Animal Behaviour 118 (2016) 27–32

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Immune activation influences the trade-off between thermoregulation and shelter use

George Todd*, Alicia Jodrey, Zachary Stahlschmidt [1]

Biology Department, Georgia Southern University, Statesboro, GA, U.S.A.

ARTICLE INFO

Article history:
Received 12 November 2015
Initial acceptance 16 December 2015
Final acceptance 29 March 2016

MS. number: A15-00971R4

Keywords:
cornsnake
ecological immunology
fever
shelter utilization
temperature
thermal preference

Animal behaviour is influenced by many environmental factors, including temperature and predation risk. Although many species utilize shelters to buffer ambient temperature and avoid predators, a trade-off can exist between thermoregulation and predator avoidance (e.g. an animal avoids a shelter that is too hot or cold at the expense of increased vulnerability to predators). Immune activation influences both thermoregulation and shelter use, yet its role in mediating a trade-off between these two important processes is unclear. Thus, we examined the dynamics of this thermoregulation–shelter use trade-off using the cornsnake, *Pantherophis guttatus*, and a repeated measures 2×2 factorial design in a thermal gradient where shelter availability and immune activation status were manipulated. Immune activation (injection of lipopolysaccharide, LPS, an endotoxin found in the cell walls of Gram-negative bacteria) did not elicit behavioural fever or change shelter use when shelter was available across the entire thermal gradient. Although snakes strongly prioritized shelter use (e.g. snakes injected with saline were observed under shelter 98% of the time), their prioritization shifted during immune challenge. Snakes injected with LPS that were forced to choose between preferred temperature and shelter use maintained thermoregulation, but they spent up to nine-fold more time exposed relative to when they were injected with saline. These results demonstrate the plasticity of the widespread trade-off between thermoregulation and shelter use. Our results also indicate that immune-challenged animals not exhibiting fever may still exhibit important shifts in the prioritization of thermoregulation; thus, we recommend a more nuanced assessment of the effects of immune activation on thermoregulatory behaviour.

The vast majority of animal species (99.9%) are ectothermic (Atkinson & Sibly, 1997) and, thus, are particularly sensitive to changes in ambient temperature. Temperature affects many life processes, including locomotion (Kubisch, Fernández, & Ibargűengoytía, 2011), digestion (Wang, Zaar, Arvedsen, Vedel-Smith, & Overgaard, 2003), growth (Yagi & Litzgus, 2013) and reproduction (El-Hafez, El-Sharkawy, & Hassan, 2014). Because ambient temperature varies spatially and temporally, thermoregulation (i.e. regulation of body temperature, T_b) is a crucial behavioural process for most animal taxa (Blouin-Demers & Weatherhead, 2001; Caillon, Suppo, Casas, Woods, & Pincebourde, 2014; Deban & Lappin, 2011; Hoecherl & Tautz, 2015; Reinert, 1993; Sauter, Crawshaw, & Maule, 2001).

Several features of the environment promote thermoregulation, including shelters (refuge structures), which can exhibit different absolute temperatures and temperature variability than ambient conditions (van den Berg, Thompson, & Hochuli, 2015; Stahlschmidt, Shine, & DeNardo, 2012). In addition to promoting thermoregulation and other behaviours (e.g. ambush hunting: Bevelander, Smith, & Kennth, 2006; Clark, 2007), shelters can promote predator avoidance for many animals, from worms (Dill & Fraser, 1996) to primates (Cowlishaw, 1997). Both facilitation of thermoregulation and predator avoidance can coincide (Roper, Bennett, Conradt, & Molteno, 2001; Schwarzkopf & Alford, 1996), but shelters are not always thermally optimal (e.g. a log exposed to constant sunlight may be too hot to promote optimal growth for an animal: Downes, 2001). Thus, a trade-off can occur where an animal is forced to choose between thermoregulation and using a shelter for protection from predators (Amo, Pilar, & Martín, 2004; Stahlschmidt & Adamo, 2013a). Although behavioural thermoregulation improves locomotive performance (Angilletta, Hill, & Robson, 2002), predator avoidance is linked to immediate

* Correspondence: G. Todd, Biology Department, Georgia Southern University, Statesboro, GA 30458, U.S.A.
E-mail address: gt00682@georgiasouthern.edu (G. Todd).
[1] Z. Stahlschmidt is now at the Department of Biological Sciences, University of the Pacific, Stockton, CA 95211, U.S.A.

http://dx.doi.org/10.1016/j.anbehav.2016.05.003
0003-3472/

survival and, thus, has been shown to be prioritized in thermoregulation–predator avoidance trade-offs (Mabille & Berteaux, 2014).

Like other important trade-offs, the thermoregulation–predator avoidance trade-off may be plastic in response to environmental conditions (Dosmann, Brooks, & Mateo, 2015; Stahlschmidt & Adamo, 2013a; Stahlschmidt, O'Leary, & Adamo, 2014). For example, pathogen exposure varies temporally and spatially (Raucher, 2002; Suwanpakdee et al., 2015), and it can influence thermoregulation and predator avoidance independently. During immune activation, many animals putatively shift their T_b to create an undesirable thermal environment for pathogens (Bicego, Barros, & Branco, 2007; Kluger, 1986). This shift in T_b often presents as fever (increase in T_b), such as in crayfish (Payette & McGaw, 2003), insects (reviewed in Stahlschmidt & Adamo, 2013b; but see Ballabeni, Benway, & Jaenike, 1995), mice (MacDonald, Begg, Weisinger, & Kent, 2012) and reptiles (Merchant, Fleury, Rutherford, & Paulissen, 2008; but see ; Zurovsky, Brain, Laburn, & Mitchell, 1987), but immune activation can have hypothermic effects on animals (Almeida, Steiner, Branco, & Romanovsky, 2006; Deen & Hutchison, 2001). Immune activation can also influence predator avoidance behaviour (e.g. cause an infected individual to react slower to predator attacks even at preferred temperature; Joop & Rolff, 2004; Lefcort & Eiger, 1993; Otti, Gantenbein-Ritter, Jacot, & Brinkhof, 2011; Rantala, Honkavaara, & Suhonen, 2010). The role of immune activation in the trade-off between thermoregulation and predator avoidance has been proposed (e.g. Nord, Sköld-Chiriac, Hasselquist, & Nilsson, 2014; Otti et al., 2011) but never explicitly tested.

