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Author for correspondence:
Karla Alujević
e-mail: alujevick@gmail.com

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Mismatches between phenotype and environment shape fitness at hyperlocal scales

Karla Alujević[1,2], Jeffrey W. Streicher[3], Raquel A. Garcia[1], Ana Riesgo[3,4], Sergio Taboada[3,5,6], Michael L. Logan[2] and Susana Clusella-Trullas[1]

[1]Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Stellenbosch 7600, South Africa
[2]Department of Biology and Program in Ecology, Evolution, and Conservation Biology, University of Nevada, Reno, NV 89557, USA
[3]Department of Life Sciences, The Natural History Museum, London SW7 5BD, UK
[4]Museo Nacional de Ciencias Naturales, Madrid, Spain
[5]Departamento de Biodiversidad, Ecología y Evolución, Universidad Complutense de Madrid, Facultad de Ciencias, 28049 Madrid, Spain
[6]Departamento de Ciencias de la Vida, EU-US Marine Biodiversity Group, Universidad de Alcalá, 28871 Alcalá de Henares, Spain

KA, 0000-0002-0321-2549; JWS, 0000-0002-3738-4162; MLL, 0000-0003-2242-1810

In the era of human-driven climate change, understanding whether behavioural buffering of temperature change is linked with organismal fitness is essential. According to the 'cost–benefit' model of thermoregulation, animals that live in environments with high frequencies of favourable thermal microclimates should incur lower thermoregulatory costs, thermoregulate more efficiently and shunt the associated savings in time and energy towards other vital tasks such as feeding, territory defence and mate acquisition, increasing fitness. Here, we explore how thermal landscapes at the scale of individual territories, physiological performance and behaviour interact and shape fitness in the southern rock agama lizard (*Agama atra*). We integrated laboratory assays of whole organism performance with behavioural observations in the field, fine-scale estimates of environmental temperature, and paternity assignment of offspring to test whether fitness is predicted by territory thermal quality (i.e. the number of hours that operative temperatures in a territory fall within an individual's performance breadth). Male lizards that occupied territories of low thermal quality spent more time behaviourally compensating for sub-optimal temperatures and displayed less. Further, display rate was positively associated with lizard fitness, suggesting that there is an opportunity cost to engaging in thermoregulatory behaviour that will change as climate change progresses.

1. Introduction

Behavioural thermoregulation is the primary mechanism by which many ectothermic species buffer temperature change in their environments [1]. Ectothermic organisms often exhibit narrow preferred body temperature ranges at which their physiological performance is optimized [2]. Using behavioural thermoregulation, ectotherms can select appropriate thermal microclimates to maintain optimal body temperatures as they cope with changing thermal conditions [3]. Thermoregulatory compensation is generally viewed as positive since it can provide immediate benefits by preventing short-term performance loss and thus buffer the negative effects of environmental change [4,5]. However, behavioural responses to changing environments may have costs that reduce fitness, and these are rarely considered.

Forecasts of the impacts of climate change often rely on coarse scale thermal data which greatly exceed the spatial scales actually experienced by organisms.

At the population level, the thermal quality of the environment is typically calculated as the absolute difference between the preferred temperature of a species and the mean operative temperature of the broader habitat [6]. However, most ectotherms experience thermal environments at small spatial scales, where individuals sometimes live their entire lives within a patch of habitat that is only several square metres in area [7]. The distributions of available microclimates in these small patches might substantially differ from climate measured at the wider scale of a population or species [8,9]. In addition, individuals within a population often show consistent differences in their physiological phenotypes [10–12] suggesting that the thermal quality of a given habitat can only be quantified relative to the physiological phenotype of the occupant. Nevertheless, the extent to which fine-scale thermal variation interacts with the physiology of individuals to shape thermoregulatory performance and behaviour is almost completely unknown (but see [13]).

The likelihood that any individual will engage in thermoregulatory behaviour is dependent on the associated costs and benefits of such behaviour [14]. To engage in thermoregulatory behaviour, the organism must pay an energetic cost that arises from movement between microclimates. Individuals must also pay an opportunity cost, as the time spent thermoregulating cannot be spent (at least entirely) on other fitness-relevant activities such as foraging, territory defence, and mate acquisition [15]. When preferred microclimates become rare, ectothermic organisms must invest more time and energy searching for optimal patches of habitat and thus the cost of thermoregulation should increase [14,16,17]. By contrast, environments with high frequencies of favourable thermal microclimates should allow for efficient thermoregulation that permits higher investment in other fitness-related tasks [14,18,19].

To test how thermal quality affects lizard behaviour and fitness, we studied a population of southern rock agamas (*Agama atra*; hereafter, rock agama). Rock agamas represent an ideal study system due to their high territoriality, allowing us to easily estimate thermal availability within the area individual lizards occupy. Dominant males actively defend territories that consist of small rock outcrops, and they stay in these territories throughout their adult lives, whereas females, juveniles and subdominant (often younger) males cross territorial boundaries [7]. Rock agamas use signalling behaviour such as head bobbing and push-ups to maintain social status, repel rivals, and secure mating opportunities [20]. Territory defence during the breeding season and the occupation of higher positions in the social hierarchy via more frequent bouts of signalling should have direct implications for reproductive success [21]. Given that environments with high frequencies of favourable thermal microclimates should allow for efficient thermoregulation that permits higher investment in other fitness-related tasks [14,18,19], we hypothesized that (i) individual male rock agamas that occupied territories with high frequencies of favourable thermal microclimates (defined relative to individuals' physiology) would thermoregulate more efficiently and (ii) males living in high-quality thermal environments would shunt time and energy savings into non-thermoregulatory behaviours that maintain social dominance, ultimately resulting in higher relative fitness. To test these predictions, we combined laboratory assays of adult male lizard physiology with detailed measurements of environmental temperatures at the scale of individual male territories to assess the thermal quality of those territories with respect to each focal lizard. We then conducted more than 150 h of behavioural observations in the field and tracked reproductive success by genotyping sires and their offspring. Our analysis represents one of the most comprehensive examinations of the relationship between behavioural thermoregulation and fitness conducted to date, with important implications for the responses of ectotherms to climate change.

