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RESEARCH ARTICLE

The structure of the thermal landscape determined behavioural and physiological responses to simulated predation risk

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Abstract

1. Although predators can deter an animal from regulating its body temperature by basking or shuttling, this response to predation should depend on the spatial distribution of thermal resources.
2. By simulating predation risk, we showed that movement, thermoregulation and corticosterone of male lizards (*Sceloporus jarrovi*) depended on the spatial distribution of shade.
3. Simulated risk caused lizards to move less, thermoregulate worse and circulate more corticosterone than they did without risk. However, a patchier distribution of shade enabled lizards to move more, thermoregulate better and circulate less corticosterone when exposed to a simulated predator.
4. In the absence of simulated risk, lizards in patchier environments moved less, thermoregulated better and circulated less corticosterone, indicating the distribution of shade also affected the energetic cost of thermoregulation.
5. This study provides the first test of a spatial theory of thermoregulation under the perceived risk of predation.

KEYWORDS
corticosterone, ecology of fear, movement, predation risk, thermal resources, thermoregulation

1 | INTRODUCTION

When forecasting how organisms will respond to changing thermal landscapes projected by climate change, biologists commonly assume that animals will shift the timing and location of activity to avoid extreme temperatures (Buckley, 2008, 2010; Elith et al., 2010; Kearney & Porter, 2009; Leroux et al., 2013). This assumption follows from the common observation that many types of animals will shuttle between sun and shade to regulate body temperature. This ability depends on the features of the environment, both abiotic and biotic. In a habitat with rocks, vegetation or hills, solar radiation will warm certain areas more than others. This thermal heterogeneity enables an animal to raise or lower its body temperature by shifting its position or orientation within the environment. An animal that can thermoregulate effectively will maximize performances such a locomotion, digestion, or defence (Angilletta et al., 2002; Glass & Harrison, 2022; Steell et al., 2019). However, biotic factors may constrain how effectively an animal can thermoregulate (Angilletta, 2009; Huey & Slatkin, 1976; Sears & Angilletta, 2015). For example, basking in the sun or shuttling between sun and shade may be maladaptive in the presence of predators (Huey & Slatkin, 1976; Pianka & Pianka, 1970).

Ecologists who study other behaviours, such as foraging, have long recognized the nonlethal costs imposed by predators (Brown, 1999; Brown & Kotler, 2004; Jones & Dornhaus, 2011; Lima, 1998a, 1998b). The ecology of fear focuses on behavioural and physiological costs of avoiding predators (Brown et al., 1999; Gaynor et al., 2020). Fear reduces foraging success, invokes hormonal responses and even increases the chance of death by causes other than predation (Clinchy et al., 2013; Schmitz et al., 1997; Smith et al., 2017; Zanette et al., 2011). Researchers studying thermoregulation have established a tradeoff between obtaining thermal resources and avoiding death (Downes & Shine, 1998; Martín & López, 2000; Polo et al., 2005). Ectotherms must seek sun and shade to thermoregulate, just as they seek food to grow (Angilletta, 2009; Cowles & Bogert, 1944). However, any animal shuttling between sun and shade is more visible, and presumably more vulnerable to predators (Lima & Dill, 1990; Skelly, 1994; Smith, 1992; Webb & Whiting, 2005). In risky environments, an animal might trade thermoregulation for safety (Beauchamp, 2015; Ito & Mori, 2010; Treves, 2000). For instance, many types of organisms respond to a predator by either fleeing to shelter or freezing in place (Cooper, 2008; Lima & Dill, 1990; Sih, 1987). Either behaviour hinders thermoregulation, because an ectotherm cools when hiding in a refuge and warms when cowering in the sun (Martín & López, 1999; Polo et al., 2005), which reduces temperature sensitive performances (Angilletta, 2009).

The trade-off between regulating temperature and avoiding predators should depend on the spatial distribution of thermal resources. The landscape-of-fear framework considers how animals navigate variation in predation risk over time and space (Gaynor et al., 2019; Laundré et al., 2001, 2010). According to this framework, an organism should adjust its behaviour according to its perceived risk in a given landscape. When resources occur throughout space, prey can play a “shell game” to reduce their risk of predation (Laundré, 2010; Mitchell & Lima, 2002). By moving among patches, prey become less predictable to a searching predator. Moreover, a patchy thermal environment enables animals to thermoregulate effectively over a greater area (Basson et al., 2017; Sears & Angilletta, 2015; Sears et al., 2016). By contrast, animals cannot play this shell game and thermoregulate effectively when thermal resources are concentrated in space. Using game theory, Mitchell and Angilletta (2009) predicted that prey should occupy a wider range of microclimates in the presence of predators, consequently reducing their thermoregulatory effectiveness. This model was partially supported by an experiment, in which newts spent less time in a warm patch when predatory dragonflies were present (Gvoždík et al., 2013). Presumably, this behaviour lowered body temperature and locomotor performance, as observed in other ectothermic animals (Batty et al., 1993; Cooper, 2000; Hesselberg & Vollrath, 2006). However, a shell game between predators and prey can only emerge when preferred microclimates occur in several places at once. Biologists have yet to develop a theory or test hypotheses about this interaction between the thermal landscape and predation risk.

To test these ideas about thermoregulation under the risk of predation, we studied the behaviour of male spiny lizards (*Sceloporus jarrovi*) in large, outdoor arenas. These lizards thermoregulate by basking and shuttling (Sears et al., 2016), but flee to a shelter when approached by a potential predator (Rusch, pers. obs.). By placing lizards in experimental arenas, we manipulated the thermal patchiness of their environment and their perceived risk of predation. The spatial distribution of microclimates was controlled with patches of shade cloth. Predation risk was simulated by flying a model of a predatory bird over each arena at random times and places. We recorded the movements and temperatures of lizards in each environment, as well as circulating corticosterone as an indicator of physiological stress (Langkilde & Shine, 2006; Rusch et al., 2018; Sapolsky et al., 2000). We predicted that lizards under simulated predation risk would thermoregulate less accurately and circulate more corticosterone compared to lizards in the control treatment. Furthermore, we predicted that lizards in a patchier thermal environment would respond to predation risk by engaging in a shell game, moving more frequently and covering more area than they would in the absence of predation risk.