Thus, we examined the dynamics of a thermoregulation–shelter use trade-off to test two hypotheses. We used the cornsnake, *Pantherophis guttatus*, to test our hypotheses because snakes utilize existing shelter (Hyslop, Cooper, & Meyers, 2009) and adjust their habitat selection to carefully regulate T_b (Aïdam, Michel, & Bonnet, 2013; Blouin-Demers & Weatherhead, 2001; Lorioux, DeNardo, Gorelick, & Lourdais, 2012; Lourdais, Guillon, DeNardo, & Blouin-Demers, 2013; McConnachie, Greene, & Perrin, 2011; Stahlschmidt et al., 2012). Like other animals (Johnson, 2002; Lefcort & Eiger, 1993), snakes may exhibit a shift in T_b and increase shelter use due to immune activation; thus, we first hypothesized that immune activation influences thermoregulation and shelter use independently (sensu Kluger, 1986; Otti et al., 2011). Under this hypothesis, we predicted that snakes would undergo fever and increase their shelter use during an immune activation. Our second hypothesis was that immune activation affects the thermoregulation–shelter use trade-off. We predicted that animals normally prioritize shelter use over thermoregulation (sensu Mabille & Berteaux, 2014) but that this prioritization is reduced during an immune challenge due to the strong effects of immune activation on thermoregulation (Almeida et al., 2006; Deen & Hutchison, 2001; MacDonald et al., 2012; Merchant et al., 2008; Payette & McGaw, 2003; reviewed in ; Stahlschmidt & Adamo, 2013b; but see ; Zurovsky et al., 1987). The results of our study will give insight into how an important environmental factor (immune activation by pathogens) influences the trade-off between two widespread behaviours (thermoregulation and shelter use).

METHODS

Animals and Maintenance

Pantherophis guttatus is a member of the family Colubridae and is commonly found throughout the southeastern United States (Dorcas & Gibbons, 2005). The experiment used 23 captive-born *P. guttatus* (1.5–2 years of age; 14 males and 9 females; body mass range 119–486 g) that were first- to fourth-generation progeny of snakes caught in Beaufort County, SC, U.S.A. Prior to trials, snakes were housed individually in translucent plastic enclosures (17 × 38 × 14 cm). To facilitate behavioural thermoregulation, subsurface heat tape at one end of the enclosures created a temperature range of 24.5–33 °C, which accommodates the preferred T_b of *P. guttatus* (Raske et al., 2012; Roark & Dorcas, 2000; Stahlschmidt, Jodrey, & Luoma, 2015). Snakes were fed frozen/thawed mice (10–20% of each snake's body mass) every 1–2 weeks and provided water ad libitum. Digestion can invoke T_b shifts in *P. guttatus* (Sievert et al., 2013) so snakes were nonabsorptive (>5 days postfeeding: Crocker-Buta & Secor, 2014) during trials. Throughout the study, snakes exhibited no inflammation or evidence of trauma at the sites of injection and, as further evidence that treatments were not overly stressful, they readily accepted food shortly after trials (see details below). All procedures were approved by the Institutional Animal Care and Use Committee of Georgia Southern University (protocol number I14004).

Thermal Gradient

Experiments were performed in thermal gradient lanes (length: 2.4 m; width: 0.3 m; height: 0.4 m) within an arena (Fig. 1). The temperature gradient was achieved with modified Flex-Watt heat tape (Calorique, West Wareham, MA, U.S.A.) placed below 2–3 cm of sand (a temperature stable substrate). Substrate temperature ranged from 20 °C to 35 °C because *P. guttatus* exhibit a preferred T_b of 26–27 °C (Raske et al., 2012; Roark & Dorcas, 2000; Stahlschmidt et al., 2015). When trials were in session, the top of the arena was covered with acrylic glass to keep snakes inside and to help stabilize the temperature of the substrate.

Experimental Procedures

To determine the role of immune activation on a thermoregulation–shelter use trade-off in *P. guttatus* ($N = 23$), we used a repeated measures 2 × 2 factorial experimental design in the thermal gradient arena. Two treatments were used, each with two levels: shelter availability (full and partial) and presence of immune status (challenge or control), as described below.

For the shelter availability treatment, we provided either shelter across the entire gradient ('full' shelter) or only at temperature extremes ('partial' shelter) (Fig. 1). In both cases, shelter consisted of a corrugated steel sheet (0.15 m wide) placed 3–4 cm above the substrate (Fig. 1). The temperature extremes in the partial shelter level were at least 2–3 °C below and above preferred T_b (20–23 °C and 29–35 °C, respectively); that is, the partial shelter level of treatment forced the snakes to choose between a shelter and their preferred temperature.

