



The Bogert Effect and environmental heterogeneity

Michael L. Logan^{1,2,3} · Jenna van Berkel³ · Susana Clusella-Trullas³

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Abstract

A classic question in evolutionary biology is whether behavioral flexibility hastens or hinders evolutionary change. The latter idea, that behavior reduces the number of environmental states experienced by an organism and buffers that organism against selection, has been dubbed the “Bogert Effect” after Charles Bogert, the biologist who first popularized the phenomenon using data from lizards. The Bogert Effect is pervasive when traits like body temperature, which tend to be invariant across space in species that behaviorally thermoregulate, are considered. Nevertheless, behavioral thermoregulation decreases or stops when spatial variation in operative temperature is low. We compared environmental temperatures, thermoregulatory behavior, and a suite of physiological and morphological traits between two populations of the southern rock agama (*Agama atra*) in South Africa that experience different climatic regimes. Individuals from both populations thermoregulated efficiently, maintaining body temperatures within their preferred temperature range throughout most of their activity cycle. Nevertheless, they differed in the thermal sensitivity of resting metabolic rate at cooler body temperatures and in morphology. Our results support the common assertion that thermoregulatory behavior may prevent divergence in traits like field-active body temperature, which are measured during periods of high environmental heterogeneity. Nevertheless, we show that other traits may be free to diverge if they are under selection during times when environments are homogenous. We argue that the importance of the Bogert Effect is critically dependent on the nature of environmental heterogeneity and will therefore be relevant to some traits and irrelevant to others in many populations.

Keywords Adaptation · *Agama* · Behavioral thermoregulation · Metabolic rate · Water loss

Introduction

A classic question in evolutionary biology centers around the extent to which behavior may constrain trait divergence among populations (Duckworth 2009; Hardy 1965; Lande 1981; Logan et al. 2018; Mayr 1959; 1963; West-Eberhard 1989; Wong and Candolin 2015). Depending on the kinds of

traits examined and methods used, some taxonomic groups, such as birds (Lapiedra et al. 2013; Nicolakakis et al. 2003; Scott et al. 2003) and arthropods (Masta and Maddison 2002), show positive associations between behavioral plasticity and rates of speciation and diversification. Others, such as lizards (Bogert 1949; Buckley et al. 2015; Grigg and Buckley 2013; Huey et al. 2003; Rato and Carretero 2015), show niche conservatism. The latter phenomenon, where behavioral compensation for environmental heterogeneity reduces the number of environmental states experienced by the organism and buffers the population against selection, has been dubbed the “Bogert Effect” (Bogert 1949; Huey et al. 2003). Research on the Bogert Effect has focused on thermoregulatory behavior in terrestrial ectotherms, particularly lizards, as these systems offer especially clear examples of the potential for behavior to compensate for environmental variability (Basson and Clusella-Trullas 2015; Kearney et al. 2009; Kearney 2013).

A consistent result has emerged from studies of lizard biology: in species that display efficient thermoregulatory

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✉ Michael L. Logan
mike.logan1983@gmail.com

¹ University of Nevada, Reno, Reno, USA

² Smithsonian Tropical Research Institute, Panamá, Panamá

³ Department of Botany and Zoology, Centre of Excellence for Invasion Biology, Stellenbosch University, Stellenbosch, South Africa

behavior, divergence in thermal physiology across space is minimal (Bogert 1949; Buckley et al. 2015; Clusella-Trullas and Chown 2014; de la Vega-Perez et al. 2013; Grigg and Buckley 2013; Huey et al. 2003; Huey and Tewksbury 2009; Kearney et al. 2009; Logan et al. 2018; Rato and Carretero 2015). For traits like field-active body temperature, this result is intuitive, because individuals can achieve their preferred temperatures by choosing suitable microsites or changing their posture and orientation, even when the mean operative temperature of the habitat as a whole is suboptimal (Brewster and Beaupre 2019; Cox et al. 2018; Fey et al. 2019; Huey 1974; Huey and Slatkin 1976; Logan et al. 2015; Muth 1977).

However, for ectotherms to be able to use behavior to minimize the impact of regional thermal variation, there must be local spatial variation in temperature that can be exploited. In other words, there must be sufficient numbers of suitable microsites available at each locality (Basson et al. 2016; Huey and Slatkin 1976; Logan 2019; Logan et al. 2013; Sears et al. 2011). Indeed, studies on the Bogert Effect tend to focus on traits (e.g., field-active body temperature) that are relevant during periods (i.e., during the day time) when local spatial heterogeneity in operative temperature is high (Grigg and Buckley 2013). These traits are expected to be conserved, because behavior can be used to compensate for regional environmental variability. Conversely, few studies consider traits that may be under selection during periods of low spatial variation in temperature (Beaupre 1995; Christian et al. 2003; Gordon et al. 2010; Milsom et al. 2008; Reyes and Milsom 2010; Tan and Schwanz 2015). For example, resting metabolic rate (RMR) sets the energy requirements of individuals at night (or when they are sheltering in retreat sites). At these times, thermal heterogeneity is typically lower and behavior is likely to contribute much less to reducing the environmental variation experienced by individuals. For this reason, we hypothesize that traits which are relevant to organisms during periods of low spatial heterogeneity in the environment may diverge among populations despite similarities in field-active body temperatures.

