

RESEARCH ARTICLE

Temperature dependence of regional heterothermy in a diminutive ectotherm

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ABSTRACT

Regional heterothermy describes when body regions differ in temperature, which can have important ramifications for performance because most biological processes are temperature dependent. However, the relationship between regional heterothermy and environmental temperature is not well known, particularly for ectotherms. The relationship between environmental heterogeneity and regional heterothermy might also yield insight into how the latter is regulated. We examined the thermal dependence of regional heterothermy (between the head and the cloaca) in live ring-necked snakes (*Diadophis punctatus*) in both the lab and the field, as well as in one dead and preserved individual. We found that the magnitude of the head–cloaca difference declined with average environmental temperature, that the relationship between head temperature and ambient temperature differed from that of cloaca temperature and ambient temperature, and that the preserved snake specimen did not display a consistent head–cloaca temperature difference. Our results suggest that (1) cloacal and head temperatures are regulated differently, (2) the head–cloaca temperature difference is not merely due to differences in the material properties of the head and cloaca, and (3) this difference may arise from altered circulation and perhaps even endogenous heat-generating mechanisms. Our results also suggest that the thermal dependence of regional heterothermy likely has ramifications for organismal function.

KEY WORDS: Thermal regulation, Ring-necked snake, Thermal biology, Thermal ecology

INTRODUCTION

Regional heterothermy, which is when body regions differ in temperature, can have important ramifications for performance because most biological processes that affect organismal function are temperature dependent. Mechanistically, regional heterothermy can occur as a result of variation in the physical properties of

different body regions (e.g. the material properties of bone versus soft tissue or the spatial distribution of circulatory system components) or can arise via physiological or behavioral regulatory mechanisms. Organisms might regulate temperatures in one body region and not another in either cool (lamnid sharks, tunas and billfishes: Dickson and Graham, 2004; leatherback sea turtles: Frair et al., 1972; grey whales: Heyning, 2001; Neill and Stevens, 1974; emperor penguins: Ponganis et al., 2003; bats: Rummel et al., 2019; green sea turtles: Standora et al., 1982) or warm ambient temperatures (e.g. chuckwalla lizards: Crawford, 1972; sea star: Pincebourde et al., 2013; African elephants: Weissenböck et al., 2010). Regional heterothermy can lead to the evolution of functional variation among body regions, or vice versa, producing patterns such as different temperature optima for muscle function in different regions of the wing in bats (Rummel et al., 2019, 2021). Regardless, the specific pattern of regional heterothermy in a given species might depend on environmental context (e.g. mean environmental temperature), which would have ramifications for understanding the regulatory basis of regional heterothermy and its function.

The relationship between regional heterothermy and environmental temperature is not well studied, particularly for ectotherms. For endotherms, regional heterothermy is often the product of the interaction between environmental temperatures and metabolically maintained core body temperature (Lovegrove et al., 1991). Work in endothermic marine vertebrates in cold environments suggests that regional heterothermy increases with cold exposure, as the result of prioritizing warm core temperatures at the expense of the extremities (lamnid sharks, tunas and billfishes: Dickson and Graham, 2004; leatherback sea turtles: Frair et al., 1972; grey whales: Heyning, 2001; Neill and Stevens, 1974; emperor penguins: Ponganis et al., 2003; green sea turtles: Standora et al., 1982). In contrast, many endothermic animals in warm terrestrial environments regulate head temperature to keep the brain cool via panting, countercurrent exchange in carotid retes, or radiation of heat with external structures such as enlarged pinnae (Crawford, 1972; Tattersall et al., 2006; Weissenböck et al., 2010). These patterns are less well understood in ectotherms, but some studies have found that important body regions maintain temperature above that of the environment in colder environments, and below that of the environment in warmer environments (Dorcas and Peterson, 1997; Tattersall et al., 2006). While we know that regional heterothermy is common, even in small ectotherms with high surface area to volume ratios (Cox et al., 2023), we still know very little about how the magnitude of regional heterothermy varies as a function of changing environmental temperatures. Does regional heterothermy decline or increase as environmental temperatures get warmer? The answer to this question not only has implications for how different body regions may function as the environment shifts but also may help us understand the extent to

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which regional heterothermy is driven by physiological versus physical mechanisms. For example, because temperature in the wings and feet of emperor penguins is closer to environmental temperature than to core temperature across a broad range of environmental temperatures, this suggests that core body temperature is regulated (Pongani et al., 2003). Accordingly, examining the temperature dependence of regional heterothermy can provide insight into how it is regulated, particularly for groups of organisms such as snakes where the mechanisms that give rise to and maintain regional heterothermy remain poorly understood.