2 | MATERIALS AND METHODS

2.1 | Collecting and caring for lizards

In April of 2013, we collected 80 adult males of *Sceloporus jarrovi* in the Chiricahua Mountains of Arizona (1500–2500 m) and transported them to the Sevilleta Field Station, located in La Joya, New Mexico (1500–1600 m). At the time of the experiment, mean maximal and mean minimal temperatures were similar between the two sites. The range of mean air temperature at the collection site was 16.7–31.1°C for July and 16.1–30°C for August. The range at the field station was 15.6–35°C for July and 15–33.3°C for August (NOAA, retrieved 2023). After bringing lizards to the field station, we recorded their masses (mean = 17.2 g, SD = 3.8 g) and snout-vent lengths (mean = 85.3 mm, SD = 5.09 mm) and clipped toes for permanent identification (Perry et al., 2011).

Each lizard was housed in a plastic terrarium (30 × 26 × 13 cm) with a paper towels for shelter. One end of the terrarium was heated underneath by Flexwatt tape (DBDPet, Mountainside, NJ, USA), creating operative environmental temperatures ranging from 20 to 42°C. These temperatures were measured with a hollow, copper model containing a K-type thermocouple (Bakken & Angilletta, 2014; Bakken & Gates, 1975). Cardboard was placed between terraria to prevent social interactions. Every other day, we offered lizards adult crickets (*Acheta domestica*) and larval beetles (*Tenebrio morio*), and misted the terraria with water. Food was coated with vitamins and calcium (Rep-Cal, Los Gatos, CA, USA). Lizards were kept for at least 2 weeks before measuring thermoregulation. Lizards that lost mass were excluded. All procedures were approved by the Animal Care and Use Committee of Arizona State University (Protocol 15-1392R). Collection of animals was approved by Arizona Department of Game and Fish (LIC# SP719140).

2.2 | Measuring preferred body temperatures

We measured preferred body temperatures in artificial thermal gradients created in plastic containers (112 × 35 × 30 cm) with a sand substrate (~1 cm deep). The containers were uniformly illuminated by fluorescent lights. To create a thermal gradient, the room temperature was maintained around 20°C and an infrared lamp (150 W; Exo-Terra, Mansfield, MA, USA) was suspended above one end of each container. Fluorescent light was provided between 0600 and 2000 h, and infrared light was added between 0700 and 1700 h. When both lights were active, the operative environmental temperatures ranged from 22 to 44°C, determined by a hollow, copper model containing a K-type thermocouple (Bakken & Angilletta, 2014, Bakken & Gates, 1975). Previous experiments have established that lizards thermoregulate effectively in this type of gradient (Rusch & Angilletta, 2017; Sears et al., 2016).

Each lizard was placed in a thermal gradient at 2000 h, when the lights were off. The next day, lizards were able to explore the gradient undisturbed. On the third day, we recorded body temperatures every 2 h, from 0800 to 1600 h. We captured each lizard by hand and inserted a quick-reading thermometer (T-4000, Miller & Weber, Inc., Queens, NY) into the cloaca. As with most studies of preferred temperature, we provided no food or water in the thermal gradients, although previous research indicates that spiny lizards prefer the same temperature when fed or fasted (Schuler et al., 2011). After these measurements, each lizard was returned to its terrarium, where food and water were offered as described above.

2.3 | Implanting temperature loggers

Five days after measuring preferred body temperatures, we surgically implanted a miniature temperature logger (mean = 1.45 g, SD = 0.05 g; Weedot, Alpha Mach, Inc., Qc, Canada) into the abdominal cavity of each lizard. Loggers were programmed to record temperature every 10 min for the duration of the experiment. Prior to surgery, each logger was coated in plastic (Plasti Dip, Plasti Dip International, Blaine, MN, USA) and then sealed in paraffin (Gulf Wax, Kalton, OH, USA). Surgical procedures followed those of Sears et al. (2016) and maintained a 99% survival rate. Two weeks after surgery, we measured preferred body temperatures again as described above. The means and standard deviations of preferred body temperature before and after surgery were nearly identical: 34.8 ± 2.1°C before surgery and 34.3 ± 2.1°C after surgery (see Section 2.8 below for details). Furthermore, the mean preferred body temperature of each lizard after surgery was within 2°C of the mean before surgery (Figure S1).

2.4 | Experimental design

To manipulate the thermal landscape, we followed methods of Sears et al. (2016). We used nine outdoor arenas (20 × 20 m), consisting of sheet metal walls and a canopy of shade cloth (80% black knitted cloth; Greenhouse Megastore, Georgetown, IL, USA). The area of each arena matched the mean area of the home range of a male spiny lizard (Ruby, 1978). Shade cloth was suspended 1.2 m above each arena on steel cables, fastened to iron posts outside the arena. The total area of shade was 36% of the arena (144 m^2), but this shade was distributed as either one patch measuring 12 × 12 m (clumped distribution) or four patches measuring 6 × 6 m (patchy distribution; Figure S2). The shade cloth in some areas created two distinct microclimates within each arena; the difference in temperature between shaded and unshaded patches ranged from 0.1 to 19.5°C, depending on the time of day and cloud cover (Figure 4, range of T_e). Operative temperatures were determined with a hollow, copper model containing a K-type thermocouple (Bakken & Angilletta, 2014; Bakken & Gates, 1975). Four arenas had the clumped distribution and five arenas had the patchy distribution. We observed nine lizards in each of eight temporal blocks for a total of 72 lizards.

To simulate predation risk, we flew models of a red-tailed hawk (*Buteo jamaicensis*) along flyways situated 2 m above the ground (Figure S3). Flyways were constructed with paracord, and a hawk model was pulled across individual flyways using fishing line (Shimano, Irvine, CA, USA) connected to an electric motor (Grainger, Lake Forest, IL, USA). With three flyways per arena, each flight occurred randomly along one of six possible trajectories. Two flights occurred per hour at random times within each hour.

We observed the temperatures and movements of lizards in outdoor arenas while manipulating thermal patchiness and perceived risk in a factorial design. Each lizard was observed for 2 days in one of six treatments: (1) a clumped distribution of shade with no simulated predation risk on either day; (2) a patchy distribution of shade with no simulated predation risk on either day; (3) a clumped distribution of shade with simulated predation risk on the first day; (4) a patchy distribution of shade with simulated predation risk on the first day; (5) a clumped distribution of shade with simulated predation risk on the second day; or (6) a patchy distribution of shade with simulated predation risk on the second day. Before each trial, lizards could explore their thermal environment for 2 days without simulated predation risk. Therefore, each trial lasted 4 days, with 2 days of exploration followed by 2 days of observation.

