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RESEARCH ARTICLE

A diminutive snake species can maintain regional heterothermy in both homogeneous and heterogeneous thermal environments

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ABSTRACT

Regional heterothermy is a pattern whereby different body regions are maintained at different temperatures, often to prioritize the function of certain body parts over others, or to maximize the function of organs and tissues that vary in thermal sensitivity. Regional heterothermy is relatively well understood in endotherms, where physiological mechanisms maintain heterogeneity. However, less is known about regional heterothermy in ectotherms, where behavioral mechanisms are more important for generating thermal variation. In particular, whether small and elongate ectotherms with high surface area to volume ratios such as diminutive snakes can maintain regional heterothermy, despite rapid thermal equilibration, is not yet known. We measured regional variation in body temperature and tested whether environmental heterogeneity is used to generate regional heterothermy in the ring-necked snake (*Diadophis punctatus*) using both field and laboratory studies. We found that ring-necked snakes have robust regional heterothermy in a variety of contexts, despite their small body size and elongate body shape. Temperature variation along the length of their bodies was not detectable when measured externally. However, snakes had higher mouth than cloacal temperatures both in the field and in laboratory thermal gradients. Further, this regional heterothermy was maintained even in ambient laboratory conditions, where the thermal environment was relatively homogeneous. Our results indicate that regional heterothermy in ring-necked snakes is not solely driven by environmental variation but is instead linked to physiological or morphological mechanisms that maintain regional variation in body temperature irrespective of environmental context.

KEY WORDS: Body temperature heterothermy, Squamate, Thermal biology, Thermal heterogeneity

INTRODUCTION

Biochemical reaction rates that underly physiological functions are strongly influenced by temperature (Shapley, 1924; Angilletta et al., 2010; Dell et al., 2011) and can be tightly linked to fitness (Knies et al., 2009; Logan et al., 2014, 2018; Gilbert and Miles, 2017; MacLean et al., 2019; Campbell-Staton et al., 2020). However, not

all physiological systems within organisms respond to temperature in the same way, and they can vary in their importance in terms of survival and fitness (Penick et al., 1996; Ponganis et al., 2003; Streicher et al., 2012; Cheslock et al., 2021). As such, some organisms exhibit regional heterothermy, which occurs when body regions are maintained at different temperatures, often to prioritize physiological function of certain organ systems or to maintain different tissues at their respective thermal optima (Clarke and Pörtner, 2010; Boyles et al., 2011). For example, many aquatic vertebrates maintain warmer core and head temperatures than external temperatures, prioritizing warmth in crucial body regions (e.g. leatherback sea turtles: Frair et al., 1972; Neill and Stevens, 1974; green sea turtles: Standora et al., 1982; grey whales: Heyning, 2001; emperor penguins: Ponganis et al., 2003; lamnid sharks, tunas and billfishes: Dickson and Graham, 2004). Some species can slow warming rates in critical body regions such as the head or vital organs when exposed to inhospitably warm temperatures, which can be accomplished by behavioral mechanisms such as panting (Crawford, 1972), manipulation of thermal windows such as the ears of African elephants (Weissenböck et al., 2010), or positioning of body parts into cooler microclimates such as in aerially exposed sea stars (Pincebourde et al., 2013). Hence, regional heterothermy is often a strategy to retain heat in cold environments and to lose heat in hot environments. For thermoregulating ectotherms, regional heterothermy may allow rapid warming of critical body areas, such as the head, to prioritize function of the central nervous system (Hammerson, 1977, 1987; Dorcas and Peterson, 1997). Regional heterothermy has been relatively well studied in endotherms, where temperature variation is maintained by physiological mechanisms (Clarke and Pörtner, 2010; Boyles et al., 2011). However, less is known about the presence and function of regional heterothermy in ectotherms, where behavior often plays a dominant role.

Although not well understood, regional heterothermy has been documented in a variety of ectotherms (e.g. lizards: Webb et al., 1972; snakes: Johnson, 1973; alligators: Spotila et al., 1977; sea stars: Pincebourde et al., 2013), and has been linked to both behavioral and physiological mechanisms. Behaviorally thermoregulating ectotherms can exploit thermal heterogeneity in the environment to generate and maintain regional heterothermy by exposing only certain body regions, such as the head, to solar radiation (Hammerson, 1977, 1987). Specialized behaviors, such as panting or gaping, can cool the head via evaporative cooling (Spotila et al., 1977). Either independently or in concert with behavior, physiological mechanisms such as patterns of blood flow, including intracardiac shunts and countercurrent heat exchange, have been implicated in regional heterothermy in ectotherms (Pough, 1976; Spotila et al., 1977). Yet, regional heterothermy might not be possible for certain body sizes and shapes, regardless of mechanism. For example, elongate ectotherms have a