2. Material and methods

(a) Study site, lizard collection and territory estimation

Our study site was in Jonaskop, Western Cape Province, South Africa (33.93 S, 19.52 E; 900 m elevation). We collected data between September 2017 and May 2018. The study period incorporated a summer breeding season (September–January) when females ovulate and produce one to two clutches [22,23] and an autumn post-breeding season when most juveniles hatch (February–May). During the study period, we captured all adult males that we encountered using the lasso technique [24], then weighed and marked them before releasing them back to the precise location where they were originally captured. Prior to release, we sutured a unique sequence of small, coloured beads to the dorsal surface of the base of each male's tail. This enabled us to identify and observe each focal male from a distance without disrupting their behaviour [25]. In addition, focal males were toe-clipped to assure identification even in the case of lost beads. This happened with only two lizards in our study, and these individuals were re-beaded and immediately released back to their territory. One toe per foot was toe-clipped for each animal which has been shown to not affect locomotor performance or body condition in multiple species of lizards [26,27]. Every time we encountered a focal male, we recorded a GPS coordinate (Garmin e-trex Vista, GARMIN International, Inc., KS, USA) at the point location of the sighting. Home ranges and core areas of individual lizards were estimated using the minimum convex polygon (MCP) method [28]. From these data, we obtained two indices—the 95% isopleth representing the 'home range' (excluding the 5% of points that were farthest from the centroid) and the 50% isopleth, which represents the 'core area' [29,30]. Since core areas represent areas where lizards are most commonly found, these correspond to the focal parts of lizards' territories that comprise the main perches used for signalling and displays, crevices used as roosting areas and refuge from unfavourable weather conditions and predators. Therefore, in this study, and at least for adult males, we considered core areas as good proxies of lizard territories, since core areas represent areas of intense and exclusive use by individuals inhabiting them [31] (see electronic supplementary material for details). Home range estimation analyses were carried out in R software using the package 'adehabitatHR' [32]. Shape files generated using the MCP method, computed for 50% core areas and 95% home ranges, were exported using the function 'writePolyShape' from the package 'maptools' and visualized using ArcGIS v.10.6.1 (ESRI, Inc., CA, USA).

(b) Measuring thermal landscapes of lizard territories

After we mapped the territories of our focal males, we used operative temperature models (OTMs; electronic supplementary material, figure S11) to assess the availability of thermal microclimates within each territory [6,9,33]. We built these models following previously established methods for rock agamas [34]. We used thin-walled copper piping (80 mm long), painted to represent the thermal reflectance properties of a typical adult rock

agama, and ends closed using cork stoppers. The spectral reflectance of the paint (Duram NS4 Grey; reflectance = 0.09) approximated the skin reflectance of rock agamas (0.10) measured using standard techniques [35]. A temperature logger (thermochron iButtons, Dallas Semiconductor, CA, USA) wrapped in non-conductive acrylic mesh was centrally suspended in each copper model. We used two iButton models (high-capacity DS1922L, ±0.5°C, 8 kB memory, and low-capacity DS1921G, ±1°C, 2 kB). To compensate for differences in storage capacity between models, high-capacity loggers were set to record temperatures every 15 min and low-capacity loggers set every 30 min for the same period of time. An even mix of these models was deployed within each lizard territory. We placed 21 OTMs within the boundaries of each of 15 focal lizard territories from December 2017 to January 2018 (summer breeding season). We placed 18 OTMs in each of a separate set of 16 focal lizard territories between March and April 2018 (autumn post-breeding season). For all focal lizards, we deployed OTMs across microsites that were ecologically relevant for the species [36,37]. We used a combination of several factors to determine model placement, capturing a broad representation of operative temperature quality and availability within territories: (i) type of substrate (rock or soil); (ii) terrain exposure (open area or crevice); (iii) sun exposure (sun, partial sun, shade); (iv) wind exposure (exposed or sheltered) and (v) orientation (N, W, S or E). This sampling strategy was chosen instead of a full random design because we wanted to ensure that the entire range (including minimum and maximum bounds) of temperatures available were represented across all territories (see [38] for effects of model distribution on thermal indices). Detailed descriptions of the distribution of OTMs deployed in each territory are given in electronic supplementary material, tables S1 and S2.

(c) Field-active body temperatures

We collected surface (skin) temperatures as body temperatures of lizards using an infrared (IR) thermal-imaging camera (FLIR-T450sc, FLIR Systems, Wilsonville, Oregon; 7.5–13.0 µm spectral range, 320 × 240 resolution), which is the only technique that allowed minimal disturbance of the study animals. Surface temperatures estimated from thermography are typically highly correlated to internal body temperature in other lizards [39,40]. We collected 256 body temperature measurements from 23 individuals (23 focal males in the summer breeding season and 17 of these same focal males sampled again in the autumn post-breeding season, overlapping the periods with OTM deployment; numbers of focal lizards varied across seasons and traits due to logistical constraints; see electronic supplementary material, figure S1, for a schematic of study design and sample sizes). Body temperatures were collected by the same person from a standardized distance (1 m from the lizard) and position (consistent angle from camera to lizard, capturing the dorsal surface) while animals were active (not hiding in crevices) over the course of their diel activity cycle (8.00–18.00 h). Emissivity was set to 0.95 on the IR camera according to standard methods (Chapter 32, FLIR T4xx user's manual, 2014, FLIR Systems, Inc.) and corresponds to values in previous lizard studies [41,42]. We measured air temperature and relative humidity multiple times each day as conditions changed using a portable weather meter (Kestrel 3000, Kestrel Meters, PA, USA) placed at a standard 1.2 m above ground-level and these values were fed as continuous inputs to the IR camera.