Each temporal block of observations followed the same design. Lizards were placed in arenas between 1230 and 1300 h to begin 48 h of exploration. After this period, each lizard was captured by hand and 50 μL of blood was collected in a heparinized glass capillary tube by rupturing blood vessels in the orbital sinus. Capillary tubes were sealed with Critoseal (Fisher Scientific, Pittsburgh, PA, USA) and stored on ice until processing. Blood was sampled within 2 min of capture to minimize the effect of handling stress on circulating corticosterone. Afterward, lizards were released in their arena and offered two larval beetles coated in vitamins and calcium; all lizards consumed at least one larval beetle. Over the next 2 days, simulated predation was applied according to the assigned treatments (described above). Body temperatures were recorded automatically by the implanted loggers, while movements were recorded manually from 0720 to 1220 h each day (see Section 2.6 below). After 2 days of observations, we collected a second blood sample from each lizard between 1230 and 1300 h, enabling us to estimate the change in circulating corticosterone during the experiment. Finally, lizards were returned to the lab and treated as described above (see Section 2.1). Table S1 summarizes the sequence of events in each temporal block of the experiment.

2.5 | Measuring operative environmental temperatures and body temperatures

Operative temperatures within arenas were estimated with two hollow, copper models of a lizard, each fitted with an internal K-type thermocouple. Temperatures were recorded automatically every 20 min (RISEPRO 4 Channel K Type Digital Thermometer, HT-9815, Bangalore India). During trials, we placed one model in full sun and the other model under a piece of shade cloth. These models were placed outside of the arenas to avoid disturbing lizards. Body temperatures were recorded by the temperature logger implanted in the abdominal cavity of each lizard. A few days after each trial, temperature loggers were surgically removed by the methods described above (see Section 2.3). Data were downloaded to a computer with a Weedot connector (Alpha Mach, Inc., QC, Canada). Only body temperatures recorded from 0720 to 1220 h were used in analyses.

2.6 | Measuring movement

We used the locations of each lizard to estimate the minimum total distance moved (m), the minimum total area used (m^2), and the probability of exposure. A Cartesian coordinate system was painted on the walls of each arena at 1-m intervals. During a trial, we recorded the location and exposure of each lizard at 20-min intervals. To estimate the minimum total distance moved, we used triangulation to calculate the distance between successive positions and summed these distances. To estimate the minimum total area used, we created a digital Cartesian coordinate system in Microsoft Excel, representing the 400-m^2 arena (20 cells × 20 cells = 400 cells, each representing an area of 1 m^2). We then plotted the series of locations and summed unique cells through which a lizard would have travelled along the shortest path between successive locations. This sum represents the minimum total area used. Lastly, we estimated a probability of exposure from the percentage of times that a lizard was observed in the sun (i.e. exposed to solar radiation).

2.7 | Quantifying the circulating concentration of corticosterone

We quantified total plasma concentration of corticosterone (free plus bound) using a commercial enzyme-linked immunoassay with a sensitivity of 32.02 $\mathrm{pg\,mL^{-1}}$ (Enzo Life Sciences, Farmingdale, NY). Blood was centrifuged within an hour of collection to separate red blood cells from plasma. Plasma was stored at −80°C until assayed (see below). Samples were analysed in duplicate on the same day, following instructions supplied with the assay kit. The assay was validated with a standard curve, constructed from serial dilutions of corticosterone (Fokidis et al., 2009). The slope of a curve produced by serial plasma dilution (4- to 64-fold) was similar to that of the standard curve. This approach enabled us to conclude that a 32-fold dilution was appropriate for our samples. Diluted samples were distributed randomly in a 96-well plate. Mean coefficients of variation within assays were 3.59%, 4.68%, 4.71% and 3.31%. The mean coefficient of variation among assays was 4.07%. Each pair of samples per lizard was run in duplicate ($n = 72$ lizards × 2 = 144 samples, and $n = 144$ samples × 2 = 288 wells).

2.8 | Statistical modelling

We modelled the effects of thermal patchiness (clumped or patchy) and simulated risk (none, first day, or second day) on the following variables: minimum total distance moved, minimum total area used, body temperature, probability of exposure, and the circulating concentration of corticosterone. Thermal patchiness, simulated risk and temporal block were considered fixed factors. Body mass was a covariate. The identity of each lizard was treated as a random intercept.

When modelling body temperature or exposure probability, we also included a fixed factor representing the operative environmental temperature. We used a principal component analysis to generate a linear combination of two highly correlated variables, the maximal operative temperature in the arena and the range of operative temperatures in the arena. The first principle component described 97% of the variation in these variables (Table S2).

We used the R Statistical Software (R-Core-Team, 2015) to fit statistical models to the data. For body temperature, we fit a general additive mixed model with the *gamm* function of the *mgcv* library (Wood, 2006). This model enabled us to characterize the nonlinear relationship between operative environmental temperatures (PC score) and body temperature. For the probability of exposure, we fit a generalized linear mixed model with the *glmer* function of the *lme4* library (Bates et al., 2015), choosing a binomial link function for discrete data (shade = 0; sun = 1). For all other dependent variables, we fit a general linear mixed model with the *lme* function of the *nlme* library (Pinheiro et al., 2012). For each model, we used the procedure described by Zuur et al. (2009) to determine the most likely random component before exploring the most likely deterministic component.

We inferred the biological significance of effects using parameters obtained by multimodel averaging. Using the *MuMIn* library (Bartoń, 2013), we fit the full model and all nested submodels, including the null model. This package computes the Akaike weight of each model—the probability that the model describes the data better than other models. Akaike weights were used to calculate a weighted average of each parameter in the full model (Tables S3–S7). All possible models were included when averaging parameter values, such that weighted effects were considered zero for any factors excluded from a model (Burnham & Anderson, 2002). The resulting values of parameters were used to calculate the most likely mean of a dependent variable for each treatment (Tables S8–S12). Model averaging eliminates the need to interpret *p* values, because all models, including the null model, contributed to the most likely value of each mean. If no model parameters were considered statistically significant, then the null model would have had the best fit and greatest Akaike weight, which did not occur in any of our analyses (Tables S3–S7). However, associated parameter *P* values from the model averaging outputs can be found in the supplemental tables (Tables S8–S12), which further support our findings.

3 | RESULTS

3.1 | Movement

Both thermal patchiness and simulated risk determined how lizards used space. Without simulated risk, lizards in clumped thermal landscapes moved 31% farther and covered 15% more area than did lizards in patchy thermal landscapes (Figures 1 and 2, boxes labelled Control). The pattern of movement reversed under a simulated risk; lizards in patchy thermal landscapes moved 37% farther and covered 41% more area than did lizards in clumped thermal landscapes (Figures 1 and 2, boxes labelled Predation D1 and Predation D2). Nonetheless, lizards in either type of landscape moved a shorter distance and covered less area in the presence of a simulated predator. Further, lizards that experienced simulated risk on the first day of observation still moved shorter distances and covered less area on the second day, compared to lizards that experienced no simulated risk (Figures 1 and 2).

