Enhanced reproductive outcomes under worst-case climate change scenarios in Red-sided Garter snakes (*Thamnophis sirtalis parietalis*)

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ABSTRACT

As global temperatures continue to increase, understanding the impacts of warming environments is increasingly relevant. Temperature is especially relevant for ectothermic organisms which depend upon consistent and predictable annual temperature cycles for reproduction and development. However, additional research is required in this area to elucidate the potential long-term impacts of climate change. To understand how projected increases in environmental temperatures may impact reproductive outcomes within natural populations of ectothermic vertebrates, we manipulated ambient temperatures during gestation in Red-sided garter snakes (*Thamnophis sirtalis parietalis*). Wild snakes were collected in the Interlake region of Manitoba, Canada during their spring mating season and allowed to mate in controlled conditions. For the duration of gestation, mated females were placed into one of two ambient thermal conditions: temperatures emulating those found in the species' natural habitat or temperatures with a consistent 5 ºC increase to match end-of-century climate change projections. We recorded observations for each litter and all neonates resulting from controlled mating trials. We observed no difference in litter sizes or birth rates between thermal conditions. Interestingly, we observed both a significant reduction in gestation length and a significant increase in neonate

body size associated with increased ambient temperatures. These results suggest that increased temperatures during gestation may confer reproductive benefits for the northern populations of this species even under the most extreme current modeled warming predictions. We discuss the broader implications of this effect, including possible negative ecological outcomes.

Key words: Climate change, Reproduction, Gestation, Ectotherm, Viviparous, Thamnophis sirtalis parietalis

1. Introduction

In a time of rapidly changing environmental temperatures, it is increasingly important to elucidate how altered thermal conditions impact the coordination of important life history stages and essential events such as mating, gestation, and foraging. Because reproductive success is a defining feature of individual fitness, factors that greatly impact reproduction among individuals within a population have the potential to dramatically affect the long-term success and survival of that population (Brommer et al, 2002; Sherman and Runge, 2002; Rabaiotti et al., 2023). Ectotherms are influenced particularly strongly by environmental conditions, especially temperature, which is frequently an important cue used to coordinate behavior and physiological processes essential to reproductive success including courtship behavior and successful incubation or gestation (Huey and Stevenson, 1979; Huey, 1982; Miles et al., 2000; Lourdias et al., 2013). Ectotherms from terrestrial ecosystems demonstrate existing plastic physiological and behavioral mechanisms to maintain homeostasis during large seasonal and acute temperature fluctuations (Huey et al., 2001; Gunderson et al., 2017; Noer et al., 2022). These extreme

temperature fluctuations have been increasingly exacerbated by recent climate change (Masson-Delmotte et al., 2021). Therefore, investigating how terrestrial ectotherms respond to these new conditions provides an excellent opportunity to explore the broader impacts of a warming climate.

The challenge of maintaining ideal thermal conditions during embryonic development is central to reproductive success among many ectotherms, though overcoming this challenge is accomplished using different strategies. Oviparous (egg laying) ectotherms must ensure consistent thermal conditions for their eggs throughout the entire period of embryonic development by utilizing complex behaviors such as careful nest building, precise nest site selection, or by maintaining thermal conditions through monitored incubation of clutches (Du and Shine, 2014). Typically, these nest sites are abandoned by the parent shortly after egg deposition, allowing the nest itself to maintain environmental conditions conducive to proper embryonic development while the mother is free to forage and rebuild depleted energy reserves (Shine, 1988; Gans et al., 1996; Huang and Pike, 2013; Doody and Refsnider, 2022). In contrast, viviparous (live-bearing) ectotherms must alter their behavior while gravid to maintain optimal internal thermal conditions throughout gestation, even to the extent of risking their own survival to prioritize ideal thermal conditions for developing embryos (Seigel et al., 1987; Charland and Gregory, 1995; Gregory et al., 1999; Lorioux et al., 2013). This behavioral tradeoff serves to illustrate the importance of an organism's thermal environment, and that maintaining proper temperature is vital for the survival and optimal development of offspring and therefore directly impacts the individual fitness of reproducing adults. Temperatures experienced during gestation are often observed to impact neonate size in viviparous squamates (Mathies and Andrews, 1997; Lourdias et al., 2004; Gao et al., 2010; King, 2022). Both greater neonate body length (Brown