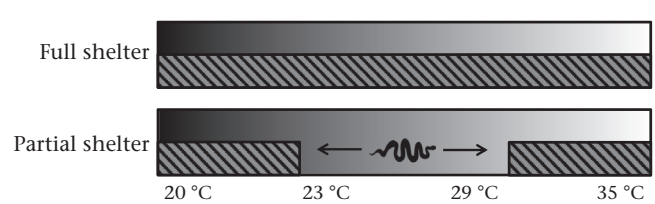

Figure 1. Diagram of shelter treatments in thermal gradient. Open area represents exposed space and diagonally patterned area represents sheltered space. Each snake underwent trials with both shelter types (full and partial) at two immune states (challenged and control). See text for details. A sample snake is shown to demonstrate how each snake had the option to move freely in any direction based on its temperature or shelter preference.

For each snake, the temperature it selected (T_s) in full and partial shelter treatments was determined in two immune states, the order of which was randomized. For each trial, snakes were given a subcutaneous injection (1 ml/kg of body mass) of phosphate-buffered saline (PBS) solution (1× concentration) or lipopolysaccharide (LPS, 10 mg of LPS dissolved in 1× PBS). Lipopolysaccharide is an endotoxin found on the membranes of Gram-negative bacteria that typically elicits a strong immune (and often febrile) response in many animals, including reptiles (Alexander & Rietschel, 2001; Burns, Ramos, & Muchlinski, 1996; Ortega, Stranc, Casal, Hallman, & Muchlinski, 1991; but see ; Zurovsky et al., 1987). The LPS was a lyophilized powder from *Escherichia coli* (Sigma-Aldrich, Rehovot, Israel, Lot no. 044M4004V) and the 1× PBS was made by adding tablets (Fisher Scientific, Fair Lawn, NJ, U.S.A., Lot no. 145850) to distilled water. Each snake was given an injection (LPS or PBS) at 2000 hours and was then allowed to habituate to its own lane within the thermal gradient overnight. We took six hourly measurements of temperature choice and shelter utilization the next day from 0800 to 1300 hours. Temperature measurements were taken using a factory-calibrated infrared thermometer (ProTemp 12, Jewell Instruments, Manchester, NH, U.S.A.; range −50–550 °C; accuracy: 1.5%; resolution: <0.1 °C). Because substrate temperature and T_b are highly related in reptiles (Lagarde et al., 2012), this method is useful in quiescent snakes and also because it is less invasive than direct methods such as cloacal probes. Snakes that were moving during an observation (<2% of observations) were not recorded for temperature. To avoid confounding olfactory signals among snakes, we mixed the sand and cleaned the shelters with 70% ethanol between trials. Each snake's trials ($N = 4$) were separated by at least 2 weeks.

Statistical Analyses

We performed analyses in SPSS (v.22 IBM Corp., Armonk, NY, U.S.A.) and determined two-tailed significance at $\alpha < 0.05$. For shelter preference, we used a binary logistic generalized linear mixed model where shelter status (full or partial) and immune status (control or LPS-challenged) were included as fixed effects, and each observation ($N = 6$ per trial for each of a given snake's four trials) was the dependent variable (0: under shelter; 1: not under shelter). Observation (but not trial) order significantly influenced shelter preference; thus, we included observation order (1–6) as a random effect. To examine whether animals had a preference for warmer or cooler shelter in the partial shelter trials, we performed chi-square goodness-of-fit tests for each immune status. For preferred T_s, we used a linear mixed model where shelter status and immune status were included as fixed effects, and each temperature observation ($N = 6$ per trial for each of a given snake's four trials) was the dependent variable. Trial (but not observation) order significantly influenced T_s; thus, we included trial order (1–4) as a random effect. To account for repeated sampling, we included animal ID as a random effect in all mixed models.

RESULTS

Shelter preference was influenced by the interaction between shelter status and immune status ($\chi^2_1 = 12$, $P = 0.001$) but not by shelter status ($\chi^2_1 = 0.47$, $P = 0.49$) or immune status ($\chi^2_1 = 2.8$, $P = 0.094$; Fig. 2) independently. When immune-challenged and given only partial shelter, snakes exhibited reduced shelter preference relative to other treatment combinations (83% versus >96% of observations under shelter; Fig. 2). When forced to choose between shelters that were cooler and warmer than their preferred shelters (i.e. during partial shelter trials), snakes strongly preferred cooler shelters when LPS-challenged (89% of observations) ($\chi^2_1 = 26$, $P < 0.001$) and when nonchallenged (99% of observations) ($\chi^2_1 = 46$, $P < 0.001$; Fig. 3). Preferred T_s was independently influenced by shelter status ($F_{1,517} = 8.6$, $P = 0.003$) and immune status ($F_{1,517} = 11$, $P = 0.001$), but these effects were largely driven by a shelter∗immune interaction ($F_{1,517} = 33$, $P < 0.001$; Fig. 3). When nonchallenged and given only partial shelter, snakes exhibited reduced T_s relative other treatment combinations (24 °C versus 27 °C; Fig. 3).

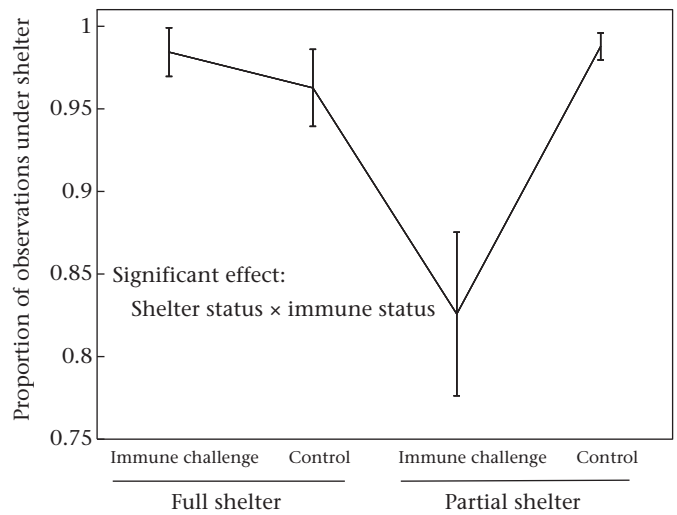

Figure 2. Effects of shelter treatment (full or partial) and immune status (challenged or control) on the proportion of observations of *P. guttatus* ($N = 23$) under refuge. Each snake underwent all combinations of trials in randomized order. See text for details about shelter and immune status treatments. Symbols represent means ± SE.