We studied two populations of the southern rock agama (*Agama atra*) in South Africa. These populations are found on isolated rock outcrops that differ in climate, yet they are thought to be accurate thermoregulators with a conserved thermal physiology (Bruton 1977; Matthee and Flemming 2002; Swart et al. 2009). We estimated the extent to which this species compensates for environmental differences among sites using thermoregulatory behavior, and then compared morphology, the thermal sensitivity of RMR, and the thermal sensitivity of total water loss (TWL) rate between populations. We hypothesized that the thermal sensitivity of physiological functions at low body temperatures may diverge between populations, because they are likely under selection at night, when spatial thermal heterogeneity

and the opportunity for behavioral compensation are minimal. Finally, we hypothesized that morphology may differ between populations due to correlated responses to climate or other sources of selection such as predation and competition (Logan et al. 2012; Muñoz and Losos 2018).

Materials and methods

Study system

Southern rock agamas are diurnal, insectivorous lizards that occupy rock outcrops throughout Southern Africa (Bruton 1977; Matthee and Flemming 2002). Males defend territories from the top of preferred boulders, and during the spring, they develop bright blue heads which they bob to attract females and deter rival males (Bruton 1977). Breeding typically occurs in the spring, between August and October, although it has been reported that some populations undergo a second breeding pulse during the dry season, between February and March (van Wyk 1984; van Wyk and Ruddock 2001).

We studied males from two populations in the Western Cape Province of South Africa from September of 2015 through December of 2016. One population was from a rock outcrop about 25 km east of the town of Touws River, on the interior (hereafter referred to as the “Touws River” population). The second population was from a rock outcrop about 10 km east of the town of Kleinmond, on the southern coast. Although these populations are separated by less than 150 km (a small fraction of the species’ total range), they experience different climates (Fig. 1). The Touws River population, living up on the central plateau, experiences hot summers and cool winters (environmental temperature is temporally variable), with low rainfall (South African Weather Service 88-year average = 230 mm/year). The Kleinmond population experiences cooler summers and warmer winters than Touws River, and environmental temperature is relatively temporally stable, likely because of its proximity to the ocean. Kleinmond also experiences higher rainfall (South African Weather Service 66-year average = 715 mm/year).

Environmental variation among sites

From October 2015 through December 2016, we deployed between 28 and 32 operative temperature models (OTMs) at each study site (following Logan et al. 2015, 2016). OTMs were built with type-M (thin-walled) copper tubing, painted to approximate the skin reflectance of a typical southern rock agama individual, and calibrated with a live individual to ensure accuracy (Fig. 1, electronic supplementary material). Data loggers (iButtons; Embedded

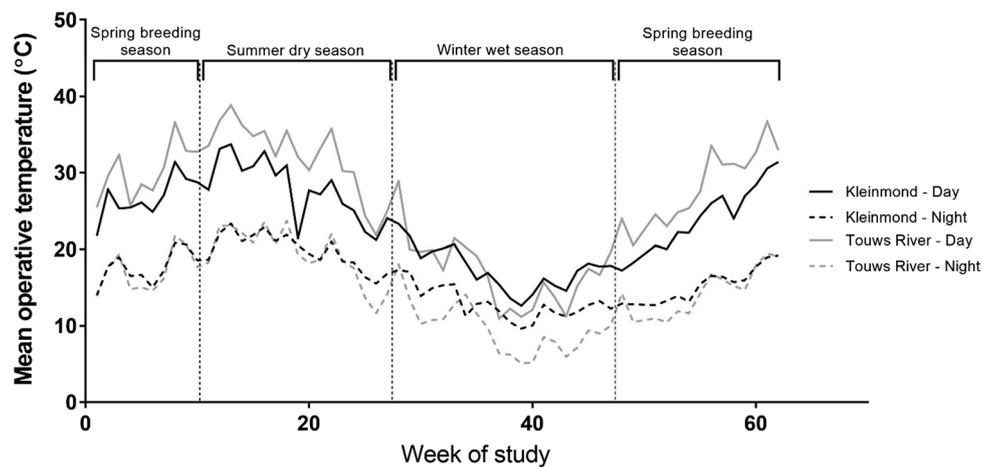


Fig. 1 Operative temperatures (weekly means) over the course of the study for Touws River (day time: gray, solid line; night time: gray, dashed line) and Kleinmond (day time: black, solid line; night time: black, dashed line). Operative temperatures are delineated by season. Daytime temperature values were averaged across all operative

temperature models between 0700 and 1900 h. Nighttime temperature values were averaged across all operative temperature models between 1900 h and 0700 h. The first week of the study corresponds to the week of October 1st, 2015. The last week of the study corresponds to the week of December 1st, 2016

Data Systems, Lawrenceburg, KY, USA) were set to record temperature every 70 min and were suspended inside each model with non-conductive acrylic mesh. We staggered the programmed start times of iButtons, such that multiple OTMs would record temperatures within any given half-hour period. Models were deployed at random heights (0–2 m at 0.5-m intervals) above the ground and random distances (0–5 m at 1-m intervals) and directions (north, south, east, or west) from haphazardly chosen locations at each study site with the goal of covering as much of each site as possible. We retrieved all OTMs every three months, downloaded the data, and redeployed them at a new set of random locations. Our operative temperature (T_e) data set consisted of approximately 400,000 individual temperature measurements across both sites. We compared differences in daily mean operative temperature between sites using ANOVA and Levene's test for differences in variances. We examined differences in thermal heterogeneity between diurnal and nocturnal periods at each study site using Levene's test for differences in the variances of all measurements collected between 0700 h and 1900 h and 1900 h and 0700 h, respectively, during the spring breeding season of 2016. For a comparison that strictly tests the hypothesis that nocturnal operative temperatures are spatially homogenous relative to diurnal operative temperatures, we also compared the variance among OTM measurements between the hours of 2400 and 0100 to the variance among measurements between the hours of 1200 and 1300 during a typical day (sunny, no precipitation) for the 2016 breeding season. All statistical analyses were conducted in SYSTAT.

