Experimental Thermoregulation in the Wedge-Snouted Lizard
(*Meroles cuneirostris*)

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ABSTRACT

Although the core temperature of lizards can vary with, for example, species, age, size, gender and season, they, in fact, maintain core temperature within an effective and safe range for biochemical and CNS processes. Desert dwelling lizards have evolved behaviors that minimize contact with dangerously hot sand, and to regulate the heat flow between the environment and their bodies. Shuttling between sun and shade, and digging are effective, but in certain situations it is disadvantageous to move, and instead, modification of posture *in situ* is employed. The postures include thermo*protective* thermoregulation, as when a lizard lifts feet and/or legs and tail off the surface to avoid getting feet burnt, and thermo*regulation* when it stands on all legs with the torso lifted high off the substrate to minimize heat flow from the substrate. These behaviors can at times conflict, as when it needs to support itself on the hot sand, while lifting the body to defend core temperature. Resolution of this conflict often is with a "thermal dance". To analyze this "thermal dance" of the Wedge-snouted lizard we experimentally investigated the core temperature at which the transition from thermoprotection to thermoregulation occurred. To quantitatively assess the behaviors, numerical score systems were developed for thermoprotection, P0-4 and thermoregulation, R1-3. During the southern hemisphere summer of 2011, two female lizards, weighing 4 g and 9 g, were captured and implanted with temperature transponders. Computerized control of the substrate temperature and simultaneous records of core temperature and videos of behavior were accomplished using an inexpensive solar-powered portable field laboratory. The system enabled arbitrary and rapid changes in substrate temperature, which allowed us to separate time from core temperature as the controlling variable of the behavior.

Our preliminary results show that there was a specific core temperature at which either of the lizards adopted thermoregulatory posture R3, >39.5°C and >41.5°C for the lizard with the higher and lower weight, respectively. The slopes of core temperature rise during the test program were similar for both lizards, thus the size difference did not affect the independent variable. Preconditioning with a higher initial substrate temperature effectively shifted the distribution of core temperatures during test. The simultaneous recording of core temperature and behavior without manually manipulating the lizard provides many opportunities for exploring behavioral thermoregulation. This compact and inexpensive laboratory setup that was developed is appropriate for student work in places with limited resources.

AIM OF STUDY

In the Wedge-snouted lizard, to

(1) experimentally determine the transition core temperature for a change in behavior from the thermo*protective* "thermal dance" to thermo*regulatory* "push-up";

(2) determine thermal mass' effect on core heating.

INTRODUCTION

Although previously thought of as "cold blooded" with a stereotyped "reflexive" behavior, reptiles are now acknowledged to have regulated body temperatures; substantial behavioral flexibility, and at least some amount of cognitive function. For example, lizards can rapidly learn to reveal a food item by dragging or knocking the correct colored cap (Leal & Powell 2011). Reptile biologists and ecologists have studied thermoregulatory behavior, in both field and experimental laboratory conditions, using infrared thermometers, thermocouples, or telemetry to measure body temperature in a variety of natural and artificial environments (Christian *et al.* 1985, Gvoñdík 2002, Herczeg *et al.* 2006). Analyses of costs and benefits of thermoregulation during different environmental and climate situations have been conducted using laboratory and field data, along with mathematical or physical models of the determinants of core temperature (Christian *et al.* 1997, Christian *et al.* 2006, Herczeg *et al.* 2006, Huey & Slatkin 1976, O'Connor 1999, Shine & Kearney 2001). One of the first and most extensive field studies of thermoregulatory behavior, which related core temperature to posture in a number of different species of snakes and lizards, was Cowles and Bogert (1944). Among the species that they studied in extreme hot and dry regions, were heliotherms (animals that use the sun to warm the body). They measured the rectal temperature of Chuckwalla lizards (*Sauromalus obesus*) and sidewinder rattle snakes (*Crotalus cerastes)*, and simultaneously recorded their body position; thus, showing that these reptiles accurately controlled their core temperature by adjusting their body position relative to the sun. Figure 1, a photo taken near our laboratory in the Namib Desert, shows a heliothermic skink precisely orienting its body to the sun for optimal heat absorption.

 A. Behavior, biology and neurophysiology of thermoregulation

At rest, endothermic animals produce sufficient metabolic heat to maintain euthermia; whereas, for ectotherms, endogenous heat production, which is 5 to 10 times lower, is insufficient to significantly influence core temperature (Seebacher 2009). As metabolism, and consequently all body functions are temperature dependent, ectotherms have evolved, in addition to direct physiological processes, behavioral regulatory mechanisms to adjust core temperature to the set-point. Because behavioral mechanisms can derive energy directly from the environment, ectotherms may need less nutritional caloric intake for effective thermoregulation. The core temperature set-point is not always constant; for endotherms, ovulation and fever are obvious examples of shifts in set-point. For ectotherms,

phenotypic plasticity has evolved to reduce cost of thermogenesis in highly variable ambient temperatures (Glanville & Seebacher 2006); thus, the core temperature may differ substantially within a population, as a function of age, gender, season, time of day, or altitude (Diaz & Cabezas-Diaz 2004, Huey & Pianka 1977, Huey & Pianka 2007, Mathies & Andrews 1997, Seebacher 2005, Seebacher & Franklin 2004).