and Shine, 2004; Kissner and Weatherhead, 2005; Addis et al., 2017) and greater body mass (Jayne and Bennett, 1990; Bronikowski, 2000; Kissner and Weatherhead, 2005) are associated with significantly increased rates of survival to adulthood in viviparous snakes. Here, we use the term “body condition” to refer specifically to the ratio of body mass to snout-vent length (SVL), which is frequently used as a proximate measure for the nutritional state and overall health of an individual.

Changes to environmental thermal conditions during gestation have been observed to impact both physiology and behavior of viviparous ectotherms to alter characteristics such as maternal energy use and basking and foraging behavior (Lourdias et al., 2002; Ladyman et al., 2003; Wang et al., 2017), gestation length (Blanchard and Blanchard, 1940; Ji et al., 2006; Wang et al., 2017; King, 2022), litter size (Lourdias et al., 2004; Zhang et al., 2010; Tang et al., 2012), and body condition and viability of neonates (Lourdias et al., 2004; Wang et al., 2017; King, 2022). Although there is often agreement regarding the influence of warmer temperatures on gestation, neonate survival, and body condition, the specific effects observed differ between studies and depend on the biological system examined. Wang et al. (2017) reported that increased ambient temperature was associated with significantly shorter gestation length, increased neonate mass, and a decrease in general activity during gestation for Toad-headed lizards (*Phrynocephalus putajatia*). Beuchat (1988), found that increased environmental temperatures during gestation were associated with a decrease in gestation length in Yarrow's spiny lizards (*Sceloporus jarrovi*), but this study also reported that sustained increased temperatures resulted in a detrimental effect on the body conditions of both mother and neonate. This latter general trend was further supported, as Tang et al. (2012) reported an increase in litter size and a decrease in gestation length associated with higher temperatures for Multi-ocellated

racerunner lizards (*Eremias multiocellata*), but that these potentially beneficial effects were lost as temperatures continued to increase. Lourdias et al. (2004) demonstrated that increased ambient temperature resulted in shorter gestation length, increased embryo viability, and caused phenotypic changes, including changes to scale patterns, in neonate European asps (*Vipera aspis*) within a population living at the northern boundary of its range. Each of these studies demonstrate that, even among animals that are able to effectively behaviorally thermoregulate, increased ambient temperatures significantly impact both the behavior of gravid females and the resulting reproductive outcomes in viviparous species.

Red-sided garter snakes (*Thamnophis sirtalis parietalis)* are viviparous ectotherms and one of the most northerly living squamate species in North America (Aleksuik and Stewart, 1971). The focal population of this study is found in the Interlake region of Manitoba, Canada near the northern extreme of the species' range. The Interlake region is particularly well suited for studies examining the effects of temperature on ectothermic vertebrates, as it is characterized by major seasonal changes to the thermal landscape. Unsurprisingly, ectotherms living within this region are well adapted to this extreme environment and demonstrate highly coordinated annual behavioral cycles primarily driven by seasonal temperature changes (Aleksiuk and Stewart, 1971; Gregory, 1974; Lutterschmidt et al., 2006). Indeed, Red-sided garter snakes spend the majority of their life in a hibernation-like state of winter dormancy known as "brumation," during which the entire population retreats into subterranean hibernacula for up to 8 months each year (Aleksiuk and Stewart, 1971; Costanzo, 1985). Brumation is a behavioral and physiological state characterized by extremely low levels of activity and greatly reduced metabolic rates in ectotherms. This state is driven by low temperatures and serves as an essential strategy for surviving extremely cold and resource-limited winter conditions (Gregory, 1982; Geiser, 2013).