DISCUSSION

Although we did not detect a strong independent effect of immune activation on thermoregulatory or shelter use behaviour (Figs. 2 and 3), which does not support our first hypothesis, we did find support for our second hypothesis that immune activation influences the trade-off between thermoregulation and shelter use. The snakes utilized shelter at least 96% of the time when shelter was readily available (Fig. 2). However, when forced to choose between thermoregulation and shelter use, immune-challenged

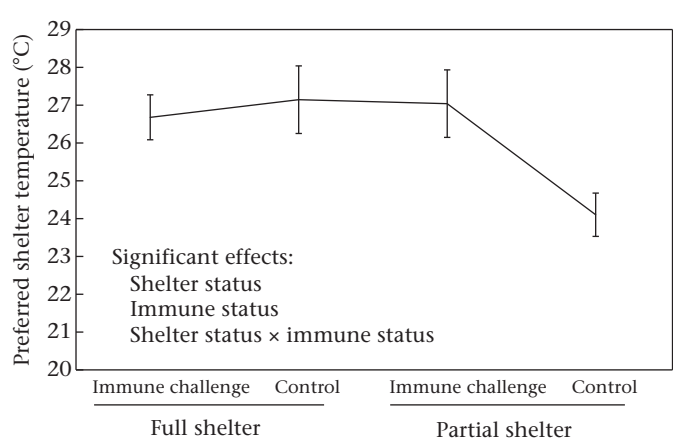

Figure 3. Effects of shelter treatment (full or partial) and immune status (challenged or control) on mean temperature selected by *P. guttatus* ($N = 23$). Each snake underwent all combinations of trials in randomized order. See text for details about shelter and immune state treatments. Symbols represent means ± SE.

snakes were more likely to maintain their T_s at the expense of shelter use, specifically, they spent nine-fold as much time exposed relative to control snakes (Fig. 2).

Across taxa, the effects of immune activation can have varying effects on temperature preference. Although fever is demonstrated by many animals (e.g. reviewed in: Kluger, 1986; Stahlschmidt & Adamo, 2013b), some species exhibit a hypothermic response to an immune activation or even no response at all, including several reptile species (Burns et al., 1996; Don, Estany, Ramos, Leoni, & Muchlinski, 1994; Laburn, Mitchell, Kenedi, & Louw, 1981; Zurovsky et al., 1987). Fever is a complex response, and several factors (e.g. the type and dosage amount of pyrogen and the timing of measurement: Stahlschmidt & Adamo, 2013b) must be considered when determining its presence or absence. Although immune activation can also influence predator avoidance behaviour and shelter preference (Joop & Rolff, 2004; Lefcort & Eiger, 1993), experiments examining fever typically do not include shelter (Burns et al., 1996; Cichón, Chadzińska, Książek, & Konarzewski, 2002; Don et al., 1994; Ortega et al., 1991; Stahlschmidt & Adamo, 2013a; Zurovsky et al., 1987). Shelter is important for a plethora of behavioural processes, such as competition (Fero & Moore, 2014; Kintzing & Butler, 2014) and oviposition site selection (Stahlschmidt & Adamo, 2013a), and thus, it may be similarly important for behavioural fever. Future studies examining fever should provide varying degrees of shelter to determine the importance of shelter in thermoregulatory responses to immune activation.

Given our results (Figs. 2 and 3) and those of others (Bonnet, Fizesan, & Michel, 2013; Stahlschmidt & Adamo, 2013b; Villén-Pérez, Carrascal, & Seoane, 2013; Webb, Pike, & Shine, 2009), the prioritization of shelter use over thermoregulation by animals may be widespread. When forced to choose between thermoregulation and shelter use, the control snakes in our study did not alter their shelter utilization (Fig. 2) and this came at the expense of their T_s (Fig. 3); that is, they prioritized shelter use over thermoregulation. Both shelter use and thermoregulation are important (i.e. an animal lacking both aspects will likely exhibit higher risk of predation and hindered physiological performance; Cowlishaw, 1997; Regal, 1966). However, a lack of shelter typically outweighs the negative effects of undesirable ambient temperature in insects (Stahlschmidt & Adamo, 2013b), reptiles (Bonnet et al., 2013; Webb et al., 2009) and birds (Villén-Pérez et al., 2013). When lacking shelter, snakes exhibit reduced energy intake (high regurgitation rate) and chronically elevated levels of corticosterone (CORT, a hormone secreted in response to stress via the hypothalamic–pituitary–adrenal, HPA, axis) (Bonnet et al., 2013; Moore & Mason, 2001). However, some snakes do not experience an increase in baseline plasma CORT levels when occupying thermally suboptimal environments (Sykes & Klukowski, 2009; but see ; Dupoue, Brischoux, Lourdais, & Angelier, 2013). Furthermore, although little data on thermal performance exist for *P. guttatus*, the swimming and striking performances of other colubrid snakes are not highly sensitive to shifts in T_b (e.g. the range of T_bs over which performance is 80% of maximal is 15–18 °C: Blouin-Demers, Weatherhead, & McCracken, 2003). Therefore, shelter use may be ultimately prioritized over thermoregulation due to its direct effect on fitness (i.e. avoiding predators increases survival), and this prioritization may be mediated at a proximate level by the HPA axis (Smith & Vale, 2006).