high surface area to volume ratio and their body temperature will rapidly equilibrate with environmental temperatures, potentially minimizing the opportunity for regional heterogeneity in temperature (Stevenson, 1985; Porter and Kearney, 2009; Cox et al., 2020). Despite this fact, regional heterothermy has been documented in several species of snakes (e.g. Australian pythons of the genera Liasis, Aspidites and Morelia: Johnson, 1973; *Masticophis flagellum*: Hammerson, 1977; *Coluber constrictor*: Hammerson, 1987; *Thamnophis elegans*: Peterson, 1987; *Thamnophis sirtalis*: Gregory, 1990; *Charina bottae*: Dorcas and Peterson, 1997; *Pituophis melanoleucus*: Ashton, 1998; *Pantherophis guttatus*: Roark and Dorcas, 2000), although never in extremely small-bodied species. Diminutive snakes have both a high surface area to volume ratio and extremely low thermal inertia, which should minimize the potential for regional heterothermy (Stevenson, 1985; Porter and Kearney, 2009; Cox et al., 2020). Whether small-bodied and elongate ectotherms can exhibit regional heterothermy, the potential function of this heterothermy, and the mechanisms by which it could be generated and maintained remain poorly understood.

We studied the environmental and organismal correlates of regional heterothermy in the ring-necked snake (*Diadophis punctatus* Linnaeus 1766). Ring-necked snakes are small bodied and found across much of temperate and subtropical North America (Ernst and Ernst, 2003), and are perhaps best known for their deimatic coloration (Cox et al., 2021). Although in parts of their range they can approach 1 m in length, individuals in most populations weigh less than 20 g and are less than 50 cm in total length (Ernst and Ernst, 2003). Previous research has found that ring-necked snakes, like other snake species (Huey et al., 1989; Webb and Shine, 1998b), thermoregulate under cover-object refuges by selecting rocks based upon their physical characteristics (Cox et al., 2018). Because they actively thermoregulate in thermally heterogeneous environments, it is possible that ring-necked snakes could generate regional heterothermy by harnessing this variation. However, whether such a small and elongate ectotherm could maintain thermal heterogeneity, despite rapid thermal equilibration, is not yet known.

We sought to test whether and how ring-necked snakes achieve regional heterothermy. First, we tested whether individual snakes display regional heterothermy, despite their small body size. To this end, we thoroughly characterized external body temperature along the length of the body, including both dorsal and ventral surfaces, as well as internal body temperature measured in the mouth and the cloaca. If ring-necked snakes have regional heterogeneity in body temperature, this implies that the thermal inertia of a large body size is not a prerequisite for regional variation in body temperature, even in an elongate ectotherm. Second, we tested for a relationship between field body temperature and environmental temperature, as well as for a relationship between regional heterothermy and environmental heterogeneity. If regional heterothermy and environmental temperature variation are linked, then this implies that snakes may be behaviorally exploiting environmental heterogeneity to generate regional heterothermy. Third, we examined the relationship between regional heterothermy in the field and organismal characteristics such as sex and parturition status to gain insight into its potential function in our study species. Fourth, we tested whether the relationship between body temperature variation and the environment persisted in a laboratory thermal gradient. We predicted that snakes would position themselves in the gradient in such a way that they maintain regional heterothermy. Finally, we tested whether regional heterothermy was maintained in an ambient (thermally homogeneous) laboratory environment. The persistence of regional heterogeneity of body temperature in a thermally homogeneous environment would imply a

role for physiological or morphological mechanisms in maintaining heterothermy.

MATERIALS AND METHODS
Collection of field data and captive maintenance
We studied a population of ring-necked snakes from an exposed, south-facing slope at 1175 m elevation in Giles County, VA, USA. Vegetation cover was minimal, with some staghorn sumac shrubs and mixed warm season grasses. Our previous work revealed that snakes use the rocks on this slope for thermoregulation, and the preponderance of gravid females in late spring and early summer suggests that this location is both a hibernaculum and oviposition site (Cox et al., 2018). We collected a total of 17 snakes for this study (n=10 females and n=7 males) between 29 June and 1 July 2022.