Heat transfer between the skin and substrate can be modulated by heart rate hysteresis: This vascular mechanism increases pulse rate during heating and augments peripheral perfusion; consequently, diurnal species can be active for a longer time by selectively absorbing heat faster than they loose it (Grigg & Seebacher 1999, Seebacher & Franklin 2001). In the Bearded Dragon (*Pogona vitticeps*) this mechanism is mediated by prostaglandins (Seebacher & Franklin 2003). Other peripheral vascular mechanisms include blood pressure changes via increased release of prostaglandin and/or nitric oxide, and near the preferred body temperature, thyroid hormones also have thermal effects similar to those in endotherms, increasing oxygen consumption and activity (Kardong 1998, Seebacher 2005, Seebacher & Franklin 2005). Thus, autonomic physiological responses may be more important for thermoregulation in many ectotherms than originally believed. The hypothalamus, and the pineal gland, may act as thermostats and melatonin may mediate changes in the thermoregulatory behavior in the circadian rhythms of reptiles (Lutterschmidt *et al.* 2003, Seebacher & Franklin 2003, Tosini 1997). In ectotherms, blood infections or extensive exercises can produce hypoxia, which reduces metabolism; slows movement, and affects the CNS set-point, all of which in turn reduce the precision of behavioral thermoregulation; for example, by impairing the selection of favorable spots for warming and cooling (Cadena & Tattersall 2009, Seebacher & Franklin 2005). In desert lizards, respiratory cooling via panting or gaping is "expensive", but it is, nonetheless, engaged at high core and/or brain temperatures (Crawford & Barber 1974, Crawford *et al.* 1977, Tattersall *et al.* 2006).s

Correspondingly, although shivering is not a common reptilian thermoregulatory mechanism, it does occur, for example, heat production by muscle shivering has been reported for egg brooding female pythons (Aubret *et al.* 2005). It is reasonable to believe that, given these examples, as neuronal and metabolic pathways are conserved among vertebrates, the differences in thermogenesis between ectotherms and endotherms maybe more quantitative than qualitative.

In contrast to thermo*regulation*, environmental temperature challenges can be met by thermo*adaptation* via elevated expression of heat shock proteins and changes in the composition of membrane lipids that sustain fluidity at lower body temperatures (Horowitz 2001, Seebacher 2005, Seebacher & Franklin 2005).

For most vertebrates, the sensory physiology and anatomy of thermoregulation is quite similar. Whether in endotherms or ectotherms, the neuronal mechanisms include peripheral heat sensors that transmit information to the CNS. Throughout the animal kingdom, from the fruit fly to mammals, different classes of thermoreceptors have evolved to respond to environmental temperatures which are either harmless or harmfully cold or hot (Caterina 2007, McKemy 2007). These neurons, which fire in either finely graded, or nearly all-or-nothing patterns, respectively, project to the hypothalamus, which is the central coordinating area for thermoregulation in vertebrates (Liu *et al.* 2006, Seebacher 2009). The transient receptor potential (TRP) ion channels have been studied mainly in mammals (Bevan 2006), but results from sequencing support homologs in other vertebrates as well as in invertebrates (Seebacher 2009, Seebacher & Murray 2007). They are expressed in somatosensory neurons in the dorsal root ganglia and in peripheral tissues and some act also as temperature sensors with a range of thresholds (Bevan 2006, Clapham 2003, Voets *et al.* 2004). By interactions with other membrane proteins they are involved in expression, stability and signaling activity of the ion channels. The vanilloid TRPV1 in heat sensitive neurons (>40°C) have been sequenced in a few reptiles species, and the melastatin TRPM8, which react to cold (<20°C), only in crocodile (Seebacher & Murray 2007).

B. Choice of experimental species and preliminary/introductory studies
The Wedge-snouted (*Meroles cuneirostris*) and Shovel-snouted lizards (*Meroles anchietae*) are small ectotherms (snout to vent 45 - 55 mm), which belong to the *Lacertidae*, a family of Old World lizards in the deserts of southern Africa. These species are endemic to the Namib Desert, and encounter both very high and low temperatures within their habitats; the sand dune substrate temperatures can reach 70°C. The specific environment of the Shovel-snouted lizard is sparsely vegetated sand dune slopes, where it lives on a mixed diet of plant and animal debris or live insects. The Wedge-snouted lizard occupies more vegetated dunes (Fig. 2) and is almost exclusively a predator of insects and beetles. Both species dive into the sand for cooling or predation protection, but the habitat of the Wedge-snouted lizard presents more opportunities to find relief from the solar heat. The lizards

also dig into the sand at night to maintain their core temperatures above that of the nighttime substrate. The Shovel-snouted lizard is notable for performing its "thermal dance", a behavior that prevents core temperature from rising to a harmful level and at the same time periodically interrupts contact between the feet and the hot sand (Lovegrove 1993). To our knowledge this behavior has not been previously described in the Wedge-snouted lizard; however, because lizards performing the thermal dance are immediately assumed to be Shovel-snouted, the two species are sometimes confused, even by experts. Although both species of lizards can very rapidly dive into the sand for cooling, conflicts can occur; for example, a lizard might need to remain unprotected from the heat of the sun in order to catch a prey; correspondingly, it might need to remain in shade and suffer a fall in core temperature in order to avoid predation.

During previous laboratory experiments 2009 in NamibRand Nature Reserve in Namibia on the Wedge-snouted lizard, we observed a change in patterns of thermal behaviors as ambient temperature in the experimental laboratory increased with the onset of the southern spring. When challenged with a hot substrate, the lizard's behavior switched from sitting on its hips and lifting only the tail and/or one leg to a more typical "thermal dance", which involved standing away from the substrate on all four limbs. The following year, using lizards of different sizes, we also observed that a smaller lizard reacted much faster to the hot surface in the experimental box than larger lizards with a greater thermal mass. These observations, led us to hypothesize that core temperature was responsible for the changed behavior pattern. However, in the test situation core temperature and time in the test were confounded; thus, this past year we designed experiments to specifically separate these variables.

At present we have not found any publications describing simultaneously recording core temperature via an internal transponder and detailed thermoregulatory and thermoprotective behaviors of a small desert lizard.

MATERIAL AND METHODS

A. Computer system and recording

An Acer 5620 laptop computer with a secondary monitor was connected to an Omega CN63100-R1-R2-F3-RSP temperature controller with an OS36-J-140f IR Surface Temperature Sensor; an 8-channel Measurement Computing USB-1408FS analog-digital interface, and a Logitech HD QuickCam Vision Pro webcam. The two screens enabled simultaneous viewing of the surface and core temperatures versus time graphs, and large high resolution video image of the lizard. The temperature sequences were generated, and video and temperature data were acquired by a custom program written in the MATLAB/SIMULINK language. All data were combined into a primary AVI video document which consisted of a video image of the lizard and embedded numerical temperature values. This recording procedure insured reliable primary data integrity.

