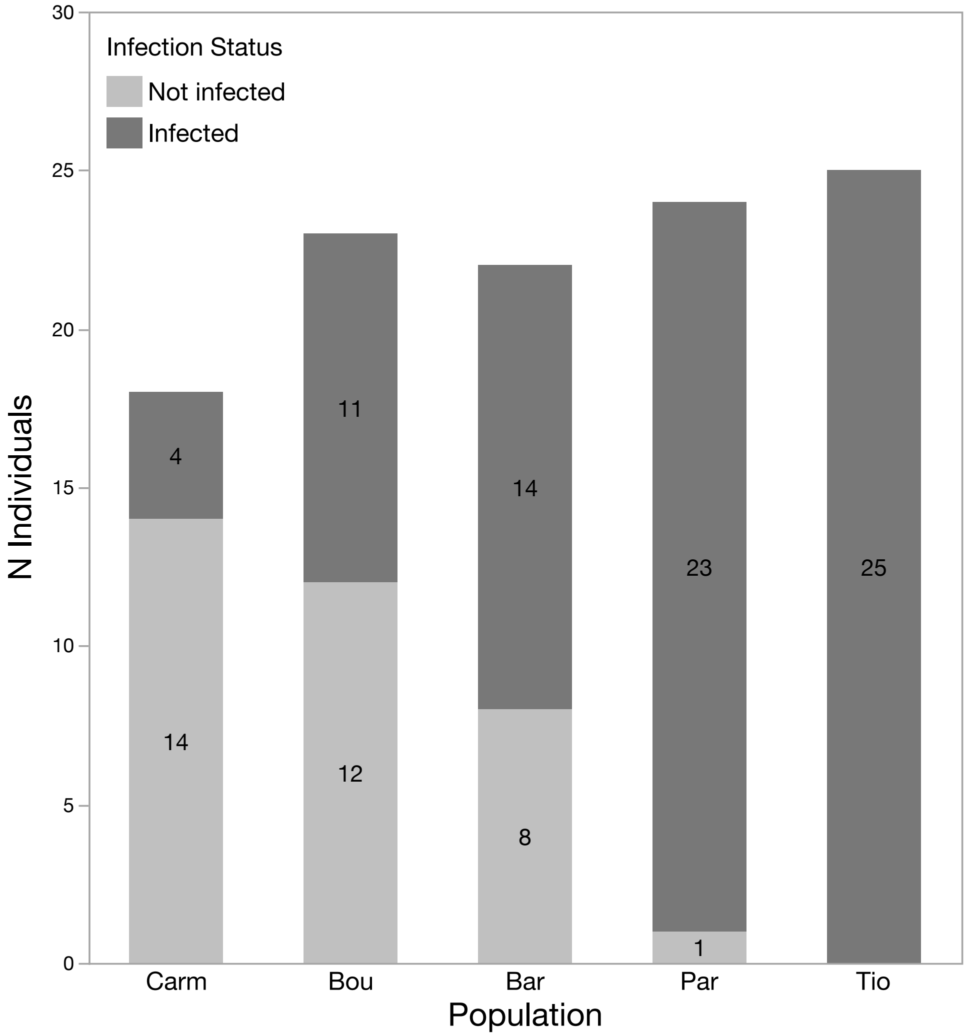

Figure 3.4. Infection status of populations with the endoparasite *Karyolysus* in order of least to most infected. 77 out of 112 individuals (68.75%) were infected and populations significantly differed in infection status ($\chi^2_4 = 51.06$, p<.0001).