Snakes were collected by turning over rocks in appropriate microhabitats. Ambient temperature during the collection period was taken from a weather station at Mountain Lake Biological Station located ca. 1 km from the field site at a similar elevation (https://mlbs.virginia.edu/meteorological-data, accessed 14 July 2022). If a rock had a snake(s) underneath it, the snake was captured and internal body temperature was measured in two different body regions by inserting thermocouples (Type K) attached to a thermometer (Omega HH800A, accuracy ±1°C) simultaneously into both the cloaca and mouth of the snake by two people. For both the mouth and cloaca, the thermocouple was inserted about 1 cm into the orifice, and care was taken to ensure that the thermally sensitive part of the probe was not in a part of the snake that was being grasped for restraint and thus potentially warmed by the handler's body heat. We also wore gardening gloves, which should further mitigate any warming. Immediately following measurement of internal body temperature, external (surface) body temperature was measured using an infrared (IR) thermometer (General Tool and Instruments IRT217, accuracy ±2°C, distance: spot ratio of 10:1, fixed emissivity of 0.95) held approximately 1 cm from the surface of the snake. An umbrella was used to shade the snake during measurement to minimize the temperature change from solar radiation. For each snake, we measured external body temperature at four positions (anterior, anterior midbody, posterior midbody and posterior) on both the dorsal and ventral surfaces. The anterior data point was approximately 1 cm posterior from the head, and the posterior data point was approximately 1 cm anterior to the cloaca. Midbody datapoints were approximately equidistant from adjoining points. The order of the temperature measurements (dorsal or ventral first, anterior or posterior first) was randomized prior to each trial. We recorded the time elapsed between capturing the snake and the final temperature measurement. It is worth noting that temperatures measured with a thermocouple may not be directly comparable to those measured with an IR thermometer, and so we did not statistically compare temperature measurements taken with different methods.

A second team measured the temperature of the underside of rocks and the underlying substrate for rocks with (n=13) and without (n=64) snakes, such that we measured the temperature characteristics of most rocks in the study area. We used a 4×4 grid, with each grid cell 2.5 cm in height and width, yielding up to 16 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. In the case of large rocks without snakes, we positioned the grid in the center of the rock. For rocks that were smaller than the grid, we only measured the temperature of the rocks and substrate, and omitted grid cells outside the rock. We used the IR thermometer to measure the temperature of the rocks and substrate by placing the

thermometer measurement point in the center of the grid cell about 1 cm from the surface that was to be measured. An umbrella was used to shade the rock and substrate during measurement to minimize temperature change from solar radiation.

Following field collection, snakes were transported to the lab, and all experiments took place within 48 h of capture. On the day of capture, we recorded snout–vent length (SVL), total length (TL), mass, sex, state of ecdysis, whether the snake was gravid and, if so, the number of eggs, and the presence of wounds or scars. We measured SVL and TL by stretching the snake along a measuring stick, and length was recorded to the nearest mm. Mass was measured using a digital scale to 0.1 g. We determined sex via the length and width of the base of the tail (males have longer tails that are wider and do not taper at the base) and by gently palpating the base of the tail to totally or partially extrude the hemipenes. We scored state of ecdysis by noting whether the snake was actively shedding skin or had opaque ocular scales and dull body coloration (which indicates that shedding is imminent). We determined whether a female was gravid by gently palpating the abdomen to count eggs. Finally, we noted any wounds or scars, including missing tail tips. Snakes were held in containers with lightly moistened paper towels to maintain humidity, and these containers were placed in an incubator overnight at a constant temperature of 22°C.

Laboratory thermal gradient

We tested the body temperature of snakes in a laboratory thermal gradient. Because these snakes are secretive and normally found under cover objects, our gradient was constructed to emulate the conditions in the field. Briefly, each gradient consisted of a plastic arena (82 cm×45 cm) that was partially filled with soil (collected from a location adjacent to our field site) to a depth of ca. 4 cm. Each plastic arena also had two tiles (45×45 cm) that slightly overlapped in the center of the arena and covered the surface of the substrate. Plastic arenas were arranged in pairs, and above each pair was a heat lamp that was focused on the adjoining corners of two adjacent arenas, creating a thermal gradient with a hot spot in one corner and the coolest spot in the opposite corner. Temperatures available to snakes in the thermal gradient ranged from 52.9±1.79°C at the warm

end to 16.3±0.21°C at the cooler end. Snakes were then introduced into the arena and allowed to crawl under the tiles at the center point of the gradient. We found that snakes moved from the initial point of introduction in most cases. Trial duration was 30 min, after which snake body temperature was measured using the same methods as in the field collection (see 'Collection of field data and captive maintenance', above). Because we only measured temperature at the end of the trial, we assumed that the body temperature at the end of the trial is indicative of mean temperature chosen in the gradient. We also characterized the temperature of the tile and substrate where the snake was found using the grids as in field collection (see 'Collection of field data and captive maintenance', above). Grids were always oriented such that the same cell was the hottest part of the gradient. Furthermore, we noted the posture of the snake as having its body coiled or uncoiled and whether its head was in contact with the substrate.