Thermoregulatory behavior

Concurrent with operative temperature measurements taken during the spring breeding seasons of 2015 and 2016, we measured field-active body temperatures (T_b) of male lizards at both sites. Lizards were captured using a slip noose and immediately probed with a cloacal thermometer. Body temperatures measured from lizards removed from crevices or lizards that had been chased for more than 1 min prior to capture were not included in the final data set. We compared thermoregulatory behavior between sites by first contrasting the mean and variance of field-active body temperature for each population using ANOVA and Levene's test, respectively. Next, we plotted individual lizard T_b s as a function of the mean T_e across all available microsites (averages of all OTM measurements) at the time of capture. A thermoconforming population is represented by a strong correlation between T_b and T_e . Thus, we take the deviation of this relationship from 1:1 and the extent to which T_b overlaps with the preferred temperature range of *A. atra* [measured in a laboratory thermal gradient for a third population (S31°46.614'; E18°46.447') as part of a previous study; van Berkel and Clusella-Trullas 2018)] to approximate the accuracy of behavioral thermoregulation in each population (Angilletta 2009; Hertz et al. 1993). This approach is essentially a visual representation of the model presented by Hertz et al. (1993).

Metabolic and water loss rates

From October 2015 through January 2016, we captured about 40 male *Agama atra* from each site ($N=86$) and brought them back to our laboratory at Stellenbosch University. We only captured males, because this species is sexually dimorphic, such that our statistical power would be reduced if we had considered both males and females (Logan et al. 2014). Individuals were maintained in small plastic terraria placed inside incubators set to a 12-h day/night cycle (UVB lighting during the day), with a ramping temperature cycle that ranged from 22 °C at night to 30 °C at mid-day. Lizards were never kept in captivity for longer than 72 h. They were not fed but were given water *ad libitum*.

After a 24-h acclimation period to laboratory conditions, we simultaneously measured RMRs (VCO_2 production) and TWL at each of three temperatures (14, 24, and 34 °C) using a calibrated LI-COR infra-red analyzer (Li-7000, LI-COR, Lincoln, NE) set in gas flow-through configuration. The gas analyzer was connected to a Sable Systems eight channel multiplexer placed inside of an incubator (MIR-153, Sanyo Electric). Depending on the number of lizards caught in the field, they were measured in batches of two to six individuals, and were prevented from seeing each other with cardboard dividers. One cuvette was always left open (no lizard) for baselining (a minimum of 1 h of measurements on an empty cuvette were used as baseline measurements for each batch of lizards at each experimental temperature). Lizards were allowed to equilibrate to the target temperature (and habituate to the cuvette) for a minimum of 1.5 h prior to the onset of measurements. The order of temperatures was randomized for each batch of lizards. The light was turned off inside of the incubator to mimic the lighting experienced inside of the crevices occupied by *A. atra* in the field when they are inactive. Air (scrubbed of CO_2 and water vapor with soda lime and silica gel, respectively) flowed through the system at a rate of 300–400 ml/min. At these flow rates, most measurements were stable after 10 min, though RMR and TWL rates were measured for each lizard for a minimum of 30 min at each experimental temperature.

We examined VCO_2 and VH_2O traces using Sable Systems Expedata software. We only included estimates of mean RMR and mean TWL for a particular lizard if we could isolate > 5 min of stable VCO_2 measurements (i.e., when the lizards did not appear active). We were able to include the average of 15–30 min of stable measurements for the majority of individuals. After log-transforming the metabolism and water loss means for normality, we compared pairwise rates between populations at each experimental temperature using ANCOVAs with log-transformed mass as a covariate. We also compared the thermal sensitivity of each trait by calculating the slope and temperature coefficient (Q_{10}) for each individual in two segments: between 14 and 24 °C

(more relevant to nocturnal conditions) and then between 24 and 34 °C (more relevant to diurnal conditions). Hereafter, we refer to these as the “lower” and “upper” slopes, and “lower” and “upper” Q_{10} s, respectively. We calculated Q_{10} using the following equation:

$$Q_{10} = (R2/R1)^{10/(T2-T1)}, \quad (1)$$

where $R1$ and $R2$ are either VCO_2 or VH_2O measured at the lower and higher temperatures in each segment, respectively, and $T1$ and $T2$ are those temperatures. Differences between log-transformed slopes and log-transformed Q_{10} s were analyzed with ANCOVAs (log-transformed mass as a covariate). Parametric analyses were performed in SYSTAT. Finally, we also compared VCO_2 and VH_2O between populations and across all temperatures in a single statistical model for each trait. We used restricted maximum-likelihood (REML) linear mixed-effects models with site, mass, temperature, and site \times temperature as fixed effects, and individual as a random effect. Mixed-model analyses were performed in JMP (SAS Institute, Cary, North Carolina, USA).