Red-sided garter snakes remain relatively inactive throughout brumation and do not feed for approximately 8-9 months each year (O'Donnell, 2004). Thus, they must rely solely upon stored energy to survive winter dormancy as well as the intense and energetically costly activities of mating and migration immediately following emergence from the hibernacula in early spring (Aleksiuk and Gregory, 1974; Aleksiuk, 1976). These energy stores must be obtained during an extremely brief summer feeding season lasting as little as 10 weeks, during which an individual must acquire all of the energy necessary for an entire year of growth, development, reproduction, and survival (Crews et al., 1987; O'Donnell et al., 2004). Moreover, the challenge of building energy reserves becomes even more difficult for reproducing females as this brief feeding period coincides with gestation – a period that is both behaviorally and energetically demanding. Thus, this limited window of time is critically important and has the potential to dramatically impact individual fitness, as a reproducing female must carefully balance activities and energy demands to simultaneously ensure survival while maximizing reproductive success.

Although climate change-induced warming has resulted in a global average temperature increase of approximately 0.6 ºC over the last 60 years, the Interlake region has been disproportionately impacted, experiencing nearly triple the global rate with recorded temperatures showing an increase of 1.7 ºC over the same period (Zhang et al., 2000; Houghton et al., 2001; Environment Canada, 2014; Bush and Lemmen, 2019). Global temperatures are predicted to continue to increase by as much as 5.3 ºC by the end of the century, which is well beyond the findings of previous work where increases of as little as 2 ºC significantly impacted gestation in viviparous ectotherms (Houghton et al., 2001; Kingsolver et al., 2013; Wang et al., 2017; Masson-Delmotte et al., 2021). With current regional warming levels so much higher than

the global average, it is reasonably likely that future regional warming will be at the higher end of global climate change model projections.

Currently, the daily mean environmental temperatures experienced by Red-sided garter snakes during the summer feeding and gestation period range from 13.8 ºC in late September to 20.1 ºC in July, and mean maximum daily temperatures reach 25 ºC (https://en.climate-data.org/north-america/canada/manitoba/inwood-108217/). Previous research focused on the Red-sided garter snakes and other members of the genus *Thamnophis* have shown that both free-ranging and captive gravid females behaviorally maintain daytime body temperatures between 26 ºC and 31 ºC – a range that typically exceeds ambient temperatures (Gibson and Falls, 1979; Arnold and Peterson, 2002; Hughes et al., 2019).

In the present study, we manipulated the thermal environment of gestating female Red-sided garter snakes by increasing ambient temperatures by an ecologically relevant 5 ºC and observed the impact of this increase on gestation length and neonate size and body condition. We predicted an increased ambient temperature of 5 ºC would significantly reduce gestation length. However, we also predicted that this thermal increase would exceed beneficial thresholds and individual gains would be lost resulting in a reduction in neonate body condition or a reduction of the number of offspring per litter (Beuchat, 1988; Tang et al., 2012).

2. Materials and methods

All experimental protocols presented were approved by the Oregon State University Institutional Animal Care and Use Committee (Animal Care and Use Protocol 5058), in accordance with the Association for the Assessment and Accreditation of Laboratory Animal

Care. This research was conducted under the authority of Manitoba Wildlife Scientific Permit No. WB20333.