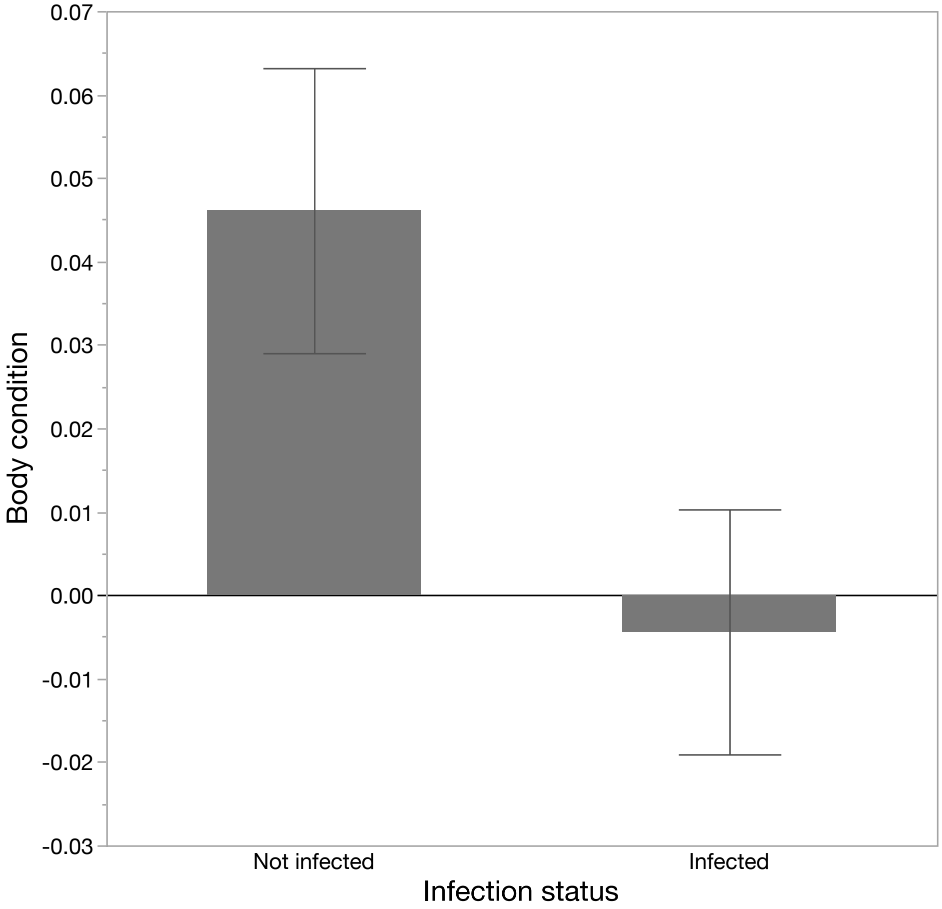

Figure 3.5. Body condition of infected vs. non-infected *Z. vivipara* individuals upon entry into the lab. Individuals infected with *Karyloysus* had significantly worse body conditions than non-infected ($F_{1,110}$=4.1940, p=0.0429).