Morphology

We measured a suite of morphological traits, including snout–vent–length (SVL), mass, tail length, hind limb length, forelimb length, head width, head depth, and scale density. SVL, tail length, limb lengths, and head dimensions were measured using a set of digital calipers (NEIKO model 01408A, resolution = 0.01 mm). Mass was measured using a digital balance (Mettler Toledo AX504; resolution = 0.001 g). Scale density was estimated with photographs taken at 30x magnification using a Leica DFC320 digital camera attached to a Leica M125 dissecting microscope (Leica Application Suite V4.4 software). Photographs were of the midpoint of the dorsal surface of each individual, and from these, we counted the number of scales within a 0.6 mm² area using ImageJ image analysis software. After log-transforming morphological variables and verifying that they met the assumptions of parametric statistics, we compared overall body size between sites by testing for differences in SVL with ANOVA. We compared other morphological variables (except scale density) between sites using ANCOVAs with SVL as a covariate.

Results

Environmental variation among sites

Temporal and spatial operative temperature distributions differed between the two study sites. Touws River was typically warmer and more thermally variable than Kleinmond (Figs. 2 and 3). When we pooled weekly means over the

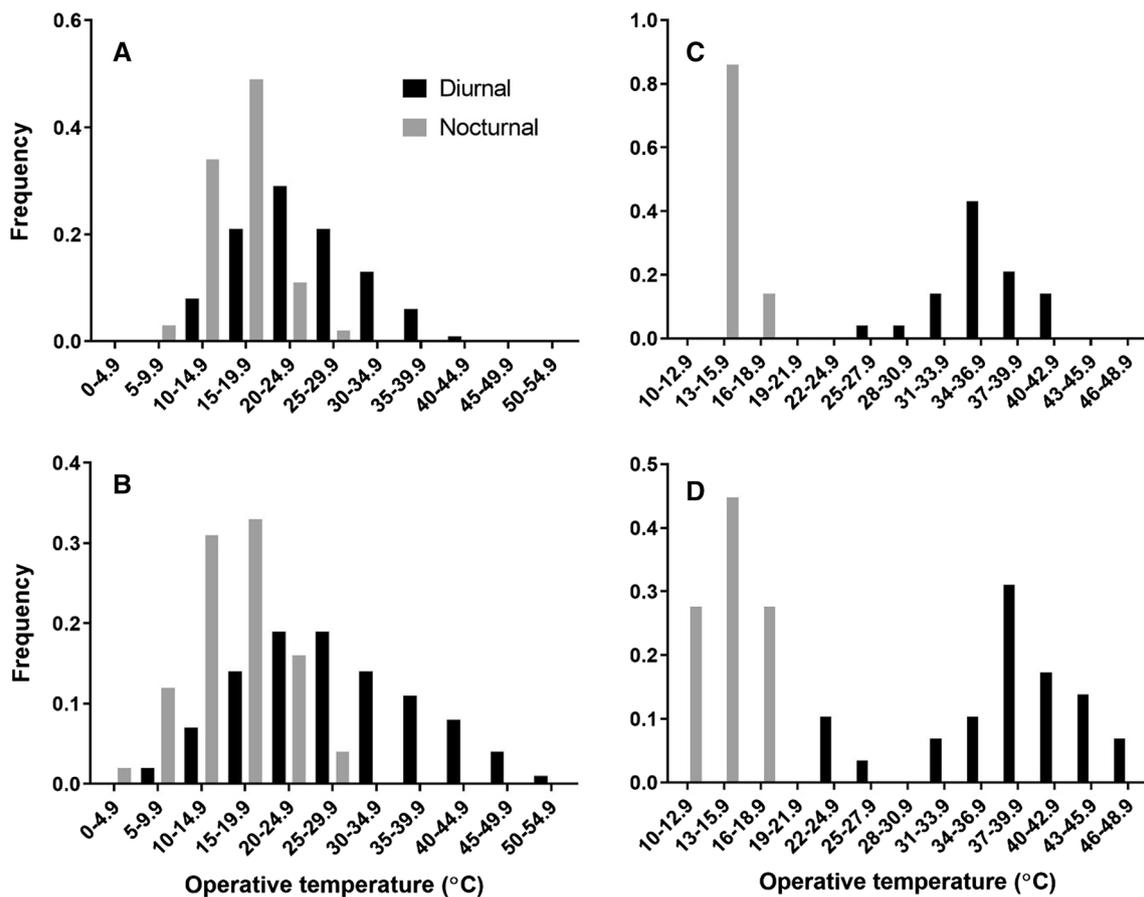


Fig. 2 A comparison of diurnal (black bars) and nocturnal (grey bars) thermal environments for the 2016 breeding season (September–December). When all measurements recorded by all OTMs across the entire breeding season are pooled, the frequency distributions for diurnal operative temperatures are broader than nocturnal tempera-

tures at both Kleinmond (a) and Touws River (b). Similarly, OTM averages between the hours of 2400 and 0100 are much more homogenous compared to OTM averages between the hours of 1200 and 1300 on a typical day during the breeding season for both Kleinmond (c) and Touws River (d)