3. *Animal Collection*

We collected free-ranging male and female Red-sided garter snakes (male n = 80, female n = 80) from the natural population at over-wintering hibernacula near the town of Inwood, Manitoba, Canada during the spring mating season between 1 May and 4 May 2018. This site contains several communal hibernacula that collectively shelter a population estimated at approximately 35,000 snakes (Shine et al., 2006). Male snakes were selected and captured after being observed actively exhibiting courtship behavior. Non-mated females were selected and captured only if their initial emergence from the hibernaculum was observed, and they were being actively courted by males. To minimize the effects of body size variation on litter size, all females selected for this study were approximately the same size, with SVLs and body masses of approx. 60 cm and 100 g, respectively. To confirm that the females had not yet mated that season, we visually inspected their cloaca for copulatory plugs upon collection. Copulatory plugs are large, gelatinous spermatophores deposited by a male inside of a female's cloaca during copulation. Copulatory plugs are substantial in size, persist for several days, and represent an easily identifiable and reliable indication that a female has recently mated (Whittier et al., 1985; Friesen et al., 2013). Any female presenting a copulatory plug, or any indication of potentially having mated, was immediately released. All animals were collected by hand and placed in cloth bags for transportation to the Chatfield Research Station, Chatfield, Manitoba. Snakes were separated by sex and housed outdoors in specially designed 1 m^3 nylon enclosures containing natural stones, hide boxes, and water dishes. Water was provided *ad libitum* for the duration of captivity, but food was not provided in the field or at the research station, as this species is

aphageous during the spring mating season (O'Donnell et al., 2004, Crews et al., 1987). Capture, handling, and transportation are not likely to have impacted results, as previous studies have consistently shown that these activities do not adversely affect survival, reproduction, or courtship behavior (Friesen et al., 2013; Friesen et al., 2014).

4. Mating Trials

To control mating conditions and timing, we used courtship arenas (opaque, cylindrical, nylon enclosures 0.5 m in diameter and 1 m tall) with heat lamps (above) and electric heating pads (below) to maintain homogenous thermal environments at ≥ 13.0 ºC – optimal temperatures to stimulate vigorous courtship behavior. Female snakes were placed individually into arenas and paired with 5 randomly selected males. All snakes were allowed to freely behave under constant observation until successful copulation occurred. Successful copulation was confirmed by visual inspection and identification of a copulatory plug. Females with copulatory plugs were separated from the males and transferred to a 1 m^3 outdoor nylon enclosure containing only mated females. Each male was allowed to mate only once. Males that successfully mated were removed from the remainder of the study to minimize any potential bias that may arise from allowing a male to sire more than a single litter of offspring. All males were marked dorsally with indelible ink to prevent recapture and released at the site of capture within 5 days of collection. All controlled matings took place within the same 48-hour period.

4.1 Experimental Conditions and Data Collection

We transported the mated females (n = 50) by car to the laboratory facilities at Oregon State University. Animals were transported in cloth bags, housed in a thermally controlled environment (air temperatures between 20 ºC and 22 ºC), and given water via a damp sponge to

prevent dehydration for the duration of the trip (< 30 h). Females were sorted by size and systematically assigned to one of two groups in order to ensure that each group contained snakes with an equivalent distribution of body sizes. Each group was then randomly assigned to different thermal regimes representing treatment and control conditions. Once assigned to a group, and for the remainder of the study, snakes were housed within microprocessor-controlled environmental chambers (Environmental Growth Chambers, Chagrin Falls, OH). The control group was housed within a chamber emulating the thermal environment found in this species' natural range during the summer feeding period, which consists of a daytime temperature of 22 ºC and a nighttime temperature of 15 ºC. The treatment group was housed in an identical chamber set to maintain ambient temperatures increased by 5 ºC, which consists of a daytime temperature of 27 ºC and a nighttime temperature of 20 ºC. Females were housed individually in 45 liter glass aquaria containing approximately 2.5cm of CareFresh™ paper fiber substrate, a water bowl, a hide box, a rock to facilitate shedding of skin, and a heat lamp situated at one end to allow behavioral thermoregulation within the constraints of the experiment. Photoperiods were identical for both treatments and emulated that of the summer feeding period in Manitoba (16 h:8 h light:dark cycle). Basking lamps provided additional heat for 4 hours per day in the center of the daylight photoperiod. Humidity was maintained at 65 % for both treatments. Maximum temperatures under the basking lamps were approximately 31 ºC for control group and 40 ºC for the treatment group. Water was provided *ad libitum*, and food (a mixture of earthworms and salmon fry supplemented with calcium) was provided once per week, during which time all snakes were allowed to eat to satiation. Snakes were maintained in these conditions for the duration of the experiment.