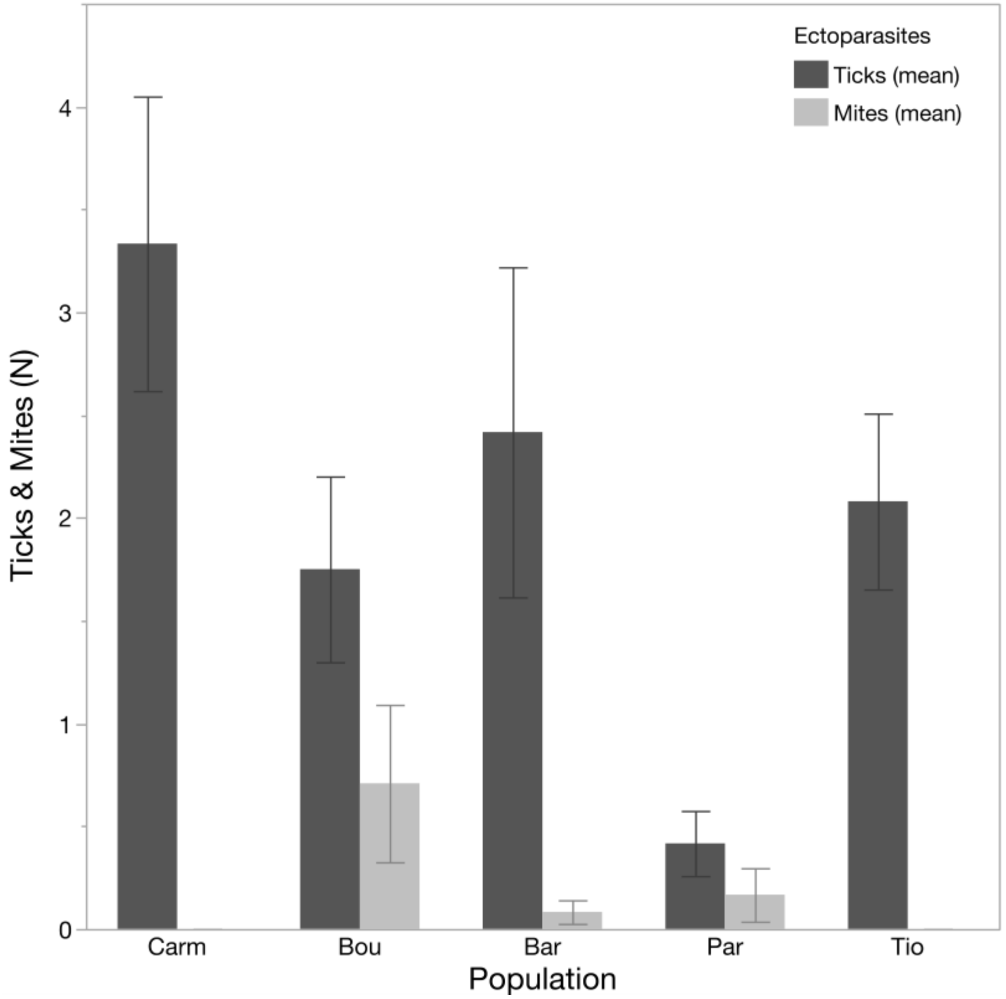

Figure 3.6. Mean ectoparasite number amongst by population in order of ascending *Karyolysus* parasite abundance.

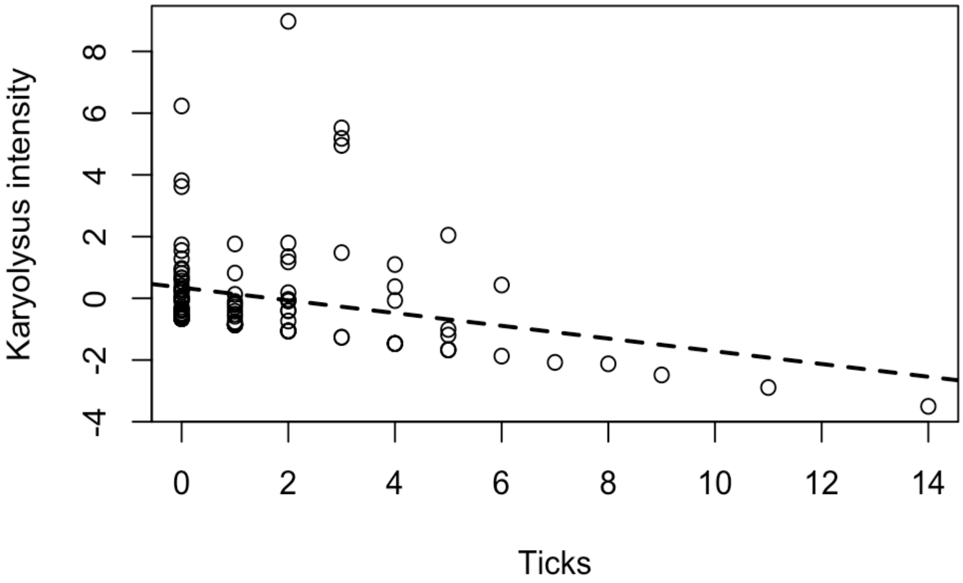

Figure 3.7. Initial intensity of *Karyolysus* infection in relation to tick load. We found a negative relationship between tick load and *Karyolysus* infection status upon entry into the lab (z=-3.0189, p=.0025).