B. Experimental chamber and substrate

The observation chamber was a 0.5 mm acrylic box in the form of a truncated trapezoidal prism with a base 15 x 5 cm, and a height of 4.8 cm. The sloping sides of the trapezoidal box effectively discouraged the lizards from climbing up the walls, and also reduced surface reflections in the video. The webcam was placed 20 cm in front of the center of a long side of the chamber, and the opposite (back) wall was covered with an optical quality first-surface mirror. The camera was equipped with a +5 diopter accessory lens to shift the autofocus distance closer, and with proper adjustment, it was possible for the single camera to continuously image the four legs and tail of the lizard. The floor of the chamber was a strong, stiff 5 x 20 cm membrane, with an ultra-low thermal mass, consisting of a 0.0025 mm etched foil heating element encapsulated between two layers of 0.05 mm Kevlar Film. The membrane was supported on the long edges by two wooden rails, one with a gap that contained a 25 mm low voltage fan, which maintained a continuous airflow beneath the membrane. The surface temperature sensor, an optical thermocouple, was mounted immediately outside of the chamber 1 cm above, and aimed down at an external section of the Kevlar membrane; it sampled the substrate temperature (T_S) with zero thermal mass and without intrusion into the lizards' space. The controller was programmed with a self-tuning PID algorithm; the set-point was determined by the MATLAB program, and was applied to the external set-point input via an analog output port of the USB-1408FS interface. The surface temperature and set-point were continuously recorded

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during a test trial. The time constant of the controller-membrane system to a step change in temperature was approximately 3 s, which meant that, in the worst case, the stable target temperature was acquired in <10 s.

C. Core temperature measurements

An implantable passive transponder, IPTT-300 from BMDS, was used to measure the core temperature of the lizard during the experiments. The micro-transponder measured 1.5 x 10 mm and was located caudally in the body cavity (see surgical procedure). The probe for the transponder was placed under the floor of the chamber, and if necessary, moved by an experimenter to remain within the ~5 cm range of the transponder. The transponder readout device lacked an interface for direct connection to the computer, and thus, core temperature readings were taken manually at ~2 s intervals, and transferred to the computer via a keyboard input where they were automatically registered in the data stream. Temperature values rarely changed by >0.2°C between readings; thus, this procedure, although awkward, was sufficiently accurate. Figure 3 shows the complete setup for the experiments.

D. Test program

In preliminary experiments we established a time-temperature program that would shift the range of core temperatures (T_C) during a trial; the effectiveness of this manipulation was evaluated experimentally.

The lizard was pre-conditioned in the experimental box at either 35°C or 45°C for a maximum of 30 min. With each trial substrate (floor) temperature (T_S) was increased to 70°C for 50 s before being returned to the conditioning temperature. Data and full-motion video were recorded, from 30 s before to 30 s after, the 70°C "test" trial.

E. Animals

The study was carried out in accordance with recommendations by the laboratory animal veterinarian at Uppsala University and approved by the ministry of Environment and Tourism, Permit Number 1555/2010 and by NamibRand Nature Reserve, Permit Number NRNR/P/011/01. Using hand nets, volunteers at NaDEET center in the southern part of the NamibRand nature reserve captured three female Wedge-snouted lizards, *Meroles cuneirostris*. The lizards were kept in

separate 25 cm diameter plastic buckets. The buckets had mosquito net lids and the floors were covered with 5 to 15 cm of sand and dry grass tufts; a regularly moistened compressed cotton ball provided water. The buckets were kept indoors to reduce the risk of attracting snakes, and were moved into direct sunlight for part of each day. Before the formal experiments began the lizards were occasionally placed in the experimental chamber to accustom to handling and the test environment. The lizards were fed termites, when available, which are their preferred food; however, because of heavy rain, termites became unavailable approximately a month after the experiments began, and other insects, such as small moths and caterpillars were substituted. The alternate foods were consumed, but not in sufficient quantity to main full body weight.

F. Surgical procedure

On the day of surgery, the lizards weighed 4.0 (L3F), 7.0 g (L1F) and 9.1 g (L2F). L1F was used only to work out the trial schemes. Each lizard was placed in a pre cooled plastic box in a refrigerator at 4°C for 25 to 30 minutes before being positioned supine on a plastic film covered pre-cooled aluminum plate. To maintain hypothermia, a small trough of polyfoam was placed over lizard until the actual surgery began. A 2- 3 mm incision was made in the skin and attached membranes from a point ~2 mm caudal to the last rib. A BMDS transponder in a smooth tip trochar was inserted caudad and the transponder ejected into the body cavity; the incision was closed by a single 4-0 Vicryl suture. The entire surgery required one minute, after which the lizard was transferred to a plastic box at 25 - 30°C. In <5 minutes the core temperature increased to >25°C and normal movements and behavior resumed. The lizard was closely monitored for at least 30 minutes post surgery. At the conclusion of the experiment, an autopsy was performed and the transponder location photographed.

G. Statistics and scaling

The central hypothesis was that the transition from "P" postures or R1 to effective thermo-regulatory postures R2 and R3 depended on core temperature. Core temperature and time-in-the test-condition were confounded; thus, core temperature was manipulated independently by changing the pre-test substrate temperature. There are thus two questions; the second is dependent on the outcome of the first: (1) was the experimental manipulation effective; (2) did T_C influence the distribution of postures? The first question is answered by a two-sample test of significance: is there

a reliable difference in T_C between a pre-test T_S of 35°C or 45°C? The second question is addressed by one-way ANOVA, and by treating T_C and R as random variables and using regression methods.