(d) Behavioural observations

From October 2017 until April 2018, we followed a standard ethological protocol [43] to construct ethograms for adult male focal lizards (25 lizards in the summer breeding season and 17 lizards in autumn post-breeding season, of which 13 were the same individuals). An observer haphazardly walked through the broader area occupied by the population of lizards until a focal male was spotted. Individual lizards were identified using binoculars based on the coloured bead markings. We conducted observations from a portable blind (1.2 m × 1.2 m × 1.0 m) made from green shade cloth containing a small opening for the observer to view the lizard. The observation blind was positioned a minimum of 20 m from the lizard's territory. The observer then followed a timed ethological protocol, monitoring lizards over the course of their diel activity cycle (8.00–18.00 h) for a maximum of 2 h or until the lizard disappeared for 30 consecutive minutes. We chose this extended observation protocol, rather than shorter observation intervals, since lizards spent long periods of time inactive or were obscured from the observer's view due to the physical complexity of their territories. During each sampling interval, we recorded the following behaviours: (i) basking, (ii) thigmothermy (heat exchange by conduction where the torso and/or head is pressed against the substrate), (iii) sitting in partial or full shade, (iv) moving (locomotion), (v) eating a prey item, (vi) head bobbing, (vii) push-ups, (viii) mating, (ix) hiding (visible but inside a crevice) or (x) not visible. Descriptions of how we categorized these behaviours are given in electronic supplementary material, table S6. We calculated the amount of time lizards invested into each type of behaviour as the proportion of total observation time (ranging from 0 to 1) during which the animal was seen engaging in that behaviour. The rate of signalling behaviour (head bobbing and push-ups per min) was calculated as the number of signalling displays divided by the observation time. Many of the behaviours we set out to observe occurred rarely. Therefore, for subsequent analyses, we focused on the subset of behaviours that, at a population level, occurred at a mean proportion or rate of at least 0.1 (electronic supplementary material, table S6).

(e) Thermal performance curves

Upon the completion of field data collection, we recaptured 19 focal males to estimate the thermal performance curves for each individual. Lizards were habituated to laboratory conditions for 5 days during which they were individually housed in glass terraria with access to a wooden crevice and a basking rock. Each terrarium was equipped with a heat bulb emitting IR radiation to enable thermoregulation and an UV light for vitamin D3 synthesis. Lizards were fed every 2 days with medium-sized crickets and mealworms (approx. 5% of lizards' average body mass and dusted in vitamin and mineral supplement) and given water ad libitum but were fasted for 24 h prior to sprint speed trials to minimize potential effects of digestion on performance [44]. We measured sprint speed at body temperatures of 15, 25, 35, 38, 42 and 44°C. To obtain target body temperatures, lizards were placed in a climate-controlled room until they equilibrated to the test temperature. We randomized the order of temperature exposures, except for the 44°C trial which was conducted for all lizards at the end of the trials to avoid heat-shock effects on subsequent trials. We confirmed that a given lizard was at the target temperature with a cloacal thermometer before the start of each trial.

Running speed was measured in an enclosed runway constructed out of wood (2 m × 0.15 m × 0.3 m) and demarcated every 25 cm [45]. Lizards ran on a layer of fine sandpaper for traction. We encouraged lizards to run by gently tapping their tails [45]. Three trials were conducted at each temperature per individual and in quick succession. We recorded trials with a high-speed digital video camera (60 fps; PowerShot G16, Canon Inc., Tokyo, Japan) and determined the maximum sprint speed over any 25 cm section using frame-by-frame analysis in the motion analysis software program Kinovea (v.0.8.15, Kinovea, France). If a lizard failed to run for at least 25 cm without stopping, we scored the sprint speed as 0 m s^{-1} [36]. We

released all focal males back to their sites of capture upon the completion of sprint speed trials.

Thermal performance curves were fitted to raw sprint speed data using TableCurve 2D curve fitting software (v.5.01, Systat Software Inc., San Jose, CA, USA) [36,46]. Curves were fitted using the fastest of three runs per temperature for each lizard. A set of biologically relevant equations (asymmetrical parabolic functions) were used to fit the performance data [46]. The critical thermal minimum (CT_{min} = 6.3°C) and critical thermal maximum (CT_{max} = 44.9°C), specific for the family Agamidae [47], were included as the lower and upper bound of the performance curve for fitting models (see electronic supplementary material). Fitted curves were compared based on Akaike's information criterion (ΔAICc), to select the best model for individual raw data [46,48]. All performance curves were fitted by third- or fourth-order polynomials. Maximal performance at the peak of the curve (P_{max}), mean performance (P_{mean}), thermal optimum (T_{opt}; temperature at which performance was maximized), performance breadth (the range of temperatures at which the lizard performed at 80% of maximal performance) and corresponding lower (B_{80} lower) and upper (B_{80} higher) threshold values were extracted from individual curves (electronic supplementary material, figure S4).

(f) Thermoregulatory accuracy

Field-active body temperatures were extracted from thermal images using the FLIR ResearchIR Max 4 software (FLIR Systems, Inc.). We obtained an estimate of body surface temperature from each image using the 'draw' tool by drawing a line along the dorsal side of the body, extending from the base of the head to the base of the tail, and averaging temperature values along the line. For each focal male, we calculated the thermoregulatory accuracy for the season by averaging the daily deviation of their mean body temperature from their thermal optimum for sprint performance across all days on which body temperatures were obtained.

(g) Thermal quality of lizard territories

We calculated full frequency distributions of operative temperatures within each territory as well as frequency distributions for each of the four microsite categories based on the level of sun exposure—full sun, partial sun, shade and crevice. We used operative temperatures during the typical activity period of rock agamas (8.00–18.00) and averaged temperatures at each 15 min interval for each category. For each focal male and season, we calculated the territory thermal quality as the mean daily number of hours that operative temperatures fell within the individual's performance breadth (B_{80}) for each microsite category and summed periods across all microsites. Performance breadths were extracted from fitted thermal performance curves as described above. We used linear mixed effects models to test if territory thermal quality predicted mean field-active body temperature or thermoregulatory accuracy. Body mass and season were included as fixed effects and lizard identity as a random effect to account for repeated measures of individuals. We used similar models to test if territory thermal quality predicted time invested in thermoregulatory behaviour or the frequency of signalling behaviour, with season and body mass included as fixed effects and lizard identity included as a random effect. In addition, we evaluated the extent to which low-quality territories tended to be too cold or too hot using linear mixed effects models with the percentage of operative temperatures that were below or above lizards' thermal performance breadths as the response variables, respectively, and territory thermal quality and season as fixed predictors, with lizard identity as a random factor. Lastly, separate linear mixed effects models were run with mean field-active body temperature and thermoregulatory accuracy as predictors of behaviour (time invested in thermoregulatory behaviour and rate of signalling behaviour), with body mass and season included as fixed effects, and lizard identity included as a random effect. Best suitable models were selected using a backward stepwise approach based on ΔAICc [48]. Due to missing data on some individuals, not all focal lizards were included in all analyses (see electronic supplementary material, figure S1, for a schematic of study design and sample sizes). All models were run in R v.4.0.3 [49].