In small-bodied, elongate ectotherms such as some species of snakes, regional heterothermy should be difficult to maintain because of both the small body size and the high surface area to volume ratio, which would confer low thermal inertia (Cox et al., 2020; Porter and Kearney, 2009; Stevenson, 1985). Yet, regional heterothermy has been documented in a taxonomically diverse array of snake species, most often by comparing the temperature of the cloaca and head (*Pituophis melanoleucus*: Ashton, 1998; *Charina bottae*: Dorcas and Peterson, 1997; *Thamnophis sirtalis*: Gregory, 1990; *Masticophis flagellum*: Hammerson, 1977; *Coluber constrictor*: Hammerson, 1987; Australian pythons of the genera *Liasis*, *Aspidites* and *Morelia*: Johnson, 1973; *Thamnophis elegans*: Peterson, 1987; *Pantherophis guttatus*: Roark and Dorcas, 2000). One pattern that has emerged from this body of research is that in warm climates, the head temperature of squamate reptiles, including snakes, is often less than that of the cloaca (e.g. *Liasis childreni*, *Liasis fuscus*, *Aspidites melanocephalus* and *Morelia spilotes*: Johnson, 1973; *Oxyuranus scutellatus*: Johnson, 1975a; *Candoia aspera*, *Candoia carinata* and *Boiga irregularis*: Johnson, 1975b; Tattersall et al., 2006), but in cool climates, the head temperature is often greater than that of the cloaca (e.g. *Pantherophis guttatus*: Dorcas and Peterson, 1997; *Thamnophis elegans* and *Thamnophis sirtalis*: Gregory, 1990; Tattersall et al., 2006). One implication of this pattern is that head temperature is regulated, while cloacal temperature conforms to ambient conditions (Dorcas and Peterson, 1997; Gregory, 1990; Tattersall et al., 2006). However, relatively few studies have examined patterns of regional heterothermy across a range of environmental conditions in the same species or population, and, as far as we are aware, the thermal dependence of regional heterothermy has never been tested in a controlled laboratory environment where physical versus regulatory mechanisms of control can be adequately parsed.

Our previous research documented the surprising presence of regional heterothermy in the extremely small-bodied ring-necked snake (*Diadophis punctatus*). This species has a broad distribution across much of North America, and is frequently found under cover objects, such as rocks or logs. Because this species has low thermal inertia as a result of its small body size (adult mass is typically less than 20 g) and an elongate body form, regional heterothermy should be difficult to maintain (Cox et al., 2023). However, we found that these snakes display pronounced regional heterothermy, where head temperatures average 2.7°C greater than that of the cloaca, a difference seemingly unlinked to behavioral thermoregulation or environmental variation (Cox et al., 2023). We know from this previous work that regional heterothermy in ring-necked snakes is not maintained through behavioral thermoregulation because it is still present in a thermally uniform laboratory environment after many hours. This suggests that regional heterothermy is maintained either by differences in the material properties of the head and cloaca or via physiological regulation (endogenous heat production and/or heat shunting mechanisms).

To develop a deeper understanding of the mechanisms that give rise to regional heterothermy in the ring-necked snake, we examined the thermal dependence of regional heterothermy in live snakes in both the lab and the field. We also measured regional heterothermy in a preserved specimen that would be incapable of physiological regulation. In doing so, we were able to test several predictions that would be true if regional heterothermy in this species is produced by physiological regulation. First, we predicted that cloacal and head temperature would be differentially regulated given their different functions. Second, we predicted that a preserved snake would have diminished or absent regional heterothermy if either the head or cloacal temperature was physiologically regulated. Third, we predicted that the relationship between head or cloaca temperature and ambient temperature would be different if head and cloaca temperatures were differentially regulated, which would manifest as lower thermal sensitivity for the head compared with the cloaca (or vice versa). Finally, we predicted that the relationship between head temperature and cloaca temperature would be reversed at temperatures near the upper thermal limit (but not near the lower thermal limit) because previous research in snakes (Dorcas and Peterson, 1997; Gregory, 1990; Tattersall et al., 2006) has suggested that cooling mechanisms might decrease head temperatures near or above thermal limits. We found that regional heterothermy declined as environmental temperature increased in both the field and the laboratory, that the head temperature changed less with increasing temperature compared with the cloaca temperature, and that regional heterothermy was not present in a dead snake. These results are consistent with differential physiological regulation of head and cloaca temperature in ring-necked snakes.

