Respiratory cooling in rattlesnakes

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Abstract

We used infrared thermography to study respiratory cooling in the rattlesnakes (Viperidae: Crotalinae) and to partition the effects of air temperature, humidity, and activity levels on head–body temperature differences. We observed a single, cooled region centered around the mouth and nasal capsule that extended across the pit membrane at air temperatures above 20 °C. Both head and body temperatures of rattlesnakes increased linearly with air temperature. Head–body temperature differentials also increased with air temperature, but declined significantly at higher relative humidities. Rattling rattlesnakes exhibited significantly greater head–body temperature differentials than did resting rattlesnakes. We suggest that respiratory cooling may provide a thermal buffer for the thermoreceptive pit organs at high air temperatures, but caution that this adaptive hypothesis must be tested with direct neural or behavioral assays.
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1. Introduction

Because the nervous system exhibits a remarkable sensitivity to fluctuations in temperature, organisms have evolved elaborate means of thermoregulating brain and sensory tissue (Schmidt-Nielsen, 1993). Certain ocean fishes utilize modified eye musculature to elevate eye and brain temperature well above water temperature (Carey, 1982; Block and Franzini-Armstrong, 1988). Harbour seals can elevate the surface temperatures of their mystacial and supraorbital vibrissal pads as much as 25 °C above water temperature, and exhibit high tactile sensitivity in waters as cool as 0 °C (Mauck et al., 2000).

Head–body temperature differences in reptiles have often been taken as evidence for the precision by which these ectotherm animals regulate temperatures. By differentially exposing certain body parts to impinging solar radiation, many reptiles elevate head temperatures relative to their bodies upon emergence from burrows (Heath, 1964; Hammerson, 1977; Gregory, 1990). They maintain these thermal gradients during activity via countercurrent heat exchangers (Heath, 1966). Localized cooling of nervous tissue may occur during exposure to heat stress or bouts of intense metabolic activity and is primarily controlled by the rate of water evaporation from respiratory passageways. When exposed to heat stress, many lizards and crocodilians increase their rate of ventilation and breathe with an open mouth (Crawford et al., 1977; Spotila et al., 1977). Panting has a marginal influence on the overall heat balance of these reptiles, but, in the desert-dwelling chuckwalla, brain temperature is stabilized at nearly 3 °C below air temperature through evaporative cooling (Crawford, 1972). Localized cooling in reptiles may be further facilitated by ancillary adaptations such as blood shunts and countercurrent heat exchangers (Heath, 1966; Webb and Heatwole, 1971), but these circulatory mechanisms alter only the distribution of cooled blood within the body.

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Evaporative cooling and associated brain cooling are dominated by one environmental variable (humidity) and one physiological variable (respiratory rate; McLean, 1974). Past researchers have only examined head–body temperatures of reptiles in relation to air temperature alone (Heath, 1964; Webb and Heatwole, 1971; Crawford, 1972; Johnson, 1972, 1973, 1975; Hammerson, 1977; Spotila et al., 1977; Dorcas and Peterson, 1997). Here, we use infrared thermography to examine head–body temperature differences in three species of rattlesnakes (the western diamondback rattlesnake, *Crotalus atrox*; the rock rattlesnake, *Crotalus lepidus*; and the black-tailed rattlesnake, *Crotalus molossus*) as a function of air temperature, humidity, and activity levels. Head–body temperature differences in rattlesnakes are of particular interest because rattlesnakes, like all vipers in the subfamily Crotalinae, possess specialized sensory organs known as facial pit organs. Pit organs are thermoreceptive organs derived from tactile receptors, and, like other sensory organs, must be maintained within a narrow temperature range (Molenaar, 1992). Because these receptors are critical during activities ranging from prey capture to microhabitat selection (Krochmal and Bakken, 2003), understanding the thermal environment in which they operate is a primary goal of this study.

2. Materials and methods

2.1. Study organism

The western diamondback rattlesnake (*C. atrox*) is a habitat generalist commonly found throughout much of the American Southwest. Twelve western diamondbacks from Texas were collected between June 1998 and October 1999. Three black-tailed rattlesnakes (*C. molossus*) and two rock rattlesnakes (*C. lepidus*) from Texas were also used in this study. (Texas Parks and Wildlife Permits #SPR-029-19 to J. Johnson, UT-El Paso; #SPR-1017-912 to T. LaDuc).