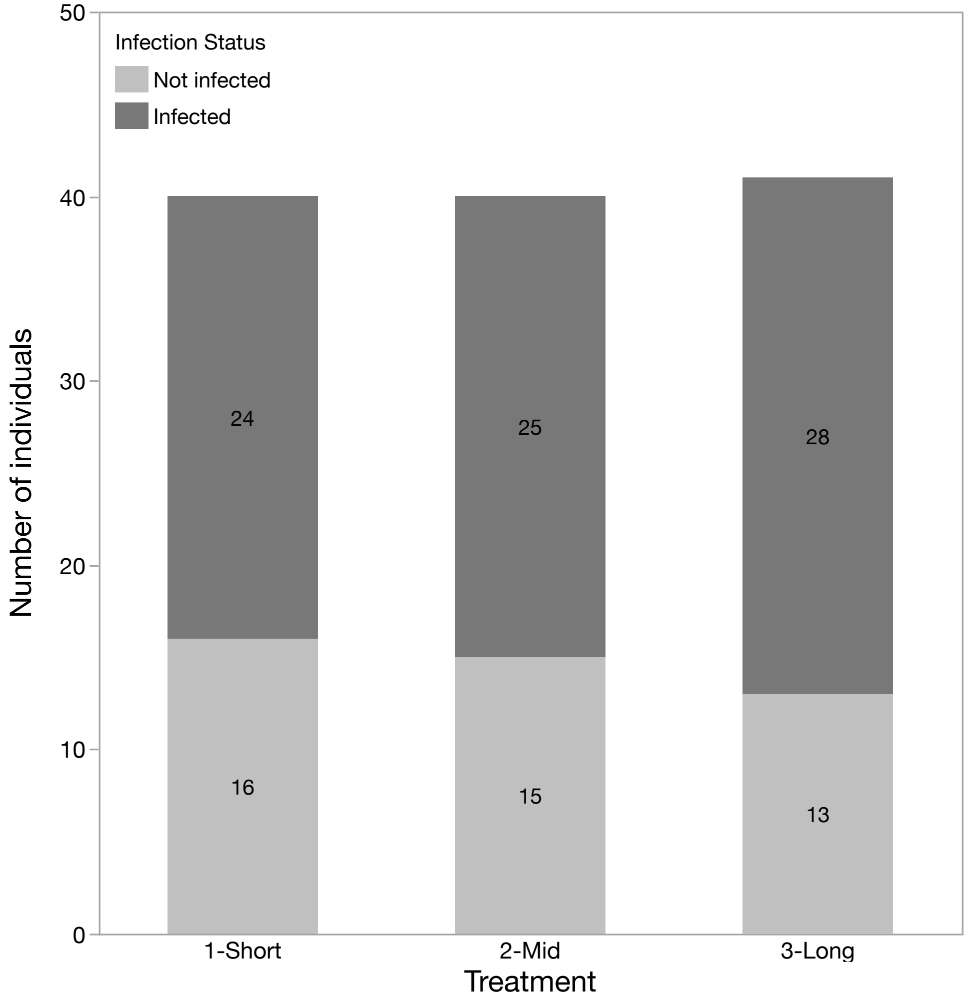

Figure 3.8. Number of individuals infected in the different treatments. Number of infected individuals did not significantly differ between treatments ($\chi^2_2 = .3245$, p=.8502).