MATERIALS AND METHODS

Study system

We studied regional heterothermy in a population of ring-necked snakes, *Diadophis punctatus* (Linnaeus 1766), in Giles County, VA, USA. This population is concentrated at a rocky hillside at an elevation of 1175 m near Mountain Lake Biological Station. Ring-necked snakes likely use this site as a hibernaculum, and this location has been the subject of previous investigations of physiology, ecology and behavior in this species (Cox et al., 2021, 2023, 2018). Most of the site is unvegetated, with some warm season grasses, mullein (*Verbascum thapsus*), staghorn sumac (*Rhus typhina*) and a few other herbaceous plants and grasses. The site is surrounded by closed canopy deciduous forest that is typical of the southern Appalachian Mountains. All research was approved by the University of Virginia Animal Care and Use Committee (Protocol 3927-04-21 to E. D. Brodie III).

Regional heterothermy in the field

We surveyed snakes at the field site in the morning (09:00–11:00 h) and afternoon (14:00–16:30 h) of both 6 and 7 June 2023. Ambient temperatures measured at the beginning of fieldwork were 20.1°C and 26.7°C in the morning and afternoon of 6 June, respectively. Ambient temperatures at the beginning of fieldwork were 25.9°C and 28.7°C in the morning and afternoon of 7 June, respectively. Ambient temperature was measured using an Acurite Humidity monitor (00632SBL/00655CASBL, Acurite, Lake Geneva, WI, USA) from a shaded spot in the field site. Snakes ($n=56$) were located by turning rocks in the field site. When a snake was located, it was quickly picked up with gardening gloves to ensure the person holding the snake did not affect the snake's body temperature, and the start time was quickly noted. Snakes were shaded by an umbrella during processing to further minimize body temperature change.

Two thermocouples (Type K) attached to a thermometer (Omega HH800A, accuracy $\pm 1^\circ\text{C}$) were inserted simultaneously into both the cloaca and mouth (hereafter head) of the snake by different people (C.L.C. and A.K.C.) and both the temperature and duration of handling (as the difference in time between picking up the snake and inserting the thermocouple) were recorded.

Immediately following discovery of the snake, we measured the temperature of the substrate and underside of the rock under which each snake was found. We used a 2×2 grid, with each grid cell 7.5 cm in height and width, yielding up to four measurements of temperature for each rock and corresponding substrate. For rocks that were larger than the grid, we centered the grid on the part of the rock and substrate where the snake was found. For rocks that were smaller than the grid, we only measured temperature of the rocks and substrate, and omitted grid cells outside the rock. We measured temperature using an infrared thermometer (General Tool and Instruments IRT217, accuracy $\pm 2^\circ\text{C}$, distance:spot ratio of 10:1, fixed emissivity of 0.95) held approximately 1 cm from the surface that was being measured. An umbrella was used to shade the rock and substrate during measurement to minimize temperature change from solar radiation. Finally, each rock was marked with a unique ID to facilitate precise release at the site of capture.

Following collection of field data, snakes were assigned unique IDs, placed in hosiery, and transported to the laboratory at Mountain Lake Biological Station. In the laboratory, we determined sex, snout–vent length (SVL), body mass, gravidity status, number of eggs if gravid, ecdysis status and whether or not the snake was scarred. We determined sex by palpating and examining the base of the tail for the presence of hemipenes. SVL was measured to the nearest mm by stretching snakes along a meter-long measuring stick. We measured body mass using a digital scale (AWS-100 Digital Scale) to the nearest 0.1 g. Snakes that were greater than 200 mm in SVL were classified as adults (Ernst and Ernst, 2003). We determined gravidity status and determined the number of eggs by gently palpating the abdomen of snakes. We determined whether a snake was near shedding or in ecdysis by the presence of an opaque ocular scale and dull body coloration. Scars were noted via visual examination of body surfaces. Following the collection of these data, snakes were placed in separate plastic containers (1.2 l in volume) with holes in the lid and with a paper towel as a refuge and substrate. Individual containers were placed in a climate-controlled room maintained at 25°C with a 12 h:12 h light:dark cycle. Two snakes died before the end of the experiment, for unknown reasons. The snakes were kept in the laboratory for 72 h and released adjacent to the rock under which they were originally captured.