(h) Single nucleotide polymorphism-based paternity inference

We collected tissue samples (tail tips) from all adult males, adult females and juveniles encountered in the population throughout the study period (September 2016–May 2018; $N = 207$; electronic supplementary material, figure S7) for genotyping analyses. We genotyped adult females in addition to adult males and offspring to increase the accuracy of allele frequency estimates. Tissues were originally preserved in ethanol and stored at −20 or −80°C [50], and then DNA extraction was performed following the magnetic beads protocol [51] (see electronic supplementary material for details). We sequenced genome-scale data using a double digest restriction site-associated DNA (ddRAD) sequencing protocol [52]. We confirmed successful library amplification on a 4200 TapeStation (Agilent, Santa Clara, CA, USA). A total of 11 Illumina index group libraries (with 17–19 samples each) were then pooled in equimolar ratios to create a single library that was sequenced using an Illumina NextSeq 500 at the sequencing facility of the Natural History Museum, London (see electronic supplementary material for library preparation protocol). Sequences were uploaded to GenBank with accession numbers SAMN35047870–SAMN35048076. Prior to identifying and scoring loci, we removed adapter contamination using the fastx tool kit [53]. Restriction site-associated DNA markers (RAD-tags) were processed using STACKS 2.2 to produce single nucleotide polymorphism (SNP) datasets [54,55]. We followed the recommended workflow using the functions 'process_radtags', 'ustacks', 'cstacks' and 'sstacks' to identify RAD-tags containing SNPs [55]. For 'ustacks', a minimum stack depth of 10 was specified (m parameter), and up to four mismatches per locus within an individual (M parameter) were allowed. For 'cstacks', we set the distance between catalogue loci (n flag) to 4. Constructed libraries were exported using the 'populations' function in STACKS. We pooled individual samples into one population and exported a given locus if it was present in at least 95% of the individuals in our sample (p parameter). To avoid site linkage, we restricted the data to the first SNP per locus (write_single_SNP parameter). Population analyses with STACKS identified 407 loci that were present in 95% of individuals and had minimum 10× coverage depth which we used for subsequent analyses. To generate composite paternity indices (CPIs), we modified the 'paternityIndex' function of Rosyara *et al.* [56] (code available at https://github.com/kalujevic/paternity). Our modified version of this R script leveraged classic equations [57] (electronic supplementary material, table S7) and enabled us to generate CPIs for father–offspring pairs when the mother's genotype was unknown. We only assigned offspring to a sire if the probability of paternity (PP) was greater than 75%. In one case where two males were assigned with high probability to have sired the same offspring, we assigned the offspring to the sire with the higher PP (100%). The ability to resolve paternity with biallelic SNPs has been shown to improve when using alleles that are more evenly distributed across individuals in the population [58]. We explored three different minor allele frequency (MAF) thresholds (no MAF, MAF = 0.10 and MAF = 0.20). Unlike the primary dataset where we were able to assign offspring to 22 different sires with high probability

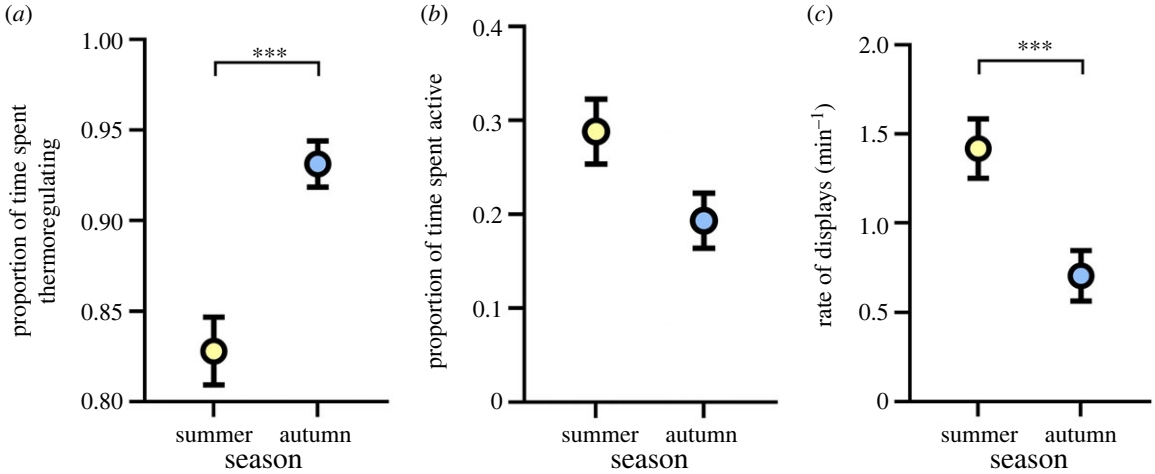

Figure 1. Seasonal differences in the proportion of time (0-1) invested in thermoregulatory behaviour (*a*), activity (*b*) and the rate of signalling behaviour (*c*). The fact that lizards spend less time thermoregulating during the summer breeding season suggests that time spent engaging in this behaviour likely comes at the cost of other behaviours that are critical for breeding success.

(PP > 75%), the MAF 0.10 and 0.20 datasets resulted in no offspring sire pairings with PP > 75%. Thus, we conducted analyses with the full dataset (no MAF) comprising 407 SNPs since maximizing the number of SNPs was demonstrably more important than removing minor frequency alleles. The number of offspring assigned to each potential sire ranged from 0 to 7, but the vast majority of sires were assigned zero, one or two offspring (53% of males sired zero offspring, 27.7% sired one, 14.9% sired two and less than 5% sired more than two offspring). Because of the low absolute number of offspring assigned to individual sires, we binned paternity assignments into two categories: males that sired some offspring or males that sired zero offspring. We then tested for significant differences in reproductive success based on the traits and territory qualities of males (e.g. body mass, territory size, territory thermal quality, thermoregulatory behaviour, etc.) using two-sample *t*-tests or Wilcoxon–Mann–Whitney tests.