3.2 | Exposure to solar radiation

Thermal patchiness and simulated risk also affected the probability that a lizard exposed itself to direct solar radiation. Without simulated risk, lizards in patchy thermal landscapes were more likely to be exposed (68%) than were lizards in clumped thermal landscapes (59%), although the difference was larger on the first day of observation (Figure 3, control box). Under simulated risk, lizards used the cover of shade cloth more often, reducing exposure to 40% or 48% in clumped or patchy thermal landscapes (Figure 3, boxes labelled Predation D1 and Predation D2). When simulated risk was removed on the second day, lizards were more likely to be exposed than they were on the first day (Figure 3, box labelled Predation D1).

3.3 | Thermoregulation

Thermal patchiness and simulated risk interacted to influence the accuracy of thermoregulation (Table 1; Figure 4). In the absence of simulated risk, lizards maintained a preferred temperature whenever the environment was heterogeneous enough to enable behavioural thermoregulation. We inferred this behaviour from the roughly asymptotic relationship between the PC score for operative environmental temperatures and the mean body temperature (Figure 4a). During each day of observation, the PC score (as defined above, in the Section 2.8) increased as solar radiation became more intense, creating a greater maximal operative temperature and a wider range of operative temperatures. When the environment had warmed sufficiently (PC score $\gtrsim 0$), lizards could maintain a body temperature in the preferred range. A simulated predator disrupted the thermoregulatory behaviours of lizards

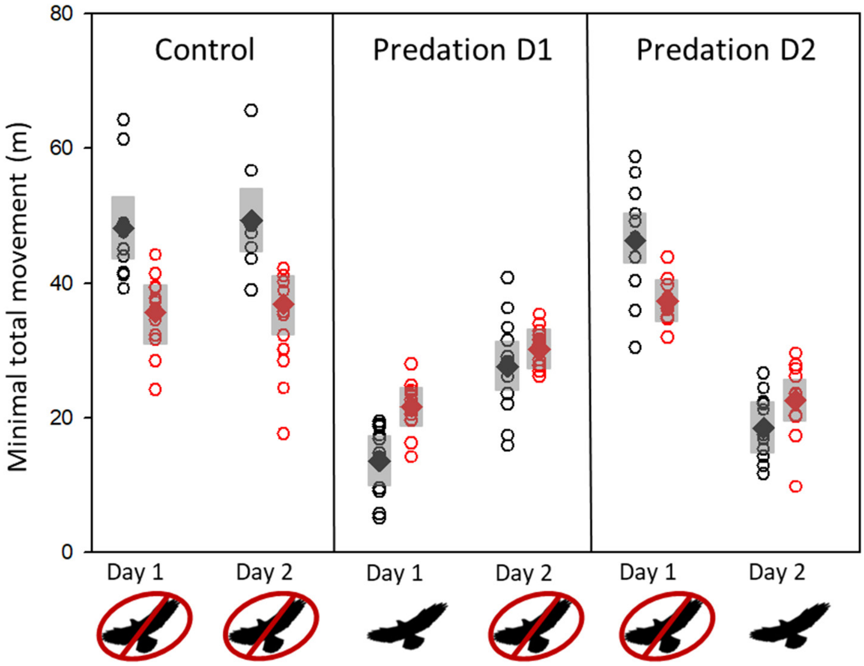

FIGURE 1 Without simulated risk, lizards in clumped arenas moved farther than did lizards in patchy arenas (box labelled Control). With simulated risk, this pattern was reversed (see boxes labelled Predation D1 and Predation D2). Furthermore, lizards that experienced simulated risk on day 1 still moved less on Day 2 than did lizards in control treatments (see data for Day 2 in box labelled Predation D1). Diamonds and grey bars denote means and standard deviations, respectively, computed by multimodel averaging. Each circle denotes the minimum total movement of a lizard. Black or red colours denote data for lizards in clumped or patchy arena, respectively.

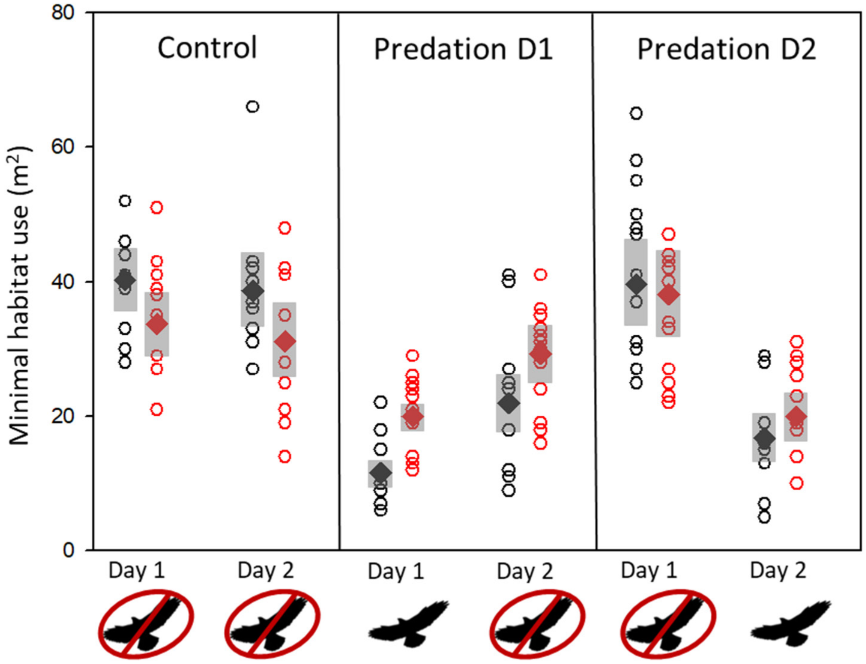

FIGURE 2 Without simulated risk, lizards in clumped arenas covered more area than lizards in patchy arenas (box labelled Control). With simulated risk, this pattern was reversed (boxes labelled Predation D1 and Predation D2). Furthermore, lizards that experienced simulated risk on day 1 still covered less area on Day 2 than lizards in control treatments did (see data for Day 2 of box labelled Predation D1). Diamonds and grey bars denote means and standard deviations, respectively, computed by multimodel averaging. Each circle denotes the area covered of a lizard. Black or red colours denote data for lizards in clumped or patchy arena, respectively.