Our second hypothesis (immune activation influences the trade-off between thermoregulation and shelter use) was supported because shelter use became deprioritized over thermoregulation due to immune activation (Figs. 2 and 3). Although this hypothesis had never been explicitly tested, others have obtained similar results for different taxa. For example, immune activation in blue tits, *Cyanistes caeruleus*, does not cause a change in T_b, but it may cause birds to sacrifice perceived safety (i.e. remain roosting in nestboxes where previous captures occurred) to maintain preferred T_b in the context of energy conservation (Nord et al., 2014). Similarly, immune activation in field crickets (*Gryllus campestris*) increases the amount of time spent exposed, and this may occur to facilitate shifts in thermoregulation (e.g. increased basking: Otti et al., 2011). Thus, the benefits of such behavioural shifts to the immune system (i.e. creating an undesirable environment for a pathogen) may begin to outweigh the costs (e.g. suboptimal temperature for other physiological processes, reduced food consumption and increased predation risk). Given the potentially widespread nature of thermoregulation–shelter use trade-offs, we encourage others to test the plasticity of this trade-off in response to immune activation using other taxa.

When forced to choose between shelters that were either cooler or warmer than their preferred shelters, snakes in our study preferred to utilize cooler shelters (Fig. 3). We offer several possible reasons for this result. First, some benefits of the warmer shelter were minimized in our study. For example, snakes were post-absorptive and, thus, did not reap the benefits of digestion related to warmer temperatures. Second, there are physiological costs to occupying and conforming to warmer microhabitats. Energy expenditure and water loss increase with increasing T_b, and thermal performance declines more rapidly at temperatures above the thermal optimum relative to temperatures below the thermal optimum (reviewed in Angilletta, 2009; Bonnet, 2011; Michel & Bonnet, 2010). Third, the thermal optimum for at least one widespread metric of performance (innate immune function: Butler et al., 2013) is lower than T_s in our study snakes (Stahlschmidt & French, n.d.). Future research should continue to examine the relationship between thermal optima for other performance traits (e.g. locomotion) and temperature-based decision making in snake taxa and beyond.

Fever responses are complex and influenced by several factors (e.g. reviewed in Stahlschmidt & Adamo, 2013b). Thus, we suggest using the following standard protocol when studying behavioural responses to immune activation in vertebrates. (1) Use the genus *Aeromonas* as the pyrogen because this group can elicit a febrile response in ectothermic vertebrates (e.g. fish: Tsai & Hoh, 1995; amphibians: Casterlin & Reynolds, 1977; reptiles: Merchant, Williams, Trosclair, Elsey, & Mills, 2007) and is associated with infections in birds and mammals (Janda & Abbott, 1998; Tomás, 2012). (2) Use a comprehensive range of sampling time points because febrile responses can range from hours (Haukenes & Barton, 2004) to days (Burns et al., 1996; Don et al., 1994) to weeks (Moore & Freehling, 2002), depending on taxon. (3) Control for shelter availability (see rationale above and below). Adopting a more standardized protocol should allow us to better understand the prevalence of fever and the role of fever in behaviours associated with shelter use.

Results from our study suggest we should re-evaluate how immune activation influences decisions related to behavioural thermoregulation. For example, we demonstrate that immune-challenged animals not exhibiting fever may still exhibit important shifts in the prioritization of thermoregulation. Our results also demonstrate that immune activation can affect the potentially widespread trade-off between thermoregulation and shelter use. In the future, researchers should continue to investigate the plasticity of this trade-off in response to other common factors, such as reproductive status, digestive state and age, or motivation for predator avoidance or foraging (e.g. by scenting areas of the arena with predators or prey, respectively; sensu Downes & Shine, 1998). We should also continue to examine this pattern across other taxa and in other contexts (e.g. the role of immune

activation in trade-offs between thermoregulation and foraging behaviour).

Acknowledgments

We thank the Department of Biology and the Office of Research Services and Sponsored Programs at Georgia Southern University for funding support. We are appreciative of Tony Mills at the Low Country Institute for the loan of animals.

References

Aïdam, A., Michel, C. L., & Bonnet, X. (2013). Effect of ambient temperature in neonate aspic vipers: growth, locomoter performance and defensive behaviors. *Journal of Experimental Zoology A, 319*, 310–318.

Alexander, C., & Rietschel, E. T. (2001). Bacterial lipopolysaccharides and innate immunity. *Journal of Endotoxin Research, 7*, 167–202.

Almeida, M. C., Steiner, A., Branco, L., & Romanovsky, A. (2006). Cold-seeking behavior as a thermoregulatory strategy in systemic inflammation. *European Journal of Neuroscience, 23*, 3359–3367.

Amo, L., Pilar, L., & Martín, J. (2004). Trade-offs in the choice of refuges by common wall lizards: do thermal costs affect preferences for predator-free refuges? *Canadian Journal of Zoology, 82*, 897–901.

Angilletta, M., Jr. (2009). *Thermal adaptation, a theoretical and empirical synthesis*. Oxford, U.K.: Oxford University Press.

Angilletta, M., Jr., Hill, T., & Robson, M. (2002). Is physiological performance optimized by thermoregulatory behavior? A case study of the eastern fence lizard, *Sceloporus undulatus*. *Journal of Thermal Biology, 27*, 199–204.

Atkinson, D., & Sibly, R. (1997). Why are organisms bigger in colder environments? Making sense of a life history puzzle. *Trends in Ecology & Evolution, 6*, 235–239.