3. Results

Individual territories differed in variance of operative temperature (T_e) within each season (electronic supplementary material, table S4) and across seasons (mean T_e variance ± s.d. in summer: 133.3 ± 12.1 and in autumn: 76.9 ± 5.1). Variance of T_e was not related to individual performance breadth in the summer breeding season ($t_8 = 1.50$, $p = 0.17$, effect size = 1.37) or in the autumn post-breeding season ($t_{14} = 0.67$, $p = 0.52$, effect size = 0.31), respectively. There was no significant difference in the number of daily hours of T_e within lizards' performance breadths between seasons ($Z = -1.26$, $p = 0.22$, effect size = −0.28; electronic supplementary material, figure S5 and table S5). Season did not have a significant effect on body temperature ($t_{13} = 0.41$, $p = 0.69$, effect size = 0.18) nor on the accuracy of thermoregulation ($t_{13} = 0.40$, $p = 0.70$, effect size = 0.12; electronic supplementary material, figure S6). However, lizards spent more time thermoregulating in the autumn post-breeding season than in the summer breeding season ($t_{12} = 4.49$, $p < 0.001$, effect size = 1.66; figure 1), but there was no significant difference in activity between the two seasons ($t_{12} = 1.77$, $p = 0.10$, effect size = −0.77). A significantly higher rate of signalling behaviour was observed during the summer breeding season in comparison to the autumn post-breeding season ($Z = -3.27$, $p < 0.001$, effect size = −1.21; figure 1), illustrating the importance of signalling behaviour to breeding success.

(a) Territory thermal quality

While the home ranges of different male lizards occasionally overlapped, individuals spent most of their time in distinct core areas (hereafter, 'territories') that overlapped only rarely (and in marginal areas; figure 2) and were largely consistent across seasons (electronic supplementary material, figure S2). Larger lizards had smaller core areas and home ranges than smaller lizards (indicating higher territoriality) in the breeding summer season ($t_{23} = -3.135$, $p < 0.001$, effect size = −49.26 and $t_{23} = -2.37$, $p = 0.027$, effect size = −172.2, respectively) while in the autumn post-breeding season, there was no significant difference between body mass and territory size ($t_{24} = -1.20$, $p = 0.24$, effect size = −10.99) or home range size ($t_{24} = -1.77$, $p = 0.09$, effect size = −107.6; electronic supplementary material, figure S3). During the summer breeding season, mean and maximum temperatures varied among territories by as much as 3°C and 11°C, respectively (electronic supplementary material, table S4). Furthermore, the spatio-temporal variability of operative temperatures differed among territories by as much as 34% (electronic supplementary material, table S4). The average lizard performance breadth (B_{80}) was 14.6 ± 3.0°C with a mean lower B_{80} threshold of 27.1°C and a mean higher B_{80} threshold of 41.6°C (electronic supplementary material, table S3). Lizards in territories of low thermal quality experienced a higher percentage of operative temperatures that were below their thermal performance breadths ($t_8 = -20.48$, $p < 0.001$, effect size = −9.42; electronic supplementary material, figure S12) and a lower percentage of operative temperatures above their thermal performance breadths ($t_8 = 4.42$, $p = 0.002$, effect size = 1.73; electronic supplementary material, figure S12).

(b) The effects of territory thermal quality on behaviour

Lizards in high thermal quality territories thermoregulated less accurately than those in low thermal quality territories ($t_8 = 2.41$, $p = 0.04$, effect size = 0.75; table 1 and figure 3). Field body temperature was not predicted by thermal quality