Posture scaling: Thermo-protective postures that reduced contact between the feet and the substrate, which prevent the feet from being burned, were scored as P0-P4 (Table IA) modeled on Sartorius *et al.* (2002). The limbs can be withdrawn to different levels from the substrate, but these subtleties were ignored to simplify scoring. In general, scoring of thermo*protective* postures reliably is difficult, as the lizards react very quickly and inconsistently to the hot substrate. Thermo*regulatory* postures, which prevent T_C from rising to a dangerous level, were scored as R1 - R3 (Table IB, Fig. 4). In our study, tail movements and withdrawal of phalanges are scored only as thermoprotective, as the limbs and tail do not appear to have an important influence on thermal regulation (Dzialowski & O'connor 2004, Herczeg *et al.* 2004). Regulatory posture R1, R2 and R3 were modified from the work of Sartorius *et al.* (2002), who developed the scale during their study of thermoregulation in two species of the *Phrynosomatidae* lizard family (North American spiny lizards), approximately the same size snout to vent as the species used in our study.

RESULTS

A. Thermoprotective postures of limbs and tail

Thermoprotective postures were scored P0 to P4 and are shown in Table IA. In general, scoring of thermoprotective postures reliably was difficult, as the lizards react very quickly and inconsistently to the hot substrate: although the postures generally followed the substrate temperature, they also appeared to depend on accumulating time on the hot surface. However, with lower T_C at the start of the trial, the withdrawal of feet, or limbs and/or tail was delayed; in contrast, even at high core temperatures, the lizards could at times remain with all feet on the substrate, for example, when "freezing", or during R3, which interferes with the "P" postures (Fig. 4, R3P0).

No thermoprotective postures involving limbs or tail were observed at a T_S <50°C. Because of the shape of the experimental box, part of one hind foot sometimes rested on the wall at the onset of the trial, which possibly could have delayed or reduced the thermoprotective activity. No specific attention was given to the use of tail in thermoregulation because Herczeg *et al.* 2004 found that tail loss in small (4 - 5 g) lizards does not affect thermoregulation. Although the withdrawal of the tail and peripheral parts of the legs occurs as a response to heat pain sensors, the thermal mass and heat transfer characteristics of the tail, in proportion to the total body surface and weight, is apparently insignificant. At thermal regulatory posture R3 the lizard sometimes uses the tail for support, but presumably, this would only slightly compromise the insulation.

B. Core temperature effects on the behavioral transition to thermoregulatory postures R2 and R3.

The core temperature T_C was related to three distinguishable naturally occurring postures, which progressively increased the thermal isolation from the hot substrate: (R1) lying flat with ventral part of the torso on the surface, (R2) sitting on the hips and forefeet, and (R3) standing on all feet with the entire body lifted away from the substrate (Table IB). Initial observations led to the hypothesis that the T_C would influence the transition to greater isolation; however, because of the elevated substrate temperature, core temperature rises during the test; thus, the variables of accumulated time on the hot substrate, and core temperature, were confounded. To experimentally separate these variables, we manipulated the initial core temperature by using either a 35°C or 45°C as the initial, and between trials, substrate temperatures. A higher initial core temperature was expected to lead to higher core temperature at corresponding time points in the trial, and that is what we found

(Fig. 5). The difference between T_C for $T_S = 45°C$ and $35°C$ is significant showing that for both lizards T_S appropriately manipulated the T_C distribution. Unpaired, two-tail Student's "t": for LF2, [$\Delta = 4.72$, df = 1590, t = 57.3, P<0.0001]; for LF3, [$\Delta = 5.67$, df = 557, t = 33.6, P<0.0001]. Our expectation was that a transition to R3, would occur when R2 failed to keep T_C within the physiological range; this was apparent for L2F, which clearly assumed posture R3 at a significantly higher T_C than for posture R2. For L3F, although the number of trials was limited, showed a trend in the same direction. For both lizards, T_C for the R3 was different from R1. For L2F, [F = 445.5, df = 2, p<.0001; for all comparisons with Fisher's PLSD, R1-R2; R2-R3; R1-R3, p<.0001]; for L3F, [F = 319.5, df = 2, p<0001; R1-R2 and R1-R3, p<.0001, R2-R3, NS]. Consistent with this, the regression analysis for L2F had an only slightly higher coefficient for a 2nd order polynomial than linear fit: [$r^2 = .66$, p<.001] vs. [$r^2 = .60$, p<.001]; whereas for L3F the curvature was greater [$r^2 = .77$, p<.001] vs. [$r^2 = .67$, p<.001] (Fig. 6). When trials began at the lower initial T_S of 35°C, none of the lizards reached the T_C that elicited the thermoregulatory posture R3 during the 70°C test period (Fig. 7). Furthermore, L3F did not adopt R2 during the trials with initial $T_S = 35$, and only adopted R2 at a $T_C \geq 41.5°C$. Notably, L2F started or switched to R2 on trials with the lower initial T_S and at a $T_C \sim 33°C$. On some of these trials this lizard appeared animated, and reacted with sprinting or digging already early during the test period.

Typically, as the T_C rose above 38°C, very fast fictive digging, or sprinting (sprint speed is dependent on body temperature (Okafor 2009), or trying to climb the wall, interfered with a direct transition from R2 to R3. Thus, at more extreme T_C, R3 was not always the first strategy the lizard adopted to retard the rise in core temperature; although increased activity, such as sprinting, might have been expected to further raise T_C, in fact, T_C did not appear to increase following intense "escape" episodes. After the bouts of activity the lizards would sometimes assume a random posture, completely motionless with closed eyes, which could persist until the end of the trial. This behavior possibly could be attributed to the extremely low thermal mass of the Kevlar substrate, which favored remaining on a spot that had already been partially 'drained' of heat, over moving to new, possibly hotter, spot. The substrate surface was slippery, and as well could have discouraged movement. A panting-like behavior was occasionally observed in the smaller lizard: twice, it opened its mouth at $T_C > 42°C$ when in posture R2 and R3. No such behavior was observed in the larger lizard, although both lizards, at times, made swallowing motions at high T_C.

C. The relationship between thermal mass, initial T_S and the rise in T_C

During the test, the rate of T_C increase was similar for both lizards (Fig 5). With the lower initial pre-conditioning $T_S = 35°C$, the rate of increase in T_C was higher than during the trials with $T_S = 45°C$. Although the surface to volume ratios of the larger lizard is smaller, the difference in size did not appear to have a large effect on thermal properties.