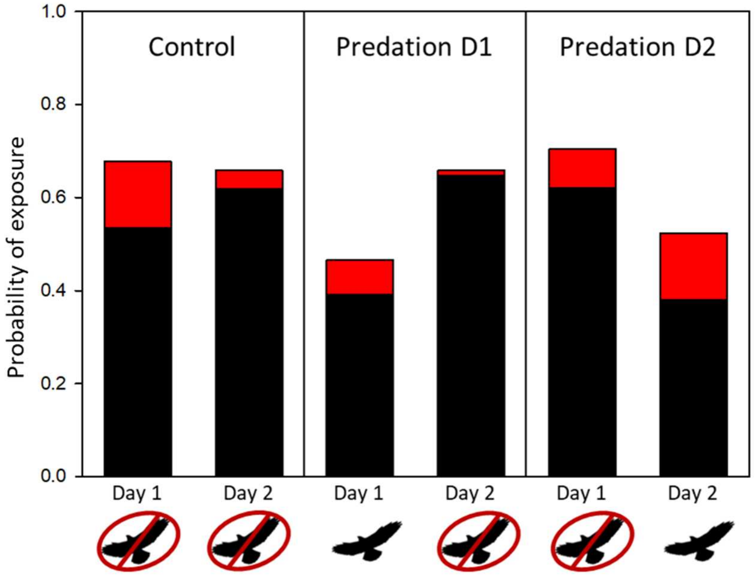

FIGURE 3 Without simulated risk, lizards in patchy arenas were more likely to expose themselves to open areas within the arena than were lizards in in clumped arenas (see box labelled Control). With simulated predation risk, all lizards were less likely to expose themselves to open areas, regardless of thermal patchiness (see boxes labelled Predation D1 and Predation D2). Lizards that experienced simulated risk on Day 1 returned to control levels of exposure on Day 2 in both clumped and patchy arenas (see data for Day 2 in box labelled Predation D1). Black and red bars denote mean probabilities of exposure with and without simulated risk, respectively, computed by multimodel averaging.

by forcing them to spend less time exposed to direct solar radiation. This effect was stronger for lizards in clumped thermal landscapes, causing the mean body temperature of these lizards to fall below their preferred range (see body temperatures for PC score between 0 and 1 in Figure 4b). Consequently, the standard deviation of body temperature increased by more than 1°C under simulated risk (Figure 5). Furthermore, when simulated risk occurred on day 1, lizards still thermoregulated less precisely on the following day than did lizards in the treatment without simulated risk. This lingering effect occurred in both clumped and patchy thermal landscapes, as evidenced by elevated standard deviations of body temperature (see data in box labelled Predation D1 in Figure 5). When risk was simulated on day 2 only, lizards maintained body temperatures similar to lizards in the control treatment on day 1 (Figure 4c). Regardless of the level of simulated risk, lizards in clumped thermal landscape thermoregulated less precisely than did lizards in patchy thermal landscapes, as evidenced by a greater standard deviation of body temperature (Figure 5).

Risk	Number of patches	Mean body temperature (°C) ± SD	% of body temperatures within preferred range
Control	1	29.6 ± 3.2	43
Control	4	30.8 ± 2.9	52
During simulated predation	1	28.6 ± 4.7	35
During simulated predation	4	30.0 ± 3.8	46
After simulated predation	1	29.3 ± 3.7	40
After simulated predation	4	30.5 ± 3.6	49

TABLE 1 Body temperatures of lizards in each treatment. The mean body temperatures were estimated by a statistical model derived from multimodel averaging. We also report the percentage of observed temperatures that were within a preferred thermal range, defined as a temperature within one standard of the preferred temperature (32.5–36.7°C).

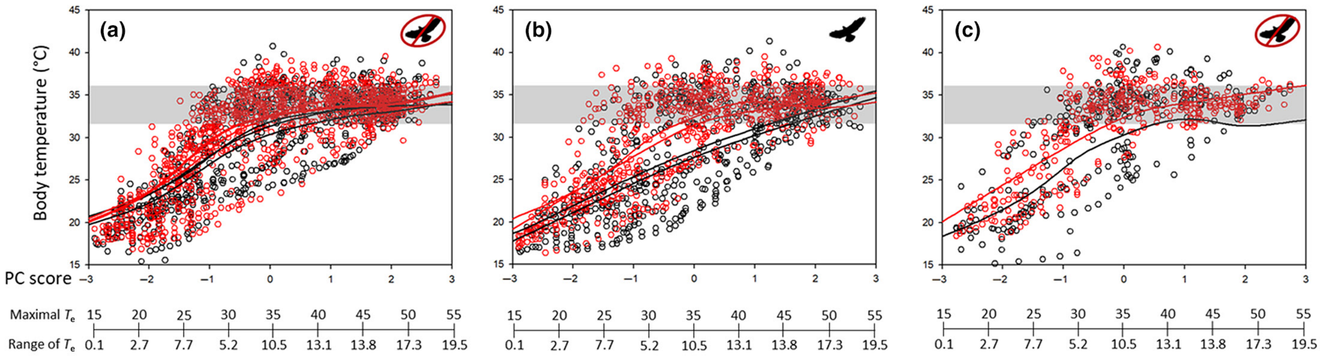

FIGURE 4 Body temperatures of lizards plotted against a principal component of environmental temperatures (Table S2). As PC1 increases, so does the maximum operative temperature (T_e) and the range of operative temperatures (i.e. the difference between the operative temperature in full sun and the operative temperature in 80% shade). Regardless of whether lizards experienced no simulated risk (a) or some simulated risk (b), lizards in patchier arenas thermoregulated more accurately. However, lizards in clumped arenas responded to simulated risk by shifting from a strategy of thermoregulation toward a strategy of thermoconforming, as evidenced by the linear relationship between operative environmental temperatures and body temperatures (b). Lizards that experienced simulated risk on Day 1 thermoregulated nearly as accurate as lizards in control treatments (c). Black and red lines denote mean body temperatures of lizards in clumped and patchy arenas, respectively, computed by multimodel averaging. Each circle denotes a body temperature of a lizard in a clumped arena (black) or patchy arena (red). The grey bar depicts the central 68% of preferred temperatures (32.5–36.7°C), respectively.

