

Thermoregulation in the dry forest anole, *Norops cupreus*

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ABSTRACT.—*Norops cupreus* inhabits lowland dry forests and gallery forests along the Pacific Coast of Central America. We used operative temperature models (OTM) to examine the thermoregulation of this species in Parque Nacional Palo Verde (Costa Rica) by conducting a field study in February 2003. *Norops cupreus* in Palo Verde appeared to thermoregulate during early morning hours, late evening, and during the afternoon to maintain their body temperature (T_b) < 33°C. Lower capture rates during the midday suggest decreased activity of *N. cupreus* during this period, perhaps because they actively searched for the coolest environmental temperature (T_e). During the rest of the day, *N. cupreus* appeared to thermoconform, with mean body temperatures tracking minimum available T_e . These results are similar to other observations reported for *N. cupreus* in Central America.

Regulation of body temperature (T_b) can be important to ectotherms because of the potentially dramatic effects of T_b on physiology and behavior; e.g., metabolic rate, reproduction, predator avoidance, and foraging (Huey 1982; Angilletta et al. 2002). Ectotherms typically regulate body temperature behaviorally by moving between appropriate thermal microclimates (Cowles and Bogert 1944), but physical adaptations, such as cardiac shunts and color change, may enhance thermoregulation in some species (Pough 2001). Regardless of the mechanism, failure to maintain body temperature within the thermal tolerance range can be detrimental and even fatal to an individual, and therefore thermoregulation should take precedence over other daily activities (Dunham et al. 1989).

Thermoregulation in ectotherms falls along a continuum between two extremes of thermoconformation and thermoregulation. Body temperature of thermoconformers passively fluctuates with ambient environmental temperature, while thermoregulators maintain a body temperature within some range regardless of environmental temperature (Huey and Slatkin 1976). The thermal environment in which the organism evolved is important for determining whether thermoregulation or thermoconformation would be the optimal life history strategy (Huey and Webster 1976). For example, if the thermal environment is such that it would be expensive (whether energetically or relative to fitness) to thermoregulate that organism may be relegated to thermoconformity (Lister 1976). Indeed, the extent of basking behavior is inversely related to associated costs for some anole species such as *A. gundlachi* and *A. cristatellus* (Huey and Webster 1976).

Thermoregulation in ectotherms is difficult to demonstrate unambiguously (Wills and Beaupre, 2001). Previous studies of thermoregulation in small ectotherms have relied on either a small variance in T_b or a slope of 1 on an isothermal plot of T_b on ambient temperature (T_a) as evidence for thermoconformity (Hertz et al. 1993). However, the standard historical protocol of comparing body temperature to ambient temperature at the site of capture can be biased and uninformative (Hertz et al. 1993). The use of operative temperature models (OTM) to determine the range of available environmental temperatures (T_e) has become more widely used to eliminate the potential bias of ambient temperature at the capture site (Wills and Beaupre 2001). OTMs are physical models, usually made of copper (reviewed in Dzialowski 2005), used to examine the thermal environment of an animal based on the physical property of heat transfer between the animal and its environment (Bakken 1976). OTMs are accurate, cost efficient, weatherproof, and easy to replicate (Dzialowski 2005).

Previous studies of the dry forest anole (*Norops cupreus*) in Costa Rica, relying on isothermal plots have shown that the lizard is active at a broad range of ambient temperatures (18.4 to 34.0° C; Clark 1973; Fitch 1973) and that the range at which *N. cupreus* is active decreases with elevation of the site (Clark 1973; Fitch 1973). Thus, we wanted to reexamine thermoregulation in *N. cupreus* using the operative temperature model approach of Hertz et al. (1993) and the randomization analysis approach of Wills and Beaupre (2001) to determine if *N. cupreus* does in fact thermoregulate and if there is a difference in thermoregulatory strate-

gy between sexes. We used randomization analysis to compare T_b and T_e to assess whether *N. cupreus* were thermoregulating or thermoconforming throughout the day. We also used randomization analysis to compare T_e between the two days of sampling. This mathematical approach has been demonstrated to be appropriate with thermal data because it counters the non-normality of data distribution and the improbability of random sampling (Wills and Beaupre 2001). We hypothesized that *N. cupreus* will thermoconform when T_e is biologically suitable and will thermoregulate when necessary and possible.