D. Autopsy

Autopsy was used to verify the position of the transponders in the abdominal cavities and showed that there were no infections or other anomalies, and that the transponders were well surrounded by gut, which is consistent with observations of the rate in increase and decrease of the core temperature. (If the transponders were more superficial we would have seen changes in T_C that were more immediately dependent on changes in posture.)

Summary preliminary results

In our experimental set up the lizards reacted to an elevated core temperature, that presumably could become dangerous to them, by trying to either escape or adopt a posture that raised the torso above the substrate: R2, in which the lizard sits with the hips on the ground or R3, in which the whole body is raised high off the ground (Fig. 4; R2P2 and R3P0) Our results indicate that there is a transition core temperature for a change in thermoregulatory behavior; whereas the thermoprotective behaviors appear to be less influenced by T_C than by heat sensors in the skin. It is reasonable that lizards experience conflict in situations where they both are on the hot sand, and at the same time need to freeze, in order to wait for prey, or avoid being seen by a predator. An alternative behavior is a sprint to a shaded, less hot substrate, which, however, could expose them to concealed predators, such as snakes. This creates a conflict, which neuronal decision mechanisms at various levels that have evolved for survival, would then resolve. Our results also experimentally illustrate that the thermal mass of the lacertid lizards used in this study does *not* noticeably affect heat absorption and thus thermoregulatory behavior.

DISCUSSION AND FUTURE EXPERIMENTS

The most obvious result from the study was the clear transition point in core temperature between thermo*protective* and thermo*regulatory* postures. Also, the difference between the two lizards for the transition was unexpected. Although T_C was consistent within each lizard, there was a difference of 2° C in absolute temperature between them.

The basis for this observation was a numerical scoring system that we developed to objectively evaluate the categories and extent of thermoregulatory postures. The system distinguishes between thermoprotection (P) and thermoregulation (R), and assigns a score of 0 - 4 for thermoprotection, and 1 - 3 for thermoregulation. The R scale is similar to that used by Sartorius *et al.* (2002); however, we added a more detailed specification of body and leg positions, and, as the tail has little role in heat conductance (Herczeg *et al.* 2004), it was not included (see Table IB). By distinguishing limb withdrawal from changes from gross body posture, we reasoned that it would be easier to distinguish the transition from heat protection of the feet to defense of physiological core temperature. We found specific core temperatures at which the switch occurred for each lizard: for the larger L2F ≥39.5°C, and smaller L3F, ≥41.5°C (Figs 6 and 7). The two lizards switched to R2 at different values of T_C: ≥39.5°C and <33°C for L3F and L2F, respectively. At the beginning of the trial L2F sometimes had R2 and remained motionless, or on other occasions was agitated with sprinting and digging movements then returning to R2. This has no obvious thermoregulatory function. Often during the high substrate temperature (T_S) tests, as T_C became elevated, the lizards often made escape-like movements (digging and sprinting) before changing posture from R1 or R2 to R3. Because a lightning storm destroyed the equipment, the number of trials for L3F is too small for statistical reliability; however, similar to L2F, the data that we obtained shows a transition at a specific value of T_C.

It would be informative to observe whether this behavior would be reduced by changing the test condition from 70°C for 50 s to a lower temperature for a longer period, for example, 60°C for 70 s. The lizards do not, in general, react to heating of the thermoreceptors in the abdominal skin by the 70°C surface, if T_C is lower than ~39; to an extent, this could be an artifact of the ultra-low thermal mass of the of the test surface. If the lizard remains in contact with the surface, it continuously draws energy away from the surface and 'locally' prevents the temperature from rising. This, to a

much lesser extent, occurs on the sand, but is probably similar to what happens when the lizard stands on dry vegetation.

Although at the onset of the experiments the two lizard's weights, 9 g vs. 4 g, and surface to volume ratios, and thus their thermal masses were substantially different, the rate of increase in T_C during the tests was similar.

We have searched for data on the core temperatures of free ranging Shovel-snouted nor the Wedge-snouted lizards, and found no descriptions of the set-point or preferred temperature range for either species, aside from "Field guide to snakes and other reptiles of Southern Africa" (Branch 1998), which notes that the Shovel-snouted can reach 44°C; however, this source gives no reference, and our attempts to contact the guide's author have been unsuccessful.

We have found a few reported core temperature values for lizards the same size and living in the same desert like habitat. The Sagebrush lizard (*Sceloporus arenicolus*), snout to vent length (SVL) 65 to 70 mm, and the Side-blotched lizard (*Uta stansburiana*), SVL ~45 mm, are desert dwelling lizards in New Mexico. Environmental midday temperature on the flats, slopes and ridges of sand dunes ranged from 45 to 50°C. Mean daytime cloacal temperature measured in the field was 34.1 ± 0.59°C and 36.5 ± 0.27°C, respectively (Sartorius *et al.* 2002); although these subjects did not have T_C >37°C, they did at times display R3. Lower mean body temperatures in pregnant, compared to postpartum, Yarrow's spiny lizards (*Sceloporus jarrovi*) have been reported (Mathies & Andrews 1997). As it is difficult to know whether a female is pregnant, particularly at an early stage, only males, if possible, should be used for future experiments.