3.4 | Corticosterone

Lizards in a clumped thermal landscape with simulated risk circulated more corticosterone than did lizards in a patchy thermal landscape without simulated risk. In the absence of simulated risk, lizards in a clumped thermal landscape circulated 74% more corticosterone than lizards in a patchy thermal landscapes (Figure 6, box labelled Control). With simulated risk, lizards in either thermal landscape circulated several-fold greater concentrations of corticosterone compared to lizards without simulated risk (Figure 6, both Predation boxes). Note, the effects of simulated predation and shade distribution was additive, not interactive. However, this amplified effect from simulated risk depended on thermal patchiness; simulated risk in a clumped thermal landscape increased circulating corticosterone by 3.5-fold, whereas simulated risk in a patchy thermal landscape increased circulating corticosterone by only 2.8-fold. The additive effects of thermal patchiness and simulated risk caused lizards in clumped thermal landscapes to circulate 47% more corticosterone than did lizards in patchy thermal landscapes (Figure 6, boxes labelled Predation D1 and Predation D2). On the day after simulated risk, lizards must have experienced a time lag in the activity of the hypothalamic–pituitary–adrenal axis, because lizards that experienced simulated risk on day 1 had more circulating corticosterone than did lizards that experienced simulated risk on day 2 (Figure 6, box labelled Predation D1).

4 | DISCUSSION

Animals move according to several important but often conflicting demands. For instance, shuttling between sun and shade prevents overheating, but such movements can attract the attention of predators (Huey, 1974; Pitt, 1999). Consequently, animals face trade-offs when making decisions, often behaving in a way that promotes survival at the expense of growth or reproduction (Brown, 1999; Gallagher et al., 2017; Lima & Dill, 1990). Because the distribution of resources and presence of predators influence the quality of a habitat, prey should behave according to the "landscape of fear" (Arthur et al., 2004; Bleicher, 2017; Brown et al., 1999). To optimize fitness, an animal should use space in a way that avoids predators while securing resources. When limiting resources are dispersed, an animal can access these resources while moving through a large area. In such a landscape, prey can play a "shell game" by moving among patches often enough to become unpredictable to predators (Laundré, 2010; Mitchell & Lima, 2002). By contrast, when limiting resources are clumped, an

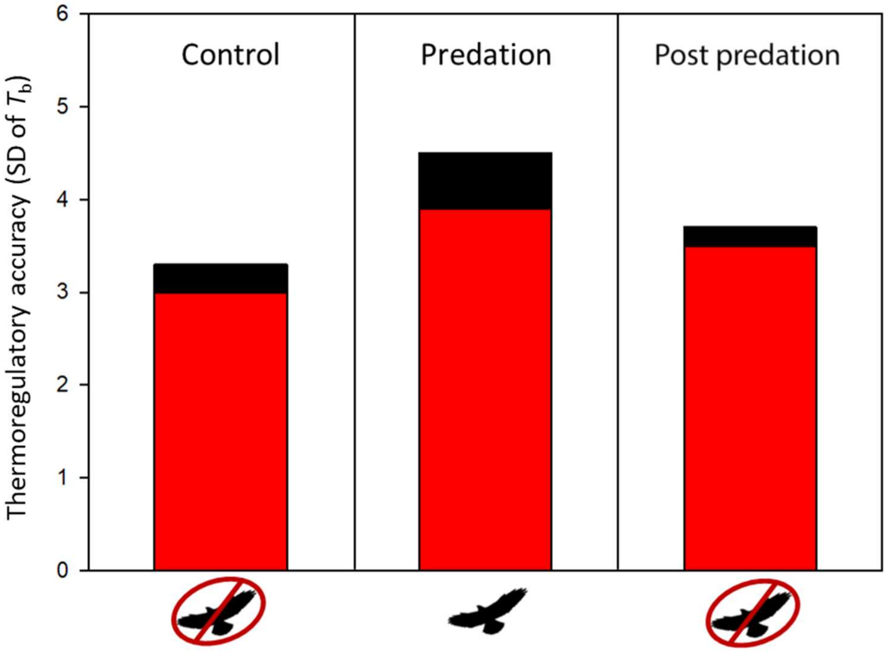

FIGURE 5 Without simulated risk, lizards in patchy arenas thermoregulated more precisely than did lizards in clumped arenas (see box labelled Control). With simulated risk, all lizards thermoregulated less precisely, regardless of thermal patchiness (see box labelled Predation). Lizards that experienced simulated risk on Day 1 did not thermoregulate as precisely as lizards in control treatments, but thermoregulated more precisely than when exposed to simulated risk (box labelled Post Predation). Black and red bars denote standard deviations of body temperature in clumped and patchy arenas, respectively, computed by multimodel averaging.

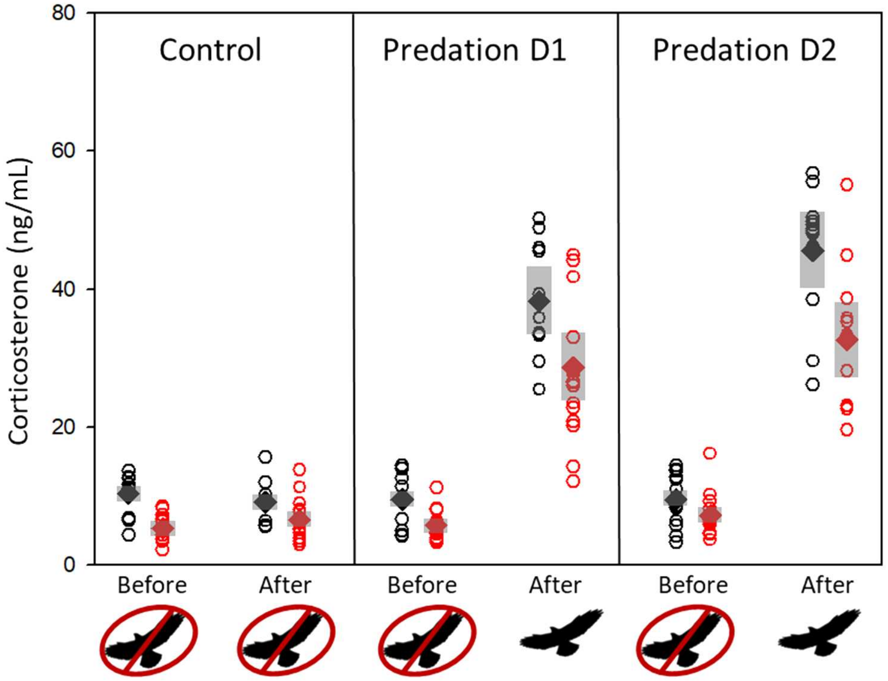

FIGURE 6 Lizards in a clumped arena circulated more corticosterone than did lizards in a patchy arena, regardless of whether they experienced simulated predation risk. However, this effect was amplified when lizards were exposed to simulated predation risk (boxes labelled Predation D1 and Predation D2). Diamonds and grey bars denote means and standard deviations, respectively, computed by multimodel averaging. Each circle denotes the circulating plasma corticosterone of a lizard. Black or red colours denote data for lizards in a clumped arena or a patchy arena, respectively.