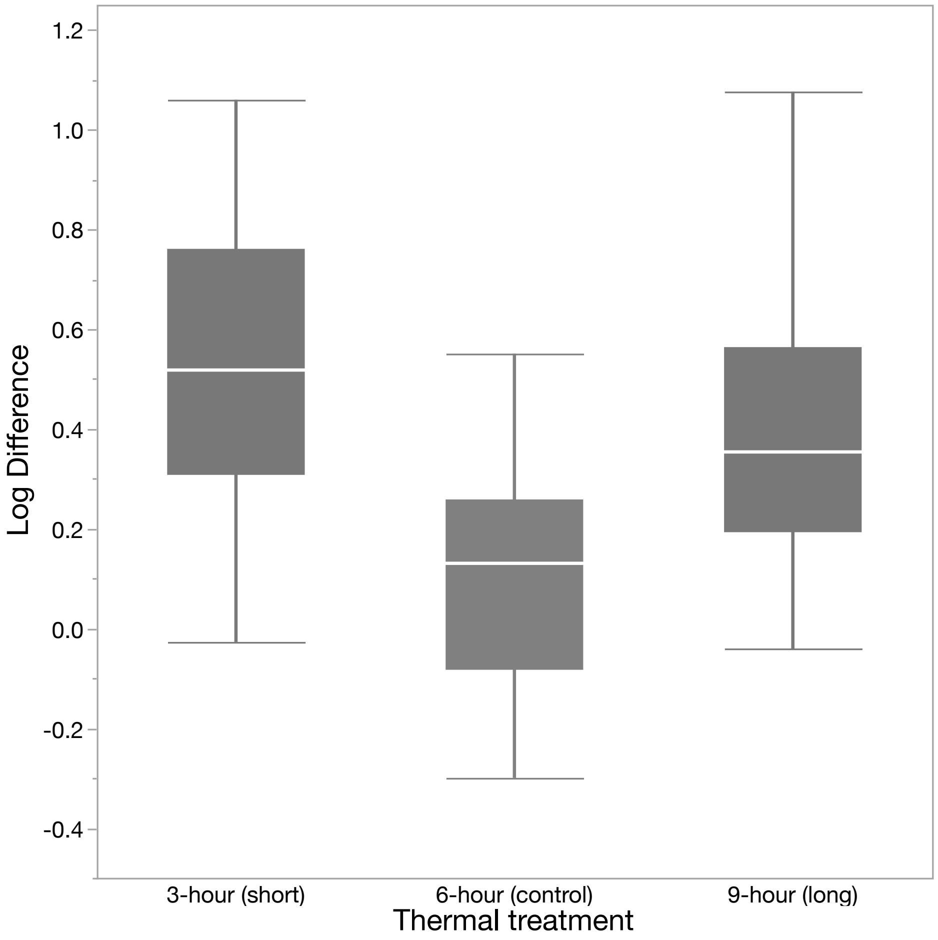

Figure 3.9. Graph showing the log difference in parasite abundance between *Karyolysus* endoparasite load pre-treatment and endoparasite load post-treatment in populations PAR, BOU and CARM combined by treatment. The control treatment had a significantly lower increase in parasite load over time in treatments than either the short or long ($F_{2,24}$= 5.55, p = 0.0105).

Synthesis

Climate change, as a result of anthropogenic disturbance, is an incredible global experimental, that while largely negative, is also an opportunity for scientists to gather information about how organisms react in the face of change. Thermoregulation is critical to ectotherm survival, and climate change threatens to impose constraints on it through extreme weather events and temperatures pushing the thermal limits of these organisms (Kearney et al. 2009; Sinervo et al. 2010). Arguably, the window when basking opportunity is most important to survival and reproductive fitness is during gestation, particularly in viviparous organisms when temperature regulation is critical to offspring viability and phenotype (Shine and Harlow 1993; Wapstra et al. 2010; Wang et al. 2017b). Thus, while the research I completed for this dissertation involves specific case studies stemming from a single lizard species, *Zootoca vivipara*, it has broad implications for how organisms may respond in the wake of global climate change.