study period, daytime operative temperatures (averaged between 0700 and 1900 h) were higher at Touws River ($F_{1,118} = 4.312, P = 0.040$), whereas nighttime temperatures (averaged between 1900 and 0700 h) were similar between sites ($F_{1,120} = 3.560, P = 0.062$). Temporal variance in both diurnal and nocturnal operative temperatures were higher at Touws River (Levene’s test for differences in variance: $F_{1,118} = 7.854, P = 0.006$ and $F_{1,120} = 8.573, P = 0.004$, respectively). Separated by season, average diurnal operative temperature was consistently higher in Touws River during the warmer months of the year (October through May, which correspond to the spring breeding season and summer dry season), but then converged with Kleinmond during the colder months (June through September, which correspond to the winter wet season). Conversely, average nocturnal operative temperature was similar between sites during the spring breeding and summer dry seasons, but was consistently lower at Touws River during the winter wet season (Fig. 1). Spatial heterogeneity in operative temperature also

differed between sites, with broader frequency distributions for Touws River across all seasons (Fig. 2). Finally, diurnal operative temperature distributions were much more spatially heterogenous than nocturnal operative temperature distributions at both Kleinmond (Levene’s test for differences in variance: $F_{29969,25947} = 3.305; P < 0.001$) and Touws River ($F_{31348,26988} = 3.438; P < 0.001$; Fig. 2). During a typical breeding season day in mid-November, 2016, we found that thermal heterogeneity between the hours of 1200 and 1300 was more than tenfold greater than between the hours of 2400 and 0100 for both Kleinmond ($\sigma^2 = 12.19$ and 0.85 , respectively; $F_{27,27} = 14.404, P < 0.001$) and Touws River ($\sigma^2 = 45.61$ and 4.09 , respectively; $F_{28,28} = 11.158, P < 0.001$; Fig. 2).

Thermoregulatory behavior

Neither the mean (Touws River = $35.3\text{ }^\circ\text{C} \pm 0.3\text{ SEM}$; Kleinmond = $34.7\text{ }^\circ\text{C} \pm 0.4\text{ SEM}$; $F_{1,100} = 1.552, P = 0.216$) nor

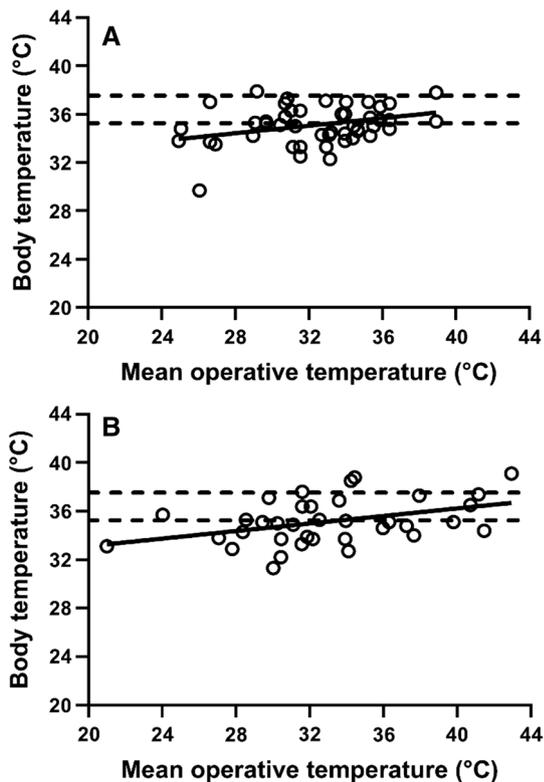


Fig. 3 Lizard field-active body temperature as a function of mean operative temperature (averaged across all OTMs within the hour that a given body temperature measurement was taken) for Kleinmond (a) and Touws River (b) populations. The preferred temperature range [estimated as the middle 50% of body temperatures chosen in a laboratory thermal gradient by 19 males from a separate, nearby population of *Agama atra*; van Berkel and Clusella-Trullas (2018)] is represented as the area between two horizontal, dashed lines in each panel

the variance (Touws River $\sigma^2 = 3.52$; Kleinmond $\sigma^2 = 4.01$; $F_{1,100} = 0.430$, $P = 0.514$) of field-active body temperatures (T_b) differed between sites. At both sites, lizards maintained T_b s very close to their preferred temperature (T_{pref}) range (mean $T_{pref} = 36.7 \text{ °C} \pm 0.1 \text{ SEM}$, 25th percentile = $36.0 \text{ °C} \pm 0.1 \text{ SEM}$, 75th percentile = $37.3 \text{ °C} \pm 0.1 \text{ SEM}$) over a wide range of T_e s (Fig. 3).