animal must occupy a smaller area, making its location predictable to predators. Although a simulated risk of predation affected the behaviour and physiology of lizards in our experiment, our results do not support the model of a predator–prey shell game. Contrary to the predicted pattern, lizards moved shorter distances and used a smaller area when exposed to a simulated predator, regardless of thermal patchiness (Figures 1 and 2). We interpret this result as a general response to fear, because lizards commonly avoid predators by running into burrows or hiding under vegetation (Cooper & Avalos, 2010; Samia et al., 2016). Although no shelters or vegetation were available in our arenas, lizards frequently ran under the shade cloth when a simulated predator passed overhead, presumably recognizing the shade cloth as a form of protection from the simulated predator.

Although seeking shelter reduces predation risk, it also reduces access to solar radiation. Consequently, an animal in a refuge must contend with a falling body temperature that will eventually reduce physiological performance (Martín & López, 1999; Polo et al., 2005). Most physiological functions become impaired at low temperatures (Angilletta et al., 2002; Huey, 1982; Stevenson et al., 1985), including an animal's ability to flee a predator (Cooper & Blumstein, 2015; Samia et al., 2016). For this reason, a cold lizard is more likely to avoid predators by crypsis than by fleeing (Cooper, 2000; Hertz et al., 1982; Irschick & Losos, 1998). Thus, lizards exposed to simulated risk in our experiment likely moved less and sought cover to reduce predation risk (Figures 1 and 3). This behavioural shift must reduce their ability to thermoregulate, because lizards exhibited a greater variance of body temperatures under simulated risk (Table 1; Figures 4 and 5). The time spent at more extreme temperatures would have restricted physiological performances such as locomotion (Herrel et al., 2007; Hertz et al., 1982) or digestion (Angilletta, 2001; McConnachie & Alexander, 2004).

Although lizards did not engage in a shell game, their behaviour aligned with the landscape-of-fear framework, which predicts an interaction between the risk of predation and the distribution of resources (Bleicher, 2017; Brown et al., 1999; Laundré et al., 2010). In a patchy environment, with well distributed refuges, an animal could hide periodically while shuttling between sun and shade. In this way, a patchier landscape should promote thermoregulation in the presence of predators. Our results supported this prediction; although simulated risk affected the behaviour and physiology of lizards in both landscapes, the magnitude of each effect was smaller in the patchier environment. When exposed to simulated risk, lizards in a patchy thermal landscape moved greater distances, covered more area and basked more frequently than did lizards in clumped thermal landscapes (Figures 1 and 3). These behaviours suggest that lizards perceived less risk in the patchier landscape, which presumably enabled more effective thermoregulation (Figures 4 and 5). In a similar experiment, a patchier thermal landscape reduced the impacts of competition on the behaviour and physiology of spiny lizards (Rusch et al., 2018). Therefore, models of thermoregulation should consider the interaction between the thermal landscape and the biotic landscape.

Anti-predator behaviours improve the chance of surviving but also impose costs (Cresswell, 2008; Daversa et al., 2021; Lima, 1998a). For instance, an animal that hides in a refuge will have less time to forage and will likely experience temperatures that slow the digestion and assimilation of food (Angilletta, 2001). Missed opportunities to acquire energy have downstream consequences for growth and reproduction, especially when these missed opportunities occur during a crucial stage of life or time of year (Brown, 1999; Lind & Cresswell, 2005; Scrimgeour & Culp, 1994). Downes (2001) quantified the long-term effects of predation risk on the growth of garden skinks. Individuals were raised to maturity in outdoor enclosures, coated with either a predator's scent or a control scent. Skinks exposed to a predator's scent became active later, moved less, and selected "safer" microhabitats than did lizards exposed to a control scent. These behavioural responses reduced opportunities to bask and forage. Although skinks gradually habituated to the olfactory cues, individuals exposed to a predator's scent grew slower and matured at a smaller size, which caused them to produce smaller offspring. Thus, antipredator behaviour, especially during an early life stage, imposes a long-term cost that likely reduces fitness below the level achieved in a safe environment (Downes, 2001). The spiny lizards that we studied primarily forage during spring and summer, storing fat needed to grow and fuel territoriality and courtship in the fall (Goldberg, 1972; Ruby, 1978). Males that grow less would suffer a disadvantage, because larger males secure more resources, attract more mates, and survive longer (Ruby, 1981; Rusch & Angilletta, 2017; Simon, 1975). Additionally, males that store less fat prior to the breeding season would not have as much energy to patrol a territory, display to females, and fight other males (Marler & Moore, 1988; Ruby, 1978). Thus, foraging opportunities early in the year can have a lasting effect on the reproductive success of lizards. Even with abundant food, predation risk can hinder thermoregulation and consequently the foraging, digestion, and growth needed to reach a competitive size by the time of breeding (Amo et al., 2007; Martín et al., 2003). Thus, patchy thermal landscapes, which afford more opportunities to thermoregulate while avoiding predators, would enhance the fitness of an animal.

In addition to reducing the movement of lizards, perceived risk also increased circulating corticosterone, the primary glucocorticoid regulating the energy use and stress response of a vertebrate (Astheimer et al., 1992; DuRant et al., 2008). This hormonal response to predation risk presumably causes animals to behave in ways that promote survival (Clinchy et al., 2013; Thaker et al., 2009; Trompeter & Langkilde, 2011). Similar hormonal responses have been observed in other species; for example, when Fijian ground frogs viewed a predatory cane toad, they circulated more corticosterone and moved less frequently, compared to frogs exposed to other frogs or inanimate objects (Narayan et al., 2013). Furthermore, Cockrem and Silverin (2002) proposed that an animal's perception of risk depends on its ability to escape. After exposing caged birds and free-ranging birds to a predator, these researchers found that caged birds greatly increased circulating corticosterone while free-ranging birds only moderately did so. These researchers argued that the groups perceived predation risk differently, because caged birds were unable to escape while free-ranging birds could escape (Cockrem & Silverin, 2002). By expanding their reasoning within the landscape-of-fear framework (Bleicher, 2017; Brown et al., 1999; Clinchy et al., 2013; Laundré et al., 2010), we propose that a thermal landscape affects a lizard's concentration of circulating corticosterone through its perception of risk. Specifically, the patchier the distribution of shade, the greater the protection from an aerial predator and the lower the sense of risk.