Thermal dependence of regional heterothermy in a preserved specimen

We collected a ring-necked snake from the study population that was found dead on the adjacent road in 2018. This snake was preserved in 10% buffered formalin and stored in 70% ethanol, and kept in the herpetology teaching collection at Florida International University (Field Number CLC1374). This individual was in the same size range (SVL=285 mm) as the other snakes in this study, and largely resembled a live snake in shape and condition. We used this specimen to evaluate whether a dead snake, devoid of any potential for physiological regulation, could still display regional heterothermy. If so, this would indicate that different material properties of the head and cloaca, which would likely remain present in a dead snake, are responsible for regional heterothermy in this species. While we anticipated the snake would eventually reach equilibration with environmental temperatures, it is possible that the

time to equilibration at the test temperature would differ between different body regions based upon different material properties of the cloaca versus the head. Additionally, the head might interact with the probe differently from the cloaca, creating variation in measured temperatures. Prior to the experiment, the snake was rinsed with water and blotted dry with paper towels. Separate thermocouples (Type K) attached to a thermometer (Omega HH800A, accuracy $\pm 1^\circ\text{C}$) were simultaneously inserted into the head and the cloaca of the specimen. We then placed the snake into an incubator (Percival Scientific) set at 25, 20 and 30°C , in that order. Preliminary trials demonstrated that body temperature of the preserved snake stabilized after 10 min in the incubator, so we recorded the final temperature used for analysis after 15 min of incubation at each trial temperature. We also confirmed that the incubator achieved the target temperature using an infrared thermometer (General Tool and Instruments IRT217, accuracy $\pm 2^\circ\text{C}$, distance:spot ratio of 10:1, fixed emissivity of 0.95).

Thermal dependence of regional heterothermy in live snakes

Experiments to determine the thermal dependence of regional heterothermy in live snakes took place the day following their capture in the field. Snakes were fasted during this period to ensure that they were post-absorptive, and we did not detect any food items in the snakes using abdominal palpation. We measured both head and cloaca body temperature for snakes that were maintained in an incubator for 1 h at 20, 25 and 30°C . To remove potential order effects, prior to measurements, snakes were assigned to group A (20, 25, 30°C), group B (25, 30, 20°C) or group C (30, 20, 25°C). We found that trial order did not impact our estimates of regional heterothermy (all $P > 0.45$) using ANOVA. For each temperature treatment, the plastic container containing the snake was placed into an incubator set to the particular temperature treatment for 1 h. We used either Percival incubators (20 and 30°C treatments) or the temperature-controlled room (25°C treatment). After 1 h had elapsed, we removed the snakes from the incubator individually and measured head and cloaca temperature simultaneously by inserting separate thermocouples in each orifice (C.L.C. and A.K.C. conducted this procedure, while wearing gloves). For this and other lab-based experiments (see below), we did not record handling duration because we could more rapidly measure these temperatures in the lab compared with the field. We were able to measure head and cloaca temperatures of all snakes within a group within 10 min, such that the time in the incubator ranged between 00:59 and 01:09 h. The ambient temperature in the laboratory was approximately 22°C . Following measurement, snakes were returned to the climate-controlled holding room (25°C) for a 1 h recovery period between trials. The holding room temperature was similar to average body temperature and thermal preference of this population of snakes (Cox et al., 2018) and within the range of environmental temperatures experienced by these snakes at this time of year (see above).

During the second day of captivity, we measured the head–cloaca temperature difference at 15 and 32°C , near the lower and upper thermal limits, respectively. Our previous work revealed that the voluntary thermal maximum for snakes in this population was $32.09 \pm 0.25^\circ\text{C}$ (mean \pm s.e.m.), and the critical thermal minimum was $11.48 \pm 0.41^\circ\text{C}$. For our experimental procedure, all snakes experienced the upper temperature last because this temperature is likely stressful and might have impacted our results at the lower temperature. The snakes were first placed in the minimum temperature (15°C) incubator, and head and cloacal temperatures were measured after 1 h. After 1 h of recovery in the holding room at

25°C, they were moved into the upper temperature (32°C) incubator, and head and cloaca temperatures were measured after 1 h. Both head and cloaca temperatures were measured as in previous experiments, with both thermocouples simultaneously inserted into the head and cloaca of the snake by two people (C.L.C. and A.K.C.). Following measurement, snakes were returned to the climate-controlled holding room at 25°C. Snakes were then released the following day to their original capture location.

Statistical methods

The head–cloaca temperature difference was calculated as the difference in temperature between the head and cloaca, such that positive values indicated that head temperature was greater than cloaca temperature and negative values indicated the opposite pattern. We also estimated the deviation between either head or cloaca temperature and ambient temperature.

For the field data, we compared duration of handling with head temperature, cloaca temperature and the difference in temperature between the head and cloaca using ordinary least-squares regression, with duration of handling as the independent variable and head temperature, cloaca temperature or the head–cloaca temperature difference as the dependent variable. We also tested whether the head–cloaca temperature difference varied with temperature of either the underside of the rock that they were found under or the substrate under the rock, and by sex, age class (juvenile versus adult), ecdysis status and presence of scars using general linear models, with head temperature, cloaca temperature or the head–cloaca temperature difference as the dependent variable and these snake characteristics as the independent variables.