Ectotherms acclimate to environmental variations in seasonal temperature. Our study took place in the late southern hemisphere summer with maximum mean air temperature in previous months above 34°C. Neither the sand temperature in the terrarium or air temperature within the experimental box was measured; it would probably be useful to do that in future studies. Free-living lizards most probably experience cooler night temperatures than our subjects, which were kept indoors to avoid attracting snakes. How these conditions would, if at all, affect the set-points or thermoregulation in general of the lizards remains to be determined. We hope to pursue the question of why the lizards in our study switched to the thermoregulatory posture R3 at a high and specific T_C. Our data suggest that the lizards have an accurate perception of core temperature, and that this perception controls thermoregulatory behavior. The degree that the thermoregulatory behavior can be modified by experience (learning) is a key question. There is increasing evidence

that lizards are capable of learning (Leal & Powell 2011), and there are data showing that in mammals regulatory plasticity occurs in a visceral and gustatory sensory structure, the NTS, for which lizards have a homolog (Tang & Dworkin 2008). The difference in core temperatures between the two lizards for transition to posture R3 was surprising. Whether this result would be consistent with additional trials with the smaller lizard was not possible to investigate this year. Is this variation in thermoregulatory behavior dependent on a learning process? The larger, and presumably older lizard, might have experienced a previous hot season with the potential for neuronal plasticity to occur. Further studies on physiological, metabolic or neuronal regulations/adaptations involved in the observed pattern of thermoregulation will be interesting. A potentially fruitful side project would be to gain more insight in ectothermal behavior and adaption in thermoregulation by replicating the study of Cowles & Bogert (1944), in an enclosed and controlled environment, with the addition of a recording of the preferred core temperature.

In addition to the specific observations, our studies confirm the advantage of using an implanted transponder, which allows core temperature recordings without intruding on thermoregulatory behavior.

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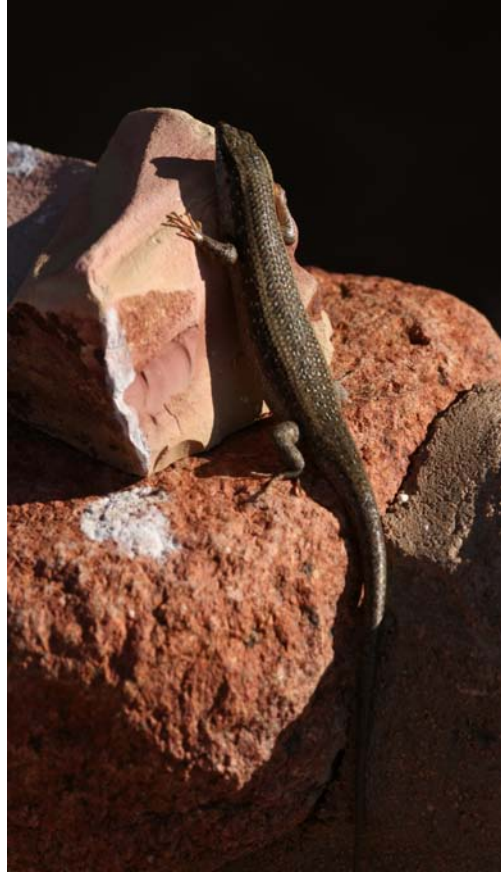
Figure 1. Southern African skink sun basking late morning. Body accurately positioned to receive maximal heat radiation from the sun

Figure 2. Foreground shows vegetated sand dunes, typical habitat of the Wedge-snouted lizard (*Meroles cuneirostris*), in the NamibRand Nature Reserve

Figure 3. Complete laboratory equipment for the experiments at NamibRand Nature Reserve

Table IA and B Numerical thermal postures

A Thermo*protective* postures

Numerical values	Posture
0	All of feet on substrate
0.5	Phalanges of one or more feet lifted off substrate
1	One or more feet flexed away from substrate
2	One leg off substrate
2.5	"2" and one or more additional feet flexed from substrate
3	Two legs off substrate
3.5	"3 " and one or more additional feet flexed from substrate
4	Full abdomen against substrate with all legs off substrate

B Thermo*regulatory* postures

Numerical values	Posture according to Sartorius *et al.* 2002	Postures modified by Wilén
1	Body and tail pressed against the substrate, head on the substrate or raised	Torso flat on substrate with neck and head on substrate or raised. Legs stretched out or resting on joint.
2	Head and chest raised, abdomen and tail pressed against the substrate	Sitting on hips with forelegs straight or bent. Rostral torso completely off substrate
3	Head and body raised above the substrate with the tail raised or resting on the substrate	Standing on two or more legs (bent or straight) with torso completely off substrate. Tail as support or lifted