Each female was inspected daily to identify births, and offspring were collected within no more than 24 hours of birth. Dates of birth and number of offspring were recorded for each litter. Neonates were weighed and measured immediately after collection. Masses were recorded to the nearest 0.01 g and SVLs were recorded to the nearest 1 mm. Body mass was divided by SVL to calculate a body condition index (BCI; g/cm) for each neonate. BCI is frequently used as a measure of approximate overall health and nutritional state as it accounts for both body mass and length for the same individual, allowing for a better description of the individual than comparing those values separately (Madsen and Shine, 2002). Measurements were collected for every litter and for every neonate until the last female gave birth in late September. All adult snakes were maintained in captivity until the following spring then returned to the location of capture. Offspring were maintained in the lab for further data collection facilitating additional studies aiming to characterize growth and development.

5. Results

Gestation duration, litter size, neonate SVL, neonate mass, and neonate body condition index (BCI) were all checked for normal distributions using the Shapiro-Wilk Normality Test, and all were found to be significantly different from a normal distribution ($P < 0.005$ for all measures). Because the data were not normally distributed, we used a non-parametric two-sample Wilcoxon rank sum test for each measure to test for significant differences between the treatment and control conditions. The median values for gestation length and neonate mass, SVL, BCI, were all found to be significantly different between groups (Figure 1, Supplementary Table 1). Median mass, SVL and BCI for neonates in the 27 ºC treatment group were all significantly

greater than those in the 22 ºC control group ($P < 0.001$ for all measures) (Figure 1: D-F). The median gestation length was significantly shorter for the 27 ºC treatment group compared to the median for the 22 ºC control group ($P < 0.001$) (Figure 1: A). Litter size was not significantly different between thermal conditions ($P > 0.74$) (Figure 1: E). Additionally, there was no significant difference in birth rates measured as the proportion of females that successfully gave birth divided by the total number of females within each group (two proportions z-test $P > 0.77$) (Figure 1: F).

6. Discussion

Our observations of gestation times accurately agreed with our initial prediction that a 5 ºC increase in temperature during gestation would result in a reduction in gestation time. Indeed, we found that gestation lengths were significantly reduced in the 27 ºC treatment conditions, even to the extent that the resulting ranges of birth dates did not overlap between the treatment and control groups. The median gestation length for the 27 ºC treatment was 21 days shorter than that of the 22 ºC control group representing a 17.8 % decrease in median gestation length. Shorter gestation lengths may increase the female's survival as gravidity imposes energetic costs as well as reduced locomotory and foraging capabilities and requires increased amounts of time engaged in active thermoregulation – all of which may ultimately result in an increased exposure and risk of predation for the gravid female (Seigel et al., 1987; Charland and Gregory, 1995; Gregory et al., 1999). As these costs in Red-sided garter snakes are imposed simultaneously with the already extremely limited opportunities for summer foraging, a reduction in gestation length would allow for both increased amount of time to forage each year and more frequent prey capture during this period ultimately resulting in increased opportunity to replenish depleted energy stores after giving birth. Because the summer feeding period is so limited, even small

increases in annual energy intake may have a large impact facilitating improved winter survival and potentially a shorter interlude between reproductive bouts, thus increasing overall fecundity (Aleksiuk and Stewart, 1971; Costanzo, 1985).