Regional heterothermy in a homogeneous environment

We also tested whether regional heterothermy would persist in the relatively constant environment of a field station laboratory in ambient conditions. Snakes were removed from the incubator and placed on a lab bench for ca. 5 h in a plastic container. We then measured the temperature of snakes using the same methods as in the field collection (see 'Collection of field data and captive maintenance', above). We measured ambient temperature in the laboratory using a thermometer (Casio gbdh100-1a7, accuracy ±1°C), and verified this temperature by using the IR thermometer to measure the temperature of the lid of the container, which was similar to ambient temperature. Finally, we noted the posture of the snake as either body coiled or uncoiled and whether its head was in contact with the substrate.

Statistical analyses

We analyzed mouth temperature, cloacal temperature and temperatures measured on multiple points on the external surface of the snake. We calculated mean internal and external temperature using the average of all internal and external temperature measurements, respectively. We also calculated mean dorsal and ventral surface temperatures using the average of all external temperatures on the dorsal or ventral surface,
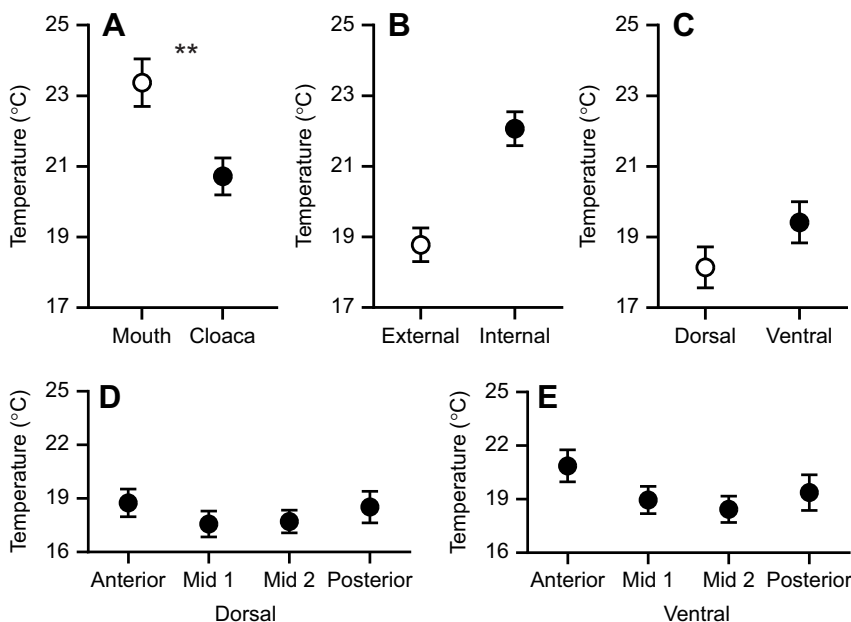
Fig. 1. Internal and external body temperature of ring-necked snakes, *Diadophis punctatus*, from southwestern Virginia, USA, measured in the field. Body temperature varied significantly between the mouth and the cloaca (A); internal and external temperatures also differed (but these values were not compared statistically; B). External temperature measured on the dorsum or ventrum (C) or dorsal and ventral regions along the length of the body (D,E) did not differ. Symbols are means±s.e.m. Asterisks denote significant differences (**$P<0.01$).

respectively. Finally, we calculated the range of internal body temperature as the difference between mouth and cloacal temperatures. We tested for the impact of handling time on mouth and cloaca temperatures, with mouth or cloaca temperature as a response variable and handling time as the independent variable. We also separately compared internal temperatures (cloaca and mouth), dorsal external temperatures, ventral external temperatures, and dorsal and ventral temperatures (taking the average of external dorsal and ventral temperatures). These comparisons were made using repeated measures analysis of variance (rmANOVA) to account for the non-independence of temperatures within a snake. During all initial analyses, we included body size as a covariate, but the body size term was never significantly associated with any measure of body temperature, and so was dropped from the final models. We compared temperatures of rocks (average temperature of rock cells), rock cells and tile cells using general linear models, with the presence of snakes as the independent variable and the mean temperature and range of temperatures of rocks, tiles, and rock and tile cells as response variables in separate models. We also used linear models to compare sex, gravidity and ecdysis (independent variables) with cloacal, mouth and range of internal temperatures (dependent variables). We

correlated mouth and cloacal temperatures and the range of internal body temperatures with environmental temperatures using both parametric (Pearson's) and non-parametric (Spearman's rank) correlation analyses. For all analyses, we used an α-level of 0.05. All statistical analyses were computed using JMP v.16.0 (SAS Statistical Institute, Cary, NC, USA).