Figure 4. Thermoregulatory (R) and thermoprotective (P) postures

R1

R1P0 All legs on substrate

R1P2 Left front leg off substrate

R2

R2P2 Right hind leg off substrate

R3P0 All feet on substrate

R3P0 All feet on substrate, but gliding

R3P1 Right hind foot lifted, but with heel on substrate
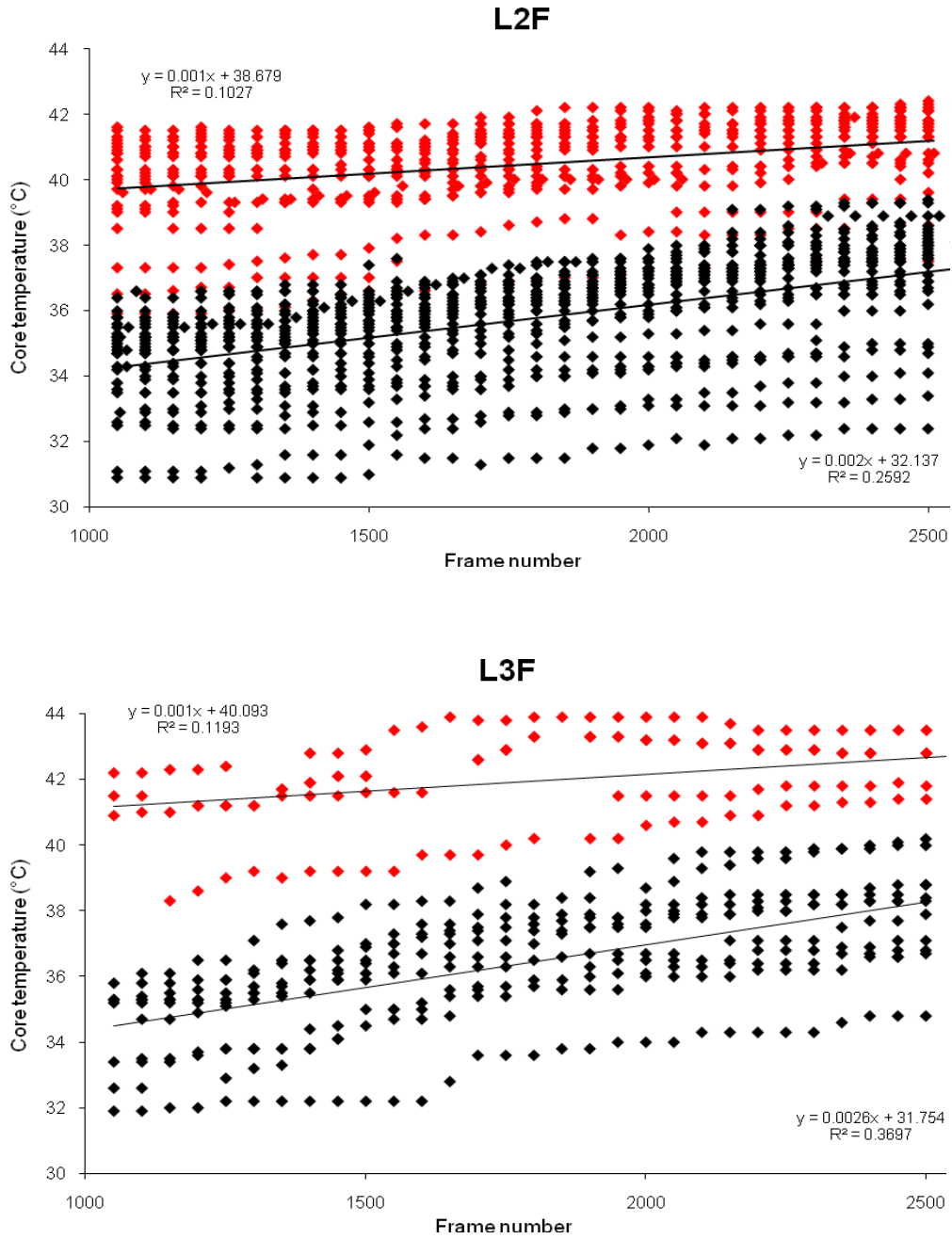
R3P4 Left front and right hind leg off substrate

L2F

$y = 0.001x + 38.679$
$R^2 = 0.1027$

$y = 0.002x + 32.137$
$R^2 = 0.2592$

L3F

$y = 0.001x + 40.093$
$R^2 = 0.1193$

$y = 0.0026x + 31.754$
$R^2 = 0.3697$

Figure 5. Manipulation of core temperature (T_C) in two Wedge-snouted lizards to test experimental set-up. Faster increase with lower initial T_C (black diamonds) compared to higher initial T_C (red diamonds)

Experimental conditions:
Lizards pre-conditioned at substrate temperature (T_S) = 45°C (red squares) and 35°C (open triangles)

Test substrate (T_S) is a 0.0025 mm etched foil heating element encapsulated between two layers of 0.05 mm Kevlar Film, 60 to 70°C during test, totally ~30 seconds

Frames from start of trial inclusive pre-conditioning (50 frames = 1.67 seconds)

Equations to the left and right in plot area represent pre-conditioning T_S 45°C and 35°C, respectively

T_C measured with a transponder implanted into abdomen of the lizard

Thermoregulatory postures; R1 Lizard with abdomen direct on substrate; R2 Lizard sitting on hips and front legs; R3 Lizard standing up on four legs, or occasionally two or three legs

Number of trials at pre-conditioning T_S 45°C and 35°C with L2F 78, initial weight 9 g

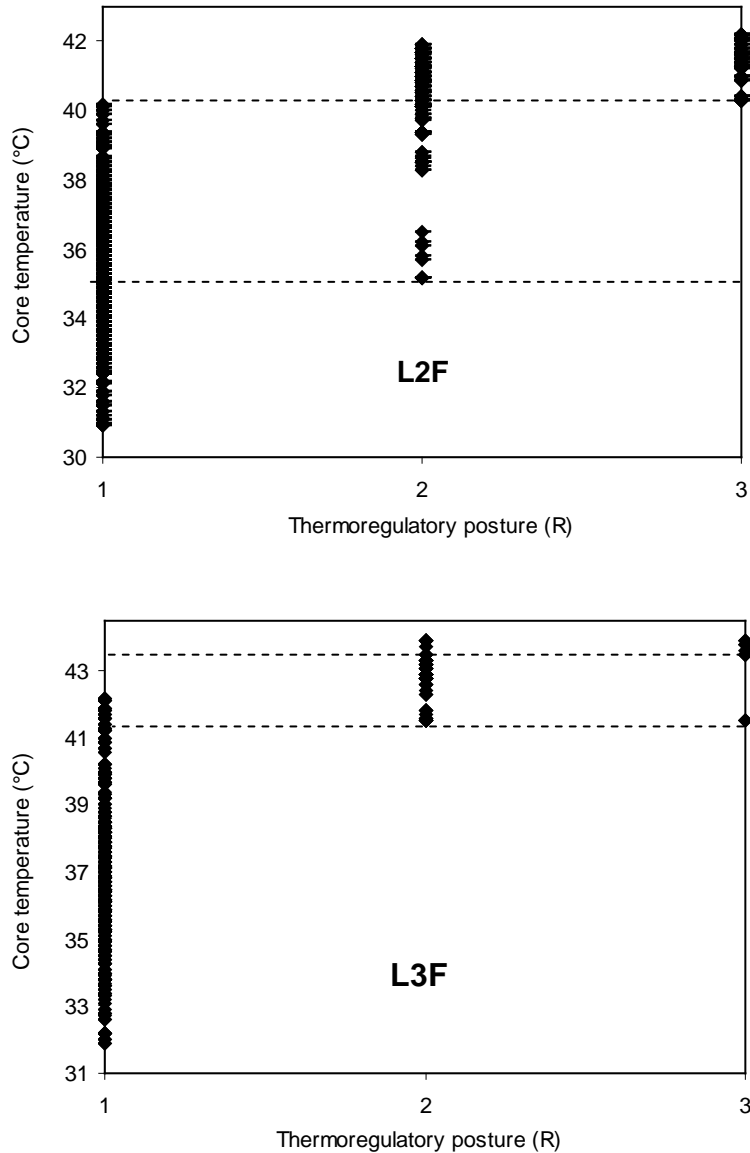Number of trials at pre-conditioning T_S 45°C and 35°C with L3F 19, initial weight 4 g