Although we observed no differences in either birth rates or average litter sizes between treatments ($P > 0.77$ and $P > 0.74$ respectively), we did observe a significant difference in the overall size and body condition of neonates. SVL and mass were both significantly greater for the 27 ºC treatment group compared to the 22 ºC control group ($P < 0.001$ for both), and this difference remained significant when combined to calculate individual BCI values ($P < 0.001$). Surprisingly, a 5 ºC increase ambient temperature both significantly decreased gestation length and, contrary to our initial predictions, significantly increased neonate size and body condition. Because larger size at birth is associated with both improved short term and lifetime survival in snakes, offspring gestated in warmer conditions will likely have increased overall fitness resulting in better parental reproductive outcomes (Bronikowski, 2000; Brown and Shine, 2004; Kissner and Weatherhead, 2005; Addis et al., 2017). However, this increase in ambient temperature had no impact on successful birth rate or the number of offspring in each litter. The observed decrease in gestation length is consistent with those previously observed among a number of other viviparous ectotherms (Blanchard and Blanchard, 1940; Ji et al., 2006; Wang et al., 2017; King, 2022).

Though not observed during this study, a common observation resulting from a moderate increase in ambient temperature is an increase in average litter size (Lourdias et al., 2004; Zhang et al., 2010; Tang et al., 2012). However, larger increases in ambient temperature have been shown to have a detrimental impact on the body conditions of the neonates, as well as potentially reversing any potentially positive impact caused by a moderate temperature increase (Beuchat,

1988; Yan et al., 2011; Tang et al., 2012). The 5 ºC increase in ambient temperature treatment used in the present study did not result in any observed detrimental impact. This suggests that a 5 ºC increase in ambient temperature is below the threshold at which any detrimental effects would begin to arise within this population. This further suggests that there may be additional potential for increased beneficial effects as temperatures increase in excess of 5 ºC, but before temperatures reach this critical, but unknown, threshold. Further research exploring a wider range of thermal conditions during gestation would be interesting, as this would expand our perspective allowing for increased ability to predict the effects of future increases in ambient temperatures and would potentially pinpoint the maximum ambient temperature threshold at which beneficial results began to reverse and detrimental impacts begin to arise.

This study did not identify that critical threshold, but these results suggest interesting implications for future warming associated with continued climate change. The response variables examined here were limited and did not directly address or account for other phenotypic affects that may have arisen from increased temperatures during gestation. While it has been well documented in oviparous ectotherms that increased thermal conditions can result in significant changes to sex ratios among offspring (Hawkes et al., 2007; Holleley et al., 2015; Noble et al., 2018), temperature likely plays little to no role in sex determination in viviparous snakes, such as Red-sided garter snakes, where sex is determined chromosomally rather than being driven by environmental temperatures (Dunlap and Lang, 1990; Matsubara et al., 2006; Viana et al., 2020; reviewed in Singchat et al., 2020). However, evidence suggests that many other developmental processes may be influenced by altered thermal conditions, and increased temperatures may result in changes to offspring phenotype such as differences in scale and bone development (Arnold and Peterson, 2002; Lourdias et al., 2004). Additionally, the impacts of

temperature during gestation on offspring traits such as the aforementioned changes to morphology, body size, and body condition are frequently associated with long-lasting impacts on survival after birth (Bronikowski, 2000; Brown and Shine, 2004; Kissner and Weatherhead, 2005; Noble et al., 2018).

Current climate change models predict future global average temperature increases of up to 5.3 ºC by the end of the century (Houghton et al., 2001; Masson-Delmotte et al., 2021). Thus, the results of this experiment show that an increase in average temperature of this degree, specifically during the brief yet crucial opportunity for feeding and gestation, is not likely to cause direct detrimental effects on the reproductive success of northern populations of Red-sided garter snakes but will potentially result in improved neonate fitness. However, the extended impacts of increasing temperature are likely to result in other adverse effects on this population. Thermal stress caused by exposure to increased temperature extremes (acute high or low temperatures) is known to result in a number of detrimental effects including misaligned behavioral timing, altered development, reduced or altered activity, and higher mortality rates (Houghton et al., 2001; Jentsch et al., 2009; Paaijmans et al., 2013; Stott, 2016). The greater thermal instability associated with climate change conditions may also lead to thermally induced alterations to energy use and metabolic rate (Friesen et al., 2015; Wilson, 2020; Holden et al., 2021). The combination of physiological and environmental changes due to increased environmental temperatures are also known to increase pathogen exposure and increase individual susceptibility to infection (Walther et al., 2001; Harvell et al., 2009; Gallana et al., 2013; Nnadi and Carter, 2021). Additionally, many species have been observed to alter seasonal migration patterns and shift territorial ranges to mitigate changes to their traditional ecosystems, ultimately resulting in disruption of ecosystems arising from novel interspecific competition and