For the laboratory data, we used a repeated-measures approach for any model that included multiple measurements per individual. For both the normal range of temperatures (20–30°C) as well as those near the thermal limits (15 and 32°C), we compared head and cloaca temperatures, the head–cloaca temperature difference, and the deviation of head and cloaca temperatures from ambient temperature at each treatment temperature using general linear mixed models with ID as a random factor, equivalent to a repeated measures ANOVA (rmANOVA). To test for differential temperature dependence of head and cloaca temperatures, we constructed a model with temperature treatment, body region and the interaction between temperature treatment and body region as independent variables, with ID as a random nested factor and body temperature as a dependent variable. For all analyses, we ensured that our data conformed to the assumptions of the statistical models. We assessed significance as $\alpha < 0.05$. All statistical analyses were conducted in JMP Pro 17 (SAS Statistical Institute, Cary, NC, USA).

RESULTS

Environmental temperature, snake characteristics and regional heterothermy

While we found no relationship between handling time and head temperature in the field ($F_{1,50}=1.3458$, $P=0.2511$), there was a significant positive relationship between handling time and cloaca temperature ($F_{1,49}=1.3458$, $P=0.0422$). This led to a significant negative relationship between handling time and regional heterothermy in the field ($F_{1,50}=1.3458$, $P=0.0020$); however, excluding all points where handling time exceeded 1 s caused the relationship between handling time and regional heterothermy to be no longer significant ($F_{1,44}=3.6292$, $P=0.0633$). For all remaining analyses of temperature and the head–cloaca temperature difference in the field, we therefore excluded all observations ($n=10$) where handling time exceeded 1 s to avoid bias from handling.

We found that environmental temperature and body size, but no other snake characteristics, impacted body temperature and the head–cloaca temperature difference of snakes in the field. Both head ($R^2=0.6561$, $P<0.0001$) and cloaca ($R^2=0.7439$, $P<0.0001$) temperature significantly increased with the temperature of the substrate under the rocks that they used as refuges (Fig. 1). Similarly, both head ($R^2=0.6830$, $P<0.0001$) and cloaca ($R^2=0.7723$, $P<0.0001$) temperature significantly increased with the temperature of the underside of the rocks that they used as refuges (Fig. 1). The head–cloaca temperature difference declined significantly with both substrate ($R^2=0.2276$, $P=0.0003$) and rock ($R^2=0.2345$, $P<0.0002$) temperatures. While there was no relationship between head temperature and either SVL ($F_{1,44}=2.4572$, $P=0.1241$) or body mass ($F_{1,44}=2.1850$, $P=0.1465$), cloaca temperature was positively associated with both SVL (marginally significant, $F_{1,44}=4.0551$, $P=0.0503$) and body mass ($F_{1,44}=4.6069$, $P=0.0375$). However, we found no significant relationship between head–cloaca temperature difference and sex ($F_{3,42}=1.8407$, $P=0.1545$), ecdysis ($F_{1,42}=1.2685$, $P=0.1545$), life stage ($F_{1,42}=1.3089$, $P=0.2589$) and the presence of scars ($F_{1,42}=0.0143$, $P=0.9054$).

Regional heterothermy in a preserved snake specimen

We found little evidence that a preserved snake specimen displayed regional heterothermy. We found that the head and cloaca temperatures during the 20°C trial were 19.4 and 20.5°C, respectively. Head and cloaca temperatures during the 25°C trial were 25.3 and 24.6°C, respectively. Finally, head and cloaca temperatures during the 30°C trial were 29.5 and 29.8°C, respectively. These differences between head and cloaca temperatures were substantially lower than those observed in live snakes and were within the expected measurement error of our thermometers.