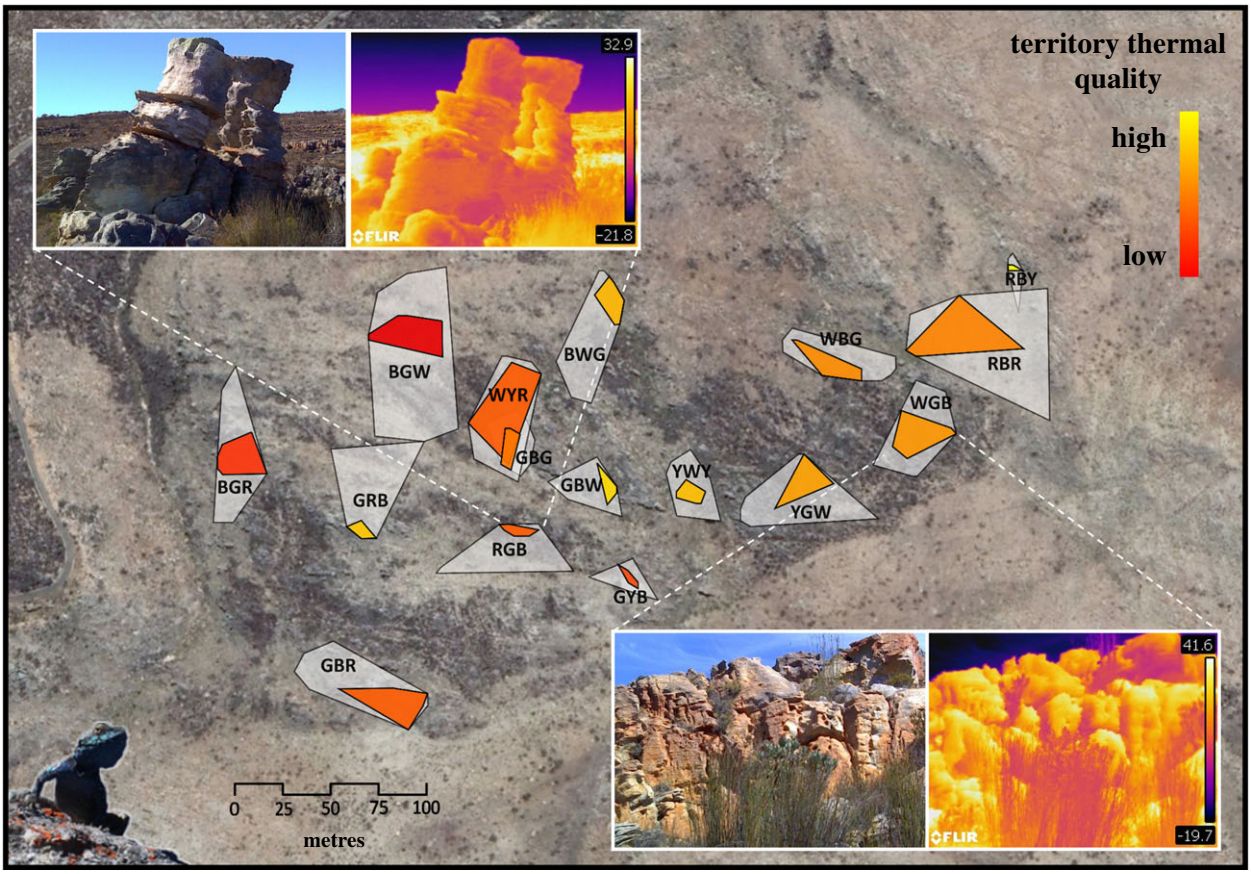

Figure 2. The average thermal quality varied among focal male rock agama territories. Home range (grey polygons) and territory (yellow to red polygons) borders were computed using the MCP approach. Individual territories (defined as 50% isopleth core areas) are colour-coded according to their thermal quality. Thermal quality is the number of hours during which operative temperatures fall within the thermal performance breadth of the territory holder and ranged from 0.5 h (low) to 6 h (high). Letter combinations correspond to individual bead markings used for lizard identification. The two pairs of inset photographs are standard RGB (left) and infrared (right) images of two rock agama territories (RGB and WGB), highlighting their spatial, structural and thermal complexity.

Table 1. Outputs of the linear mixed effects models testing for the effect of thermal quality, body mass and season on field body temperature and thermoregulatory accuracy. Best suitable models were selected using a backward stepwise approach based on Akaike's information criterion.

	β	s.e.	d.f.	*t*-value	*p*-value
body temperature					
thermal quality	−3.860	1.863	7	−2.072	0.077
body mass	−0.431	0.200	13	−2.160	0.050
thermal quality × body mass	0.176	0.079	7	2.229	0.061
thermoregulatory accuracy					
thermal quality	0.750	0.311	8	2.414	0.042

($t_7 = -2.07$, $p = 0.08$, effect size = −3.86; table 1). Lizards occupying higher quality territories spent significantly less time thermoregulating using thigmothermy (quantified as the proportion of time lizards spent with their body being in direct contact to substrate to enhance heat conduction, which is a thermoregulatory behaviour that is easy to distinguish from non-thermoregulatory movement; $t_6 = 3.26$, $p = 0.02$, effect size = 1.11; table 2 and figure 3). Territory thermal quality did not predict lizard activity ($t_6 = 0.98$, $p = 0.36$, effect size = 0.015; table 2) but did have a significant effect on time spent basking ($t_6 = -3.25$, $p = 0.02$, effect size = −0.96; table 2). This relationship was mediated by body mass ($t_6 = 3.43$, $p = 0.01$) where smaller and larger lizards spent more time basking in territories of lower and higher thermal quality, respectively. Lizards in territories of high thermal quality also signalled at higher rates (head bobbing $t_4 = -2.92$, $p = 0.04$, effect size = −0.28; push-ups $t_5 = -2.06$, $p = 0.09$, effect size = −1.27; table 3 and figure 3). Body temperature and thermoregulatory accuracy did not predict time spent thermoregulating ($t_9 = -1.59$, $p = 0.15$, effect size = −0.02 and $t_{10} = -0.04$, $p = 0.97$, effect size = 0.001, respectively) or rate of displays ($t_{10} = -0.03$, $p = 0.97$ and $t_{10} = -0.90$, $p = 0.39$, effect size = −0.04, respectively).

(c) The effects of thermal quality and behaviour on fitness

Males that sired offspring occupied territories with significantly higher thermal quality during the breeding season compared to males that did not sire offspring ($t_{5.87} = 2.44$,

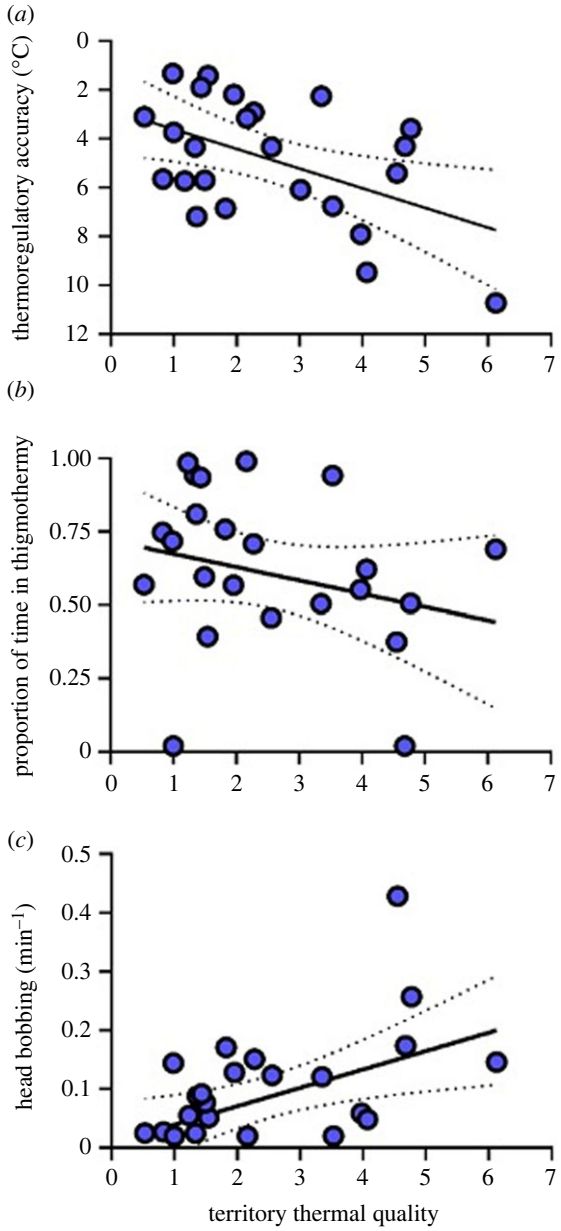

Figure 3. The relationship between territory thermal quality, thermoregulatory behaviour and signalling rates in male rock agamas. Focal males in territories of higher thermal quality (*a*) thermoregulate less accurately, (*b*) invest less time in thermoregulation (thigmothermy) and (*c*) have higher rates of signalling behaviour. Territory thermal quality is calculated as the number of hours during the daily activity period when operative temperatures fall within the resident lizard's performance breadth. Thermoregulatory accuracy is expressed as the absolute difference between mean field-active body temperature and the thermal optimum for sprint performance. Thus, higher values equate to lower accuracy of thermoregulation, and we have, therefore, inverted the *y*-axis in (*a*). The lines of best fit and 95% confidence intervals are superimposed on the raw data. Only significant relationships are shown (for all results, see tables 1–3).