Rattlesnakes were maintained in two isolated reptile rooms at the University of Texas at Austin. All snakes were kept in a 12 h:12 h L:D cycle at 27–30 °C. Each animal was housed individually in either 38 or 76 L glass aquaria. Water was provided ad libitum. Snakes were fed mice, both alive and dead, on regular 2-week intervals. All snakes were held in captivity for at least 6 months prior to filming (UT Animal Welfare Committee #99031201).

2.2. Thermographic measurements

Individual experimental animals were placed in a filming chamber that consisted of a 38 L glass aquarium modified to allow either the insertion of the lens of an AVIO Handy Thermo TVS-110 camera (temperature resolution of 0.2 °C; filming rate of 10 frames/s), or a Sony CCD-TRV16 camcorder. The interior of the aquarium was lined with cardboard panels that tapered away from the camera, thus serving to constrain the snake within the camera's field of view approximately 30 cm from the lens. Lighting for the camcorder was provided via overhead fluorescent bulbs, which yield a negligible amount of radiant heat energy. A digital thermometer/hygrometer (Mannix LAM880D) was placed inside the filming chamber to simultaneously monitor air temperature and relative humidity (RH). Air temperature (T_a) was controlled by placing the filming chamber in a HotPoint environmental room.

Snakes were allowed to acclimatize in the filming chamber for 30–60 min, and only thermograms in which body temperature was within 2.5 °C of air temperature were used. In only 7 out of 101 thermograms used in this study did this deviation exceed 2 °C, and body temperature was elevated on average 0.19 °C (±0.11 S.E.M.) above air (not significantly different from zero; two-sided *t*-test, $t_{100}=1.74$, $p>0.05$).

To examine the effects of humidity on head–body temperature differences, thermographic images were recorded at air temperatures within 2 °C of 15, 20, 25, 30, 35, and 40 °C. Humidity was modified by misting water in the aquarium. The initial temperature of the chamber was selected randomly, and the direction of temperature cycling was varied to reduce possible biases due to thermal history of the animal. Emissivity of rattlesnakes was assumed to be 95%, the value reported for human skin (Çengel, 1997). Because of the difficulty in identifying anatomical features in thermograms, body temperatures (T_b) were derived from up to three measurements along the body, whereas head temperatures (T_h) were taken from the center of cooling on the rostrum. Values of T_{diff}, the maximum head–body temperature difference, were calculated as $T_b - T_h$.

To examine the effects of activity level on head–body temperatures, the same 12 western diamondback rattlesnakes used in the above experiment were examined both at rest and during rattling subsequent to visual or tactile stimulation. Thermograms were recorded at 15, 20, 25, and 30 °C. Thermograms from each snake at each temperature were recorded within 3% RH of one other, and humidity levels between thermograms of a given snake at rest or rattling did not significantly differ (repeated measures ANOVA, $F_{1,11}=0.02$, $p=0.8939$).

2.3. Statistics

Interspecific differences in T_{diff} were tested with an analysis of covariance (ANCOVA), with T_a, RH, and species identity as independent variables. A multiple regression was used to examine the relationship between T_{diff}, T_a, and RH. In order to test the effects of rattling in *C. atrox*, a repeated-measures ANOVA was used modeling stimulus and temperature class as within-subject sources of
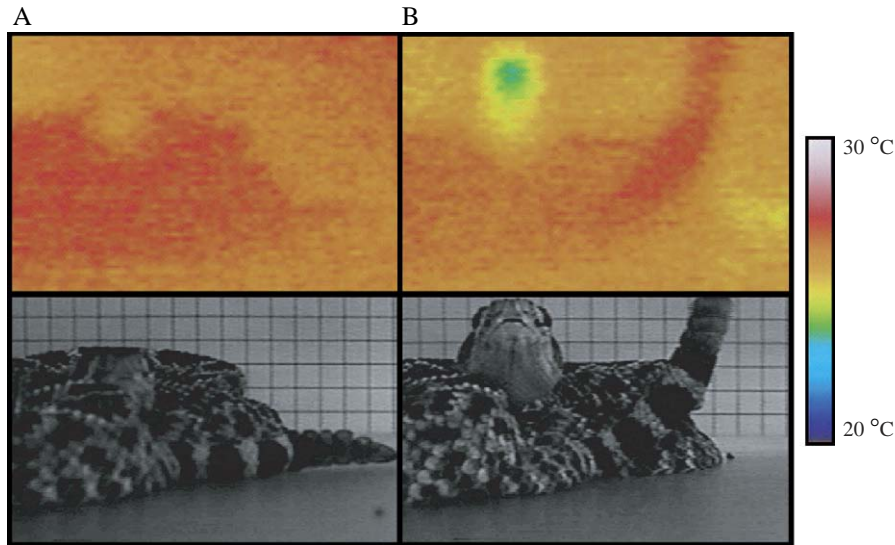
Fig. 1. Frontal thermograms (top) and video images (bottom) of a western diamondback rattlesnake (*C. atrox*) while at rest (A) and during rattling (B). Air temperature is 25 °C.