RESULTS
Regional heterothermy in ring-necked snakes in the field
We found substantial evidence of regional heterothermy in wild ring-necked snakes. Mouth temperature was significantly greater (2.7°C) than cloacal temperature ($F_{1,15}$=13.2774, P=0.0022; Fig. 1A). Handling time did not significantly impact either mouth ($F_{1,17}$=0.5487, P=0.4703) or cloacal temperature ($F_{1,15}$=3.7719, P=0.0711). Average external body temperature was lower (3.4°C) than average internal body temperature (Fig. 1B), although these values were not compared statistically because of the different methods of measurement. The average external body temperature measured on the dorsum was not significantly different from that measured on the ventral surface of the snake ($F_{1,15}$=3.7286, P=0.0714; Fig. 1C). We found that external body temperature
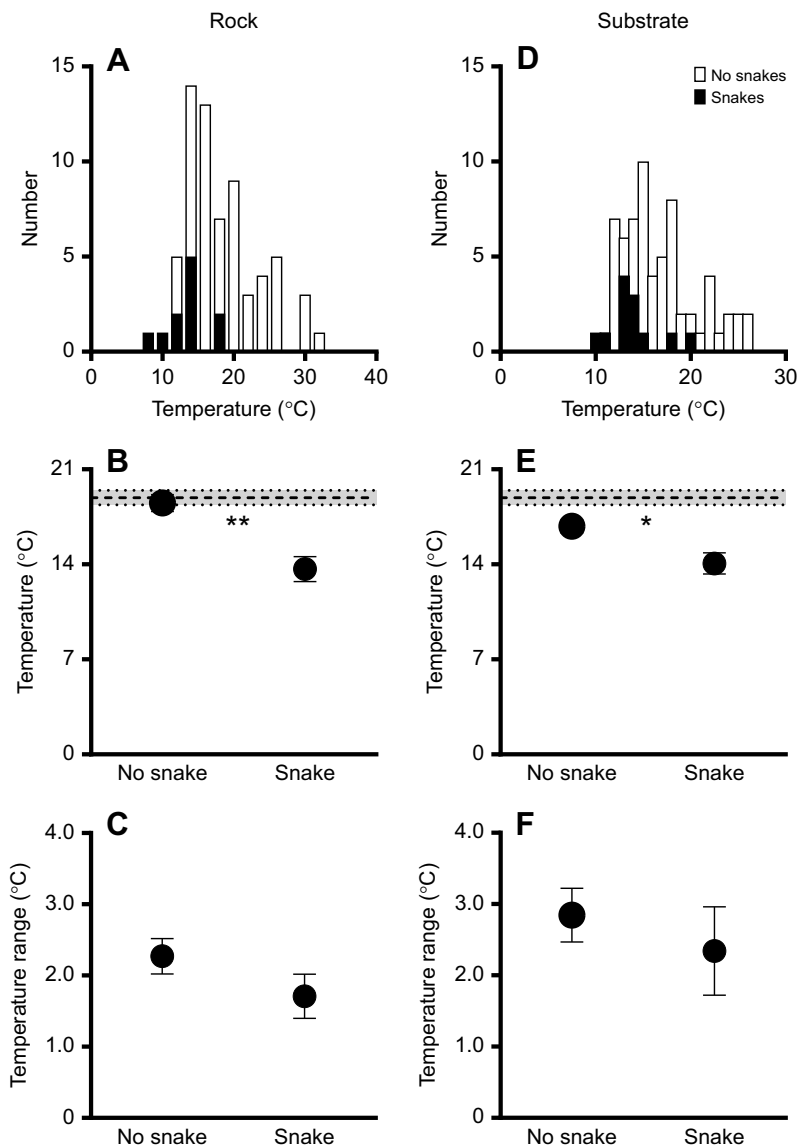
Fig. 2. Temperature distribution of the underside of rocks and the substrate below, with and without ring-necked snakes, measured in the field. Rocks with snakes were a subset of all rocks that had cooler undersides (A), and the difference in mean underside temperature between rocks that had snakes and those that did not was significant (B). However, the range of temperatures under rocks with snakes versus those without did not differ significantly (C). The substrate under rocks with snakes was also a cooler subset of substrates under all rocks (D), and the difference in mean temperature between substrates with snakes and those without snakes was significant (E). The range of temperatures of the substrate under rocks with snakes versus without did not differ significantly (F). Symbols are means±s.e.m. The dashed line in B and E indicates average ambient temperature, while the shaded area between the dotted lines encompasses the s.e.m. Asterisks denote significant differences (*P<0.05, **P<0.01).

Table 1. Relationship between internal body temperature and ambient, rock and substrate temperatures

	Ambient temperature	Rock temperature	Substrate temperature
Mouth temperature			
Correlation coefficient	0.1536	0.4525	0.3624
P-value	0.5563	0.1205	0.2030
Spearman ρ	0.0722	0.1620	0.2124
P-value	0.7831	0.5640	0.4472
Cloacal temperature			
Correlation coefficient	**0.6916**	0.4764	0.3353
P-value	**0.0021**	0.0998	0.2412
Spearman ρ	**0.6406**	**0.6043**	**0.5576**
P-value	**0.0056**	**0.0170**	**0.0308**

Correlation coefficients (Pearson and non-parametric Spearman) and P-values for the relationship between ambient temperature and both mouth and cloacal temperature when a snake was captured, mean temperature of the rock underside and mean temperature of the substrate. Significant values are in bold.