$p = 0.05$, effect size = 1.43; figure 4), despite a trend towards lower thermoregulatory accuracy in these high-quality territories ($t_{15} = 1.49$, $p = 0.15$; figure 4). Males that sired offspring also displayed to conspecifics at significantly higher rates (push-ups $t_{22} = -2.92$, $p < 0.01$, effect size = 1.27; head bobbing $Z = -2.27$, $p = 0.02$, effect size = 1.15; figure 4) during the summer breeding season. Lizards that sired offspring were larger in body size ($t_{15} = 2.41$, $p = 0.03$, effect size = 1.17; electronic supplementary material, figure S8), but body size was not related to territory thermal quality ($t_{14} = 1.86$, $p = 0.08$, effect size = 0.25; electronic supplementary material, figure S9) or rate of head bobbing ($t_{16} = 1.63$, $p = 0.13$, effect size = 0.10). Larger lizards did have a higher rate of push-ups ($t_{16} = 2.65$, $p = 0.02$, effect size = 0.11). Contrary to the expectation that lizards that sire offspring would occupy larger geographical areas, reproductive success was not associated with either home range ($Z = -1.42$, $p = 0.18$, effect size = −0.78) or territory size ($Z = -0.23$, $p = 0.86$, effect size = −0.46; electronic supplementary material, figure S10).

4. Discussion

Faced with ongoing changes in their thermal environments, many ectothermic organisms are using behavioural thermoregulation to buffer the negative thermal effects they are experiencing in their habitats [59,60]. While this strategy might provide immediate benefits by preventing short-term performance loss, it is less well understood how costly these behavioural adjustments are with respect to fitness. After combining physiological data of thermal performance with hundreds of hours of behavioural observations in the field and hundreds of thousands of fine-scale estimates of environmental temperature, we found that the relative fitness of lizards was predicted by the thermal quality of their territories, and this effect remained after accounting for variation in body and territory size. Territory thermal quality likely played a key role in determining the fitness of individuals by mediating the opportunity costs of thermoregulation whereby lizards in low-quality territories had less time to signal conspecifics because they were forced to spend more time thermoregulating.

Several studies have demonstrated the effect of regional thermal quality on thermoregulatory performance across populations or seasons [15,61–63], or have used experimental or modelling approaches to illustrate similar patterns [17,18]. However, our results indicate that variation in thermal quality at the fine scale of individual animal territories is associated with variation in fitness, and is likely mediated by opportunity costs. The fact that thermal quality of territories predicted fitness, presumably via maximization of energy gained, is in line with expectations from the cost–benefit model of thermoregulation. The theoretical expectation underlying this relationship is that accuracy of thermoregulation should increase with territory thermal quality [14]. By contrast, in our study, rock agamas thermoregulated less accurately in territories of high thermal quality. This likely occurred because the risks of reduced performance were lower in high-thermal-quality territories and thus lizards were able to focus less on thermoregulating accurately. In other words, territories of high thermal quality permitted the territory holder to 'ignore' the thermal environment over longer periods of time, which freed up these animals to spend more time engaging in competitive and courting behaviours. Similar results have been observed between thermoregulatory behaviour and thermal quality on broader geographical scales. Lymburner & Blouin-Demers [63] showed that Yarrow's spiny lizards (*Sceloporus jarrovii*) thermoregulate more efficiently at high-elevation sites where thermal quality is lower and risk of reduced performance is higher. Ornate tree lizards (*Urosaurus ornatus*) also thermoregulated less accurately in high-thermal-quality habitats [64]. In a comparative global analysis conducted on 20

Table 2. Outputs of the linear mixed effects models testing for the effect of thermal quality, body mass and season on time spent active, time spent basking and time spent in thigmothermy. Best suitable models were selected using a backward stepwise approach based on Akaike's information criterion.

	β	s.e.	d.f.	*t*-value	*p*-value
activity					
thermal quality	0.015	0.015	6	0.982	0.364
season	0.081	0.032	6	2.536	0.044
basking					
thermal quality	−0.970	0.298	6	−3.249	0.018
body mass	−0.102	0.031	13	−3.264	0.006
thermal quality × body mass	0.043	0.013	6	3.425	0.014
thigmothermy					
thermal quality	1.112	0.342	6	3.257	0.017
body mass	0.118	0.036	13	3.310	0.006
thermal quality × body mass	−0.050	0.014	6	−3.442	0.014

Table 3. Outputs of the linear mixed effects models testing for the effect of thermal quality, body mass and season on signalling behaviour (head bobbing and push-ups). Best suitable models were selected using a backward stepwise approach based on Akaike's information criterion.

	β	s.e.	d.f.	*t*-value	*p*-value
head bobbing					
thermal quality	−0.284	0.097	4	−2.925	0.043
body mass	−0.023	0.010	13	−2.240	0.043
season	−0.020	0.059	4	−0.347	0.746
thermal quality × body mass	0.013	0.004	4	3.083	0.037
thermal quality × season	0.038	0.019	4	2.001	0.116
push-ups					
thermal quality	−1.272	0.618	5	−2.058	0.095
body mass	−0.079	0.063	13	−1.262	0.229
season	0.565	0.120	5	4.728	0.005
thermal quality × body mass	0.058	0.026	5	2.218	0.077

lizard species, Nadeau & Blouin-Demers [65] showed that lizards in poor thermal quality environments thermoregulated more effectively. Neel & McBrayer [66] showed that, when experiencing unfavourably hot thermal environments, Florida scrub lizards (*Sceloporus woodi*) regulate their body temperatures with high precision. Consistent findings across multiple lizard species suggest that the assumptions of the original cost–benefit model of thermoregulation are insufficient to explain lizard investment in thermoregulation. Rather, energetic costs of thermoregulation need to be evaluated with respect to the risks of reduced performance since physiological disadvantages of thermoconformity appear to be small under thermally benign circumstances.