variation. All statistical analyses were performed using SAS or StatView (SAS Systems).

3. Results

A single cooled rostral region was visible from both lateral and frontal thermograms at temperatures 20 °C or higher (Fig. 1). In the middle range of temperatures examined (25–35 °C), cooled regions were centered around the mouth and nasal capsule and extended across the pit membrane, with surrounding areas grading into the body temperature more caudally within the head (i.e., posterior to the orbits; see Fig. 1).

We pooled all rattlesnake species because the relationship between T_{diff}, RH, and T_a, did not differ among the three species tested (ANCOVA: $F_{2,52} = 1.224$, $p = 0.3025$). T_b was directly proportional to T_a ($R^2 = 0.967$, $F_{1,59} = 1705.381$, $p < 0.0001$). T_h showed a significant linear increase with T_a ($R^2 = 0.963$, $F_{1,59} = 1549.534$, $p < 0.0001$), but the slope was significantly less than one (one-sided t-test: $t_{62} = 6.26$, $p < 0.0001$). Thus, the differential between the T_b and T_h increased at higher T_a (Fig. 2).

In a multiple regression model using T_a and RH as independent variables, RH was not a significant predictor of T_b or T_h ($p > 0.1$). However, T_{diff} was significantly affected by both T_a and RH (multiple regression: $T_{diff} = -0.063RH + 0.101T_a + 3.18$; $R^2 = 0.667$, $F_{2,58} = 58.059$, $p < 0.0001$). T_{diff} increased by approximately 1 °C for a 10 °C increase in air temperature, but a 10% increase in relative humidity
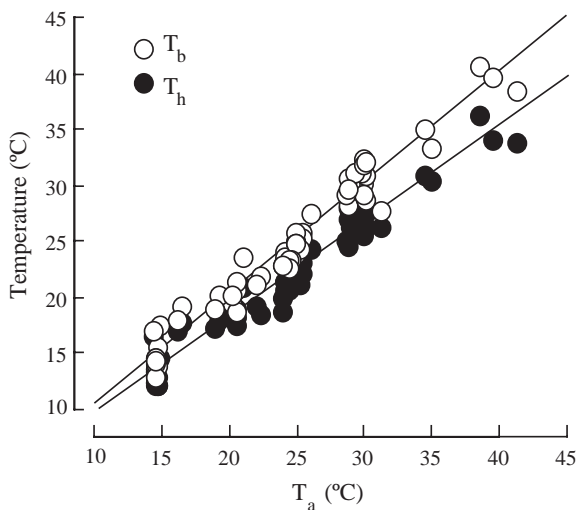