Metabolic and water loss rates

Resting metabolic rate (VCO_2) was greater for Touws River lizards at 24 °C ($F_{1,66} = 21.030$, $P < 0.001$), but did not differ between populations at 14 °C ($F_{1,71} = 2.580$, $P = 0.113$) or 34 °C ($F_{1,71} = 1.827$, $P = 0.181$; Fig. 4). TWL (VH_2O) did not differ between populations at any temperature (14 °C: $F_{1,73} < 0.001$, $P = 0.993$; 24 °C: $F_{1,66} = 3.616$, $P = 0.062$; 34 °C: $F_{1,74} = 0.422$, $P = 0.518$). The lower slope for RMR (slope between 14 and 24 °C) was greater for Touws River ($F_{1,61} = 25.103$, $P < 0.001$), whereas the upper slope (slope between 24 and 34 °C) did

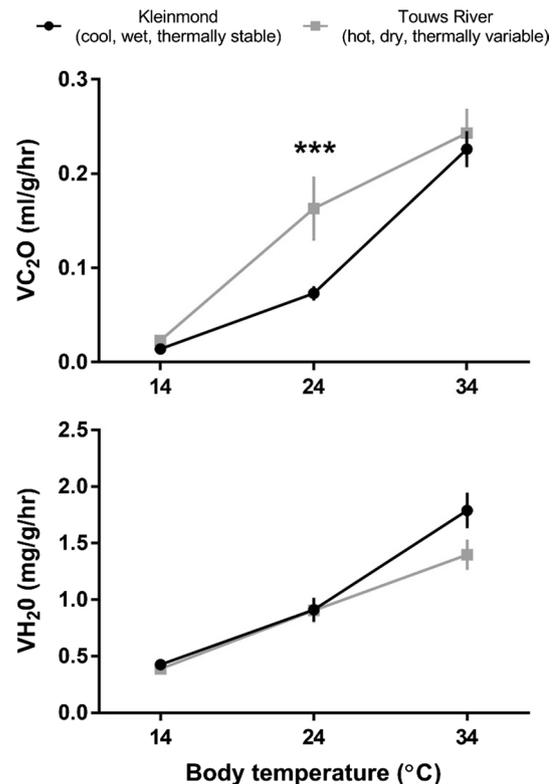
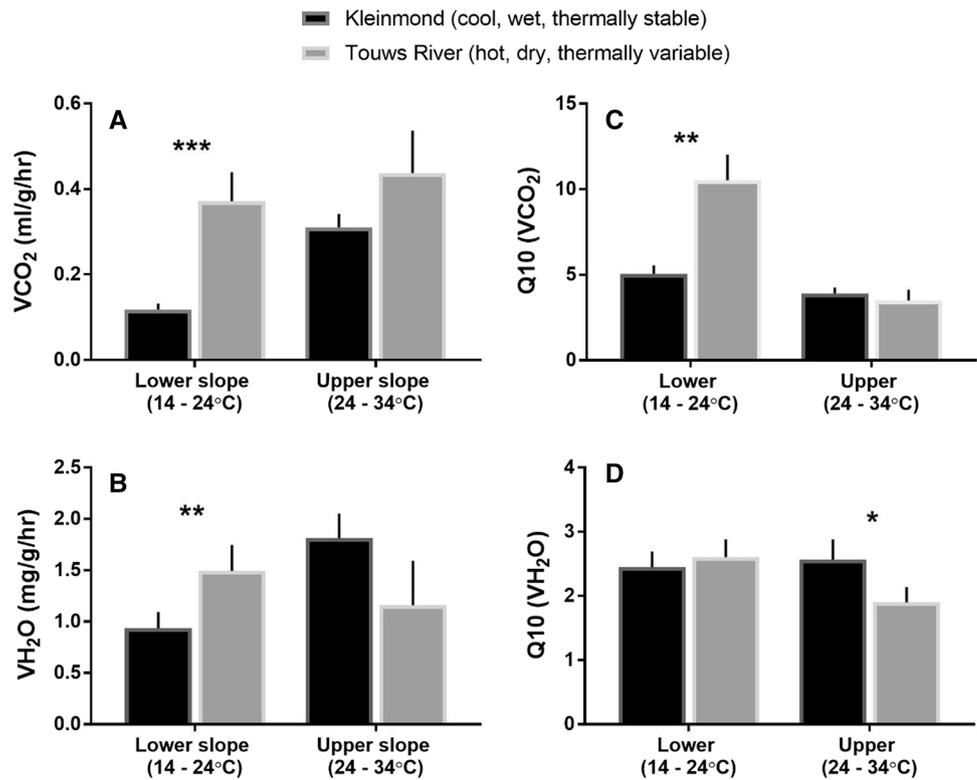


Fig. 4 Resting metabolic rate (top panel) and total water loss rate (bottom panel) at three body temperatures for male lizards from Kleinmond (black line) and Touws River (gray line). Error bars represent standard errors. Three asterisks denote significance to the ten thousandths decimal place ($P \leq 0.0009$)

not differ between populations ($F_{1,56} = 0.569$, $P = 0.454$; Fig. 5). The lower slope for TWL was greater for Touws River ($F_{1,56} = 7.350$, $P = 0.009$), whereas the upper slope did not differ between sites ($F_{1,61} = 5.965$, $P = 0.747$; Fig. 5). The lower Q_{10} for RMR was greater for Touws River ($F_{1,60} = 11.131$, $P < 0.001$), whereas the upper Q_{10} did not differ between sites ($F_{1,59} = 3.843$, $P = 0.055$; Fig. 5). The upper Q_{10} for TWL was higher for Kleinmond ($F_{1,62} = 4.740$, $P = 0.033$), whereas the lower Q_{10} did not differ between sites ($F_{1,63} = 0.248$, $P = 0.620$; Fig. 5). For VCO_2 , linear mixed-model testing for differences between populations (across all temperatures) revealed significant effects of site (greater VCO_2 in the Touws River population; $F_{1,56} = 17.522$, $P < 0.001$), temperature (VCO_2 increases with body temperature in both populations; $F_{2,128} = 294.237$, $P < 0.001$), and temperature \times site (VCO_2 increases faster with body temperature in the Touws River population; $F_{2,128} = 3.467$, $P = 0.034$). For VH_2O , linear mixed-model testing for differences between populations (across all temperatures) revealed significant effects of mass (VH_2O increases with mass in both populations; $F_{1,87} = 8.229$, $P = 0.005$) and temperature (VH_2O increases