MATERIALS AND METHODS

Norops cupreus is a small gray to brown lizard with variable pattern on the dorsum (Savage 2002). Sexual dimorphism is present between males and females with males measuring 38-57 mm SVL and females measuring up to 51mm SVL (Savage 2002). *Norops cupreus* inhabits disturbed and converted lowland dry forest and gallery forest, along the Pacific Coast of Central America from Guatemala to Costa Rica (Fitch 1973).

Fieldwork was conducted 3, 4, and 7 February 2003 at Parque Nacional Palo Verde, Guanacaste Province, Costa Rica. Forty-six operative temperature models were placed along a 23 meter transect along the waterhole trail (WGS84: 10°21'22.05"N 85°19'43.17"W). One operative temperature model was placed haphazardly every meter on each side of the transect. If the probe hit a tree it was placed in that tree (39%) and if it landed on leaf litter it was placed where it fell (61%). The haphazard placement of thermal probes appeared to produce a representative sample of the available environment. The temperature of each operative temperature model was recorded manually every 20 minutes with an Omega Type T Thermal Couple Reader (Norwalk, CT). Operative temperature models were constructed from quarter inch copper tubing cut to 6 cm. A copper-cadmium thermal couple wire was inserted into each copper tube. The copper tubing was plugged with aluminum foil and spray-painted to represent lizard body color and reflectivity (Hertz et al. 1993). Operative temperature models were calibrated to wild lizards for temperatures between 22° C and 38.1° C. Time to equilibration for the models was less than one minute so thermal inertia was minimal.

We recorded T_e data from 09:20–12:00 to 13:30–15:30 on 3 February, 08:00–09:20 and 15:30–16:20

on 4 February, and lizard T_b data from 08:00–12:00 and 13:30–15:30 on 3 February and 15:30–16:20 on 4 February (experimental period one). Environmental temperature data were compared for overlapping time periods to ensure that combining samples from the two days into one experimental period was justified. T_e and T_b were also collected from 08:20–11:20 and 13:30–16:20 on 7 February (experimental period two).

We sampled *N. cupreus* within a 4 ha area around the operative temperature model transect by visual searching. We recorded body temperature within 10 sec of capture using a cloacal insertion probe and Omega Type T Thermal Couple Reader (Norwalk, CT). We recorded time of capture, sex, snout-vent length (SVL), and habitat. To avoid recapturing the same animal, lizards were marked with non-toxic paint and released. Painting is a non-permanent and non-invasive method that allows individual recognition until the lizards go through the shedding process (Dodd 2016).

We used a randomization approach to examine potential differences between the environmental temperature profiles available to *N. cupreus* over the two days of this study. Our algorithm calculated the arithmetic mean for environmental temperature (T_e) during each time block for each day; time block 08:00 and 11:40 were excluded because no data were available for experimental period one. The observed difference (D_o) between mean T_e for each time block was calculated ($D_o = \text{mean}_{\text{Day 1}} - \text{mean}_{\text{Day 2}}$) for each of 20 time blocks. The algorithm next generated a set of 1,000 random differences (D_r) for each time block by shuffling environmental temperatures between days within a time block, recalculating the arithmetic means, and taking D_r for each time block. Statistical significance was determined by counting the number of randomized differences that were less than, equal to, or greater than the observed difference for a given time block. One-tailed probabilities were calculated because some sampling distributions of the difference between days were not symmetrical; the sign of the observed difference between days was used to determine the direction of the test and statistical significance was determined using $\alpha = 0.025$.

We used goodness of fit to a uniform distribution to test for significant differences in capture rates during different periods of day. We used separate two-factor ANOVAs to determine if there were differences in sex

and day of the capture in size (SVL) and body temperatures.