Fig. 2. Head (T_h) and body (T_b) surface temperatures recorded from three species of rattlesnakes (genus *Crotalus*) at different air temperatures (T_a). The fitted least-squares regression lines are: $T_h = 0.880T_a + 0.451$ and $T_b = 1.02T_a - 0.276$.
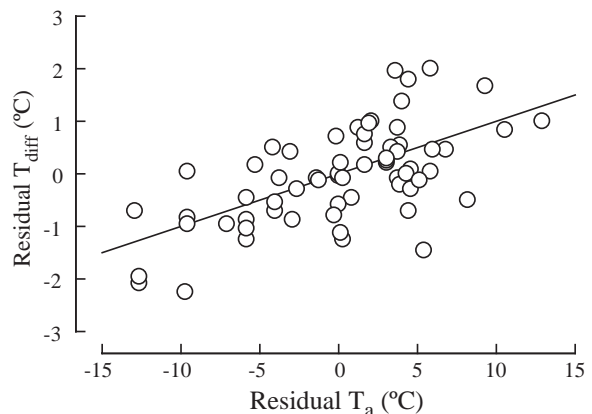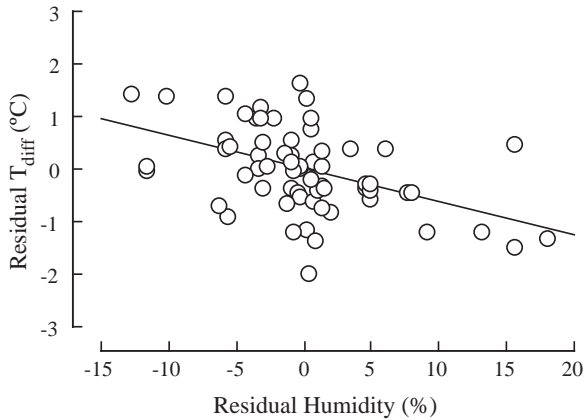

Fig. 3. Head–body temperature difference (Residual T_{diff}) recorded from three species of rattlesnakes (genus *Crotalus*) as a function of air temperature (Residual T_a). The effects of humidity were statistically removed by using residuals from regressions of T_{diff} and T_a on humidity. The fitted least-squares regression line is: $T_{diff} = 0.101T_a + 0.00$ ($R^2 = 0.397$).

Fig. 4. Head–body temperature difference (Residual T_{diff}) recorded from three species of rattlesnakes (genus *Crotalus*) as a function of relative humidity (Residual RH). The effects of air temperature were statistically removed by using residuals from regressions of T_{diff} and RH on air temperature. The fitted least-squares regression line is: $T_{diff}=-0.063RH+0.00$ ($R^2=0.218$).

reduced this difference by approximately 0.6 °C. We used residuals from least-squares regressions to visualize the independent effects of T_a on T_{diff} (Fig. 3) and RH on T_{diff} (Fig. 4).

Over the studied range of T_a, resting western diamond-back rattlesnakes exhibited a mean T_h of 1.9±0.2 °C below T_b, whereas rattling snakes exhibited a mean T_h of 2.9±0.1 °C below T_b (see Fig. 1). Rostral temperatures dropped within seconds of the initial bout of rattling and remained low during the entire rattling period. Results from 12 western diamondback rattlesnakes over four temperature classes (15, 20, 25, 30 °C) indicated highly significant effects of rattling ($F_{1,11}=23.20$, $p=0.0005$, Fig. 5) and air temperature ($F_{3,27}=23.76$, $p<0.0001$). Interaction between temperature class and rattling class was non-significant ($F_{3,25}=1.69$, $p=0.1945$). Post-hoc tests using Tukey–Kramer alpha-level protection indicated significant rattling effects on head temperature at 25 and 30 °C ($F_{1,26}=11.47$, $p=0.0023$ and $F_{1,26}=17.25$, $p=0.0003$), but not at 15 and 20 °C ($F_{1,26}=2.40$, $p=0.1340$ and $F_{1,26}=1.10$, $p=0.3036$). Further tests of regression trends revealed a significant linear ($F_{1,27}=69.02$, $p<0.0001$) but not quadratic ($F_{1,27}=0.39$, $p=0.5394$) contrast, indicating a linearly increasing slope (see Fig. 5).