Table 2. Relationship between internal body temperature range and ambient, rock and substrate temperature ranges

	Ambient temperature	Rock temperature	Substrate temperature
Correlation coefficient	−0.3557	0.3414	0.1643
P-value	0.1611	0.2129	0.5747
Spearman ρ	−0.3809	0.4829	0.1556
P-value	0.1315	0.0683	0.5772

Correlation coefficients (Pearson and non-parametric Spearman) and P-values for the relationship between the range of internal body temperatures (difference between mouth and cloacal temperatures) and ambient temperature when a snake was captured, the range of temperatures of the rock under which they were found, and the range of temperatures of the substrate where they were found.

did not differ significantly among body regions when measured on the ventral ($F_{3,13}$=2.2015, P=0.1333; Fig. 1D) or dorsal surfaces ($F_{3,13}$=1.5463, P=0.2465; Fig. 1E).

Relationship between body and environmental temperatures in the field

The underside of rocks with snakes was significantly cooler (4.9°C) than that of rocks without snakes (Fig. 2B; $F_{1,73}$=9.7498, P=0.0026), but did not significantly differ in the range of temperatures (Fig. 2C; $F_{1,73}$=0.8272, P=0.3661). The substrate was significantly cooler (2.7°C) under rocks with snakes than under rocks without snakes (Fig. 2E; $F_{1,74}$=5.1027, P=0.0268), but did not vary significantly in the range of temperatures (Fig. 2F; $F_{1,74}$=0.3031, P=0.5836).

We found that mouth temperature, but not cloacal temperature, was decoupled from environmental temperature. There was no relationship between mouth temperature and ambient, rock or substrate temperatures (Table 1). Similarly, there was no relationship between internal body temperature range and ambient, rock or substrate temperature ranges (Table 2). However, there was a positive correlation between cloacal temperature and ambient, rock and substrate temperatures (Table 1).

Relationship between snake attributes and body temperature in the field

Male and female snakes had similar mouth temperatures in the field ($F_{1,15}$=0.2380, P=0.6327). However, cloacal temperatures were significantly higher (2.3°C) in males than in females ($F_{1,15}$=6.0232, P=0.0268). Gravid females did not have different mouth ($F_{1,15}$=0.0659, P=0.8039) or cloacal temperatures ($F_{1,15}$=0.7089, P=0.4242) from those of non-gravid females, although this result should be interpreted with caution, given that only three females were gravid in our sample. Ecdysis was not significantly associated with either mouth ($F_{1,15}$=0.5697, P=0.4621) or cloacal temperature ($F_{1,15}$=0.9986, P=0.3335).

Regional heterothermy in a laboratory thermal gradient

We found that snakes maintained a difference between mouth and cloacal temperatures when placed in a laboratory thermal gradient, just as we observed in the field. All snakes spent the entirety of their trials under tiles, which held a truncated range of temperatures (Fig. 3). Snakes avoided the hottest and coldest parts of the thermal gradient (Fig. 3). Cells that contained snakes had significantly cooler tile ($F_{1,15}$=7.5361, P=0.0150) and substrate temperatures ($F_{1,15}$=6.6419, P=0.0210) than cells without snakes (Fig. 3). In general, snakes maintained body temperatures in the laboratory that were higher than those in the field (Figs 1 and 4). However, mouth temperature was still significantly warmer (2.0°C) than cloaca temperature (Fig. 4; $F_{1,15}$=14.4368, P=0.0017). Internal body temperature was also warmer than external body temperature, although this was not statistically tested (Fig. 4). External body temperature did not differ significantly between dorsal and ventral surfaces (Fig. 4; $F_{1,15}$=0.1879, P=0.6708). Similarly, we found no regional variation in external body temperature for dorsal ($F_{3,13}$=0.0990, P=0.9592) or ventral surfaces ($F_{3,13}$=0.9507, P=0.4448) (Fig. 4). Neither mouth temperature ($F_{1,14}$=3.0096, P=0.1047) nor cloacal temperature ($F_{1,14}$=0.2998, P=0.5927) in the gradient varied based on whether the head was in contact with the substrate. Similarly, neither mouth ($F_{1,14}$=0.8507, P=0.3706) nor cloacal temperature ($F_{1,14}$=0.0247, P=0.8774) depended on whether the body was coiled.