While animals in habitats of low thermal quality can experience negative fitness consequences because of reduced performance, to our knowledge, our study is the first to link this phenomenon to fitness via opportunity costs in natural conditions. The fact that individuals in high-quality thermal territories had higher fitness suggests that the benefit of increased investment into maintaining social dominance outweighs the low-performance cost of inaccurate thermoregulation. Lizard body temperatures remained close to the thermal optima of individuals regardless of the behavioural decisions they made. On the other hand, thermoregulatory behaviours in territories of low thermal quality likely came at a fitness cost, as these individuals spent less time displaying to conspecifics and were much less likely to sire offspring. Because low thermal quality territories consisted of operative temperatures that were often below the thermal performance breadths of the lizards that occupied these territories, climate warming may initially benefit this species by reducing the time and energy required for basking. This is perhaps not surprising for a species of lizard that occupies a cooler, temperate environment. Many studies have suggested that higher latitude ectotherms may initially benefit from climate warming [67–69]. Regardless, our data illustrate the general principle that low-quality thermal environments have thermoregulatory costs, and it is likely that climate change will increase these costs in many species, especially those that live in environments which are already close to their upper thermal tolerance limits.

Although our results suggest that territory thermal quality is an important mediator of lizard fitness through its effects on thermoregulatory behaviour, other variables that we did not consider might affect lizard fitness. For example, higher predation risk associated with social dominance and

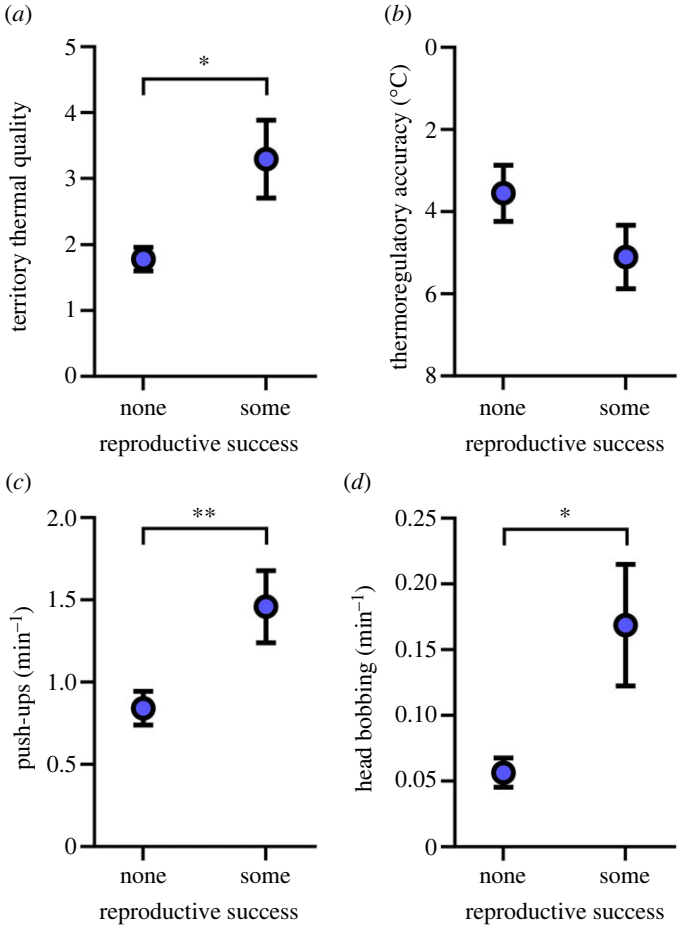

Figure 4. Factors affecting fitness in male rock agamas. Focal males had higher reproductive success when they (*a*) occupied territories of higher thermal quality, (*b*) thermoregulated less accurately (although this trend was not statistically significant), (*c*) had higher push-up rates and (*d*) had higher head bobbing rates. Reproductive success was 'none' for males that sired zero offspring and 'some' for males that sired at least one juvenile. Error bars represent standard errors and significant differences are indicated with asterisks (*$p < 0.05$; **$p < 0.01$).

territoriality of dominant males [70], or predation risk associated with prolonged behavioural thermoregulation in lizards in lower thermal quality territories, potentially affect investment into different behaviours [71]. While our data indicate that males in high-thermal-quality territories have more time for maintaining social dominance, thus increasing their appeal and mating opportunities, female abundance and female choice might also represent important factors mediating the relationship between thermal quality and fitness. Lastly, independent of territory thermal quality, males that sired offspring were larger in body size suggesting that other aspects of lizard morphology and ecology likely impact fitness in this species. Nevertheless, territory thermal quality was not correlated with lizard body mass, indicating that thermal environments at very fine spatial scales play an important role in shaping thermoregulatory behaviour, territory defence and mate acquisition, and ultimately fitness. Future studies that include females and have larger sample sizes are needed to parse out the relative importance of different variables with high confidence.

Thermoregulatory behaviour is primarily seen as a mechanism that buffers the effect of changing thermal environments on ectotherms while reducing the strength of selection and slowing rates of physiological evolution [4,72]. As habitats become warmer and more thermally variable, the first compensatory response of many species will be to increase the time they spend thermoregulating. While behavioural buffering is normally included as a fitness-maintaining process in forecasts of the effects of climate change on populations [5], our results indicate that behavioural thermoregulation might come at a severe cost, decreasing the time and energy available for competitive and courtship behaviours, and ultimately resulting in reduced reproductive success. Therefore, while behavioural buffering of sub-optimal temperatures might increase survival probability in the short term, this study demonstrates that this might result in an opportunity cost that reduces long-term reproductive output and hastens population decline. Accurate forecasts of the biological effects of rapid climate change will require consideration of the opportunity cost paid by organisms that behaviourally thermoregulate.

Ethics. Animal collection, maintenance and experiments were approved by CapeNature (permit no. AAA007-00206-0056) and the Research Ethics Committee: Animal Care and Use, protocol SUACUD14-00061.

Data accessibility. Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.v41ns1s25 [73].

Additional information is provided in the electronic supplementary material [74].

Authors' contributions. K.A.: conceptualization, data curation, formal analysis, investigation, methodology, project administration, visualization, writing—original draft; J.W.S.: conceptualization, data curation, formal analysis, methodology, supervision, writing—original draft, writing—review and editing; R.A.G.: formal analysis, writing—review and editing; A.R.: methodology, resources, writing—review and editing; S.T.: methodology, resources, writing—review and editing; M.L.L.: conceptualization, funding acquisition, methodology, resources, supervision, visualization, writing—review and editing; S.C.-T.: conceptualization, funding acquisition, methodology, resources, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. The authors declare no competing interests.

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