Ballabeni, P., Benway, H., & Jaenike, J. (1995). Lack of behavioral fever in nematode-parasitized *Drosophila*. *Journal of Parasitology, 81*, 670–674.

van den Berg, F. T., Thompson, M. B., & Hochuli, D. F. (2015). When hot rocks get hotter: behavior and acclimatization mitigate exposure to extreme temperatures in a spider. *Ecosphere, 6*, 88.

Bevelander, G., Smith, T. L., & Kennth, V. K. (2006). Microhabitat and prey odor selection in the foraging pigmy rattlesnake. *Herpetologica, 62*, 47–55.

Bicego, K., Barros, R., & Branco, L. (2007). Physiology of temperature regulation: comparative aspects. *Comparative Biochemistry and Physiology, 147*, 616–639.

Blouin-Demers, G., & Weatherhead, P. J. (2001). Habitat use by black rat snakes (*Elaphe obsolete obsolete*) in fragmented forests. *Ecology, 83*, 2882–2896.

Blouin-Demers, G., Weatherhead, P. J., & McCracken, H. A. (2003). A test of the thermal coadaptation hypothesis with black rat snakes (*Elaphe obsolete*) and northern water snakes (*Nerodia sipedon*). *Journal of Thermal Biology, 4*, 331–340.

Bonnet, X. (2011). The evolution of semelparity. In B. G. M. Jamieson (Ed.), *Reproductive biology and phylogeny of snakes* (pp. 645–672). Enfield, NH: Science.

Bonnet, X., Fizesan, A., & Michel, C. (2013). Shelter availability, stress level and digestive performance in the aspic viper. *Journal of Experimental Biology, 216*, 815–822.

Burns, G., Ramos, A., & Muchlinski, A. (1996). Fever response in North American snakes. *Journal of Herpetology, 2*, 133–139.

Butler, M. W., Stahlschmidt, Z. R., Ardia, D. R., Davies, S., Davis, J., Guillette, L. J., Jr., et al. (2013). Thermal sensitivity of immune function: evidence against a generalist–specialist trade-off among endothermic and ectothermic vertebrates. *American Naturalist, 181*, 761–774.

Caillon, R., Suppo, C., Casas, J., Woods, A., & Pincebourde, S. (2014). Warming decreases thermal heterogeneity of leaf surfaces: implications for behavioural thermoregulation by arthropods. *Functional Ecology, 28*, 1449–1458.

Casterlin, M. E., & Reynolds, W. W. (1977). Behavioral fever in anuran amphibian larvae. *Life Sciences, 20*, 593–596.

Cichón, M., Chadzińska, M., Książek, A., & Konarzewski, M. (2002). Delayed effects of cold stress on immune response in laboratory mice. *Proceedings of the Royal Society B: Biological Sciences, 269*, 1493–1497.

Clark, R. W. (2007). Public information for solitary foragers: timber rattlesnakes use conspecific chemical cues to select ambush sites. *Behavioral Ecology, 18*, 487–490.

Cowlishaw, G. (1997). Refuge use and predation risk in a desert baboon population. *Animal Behaviour, 54*, 241–253.

Crocker-Buta, S., & Secor, S. (2014). Determinants and repeatability of the specific dynamic response of the corn snake, *Pantherophis guttatus*. *Comparative Biochemistry and Physiology, Part A, 169*, 60–69.

Deban, S. M., & Lappin, A. K. (2011). Thermal effects on the dynamics and motor control of ballistic prey capture in toads: maintaining high performance at low temperature. *Journal of Experimental Biology, 214*, 1333–1346.

Deen, C., & Hutchison, V. (2001). Effects of lipopolysaccharide and acclimation temperature on induced behavioral fever in juvenile *Iguana iguana*. *Journal of Thermal Biology, 26*, 55–63.

Dill, L. M., & Fraser, A. (1996). The worm re-turns: hiding behavior of a tube-dwelling marine polychaete, *Serpula vermicularis*. *Behavioral Ecology, 2*, 186–193.

Don, M., Estany, A., Ramos, A., Leoni, R., & Muchlinski, A. (1994). Re-examination of the afebrile response in two lizard species (*Varanus exanthematicus* and *Gerrhosaurus major*). *Journal of Herpetology, 3*, 385–388.

Dorcas, M., & Gibbons, W. (2005). *Snakes of the southeast*. Athens, GA: University of Georgia Press.

Dosmann, A., Brooks, K. C., & Mateo, J. M. (2015). Evidence for a mechanism of phenotypic integration of behaviour and innate immunity in a wild rodent: implications for animal personality and ecological immunology. *Animal Behaviour, 101*, 179–189.

Downes, S. (2001). Trading heat and food for safety: costs of predator avoidance in a lizard. *Ecology, 82*, 2870–2881.

Downes, S., & Shine, R. (1998). Heat, safety or solitude? Using habitat selection experiments to identify a lizard's priorities. *Animal Behaviour, 55*, 1387–1396.

Dupoue, A., Brischoux, F., Lourdais, O., & Angelier, F. (2013). Influence of temperature on the corticosterone stress-response: an experiment in the Children's python (*Antaresia childreni*). *General and Comparative Endocrinology, 193*, 178–184.

El-Hafez, A. A., El-Sharkawy, M. A., & Hassan, K. A. (2014). Consequential effects of high temperature on biological characteristics influencing the efficacy of *Trichogramma evanescens* Westwood and its progeny. *Egyptian Journal of Biological Pest Control, 24*, 57–63.

Fero, K. C., & Moore, P. A. (2014). Shelter availability influences social behavior and habitat choice in crayfish, *Orconectes virilis*. *Behaviour, 151*, 103–123.