In **Chapter 1,** I investigated female thermoregulatory behavior in response to novel basking opportunities. We found evidence that females from more variable thermal environments had significantly larger responses between treatments, supporting the climatic variability hypothesis (Gutiérrez-Pesquera et al. 2016). My research indicates that these lizards are capable of adjusting their thermoregulatory behavior in the short-term in response to treatments in the laboratory but are clearly influenced by the historical effects (either by short-term acclimitization or by longer-term adaptation, citation) of their home population in that response. Thus, populations experiencing greater thermal variability may have an advantage adjusting to novel climate extremes and weather events into the future.

In **Chapter 2,** I investigated how basking opportunity would impact offspring phenotype and viability. Elevated temperatures during development, predicted to occur as a result of climate change, regardless of reproductive mode, can have drastic and potentially long lasting consequences including imbalanced sex ratios and morphological size differences (Sinervo 1990a; Shine and Harlow 1993; Elphick and Shine 2002; Reedy et al. 2013; Refsnider and Janzen 2016; Noble et al. 2018). Ultimately, I found offspring phenotype was significantly affected by basking treatments with evidence of local population differences and sex-dependent outcomes in juveniles. However, I did not find significant evidence for strong maternal effects on progeny thermal preference. These results demonstrate the environmental variables surrounding gestation and the offspring life-history stage are critical in drawing conclusions on how robust a population and ultimately, a species, may be in response to climate change.

I also found context-dependent differences between populations in **Chapter 3** in their ability to fight off parasites as a result of basking opportunity. We found three of the five populations sampled followed the same pattern of having increased parasite abundances over time in the long treatment vs the short and control treatment. This has concerning implications for how increasing global mean temperature may impact these species and reinforces previous work showing increased parasite abundances in warmer environments for this species (Oppliger et al. 1996). These findings can hopefully help reinforce patterns in the thermal relationships between parasites and diseases to better prepare for and anticipate changes in disease-spread in the wake of climate change.

Patterns of selection are expected to change as novel thermal challenges arise from climate change. A critical next step is to encourage genetic studies to test whether adaptive evolution is occurring in response to shifting thermal environments. Estimates of heritability and genetic variation of a population would provide insights into the relative roles of phenotypic plasticity, adaptive evolution, or a combination. There is a dearth of studies linking climate change to direct genetic changes, so moving forward, scientists should consider analyzing the molecular underpinnings to move the field in an important direction (Merilä and Hendry 2014). In addition, this dissertation focused solely on pregnant females, and while this focus was important for the study, future work considering both sexes and pregnant/non-pregnant females would provide more answers to just how flexible (or conserved) these findings are, as well cascading effects of the demonstrable maternal origin x treatments impacts on progeny traits that could ultimately affect progeny survival and reproduction in nature in sex-dependent fashion (Sinervo et al. 1992; Sinervo and Svensson 1998; Mills et al. 2008a; Sinervo and McAdam 2008).

These results could aid in the development of new models using a framework of climate change modeling on ecosystem interactions and ecophysiology such as hosts and their parasites (Friedman et al. 2017) with a population genetic framework for maternal-effects inheritance (Kirkpatrick and Lande 1989) and selection (Bijma et al. 2007). The multi-level maternal-effect model of Bijma et al. (2007) is very suitable in the case of *Z. vivipara*.

When taken together, these chapters highlight the complex challenges gestating females face as a result of climate change. Not only must a female be able to respond to changes in her environment, but she must then allocate energy in such a way that ensures her offspring are viable while battling her own parasitic infections. This research highlights the amount of intraspecific variation that can exist within a single species in thermoregulatory behavior, offspring phenotype, and host-parasite interactions in response to environmental perturbations. My dissertation is a piece of the puzzle adding to the body of knowledge that improves our understanding of how climate change is affecting and re-shaping how organism's function. The results of this dissertation will hopefully encourage further research while adding to the growing body of knowledge that anthropogenic climate change has the power to seriously impact life on our planet.

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