Regional heterothermy in a homogeneous thermal environment and the role of body posture

Even in the relatively constant environment of the lab, we found that mouth temperature was significantly higher (1.1°C) than cloacal temperature ($F_{1,14}$=11.3597, P=0.0050). Neither mouth ($F_{1,14}$=1.1772, P=0.2962) nor cloacal temperature ($F_{1,14}$=1.1772, P=0.2962) in the lab depended on whether the head was in contact with the substrate. Similarly, neither mouth ($F_{1,14}$=2.2992, P=0.1517) nor cloacal temperature ($F_{1,14}$=0.5022, P=0.4921) in the lab depended on whether the body was coiled. Whether in the field, a laboratory thermal gradient or the ambient lab environment, snakes maintained similar patterns of regional heterothermy despite substantially different environmental temperatures among these settings (Fig. 5).

DISCUSSION

We found that ring-necked snakes have robust regional heterothermy, despite their small body size and elongate body shape. While temperature variation along the length of their bodies was not detectable when measured externally, snakes maintained higher mouth than cloacal temperatures. This pattern was apparent in both the field and laboratory, regardless of the extent of thermal heterogeneity available for snakes to exploit. Thus, our data indicate that regional heterothermy in ring-necked snakes is not solely driven by environmental variation, but instead is linked to physiological or morphological mechanisms such as blood circulation or differing thermal properties (e.g. conductance) of different parts of the body.
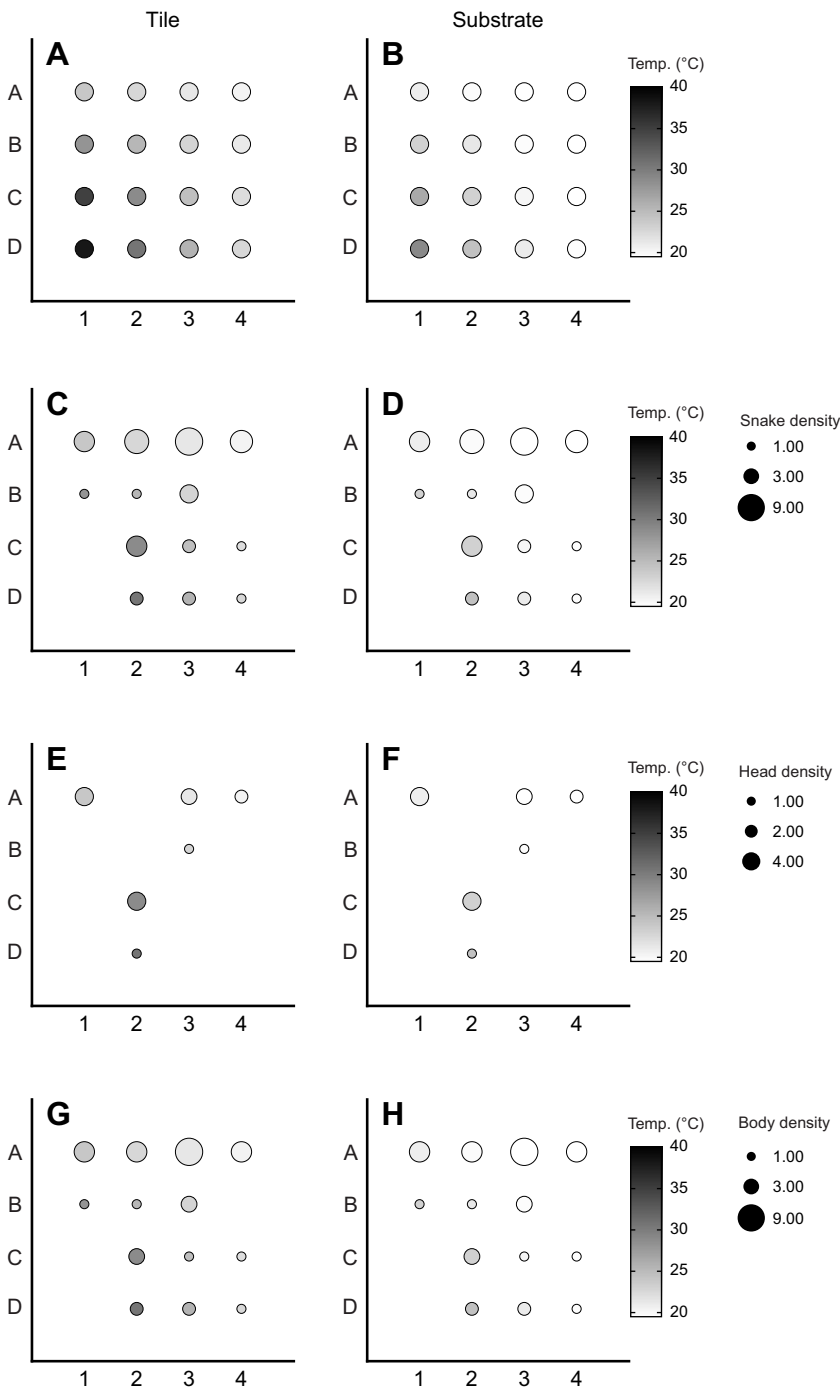
Fig. 3. Temperature gradient in the arena for thermal preference trials of ring-necked snakes. The temperature gradient was measured underneath the tiles (gradient cells; A) and for the substrate beneath those gradient cells (B) in the laboratory. Snakes avoided the hottest and some of the coldest parts of the gradient (C,D). Snakes typically kept their heads in warmer parts of the gradient (E,F) compared with the rest of their bodies (G,H). Mean temperature of both the tile and substrate of gradient cells that contained snakes was significantly cooler than that of cells without snakes (see Results). The x- and y-axis labels denote the rows and columns of gradient cells as recorded during our experiment.