Temperature dependence of regional heterothermy in a controlled environment

We found that the head–cloaca temperature difference generally decreased with increasing temperature, which was driven by head temperature becoming more similar to ambient temperature as ambient temperature increased (Fig. 2A,B). Because of the relationship between body size and the head–cloaca temperature difference, we excluded juveniles ($n=13$) from all analyses of temperature measurements in the lab. We found that head temperature was significantly higher than cloaca temperature at all five temperatures (15°C, $F_{1,42}=128.6553$, $P<0.0001$; 20°C, $F_{1,42}=204.344$, $P<0.0001$; 25°C, $F_{1,42}=224.6974$, $P<0.0001$; 30°C, $F_{1,42}=55.4693$, $P<0.0001$; 32°C, $F_{1,42}=159.5028$, $P<0.0001$). Notably, cloaca temperatures at 30 and 32°C were very similar (Fig. 2A), despite the increase in ambient temperature. The head–cloaca temperature difference decreased with increasing ambient temperature between 20°C and 32°C ($F_{4,39}=19.21$, $P<0.0001$), with the head temperature an average of 2.8°C greater than that of the cloaca at 20°C and 1.7°C greater at 30°C (Fig. 2A,B). However, the head–cloaca difference at 15°C (2.3°C) was less than the difference at 20°C. We also found that the difference between head temperature and ambient temperature decreased significantly ($F_{4,39}=51.4758$, $P<0.0001$) with increasing ambient temperature, so that head temperature was similar to ambient temperature at 32°C (Fig. 2C). In contrast, while cloaca temperature varied significantly among ambient temperatures ($F_{4,39}=112.3695$, $P<0.0001$), cloaca temperature was slightly above ambient temperature (~0.5°C) between 15 and 20°C, and cloaca temperature was below ambient temperature at 30°C and 32°C (Fig. 2C).

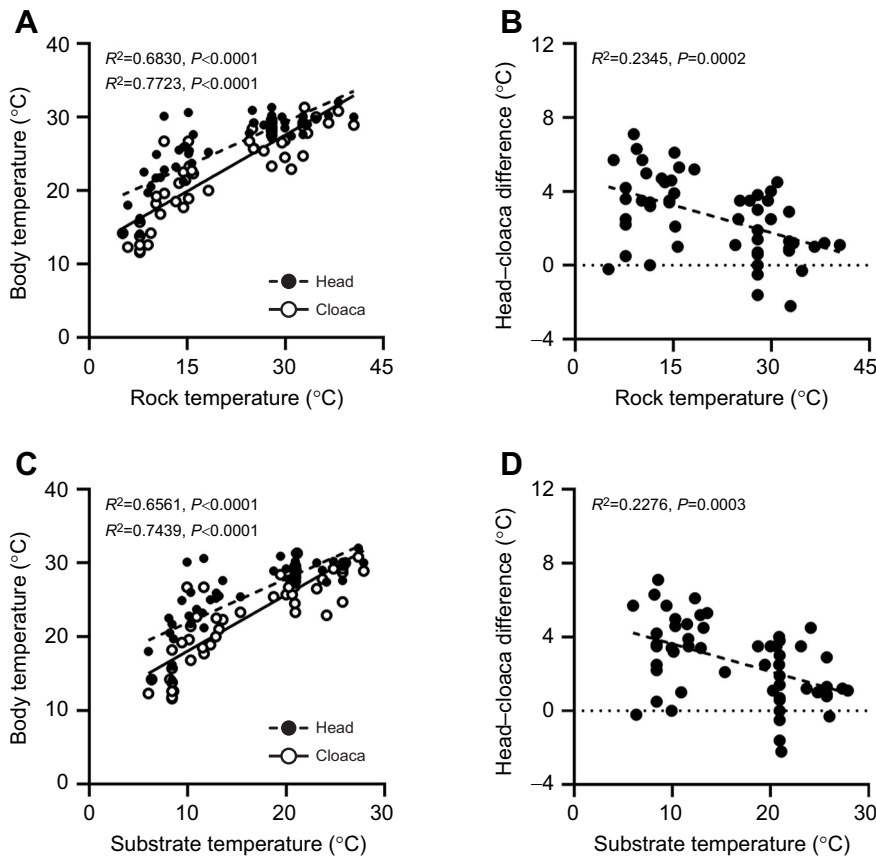


Fig. 1. Relationship between environmental temperature and both head and cloaca body temperature and regional heterothermy of ring-necked snakes ($n=57$) in the field. (A,C) Head and cloaca body temperature versus environmental temperature (A, rock; C, substrate). (B,D) Regional heterothermy, defined as the difference in temperature between the head and the cloaca, versus environmental temperature (B, rock; D, substrate). Regional heterothermy tends to decrease with increasing environmental temperature. Statistical insets are based upon linear regression analysis.

Decreasing regional heterothermy with increasing ambient temperature seems to be driven by differences in the temperature dependence of head and cloacal temperatures. We found that both body region (head or cloaca) and temperature treatment impacted body temperature (body region: $F_{4,430}=643.9933, P<0.0001$; temperature treatment: $F_{4,430}=2266.103, P<0.0001$), as would be predicted. However, we also found that the impact of temperature treatment varied based upon body region (interaction between body region and temperature treatment: $F_{4,430}=12.3086, P<0.0001$). Head temperature changed less between 20 and 30°C (7.1°C; Table 1) than did cloacal temperature (8.2°C; Table 1).