alterations to the availability of prey species or trophic effects (Parmesan, 2006; Gilman et al., 2010; Urban et al., 2012; Salinas-Ramos et al., 2020). Although we observed effects of increased temperatures that appeared likely beneficial to offspring, it is possible that that the lifetime reproductive potential of mothers would in fact be reduced as a result of long-term exposure to increased temperatures or repeated bouts of rapid gestation. Thus, future research regarding changes to ambient temperatures during gestation may benefit from examining both the immediate and long-term health impacts of mothers, such as impacts on body condition, future fecundity, parasite load, and subsequent winter survival, in addition to the long-term survival and impacts on the physiology and long-term reproductive potential of offspring as well.

Higher temperatures caused by future warming have the potential to change the shape of this population, resulting in offspring that not only develop faster, but are born larger. In the present study, we observed no apparent tradeoff or cost to the overall number of offspring in each litter. This has the potential to dramatically increase the survival rates of future generations, while increasing the time each year spent foraging for both the mothers and the resulting offspring (Seigel et al., 1987; Charland and Gregory, 1995; Brown and Shine, 2004; Kissner and Weatherhead, 2005). This species already has extremely large population sizes, with this den site alone numbering in the tens of thousands, and the potential for rapid increases in population could result in large impacts on their ecosystem spanning trophic levels (Shine et al., 2006).

The myriad impacts of climate change on an ecosystem are overwhelmingly complicated and interconnected. Thus, it is exceedingly difficult to develop models that accurately predict comprehensive, species-specific outcomes in response to altered thermal landscapes. The present study examined the effect of altered temperatures during gestation within a single large population and thus represents only a single piece of the complex overall puzzle. As global

temperatures continue to rise, additional research examining the effects of increasing temperature on the behavior and physiology of individual species, as well as studies encompassing entire ecosystems, will provide the invaluable insight necessary to further our understanding of how temperature affects ecosystems and, more importantly, will enhance our ability to predict the impacts of future climate change on the global ecosystem.

7. Conclusions

Our research demonstrated that, even under the worst-case scenario climate change model predictions, Red-sided garter snakes may see improved reproductive outcomes at the northern edge of their range. Reduced gestation length has the potential to facilitate increased fitness for both mother and offspring with no observed cost to reproductive outcomes.

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Supplementary Table 1: Median effect of a 5 ˚C increase in ambient temperature on gestation length and neonate body condition in Red-sided garter snakes.

	Mass (g)	SVL (cm)	BCI (g/cm)	Gestation (days)	Litter size
Treatment (27 ºC)	1.47 (SD 0.23)	15.78 (SD 0.76)	0.095 (SD 0.01)	97.0 (SD 3.2)	21.0 (SD 6.5)
Control (22 ºC)	1.33 (SD 0.21)	15.25 (SD 1.10)	0.085 (SD 0.01)	118.0 (SD 7.1)	18.0 (SD 8.7)
Wilcoxon (*P*-value)	<0.001	<0.001	<0.001	<0.001	>0.74 (ns)