We used a randomization algorithm programmed in R (R Core Team 2009) to evaluate the relationship between mean environmental temperatures (T_e) and mean lizard body temperature (T_b) within a time block (see Wills and Beaupre 2000). Our algorithm calculated the arithmetic mean for environmental (T_e) and body temperature (T_b) then calculated the observed difference between means ($D_o = \text{mean } T_b - \text{mean } T_e$) for each of 22 time blocks. The algorithm next generated a set of 10,000 random differences (D_r) for each time block by shuffling temperature designations (T_e or T_b) across the entire data set and recalculating the arithmetic means ($T_{e,\text{random}}$ and $T_{b,\text{random}}$) and taking D_r for each time block. We determined statistical significance within each time block by counting the number of D_r equal to or more extreme than D_o and dividing that quantity by 10,001 (the number of D_r plus D_o).

RESULTS

We captured 121 (79 M:42 F) and 153 (96 M:57 F) *Norops cupreus* on days one and two, respectively. Capture efficiency by sex was independent of the day of the study ($\chi^2 = 0.19$, $df = 1$, $p = 0.66$). We captured the fewest lizards (22 individuals) during the 13:00–15:00 period, while we captured the greatest number of lizards (47 individuals) during the 15:00–16:20 sampling period. In general, we captured more lizards than expected (based on a uniform distribution) during the early sampling intervals (08:00–09:00) and later sampling intervals (15:00–16:20) but fewer than expected during the middle portion of the of the day (09:20–14:40) when days were combined ($\chi^2 = 39.31$, $df = 21$, $P = 0.009$) and on the second sampling day ($\chi^2 = 41.55$, $df = 21$, $P = 0.005$). Although we observed the same general pattern on the first sampling day, capture efficiency was not significantly different across all time periods ($\chi^2 = 23.63$, $df = 19$, $P = 0.211$).

Male *N. cupreus* were larger than females (Sex: M SVL = 3.9 ± 0.48 cm; F SVL = 3.6 ± 0.32 cm; $F_{1,1} = 75.6$, $P < 0.0001$), yet we captured similarly-sized individuals on both days (Day: $F_{1,1} = 0.57$, $P > 0.05$), and the difference between males and females was consistent between days (Sex*Day: $F_{1,242} = 0.56$, $P > 0.05$). Size differences between males and females did not equate to differences in T_b (Sex: M $T_b = 31.7 \pm 1.23^\circ\text{C}$; F $T_b = 31.6 \pm 1.04^\circ\text{C}$; $F_{1,1} = 1.15$, $P > 0.05$). Although T_b varied

with day ($F_{1,1} = 17.7$, $P < 0.001$), the effect for males and females was similar between days (Sex*Day: $F_{1,242} = .259$, $P > 0.05$), suggesting that differences in T_b were a response to daily variation in environmental temperatures (see below).

Mean environmental temperatures varied considerably during both days of this study, yet the environmental temperature profile was similar between the two days (Fig. 1). The maximum recorded temperature was 35.5°C at 11:40 on day 1, and 35.8°C at 11:20 on day 2. Comparison of each time period between days, however, revealed significant variation. Specifically, day 1 was significantly cooler than day 2 from 08:00 until 09:40 and significantly warmer than day 2 from