DISCUSSION

We found evidence that ring-necked snakes differentially regulate head and cloaca temperature using physiological (endogenous) mechanisms. We found that head temperature differed more from ambient temperature and changed less with increasing temperature compared with cloaca temperature in both laboratory and field settings. These same patterns were not present in a dead (preserved) snake, suggesting that differences in head and cloaca temperatures did not arise from differences in the material properties of the head and cloaca. If ring-necked snakes can indeed regulate their head temperature independently of the ambient environment, this may

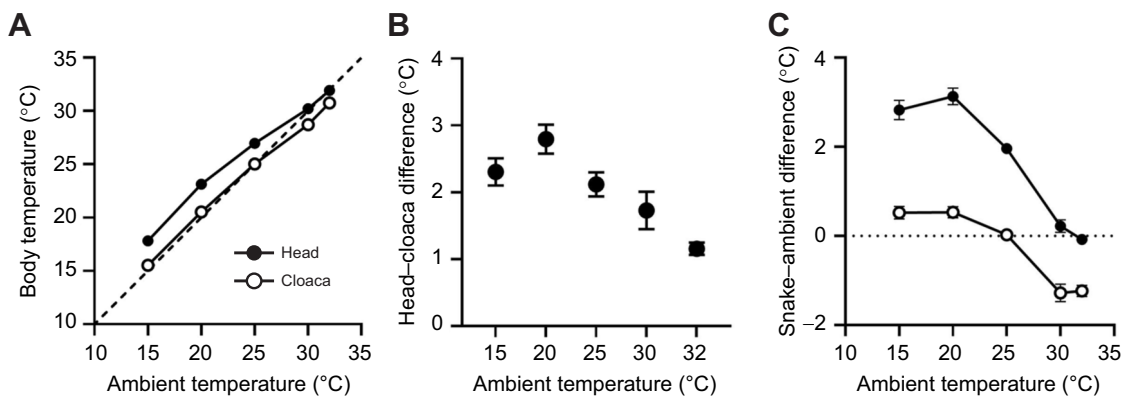


Fig. 2. Relationship between ambient temperature in an incubator and body temperature, regional heterothermy and body–ambient temperature difference of adult ring-necked snakes ($n=44$). (A) Head and cloaca body temperature, (B) head–cloaca temperature difference and (C) body–ambient temperature difference versus ambient temperature. The difference between head temperature (but not cloaca temperature) and environmental temperature decreases with increasing temperature, such that regional heterothermy decreases with increasing temperature. Symbols are means \pm s.e.m. Error bars are obscured when the s.e.m. is smaller than the symbols.

Table 1. Temperature of the head and the cloaca at five ambient temperatures

Ambient temperature (°C)	<i>n</i>	Head	Cloaca
15	43	17.83±0.21	15.52±0.14
20	43	23.13±0.19	20.53±0.13
25	43	26.96±0.10	25.03±0.09
30	43	30.22±0.14	28.72±0.20
32	43	31.92±0.12	30.77±0.13

Data are means±s.e.m.

function to maintain neural function in the brain and anterior central nervous system across a broad range of environmental temperatures.

Although some previous work has highlighted the importance of head temperature to neural function in squamate reptiles (Andry et al., 1971; Tattersall et al., 2006), research in this area is surprisingly sparse. In some lizard species, high temperatures can impact neuronal development and cognition (Dayananda and Webb, 2017; Sanger et al., 2021), and thermal stress can induce expression of cellular protective heat shock proteins such as HSP70 at multiple life stages (Akashi et al., 2016; Simoniello et al., 2016). Far less is known about how variation in temperature within the normal activity range impacts the function of the brain in ectotherms, although presumably brain function is diminished by cool temperatures (Angilletta, 2009; Angilletta et al., 2019). One of the challenges for studying the temperature dependence of brain function is the absence of performance assays that would be practical for measurements across multiple temperatures. Future work that solves these issues will help clarify the potential adaptive function of regional heterothermy in squamates.