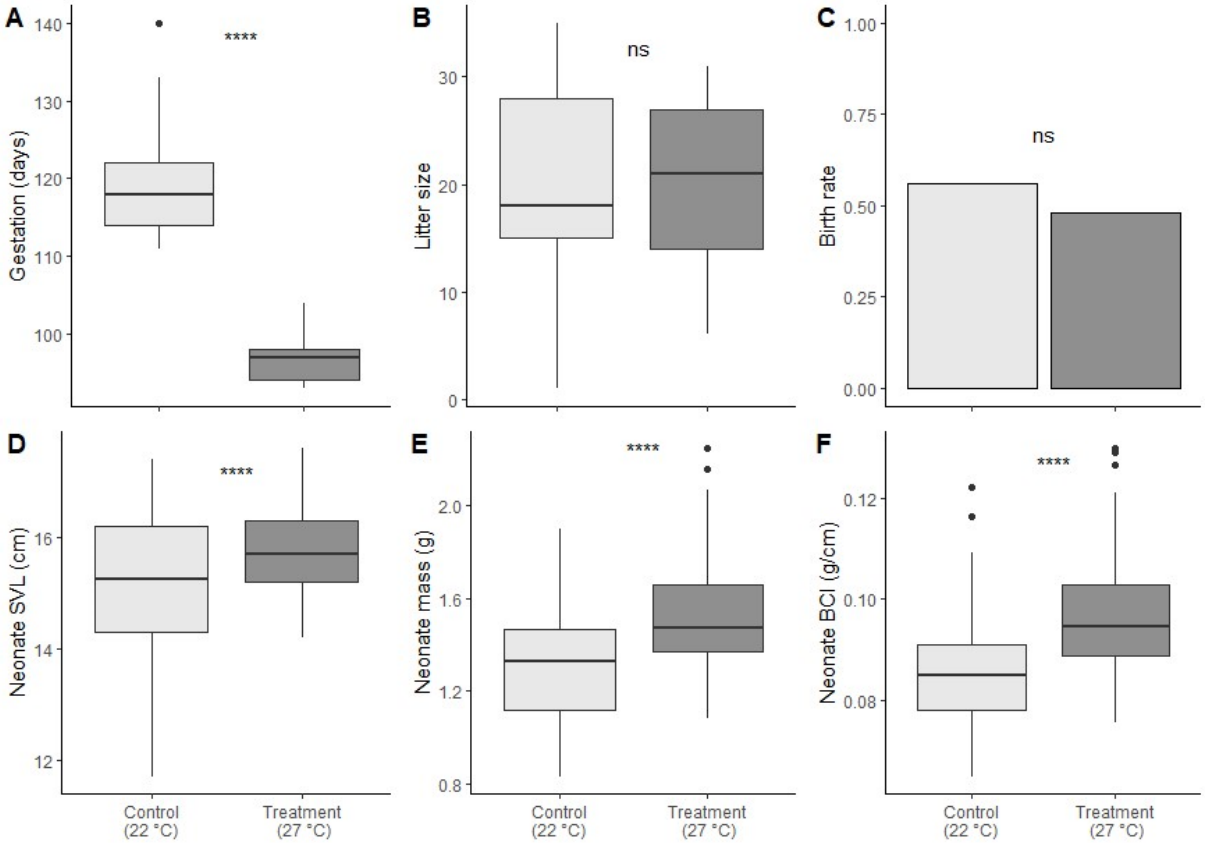

Fig. 1. Reproductive outcomes under a 5 ºC increase in ambient temperature during gestation. Plots A-E show comparison between control (ambient daytime temperature 22 ºC) and treatment (ambient daytime temperature 27 ºC) conditions. All comparisons made using a nonparametric two-sample Wilcoxon rank test except for plot C. A: Gestation length in days as measured from the day of confirmed copulation to the day of birth ($P < 0.001$). B: Litter size for each female that successfully gave birth ($P > 0.74$). C: The proportion of mated females that gave birth were compared using a two proportions z-test ($P > 0.77$). D: Neonate snout-vent lengths in cm ($P < 0.001$). E: Neonate mass in grams ($P < 0.001$). F: Neonate body condition index of mass to SVL ratio ($P < 0.001$).