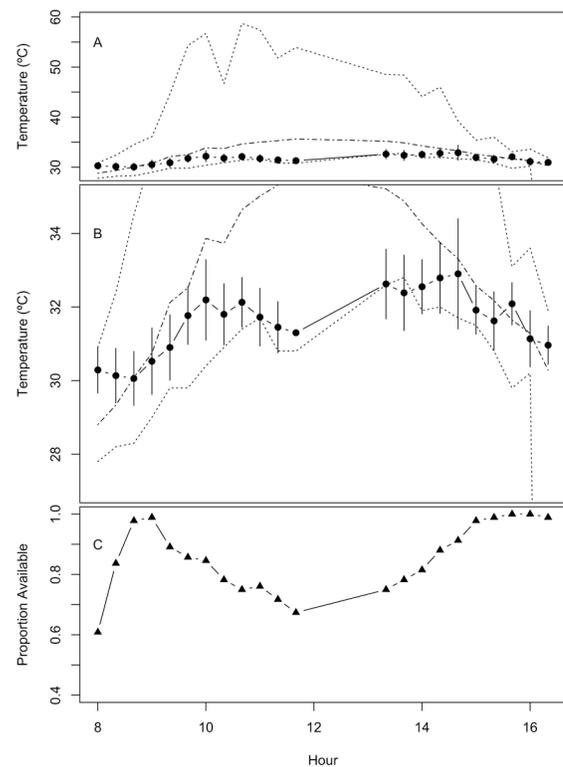


FIG. 1. Maximum and minimum environmental temperature (T_e) and lizard body temperature (T_b) as a function of time (A & B), and proportion of observed T_e available to *N. cupreus* as a function of time (C). Proportion of available temperatures is taken as $(T_{b,\text{min}} \leq nT_e \leq T_{b,\text{max}}) \div NT_e$, where $T_{b,\text{min}}$ is the minimum body temperature observed over all times, $T_{b,\text{max}}$ is the maximum body temperature observed over all times, nT_e is the number of T_e observations between minimum and maximum T_b , and NT_e is the total number of T_e observations made during the sampling period.

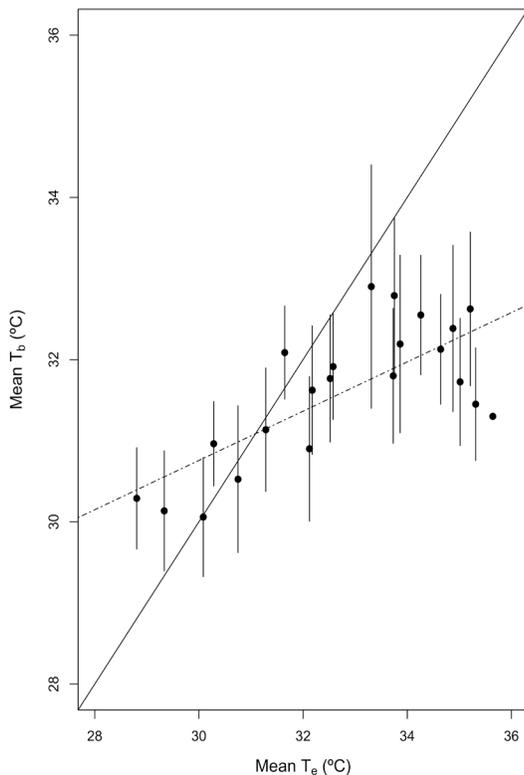


FIG. 2. Mean body temperature (T_b) as a function of mean environmental temperature (T_e). The isothermal line indicates unity between T_e and T_b . *N. cupreus* appear to regulate body temperature from $\sim 30^\circ$ to $\sim 33^\circ$ C.

14:20 until 16:20 (data not shown). Temperatures were similar between the two days from 10:00 until 14:00. Although the statistical differences between days likely arose from the collection and redeployment of OTMs between days, we analyzed both the individual and pooled data sets.

Plots of T_e max, T_e min, and T_b for the entire sampling period indicate that lizard T_b most closely follows T_e min (Fig. 1B), and lizard T_b falls significantly below mean T_e as temperatures increase throughout the day whether days are considered individually or when they are pooled (Fig. 1). Mean lizard T_b increases significantly as mean T_e increases ($b = 0.304$, $t_{20} = 4.78$, $P < 0.0001$ under $H_0: b = 0$) but does not increase solely as a function of T_e ($b = 0.304$, $t_{20} = 10.95$, $p < 0.0001$ under $H_0: b = 1$) reaching a maximum threshold of approximately 33°C (T_e) suggesting that *N. cupreus* thermoregulate to maintain $T_b \leq 33^\circ\text{C}$ (Fig. 2). When days are considered individually, mean lizard T_b increases

significantly with mean T_e during each day (under $H_0: b = 0$; Day 1: $b = 0.462$, $t_{18} = 5.55$, $P < 0.0001$; Day 2: $b = 0.230$, $t_{20} = 3.17$, $P < 0.0049$). As with the pooled data, mean T_b is not solely a function of mean T_e (under $H_0: b = 1$; Day 1: $b = 0.462$, $t_{18} = 6.46$, $P < 0.001$; Day 2: $b = 0.230$, $t_{20} = 10.62$, $P < 0.0001$).