Fig. 5 Comparisons of lower (14–24 °C) and upper (24–34 °C) slopes (a, b), and lower and upper Q10s (c, d), for both resting metabolic rate (a, c) and total water loss rate (b, d) between populations (Kleinmond = black bars; Touws River = gray bars). Error bars represent standard errors. One, two, and three asterisks denote significance to the hundredth ($P < 0.05$), thousandth ($P \leq 0.009$), and ten thousandths ($P \leq 0.0009$) decimal places, respectively



with temperature in both populations; $F_{2,145} = 202.375$, $P < 0.001$).

Morphology

Male *Agama atra* from Touws River were larger in body size than those from Kleinmond (populations differed in SVL; $F_{1,85} = 69.134$, $P < 0.001$). After accounting for body size, Touws River lizards had less dorsoventrally flattened heads ($F_{1,83} = 18.215$, $P < 0.001$), longer hind limbs ($F_{1,83} = 35.169$, $P < 0.001$), and longer forelimbs ($F_{1,83} = 27.965$, $P < 0.001$), but mass ($F_{1,83} = 0.265$, $P = 0.860$), head width ($F_{1,83} = 0.203$, $P = 0.716$), and tail length ($F_{1,83} = 2.666$, $P = 0.106$) did not differ between populations. Finally, Touws River lizards had lower scale densities ($F_{1,83} = 50.544$, $P < 0.001$; raw means and standard errors are presented in Table 1).

Discussion

Terrestrial ectotherms, especially lizards, have been used many times to illustrate the power of behavior to constrain phenotypic divergence—the so-called “Bogert Effect” (Bogert 1949; Grigg and Buckley 2013; Huey et al. 2003). When populations are distributed across space, such that local climatic regimes differ, individuals can maintain similar body temperatures by actively seeking out preferred microsites within the larger available habitat, or by

Table 1 A comparison of morphological traits between our two study populations (males only, raw means ± standard errors)

	Kleinmond (mean ± Std. Err.)	Touws River (mean ± Std. Err.)
Snout–vent–length (mm)	81.31 ± 0.71	94.24 ± 1.42
Mass (g)	20.03 ± 0.49	31.63 ± 1.43
Tail length (mm)	102.30 ± 1.55	115.72 ± 2.31
Hind limb length (mm)	40.80 ± 0.35	48.00 ± 0.56
Forelimb length (mm)	32.08 ± 0.28	37.44 ± 0.42
Head width (mm)	17.50 ± 0.16	19.85 ± 0.30
Head depth (mm)	10.48 ± 0.11	11.30 ± 0.19
Scale density (#/0.6 cm²)	79.81 ± 1.91	61.21 ± 1.87

Bold variables were significantly different between populations. Snout–vent–length (SVL) and scale densities were compared using ANOVA, whereas all other variables were log-transformed and compared using ANCOVA with SVL as a covariate to account for the effects of body size. Relative to body size, hind limb length, forelimb length, and head depth were all larger in males from Kleinmond

remaining active at selected times of the day (Gunderson and Leal 2016; Huey 1974; Huey and Slatkin 1976; Logan et al. 2014, 2015). Indeed, we found that body temperatures were similar between two populations of southern rock agamas living under different climatic regimes. Although daytime operative temperatures were significantly warmer and more variable at Touws River (Figs. 2 and 3), lizards were able to maintain body temperatures very close to their preferred

temperature across a nearly 25 °C range at both sites (note that we did not measure preferred temperature for our focal populations and assumed that they were similar to a third population not included in this study; Fig. 3). This suggests that individuals are accurate behavioral thermoregulators during daylight hours, and that there is a potential role for the Bogert Effect on the evolution of body temperature in this species.

Nonetheless, we found that the thermal sensitivity of RMR diverged between our study populations. Mixed-models including estimates of RMR across our full range of experimental temperatures revealed that RMR increases more rapidly with body temperature in Touws River lizards. Nevertheless, this pattern was likely driven by the higher thermal sensitivity of RMR in Touws River lizards at body temperatures typical of times of the day when thermal heterogeneity is relatively low. For example, when we analyzed the thermal sensitivity of RMR separately for diurnal versus nocturnal body temperatures, we found no differences between populations in the thermal sensitivity of RMR at the body temperatures (between 24 and 34 °C) most likely experienced by individuals during the daytime (Fig. 5). The spatial heterogeneity of the operative thermal environment was lower at night and nocturnal operative temperatures differed between sites. This was especially true during the winter wet season, where weekly temperature averages were regularly 5 °C cooler at Touws River, and more temporally variable, than they were at Kleinmond (Fig. 1). Because of a lack of solar radiation, operative thermal environments are spatially homogenous at night, suggesting that nocturnal thermoregulation is prohibitively costly (Fig. 2). Moreover, diurnal species are likely to be relatively inactive at night, further reducing the opportunity for behavioral buffering of thermal variation. Differences between populations in the thermal sensitivity of RMR, which were only apparent at the body temperatures experienced during nocturnal and early morning/late evening hours (between 14 and 24 °C), were likely due to the differences in nocturnal operative temperatures between sites.