4. Discussion

Thermograms of rattlesnakes revealed that the region of cooling was centered around the mouth and nasal capsule, suggesting that substantial heat loss occurs via evaporative cooling from respiratory passageways. In support of this hypothesis, we found that head–body temperature differences in rattlesnakes were greatest at low relative humidity. Historically, head–body temperature differences in reptiles have been assumed to reflect localized physiological

regulation of neural tissue (Heath, 1966). However, Pough and McFarland (1976) called this assumption into question by revealing that such differences could arise via physical heat transfer alone. By comparing panting and non-panting lizards, Crawford et al. (1977) provided the first definitive evidence that evaporative cooling through panting was, in fact, the primary means of establishing head–body temperature differences. Although panting in snakes has been described for several species of Australian pythons (Johnson, 1975) and a North American colubrid (Jacobson and Whitford, 1971), we never observed open mouths in rattlesnakes, even for the few individuals exposed to air temperatures of 40 °C. Panting may increase evaporative cooling in some animals, but it is not required for the establishment of head–body temperature differences in lizards (de Cock Buning, 1984) or snakes (Gregory, 1990; Johnson, 1975).

In addition to humidity, we infer that respiratory rate has a significant impact on head–body temperature differences in rattlesnakes. Observed increases in respiratory cooling with increasing temperature likely derive from the dependence of oxygen consumption on temperature in rattlesnakes (Schaeffer et al., 1996). However, ventilation rate in rattlesnakes does not increase in direct proportion with metabolism: it actually declines relative to oxygen consumption at higher temperatures (Wang et al., 1998). Head–body temperature differences are also influenced by rattling behavior of snakes, independent of air temperature. Increased locomotor demands during rattling lead to increased oxygen consumption (Schaeffer et al., 1996) and to corresponding increases in respiratory cooling. These results suggest that behavioral modification of respiratory rate (i.e., of breathing frequency and tidal volume) could be one route by which rattlesnakes might actively regulate their head temperature when responding to thermal stress. Formally testing this hypothesis would require demonstration of independence of ventilation rate from air temperature and rates of oxygen consumption under conditions of high thermal stress.
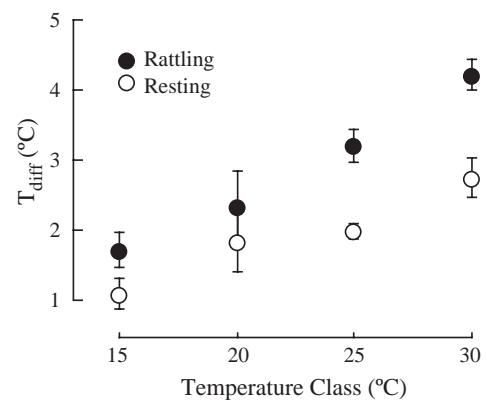

Fig. 5. Head–body temperature difference (T_{diff}) for resting and rattling western diamondback rattlesnakes at four air temperature classes.

We have demonstrated the influence of humidity on head–body temperature differences, and a logical route of investigation would next involve monitoring ventilation rate and oxygen consumption at constant air temperature but variable humidities. For instance, at 35 °C and 10% RH, head temperature should be about 29 °C. At 35 °C and 90% RH, head temperature should rise to 34 °C. Rates of oxygen consumption should not vary between these two treatments, but a large increase in respiratory ventilation rate would be expected. Indeed, camels, which experience large fluctuations in daily temperatures, do not pant but instead increase their breathing frequency independent of rates of oxygen consumption when exposed to thermal stress (Schmidt-Nielsen et al., 1981).

These results may have further significance for the sensory physiology of the rattlesnake pit organ. Although an entire class of infrared imaging cameras employs cryogenic elements, de Cock Buning (1984) has argued that cooling of the pit organs would not increase their thermal resolution. However, a complex network of capillaries on the pit membrane is at least suggestive of a heat exchanger, in which case temporal resolution of the pit organ may depend on the periodic influx of cooled blood to "erase" thermal afterimages (Amemiya et al., 1999). A second possible function of cool pits relates to their operational temperature range. In studying isolated pit membrane preparations, Bullock and Diecke (1956) indicate that their preparations worked best between 17 and 29 °C. If this temperature range represents an actual constraint for living rattlesnakes, then the thermal preferenda of many southwestern U.S. rattlesnakes, ~30 °C (Cowles and Bogert, 1944; Beck, 1996), lie at the edge of the temperature range of the pit organ. However, because of the respiratory cooling observed in this study, we expect rostral temperatures to be as low as 25 °C, placing pit organ temperatures well within their thermal limit. Future studies could usefully employ behavioral and neural assays to test the possible evolutionary significance of respiratory cooling in rattlesnakes.

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