The sampling intervals of 08:00 and 11:40 presented the smallest proportion of available environmental temperatures (61% and 67%, respectively) when days were considered separately, and the 10:00–11:59 sampling block presented the lowest percentage of environmental temperature availability (75%) when days were combined. The sampling intervals 15:40 and 16:00 presented the largest proportion of available environmental temperatures (100% during each interval) when days were considered separately, and the 15:00–16:59 presented the greatest percentage of environmental temperature availability (99%) when days were combined (Fig. 1C).

DISCUSSION

Norops cupreus in our study showed sexual size dimorphism, which has been previously observed within this species (Fitch 1973). Although *N. cupreus* sampled for this study were sexually dimorphic, there was no difference in T_b between sexes, a result consistent with previous observations of this species (Fitch 1973). Most anoline lizards examined do not show significant difference in T_b between male and female (summarized in Woolrich-Piña et al. 2015). Our results supported this observation because both sexes showed the same daily variation of body temperature.

Norops cupreus at Palo Verde appeared to thermoconform during morning (08:45–10:00) and late afternoon (14:45–16:00) when T_e was suitable (between 30.5°C and 33°C) and to thermoregulate in early morning (08:00–08:45) and late afternoon (16:00–16:30; Fig. 2) when T_e is low or high. This pattern of thermoregulation was similar to *N. cupreus* in dry lowland forest at Taboga (Costa Rica) and was thought to be due to lizards hastening warming in morning and retarding cooling in late afternoon by basking (Clark 1973).

During midday, when average T_e was elevated, *N. cupreus* thermoregulated, presumably by utilizing microhabitats that exhibited T_e closer to minimal T_e . Body temperatures of *N. cupreus* more closely tracked minimum T_e at Palo Verde, similar to Taboga where *N. cupreus* tended to have body temperatures below ambi-

ent at the upper extreme (Clark 1973). *N. cupreus* had previously been described as a thermoconformer at a high elevation site where the T_c range fell near the apparent preferred temperature, while a thermoregulator at low elevation sites where range was greater and included warmer temperatures than those preferred by *N. cupreus* (Fitch 1973). Thermoconforming species have been shown to constitute a major component of the diversity of neotropical lizards (Huey et al. 2009). Our findings from the low elevation Palo Verde site support those previous findings.

During the middle of the day (11:30–13:30) T_c appeared to constrain microhabitat availability rendering up to 30 % unavailable during the hottest part of the day, as evidenced by lower capture rates presumably because *N. cupreus* were selecting for coolest T_c available. In Taboga, *N. cupreus* showed similar behavioral patterns by being inactive when air temperatures were above 31° C and had an activity range midpoint of 25.2° C (Clark 1973). Activity pattern and thermoregulation are greatly affected by environmental temperatures and to the same extent, by spatial heterogeneity (Sears and Angilletta 2015).

At Palo Verde *N. cupreus* thermoregulated to maintain $T_b < 33^\circ\text{C}$. The average T_b , and presumably the preferred temperature, of *N. cupreus* was reported to correspond to air temperature that is about 26° C in a low elevation site (Fitch 1973). Thus, the average T_b from our low elevation study site was similar to those reported previously, but was also comparable to those reported for high elevation sites. Indeed, mean body temperature was reported to be 28.8° C for females and 29.4° C for males at San José (Fitch 1973) and 26.2° C for all individuals at Ciudad Universitaria, an upland site (Clark 1973).

In conclusion, our study using operative temperature models supports previous reports of thermoregulation based on isothermal plot of T_c versus T_b in *N. cupreus* from low elevation, dry forests. Our findings contribute to the growing body of literature that enforces the importance of understanding the thermal ecology of a population relative to the landscape when addressing population and species level questions related to physiology, ecology, evolution, and even more in the current context of global warming. The Intergovernmental Panel on Climate Change (2016) predicted the global temperature to increase by 1.5° C (compared to

pre-industrial levels) between 2030 and 2052. *Norops cupreus*, like all the tropical forest lizards (Huey et al. 2009), is very likely to be impacted by this rise in temperature since they already select the coolest T_c available. A shift in thermoregulatory behavior could aid this species over the short-term (Huey et al. 2012) but they will remain vulnerable over the long-term (Buckley et al. 2015). More research is needed to understand how global climate change will impact tropical ectotherms, especially considering lizard population declines have already been witnessed in Costa-Rica (Whitfield et al. 2007).

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