Head temperature in squamate reptiles can be regulated by behavioral thermoregulation, endogenous physiological processes, or a combination of the two (Porter and Witmer, 2015; Tattersall et al., 2006). Cooling the head can be accomplished by respiratory evaporative cooling such as gaping, panting or water vapor loss through the nasal passages (Tattersall et al., 2006). Basking with the head exposed and the body in a thermal refugium is the primary means whereby behavioral thermoregulation can cause regional heterothermy (Gregory, 1990; Heath, 1964). Physiologically, increasing peripheral blood flow during basking can raise body temperature, which could generate regional heterothermy if increased peripheral flow was limited to certain body regions (Bartholomew and Tucker, 1963; Dzialowski and O'Connor, 1999; Seebacher, 2000). Blood flow patterns can also be altered in such a way as to generate and maintain regional heterothermy. For example, there is a muscle in the jugular vein in some lizard species that can restrict venous blood returning from the head to the body, which can help maintain elevated head temperatures (Heath, 1964, 1966). Finally, counter-current heat exchange between the carotid arteries and jugular veins can help maintain regional heterothermy (Crawford, 1972; Heath, 1964, 1966). We found that head and cloacal temperatures did not differ from one another across different ambient temperatures in fluid-preserved snake, consistent with physiological regulation of regional heterothermy. It is important to note that we only measured body temperature in a single preserved snake, and future research should both increase sample size and assess regional heterothermy in recently dead snakes that have not been preserved. However, while our data cannot directly reveal the mechanism underlying the maintenance of regional heterothermy in ring-necked snakes, previous work in squamates suggests that blood circulation patterns may be responsible for the observed differences in head and cloaca

temperatures (Crawford, 1972; Heath, 1964, 1966; Tattersall et al., 2006). This is further bolstered by the proximity of the head to the heart in this species (Seymour, 1987), which suggests that shunting of blood to the head may be an efficient way to increase head temperature compared with that of the posterior of the body.

Unlike a previous study in a related species (Dorcas and Peterson, 1997), we did not observe a reversal of head and cloacal temperatures near the thermal limits (i.e. the 15 and 32°C trials) in ring-necked snakes. This may be because we used the voluntary thermal maximum (VT_{max}) as a proxy for the upper thermal limit, but VT_{max} is by definition below the 'true' upper thermal limit, which is usually measured as the critical thermal maximum or the upper lethal temperature (Camacho and Rusch, 2017; Cox et al., 2020; Logan et al., 2021; Neel et al., 2020). Hence, we may not have placed snakes in a warm enough environment to instigate cooling of the head simply because VT_{max} may be close to the thermal optimum for brain performance in ring-necked snakes. It is worth noting that cloaca temperature at the warmest temperatures (30 and 32°C) was lower than the ambient temperature, which could have been caused by evaporative cooling from the cloaca.

Perhaps the most intriguing result of our study was the fact that head temperature remained substantially higher than ambient temperature, even after prolonged exposure to a constant laboratory environment. Surprisingly, after 24 h at 25°C in the temperature-controlled holding room, snakes still had head temperatures that were 2.1°C warmer than ambient. This deviation from ambient temperature after such a long period in a thermally constant environment without a heat source seems difficult to explain solely by invoking cardiovascular adaptations. Although postprandial thermogenesis from specific dynamic action has been documented in snakes and lizards (Brown and Au, 2009; Stuginski et al., 2011; Tattersall et al., 2004), and in principle could be the source of warmer blood in the head, this seems unlikely to explain our results because our study animals were fasted. Thus, we hypothesize that ring-necked snakes employ some limited form of metabolic heat production, perhaps combined with the shunting of blood to the head, to elevate head temperature, and further experiments are required to understand this phenomenon.

Our work demonstrates that regional heterothermy in a diminutive snake persists across ecologically relevant temperatures, but declines as temperature increases, implicating physiological regulation of head and cloacal temperatures. These results have several implications for thermal biologists who study ectotherms, particularly vertebrate ectotherms, which tend to be larger in body size and have closed circulatory systems. First, the anatomical location where temperature is measured is likely important (Gregory, 1990; Hammerson, 1977; Peterson, 1987), even in small-bodied ectotherms with minimal thermal inertia (Cox et al., 2023; this study). Specifically, we found that the relationship between environmental temperature and head temperature differed from the relationship between environmental and cloacal temperature. While the anatomical location of measurement might not be important for all studies, it is likely to be important in some contexts and should be considered as part of experimental design. Second, accounting for regional heterothermy in small ectotherms might give a more holistic and nuanced understanding of how these animals use their environment. Finally, because of the crucial role of temperature for organismal function, our work suggests that the relationship between environmental temperature and regional heterothermy might have ramifications for performance. Future work that studies the mechanisms underlying elevation of head temperature in cooler environments and how regional heterothermy influences

fitness will enrich our understanding of the ecophysiology of ectotherms.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

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Data availability

Data are available from figshare: <https://doi.org/10.6084/m9.figshare.26866831.v1>

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