In the absence of simulated risk, plasma corticosterone was likely linked to energetic demands of movement and thermoregulation. Because lizards moved more and thermoregulated worse in clumped thermal landscapes (Figure 6, box labelled Control), they probably spent more energy than lizards in patchy thermal landscapes did (Basson et al., 2017; Sears & Angilletta, 2015). Thus, a surge in corticosterone might have mobilized the energy needed to cover more ground (Girard & Garland, 2001; Gleeson et al., 1993; Rees et al., 1985). Alternatively, corticosterone might have caused the greater activity rather than having been a response to this activity. If so, the effect of corticosterone must depend on a threshold. Without simulated risk, greater movement accompanied more corticosterone (Figures 1 and 6, boxes labelled Control). However, with simulated risk, less movement accompanied more corticosterone (Figures 1 and 6, boxes labelled Predation D1 and Predation D2). Therefore, low and high levels of corticosterone must have opposing effects on activity if variation in corticosterone drove variation in movement. A previous study of birds revealed evidence for a nonlinear effect of corticosterone, where a slight artificial elevation of corticosterone increased activity but a larger artificial elevation decreased activity (Breuner & Wingfield, 2000). Further investigation is needed to determine whether corticosterone is the cause or effect of thermoregulatory behaviour.

We have shown that behavioural and physiological responses of spiny lizards depended on the interaction between thermal patchiness and predation risk. Our results underscore the need to consider abiotic and biotic factors simultaneously when predicting how species will respond to climate change as many models project changes in environmental structures (Post, 2013; Zarnetske et al., 2012), which consequently affects the distribution of thermal resources. If predation risk limits access to thermal resources, the performance, dispersal, and ultimately survival of an animal will depend on the distribution of these resources. Thus far, researchers investigating the effects of climate change have mainly focused on measuring changes in the mean or variance of temperature rather than the spatial distribution of temperatures (Buckley et al., 2010; Kearney & Porter, 2009; Sinervo et al., 2010). While existing models reveal costs of and constraints on activity (Gunderson & Leal, 2016), they ignore other factors that influence these costs and constraints, such as the covariation between thermal resources and predation risk (Lima, 1998a; Sears & Angilletta, 2015; Sears et al., 2016). Thus, great potential exists to extend current approaches, or develop new ones, that incorporate spatial structure and species interactions when forecasting impacts of climate change (Kissling et al., 2012; Levy et al., 2016; Sears et al., 2016). Ultimately, biologists can use spatially-explicit models of thermoregulation to understand the behaviours of animals across landscapes in current and projected climates (Angilletta, 2009; Basson et al., 2017; Sears & Angilletta, 2015).

AUTHOR CONTRIBUTIONS

All authors conceived and designed the study. Travis W. Rusch collected animals, performed surgeries, and collected data. Travis W. Rusch and Michael J. Angilletta Jr conducted statistical analyses. Travis W. Rusch and Michael J. Angilletta Jr wrote the first draft of the manuscript. All authors revised further versions of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest.

DATA AVAILABILITY STATEMENT

All raw data have been archived at the Dryad Digital Repository (DOI: https://doi.org/10.5061/dryad.7pvmcvf0c). All R scripts will be made available upon request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. On average, lizards preferred the same body temperatures before and after surgical implantation of temperature loggers. Black symbols represent the mean temperature of each lizard in a thermal gradient. Red symbols and grey bars denote means and standard deviations computed by multimodel averaging.

Figure S2. Large, outdoor arenas (400 m^2) were used to manipulate the thermal landscape. Each arena contained one of two levels of thermal patchiness: 1 large patch of shade (a) or 4 small patches of shade (b).

Figure S3. Schematic of aggregated (1-patch) thermal arena with cable flyways. Note, only one predator.

Table S1. Outline of experimental design indicating when specific events occurred.

Table S2. Principal components describing the covariation between operative environmental temperatures.

Table S3. A ranking of mean minimal total movement models based on the likelihood of being the best model. For each model, we provide the Akaike weight, which equals the probability that the model describes the data better than other models. Only models with an Akaike weight of at least 1% are listed, in addition to the full model and the null model. All likely models included effects of day, patches, and treatment, as well as their interaction. Each model also contained an intercept and a random term associated with individual lizards.

Table S4. A ranking of mean minimal habitat use models based on the likelihood of being the best model. For each model, we provide the Akaike weight, which equals the probability that the model describes the data better than other models. Only models with an Akaike weight of at least 1% are listed, in addition to the full model and the null model. All likely models included effects of day, patches, and treatment, as well as their interactions. Each model also contained an intercept and a random term associated with individual lizards.

Table S5. A ranking of mean body temperature models based on the likelihood of being the best model. For each model, we provide the Akaike weight, which equals the probability that the model describes the data better than other models. Only models with an Akaike weight of at least 1% are listed, in addition to the full model and the null model. All likely models included effects of day, patches, PC1, and treatment. Each model also contained an intercept and a random term associated with individual lizards.

Table S6. A ranking of mean probability of exposure models based on the likelihood of being the best model. For each model, we provide the Akaike weight, which equals the probability that the model describes the data better than other models. Only models with an Akaike weight of at least 1% are listed, in addition to the full model and the null model. All likely models included effects of day, patches and treatment, as well as the interaction of day and treatment. Each model also contained an intercept and a random term associated with individual lizards.

Table S7. A ranking of mean circulating corticosterone models based on the likelihood of being the best model. For each model, we provide the Akaike weight, which equals the probability that the model describes the data better than other models. Only models with an Akaike weight of at least 1% are listed, in addition to the full model and the null model. All likely models included effects of measure, patches and treatment, as well as the interaction of measure and treatment. Each model also contained an intercept and a random term associated with individual lizards.

Table S8. Coefficients, standard errors and *p*-values for the model of minimal total movement, based on full model averaging.

Table S9. Coefficients, standard errors and *p*-values for the model of minimal habitat use, based on full model averaging.
Table S10. Coefficients, standard errors and *p*-values for the model of probability of exposure, based on full model averaging.
Table S11. Coefficients, standard errors and *p*-values for the model of body temperature, based on full model averaging.
Table S12. Coefficients, standard errors and *p*-values for the model of corticosterone, based on full model averaging.

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