Figure 6. Relationship between core temperature (T_C) and thermoregulatory postures, R1, R2 and R3 for L2F and L3F during test trials. Definitions of the thermoregulatory postures: R1, horizontal with the entire ventral torso in direct contact with the substrate; R2, sitting on hips and front legs with the base of the tail in contact with the substrate; R3, standing on extended legs with no contact between the substrate and torso. Dotted lines show the approximate T_C lower boundaries for the different postures. For both lizards, T_C for the R3 is different from R1. For L2F, [F = 445.5, df = 2, p<.0001; for all comparisons p<.0001]; for L3F, [F = 319.5, df = 2, p<0001; R1-R2 and R1-R3, p<.0001, R2-R3, NS]. Consistent with this, the regression analysis for L2F had an only slightly higher coefficient for a 2nd order polynomial than linear fit: [r^2 = .66, p<.001] vs. [r^2 = .60, p<.001]; whereas for L3F the curvature was greater [r^2 = .77, p<.001] vs. [r^2 = .67, p<.001].

The substrate was a 0.0025 mm etched copper foil heating element encapsulated between two layers of 0.05 mm Kevlar Film which was at >60; ≤70°C during test. T_C was measured with an implanted transponder.
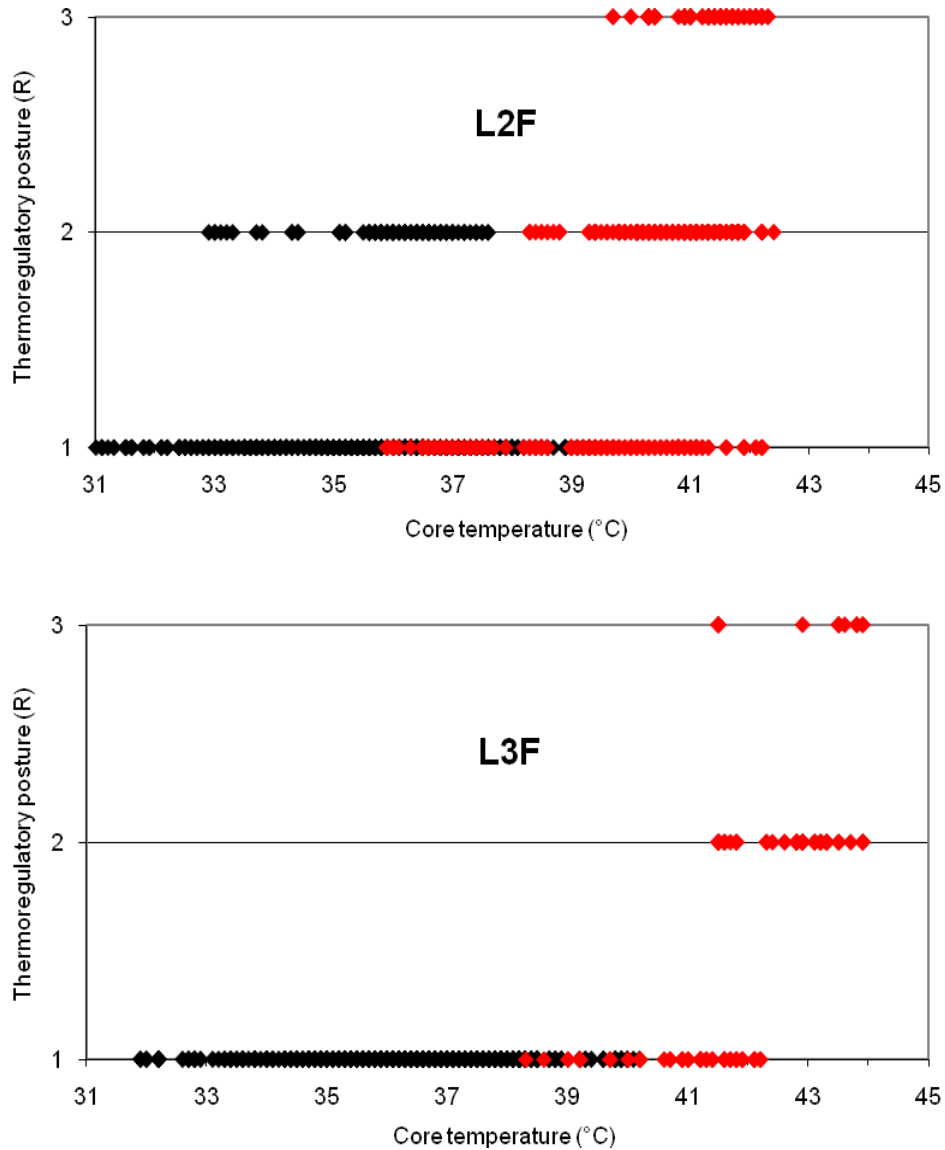
Figure 7. Core temperatures (T_C) at which Wedge-snouted lizards under experimental conditions change thermoregulatory postures (R). L2F and L3F adopt posture R3 >39.5°C and >41.5°C, respectively

Experimental conditions:
Lizards pre-conditioned at substrate temperature (T_S) = 45°C (red diamonds) and 35°C (black diamonds). Substrate consists of a 0.0025 mm etched foil heating element encapsulated between two layers of 0.05 mm Kevlar Film. Thermoregulatory postures; R1 Lizard with abdomen direct on substrate; R2 Lizard sitting on hips and front legs; R3 Lizard standing up on four legs, or occasionally two or three legs. T_C measured with a transponder implanted into abdomen of the lizard. Initial weight of L2F and L3F; 9 g and 4 g, respectively. Number of trials at pre-conditioning T_S 45°C and 35°C with L2F 78, initial weight 9 g. Number of trials at pre-conditioning T_S 45°C and 35°C with L3F 19, initial weight 4 g