At cooler temperatures (between 14 and 24 °C), metabolic rates, and therefore energetic demands, increase more rapidly with a given increase in body temperature for lizards from Touws River. Why these differences have arisen is unclear. It is possible that selection has favored higher thermal sensitivity of metabolic rate in the Touws River population, because a more rapid increase in energy metabolism with temperature confers an ecological advantage. For example, a higher thermal sensitivity of metabolic rate could increase an organism's capacity to escape predators who become more active during warm nights (Brokordt et al. 2006; Fu et al. 2015; Rawlings 1994), or increase competitive abilities early in the morning. These differences may have evolved or may be the result of plastic responses to

environmental variation (adaptive or non-adaptive) (Le Lann et al. 2011; Norin et al. 2016). Regardless, it is evident that the energetic physiology of these populations has diverged, and these differences likely have ecological consequences (Burton et al. 2011; Watson and Burggren 2016).

In contrast to the pattern which we observed for RMR, the thermal sensitivity of TWL did not differ between populations when all experimental temperatures were included in a single analysis. However, when water loss rates were analyzed separately for body temperatures that are typical of diurnal versus nocturnal hours, the populations differed at both warmer and cooler body temperatures. In general, the thermal sensitivity of water loss was low for the Kleinmond population at temperatures below 24 °C and either increased (slope) or stayed the same (Q_{10}) for temperatures above 24 °C (Fig. 5). By contrast, the thermal sensitivity of water loss for the Touws River population was relatively high at temperatures below 24 °C and decreased at temperatures above 24 °C. In other words, as lizards from the wetter environment of Kleinmond warm up during the day, their water loss also increases, whereas water loss actually decreases per unit increase in temperature during daytime conditions in Touws River lizards. Taken together, our results suggest that lizards from Touws River compensate (possibly through physiological mechanisms or differences in morphology) for changes in body temperature that would otherwise result in unsustainable increases in water loss during the hot, dry days they experience.

Our study populations differed in many of the morphological variables which we considered. Male *A. atra* from Touws River were about 50% larger in mass and 20% larger in SVL than males from Kleinmond (Table 1). Scale density was lower for the Touws River population, and scale density positively correlates with cutaneous water loss rates in many lizard species (Calsbeek et al. 2006; Soulé and Kerfoot 1972; Malhotra and Thorpe 1991; Wegener et al. 2014). Combined with lower thermal sensitivity of water loss rates at warmer body temperatures (see above), lower scale densities of Touws River lizards strongly suggest that this population is adapted to a particularly arid environment. When we accounted for body size, head depth, hind limb length, and forelimb length differed between populations. Head dimensions and limb lengths are related to performance traits like bite force and locomotor performance (Husak et al. 2006; Miles 2004; Sagonas et al. 2014; Wittorski et al. 2016), evidence that ecological variables like predation and competition may differ between localities. Thus, these traits may have diverged due to alternative selection pressures. Moreover, as Muñoz et al. (2014) and Muñoz and Losos (2018) pointed out, evolutionary stasis in physiology can lead to evolutionary lability in morphology. In other words, precise thermoregulation in differing operative environments

may require shifts in habitat use (or posture) that select for alternative morphologies. Differences in morphology between our study populations may be a result of any number of selective forces, including competition, predation, or even thermoregulatory behavior itself. Differences may also be a result of phenotypic plasticity, as environmental differences between sites can give rise to plastic shifts in traits that are frequently (but not always) adaptive (Losos 2009; Losos et al. 2000).

What is the role of behavior in phenotypic divergence? Studies on lizard thermoregulation have been used to argue that behavior dampens trait divergence by reducing the number of environmental states experienced by allopatric populations. However, the majority of lizard populations experience periods of time (mainly at night) when local thermal landscapes are spatially homogenous, and thermoregulation becomes prohibitive. Traits like RMR, which may disproportionately impact fitness during these times, are then free to diverge. Indeed, a recent meta-analysis showed that metabolic rates often diverge between reptile populations (and between seasons within populations) but only at body temperatures that are below those that are typical of activity (Berg et al. 2017). Similarly, Muñoz et al. (2014) and Muñoz and Bodensteiner (2019) showed increased divergence in traits that are more likely to be under selection at night. Likewise, we showed here that populations of agamid lizards in southern Africa differ in the thermal sensitivity of metabolic and water loss rates despite having similar field-active body temperatures. The potency of the Bogert Effect is critically dependent on the relationship between fitness-related traits and environmental heterogeneity.

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Author contributions statement MLL and SCT designed the study. MLL and JVB collected the data. MLL and SCT analyzed the data. MLL, SCT, and JVB wrote the paper.

Data availability All data supporting this study are archived in the Dryad Digital Repository: <https://doi.org/10.5061/dryad.5mkkwh71p>.

Compliance ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Statement of human and animal rights All applicable institutional and/or national guidelines for the care and use of animals were followed. All research was carried out under Stellenbosch University animal ethics protocol no. SU-ACUD14-00110 and under CapeNature permit no. 0056-AAA007-00156.

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