Haukenes, A., & Barton, B. (2004). Characterization of the cortisol response following an acute challenge with lipopolysaccharide in yellow perch and the influence of rearing density. *Journal of Fish Biology, 64*, 851–862.

Hoecherl, N., & Tautz, N. (2015). Thermoregulation of individual paper wasps (*Polistes dominula*) plays an important role in nest defence and dominance battles. *Science of Nature, 102*, 32.

Hyslop, N., Cooper, R., & Meyers, M. (2009). Seasonal shifts in shelter and microhabitat use of *Drymarchon couperi* (eastern indigo snake) in Georgia. *Copeia, 2009*, 458–464.

Janda, J., & Abbott, S. (1998). Evolving concepts regarding the genus *Aeromonas*: an expanding panorama of species, disease presentations, and unanswered questions. *Clinical Infectious Diseases, 27*, 332–344.

Johnson, R. (2002). The concept of sickness behavior: a brief chronological account of four key discoveries. *Veterinary Immunology and Immunopathology, 87*, 443–450.

Joop, G., & Rolff, J. (2004). Plasticity of immune function and condition under the risk of predation and parasitism. *Evolutionary Ecology Research, 6*, 1051–1062.

Kintzing, M. D., & Butler, M. J. I. V. (2014). The influence of shelter, conspecifics, and threat of predation on the behavior of the long-spined sea urchin (*Diadema antillarum*). *Journal of Shellfish Research, 33*, 781–785.

Kluger, M. (1986). Is fever beneficial? *Yale Journal of Biology and Medicine, 59*, 89–95.

Kubisch, E., Fernández, J., & Ibargűengoytía, N. (2011). Is locomotor performance optimized at preferred body temperatures? A study of *Liolaemus pictus argentinus* from northern Patagonia, Argentina. *Journal of Thermal Biology, 36*, 328–333.

Laburn, H., Mitchell, D., Kenedi, E., & Louw, G. (1981). Pyrogens fail to produce fever in a cordylid lizard. *American Journal of Physiology, 241*, R198–R202.

Lagarde, F., Louzizi, T., Slimani, T., El Mouden, H., Ben Kaddour, K., Moulherat, S., et al. (2012). Bushes protect tortoises from lethal overheating in arid areas of Morocco. *Environmental Conservation, 39*, 172–182.

Lefcort, H., & Eiger, S. (1993). Antipredatory behavior of feverish tadpoles: implications for pathogen transmission. *Behaviour, 126*, 1–2.

Lorioux, S., DeNardo, D., Gorelick, R., & Lourdais, O. (2012). Maternal influences on early development: preferred temperature prior to oviposition hastens embryogenesis and enhances offspring traits in the Children's python, *Antaresia childreni*. *Journal of Experimental Biology, 215*, 1346–1353.

Lourdais, O., Guillon, M., DeNardo, D., & Blouin-Demers, G. (2013). Cold climate specialization: adaptive covariation between metabolic rate and thermoregulation in pregnant vipers. *Physiology & Behavior, 119*, 149–155.

Mabille, G., & Berteaux, D. (2014). Hide or die: use of cover decreases predation risk in juvenile North American porcupines. *Journal of Mammalogy, 95*, 992–1003.

MacDonald, L., Begg, D., Weisinger, R., & Kent, S. (2012). Calorie restricted rats do not increase metabolic rate post-LPS, but do seek out warmer ambient temperatures to behaviorally induce a fever. *Physiology & Behavior, 107*, 762–772.

McConnachie, S., Greene, S., & Perrin, M. (2011). Thermoregulation in the semi-aquatic yellow anaconda, *Eunectes notaeus*. *Journal of Thermal Biology, 36*, 71–77.

Merchant, M., Fleury, L., Rutherford, R., & Paulissen, M. (2008). Effects of bacterial lipopolysaccharide on thermoregulation in green anole lizards (*Anolis carolinensis*). *Veterinary Immunology and Immunopathology, 125*, 176–181.

Merchant, M., Williams, S., Trosclair, P. L., III, Elsey, R. M., & Mills, K. (2007). Febrile response to infection in the American alligator (*Alligator mississippiensis*). *Comparative Biochemistry and Physiology Part A, 148*, 921–925.

Moore, J., & Freehling, M. (2002). Cockroach hosts in thermal gradients suppress parasite development. *Oecologia, 133*, 261–266.

Moore, I., & Mason, R. (2001). Behavioral and hormonal responses to corticosterone in the male red-sided garter snake, *Thamnophis sirtalis* parietalis. *Physiology & Behavior, 72*, 669–674.

Nord, A., Sköld-Chiriac, S., Hasselquist, D., & Nilsson, J. (2014). A tradeoff between perceived predation risk and energy conservation revealed by an immune challenge experiment. *Oikos, 123*, 1091–1100.

Ortega, C. E., Stranc, D. S., Casal, M. P., Hallman, G. M., & Muchlinski, A. E. (1991). A positive fever response in *Agama agama* and *Sceloporus orcutti* (Reptilia: Agamidae and Iguanidae). *Journal of Comparative Physiology B, 161*, 377–381.

Otti, O., Gantenbein-Ritter, I., Jacot, A., & Brinkhof, M. (2011). Immune response increases predation risk. *Evolution, 66*, 732–739.

Payette, A., & McGaw, I. (2003). Thermoregulatory behavior of the crayfish *Procambarus clarki* in a burrow environment. *Comparative Biochemistry and Physiology Part A, 136*, 539–556.

Rantala, M. J., Honkavaara, J., & Suhonen, J. (2010). Immune system activation interacts with territory-holding potential and increases predation of the damselfly *Calopteryx splendens* by birds. *Oecologia, 163*, 825–832.

Raske, M., Lewbart, G., Dombrowski, D., Hale, P., Correa, M., & Christina, L. (2012). Body temperatures of selected amphibian and reptile species. *Journal of Zoo and Wildlife Medicine, 43*, 517–521.

Raucher, R. S. (2002). *Quantifying public health risk reduction benefits*. Denver, CO: AWA Research Foundation: American Water Works Association.

Regal, P. (1966). Thermophilic response following feeding in certain reptiles. *Copeia, 1966*, 588–590.

Reinert, H. (1993). Habitat selection in snakes. In R. A. Seigel, & J. T. Collins (Eds.), *Snakes: Ecology and behaviour* (pp. 201–240). New York, NY: McGraw–Hill.

Roark, A., & Dorcas, M. (2000). Regional body temperature variation in corn snakes measured using temperature-sensitive passive integrated transponders. *Journal of Herpetology, 34*, 481–485.

Roper, T., Bennett, N., Conradt, L., & Molteno, A. (2001). Environmental conditions in burrows of two species of African mole-rat, *Georhychus capensis* and *Cryptomys damarensis*. *Journal of Zoology, 254*, 101–107.

Sauter, S. T., Crawshaw, L. I., & Maule, A. G. (2001). Behavioral thermoregulation by juvenile spring and fall Chinook salmon, *Oncorhynchus tshawytscha*, during smoltification. *Environmental Biology of Fishes, 61*, 295–304.

Schwarzkopf, L., & Alford, R. (1996). Desiccation and shelter-site use in a tropical amphibian: comparing toads with physical models. *Functional Ecology, 10*, 193–200.

Sievert, L., Suita, Y., Mayes, D., Bontrager, L., Tuttle, M., & Everly, A. (2013). Specific dynamic action, postprandial thermophily, and the impact of temperature on gastric digestion in the corn snake *Pantherophis guttatus*. *Zoological Studies, 52*, 33. http://dx.doi.org/10.1186/1810-522X-52-33.

Smith, S. M., & Vale, W. W. (2006). The role of the hypothalamic–pituitary–adrenal axis in neuroendocrine responses to stress. *Dialogues in Clinical Neuroscience, 8*, 383–395.

Stahlschmidt, Z.R., & French, S.S. (n.d.). *Thermal performance of innate immune function in the cornsnake,* Pantherophis guttatus. Manuscript in preparation.

Stahlschmidt, Z. R., & Adamo, S. (2013a). Warm and cozy: temperature and predation risk interactively affect oviposition site selection. *Animal Behaviour, 86*, 553–558.

Stahlschmidt, Z. R., & Adamo, S. (2013b). Context dependency and generality of fever in insects. *Naturwissenschaften, 100*, 691–696.

Stahlschmidt, Z. R., Jodrey, A. D., & Luoma, R. (2015). Consequences of complex environments: temperature and energy intake interact to influence growth and metabolic rate. *Comparative Biochemistry and Physiology A, 187*, 1–7.

Stahlschmidt, Z. R., O'Leary, M. E., & Adamo, S. (2014). Food limitation leads to risky decision making and to tradeoffs with oviposition. *Behavioral Ecology, 25*, 1–5.

Stahlschmidt, Z. R., Shine, R., & DeNardo, D. (2012). The consequences of alternative parental care tactics in free-ranging pythons (*Liasis fuscus*) in tropical Australia. *Functional Ecology, 26*, 812–821.

Suwanpakdee, S., Kaewkungwal, J., White, L. J., Asensio, N., Ratanakorn, P., Singhasivanon, P., et al. (2015). Spatio-temporal patterns of leptospirosis in Thailand: is flooding a risk factor? *Epidemiology and Infection, 143*, 2106–2115.

Sykes, K., & Klukowski, M. (2009). Effects of acute temperature change, confinement, and housing on plasma corticosterone in water snakes, *Nerodia sipedon* (Colubridae: Natricinae). *Journal of Integrative Biology A, 311*, 172–181.

Tomás, J. (2012). The main *Aeromonas* pathogenic factors. *ISRN Microbiology, 2012*, 256261. http://dx.doi.org/10.5402/2012/256261.

Tsai, C. L., & Hoh, K. H. (1995). Effect of indomethacin on survival of *Aeromonas hydrophila* infected tilapia, *Oreochromis mossambicus*. *Zoological Studies, 34*, 59–61.

Villén-Pérez, S., Carrascal, L., & Seoane, J. (2013). Foraging patch selection in winter: a balance between predation risk and thermoregulation benefit. *PLoS One, 8*, e68448. http://dx.doi.org/10.1371/journal.pone.0068448.

Wang, T., Zaar, M., Arvedsen, S., Vedel-Smith, C., & Overgaard, J. (2003). Effects of temperature on the metabolic response to feeding in *Python molurus*. *Comparative Biochemistry and Physiology Part A, 133*, 519–527.

Webb, J. K., Pike, D. A., & Shine, R. (2009). Olfactory recognition of predators by nocturnal lizards: safety outweighs thermal benefits. *Behavioral Ecology, 21*, 72–77.

Yagi, K., & Litzgus, J. (2013). Thermoregulation of spotted turtles (*Clemmys guttata*) in a beaver-flooded bog in southern Ontario, Canada. *Journal of Thermal Biology, 38*, 205–213.

Zurovsky, Y., Brain, T., Laburn, H., & Mitchell, D. (1987). Pyrogens fail to produce fever in the snakes *Psammophis phillipsii* and *Lamprophis fuliginosus*. *Comparative Biochemistry and Physiology A, 4*, 911–914.