In the field, we found that snakes maintained higher body temperatures than air, rock or substrate temperatures. There are several mechanisms that might explain this finding. First, while rock and substrate temperatures were measured at the site of collection, ambient temperature was collected from a nearby weather station. Because the collection site is on an exposed bluff, local temperatures were likely higher than those measured by the weather station in the nearby forest. Second, while all snakes except for one were collected underneath rocks, they may have parts of their body or head exposed to solar radiation, which could increase body temperature above ambient temperature. Finally, the equilibrium body temperatures of animals in the field often deviate from ambient or environmental temperatures as a result of the

dynamics of heat exchange between individuals and their environments (Bakken, 1992; O'Connor, 2000; O'Connor et al., 2000; Dzialowski, 2005). Regardless, our results suggest that these small snakes are able to exploit solar radiation and spatial heterogeneity to maintain warm temperatures relative to their retreat sites.

Our results also suggest that there is seasonal variation in body temperature in the field, but not in a thermal gradient, in this population of ring-necked snakes. Many snake species, including ring-necked snakes, use surface cover objects for feeding, reproduction and thermoregulation (Huey et al., 1989; Webb and Shine, 1998a,b; Ernst and Ernst, 2003; Cox et al., 2018). Previous work (Cox et al., 2018) conducted about a month earlier in the
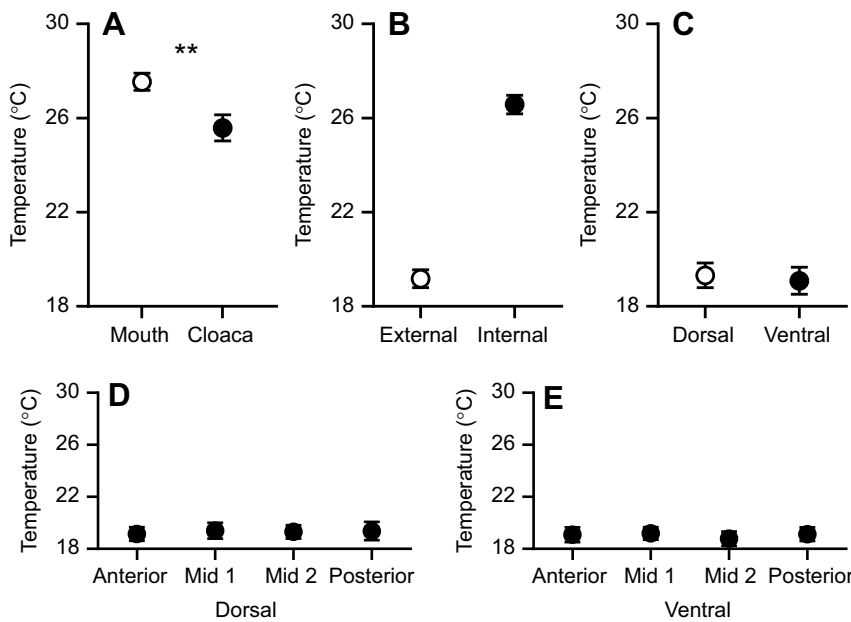
Fig. 4. Internal and external body temperature of ring-necked snakes, measured in a laboratory thermal gradient. Body temperature differed significantly between the mouth and the cloaca (A); internal and external temperatures also differed (but these values were not compared statistically; B). External body temperature did not differ between the dorsum and ventrum (C), or across dorsal and ventral regions of the body (D,E). Symbols are means±s.e.m. Asterisks denote significant differences (**$P<0.01$).

calendar year than the current study found that mean field-active body temperatures were higher (27.7°C) than the values we report here (21°C), although preferred body temperatures in a gradient were similar. Hence, both the thermal characteristics of the environment earlier in the year and the reproductive biology of ring-necked snakes in this population might explain the differences between these two studies. Sex differences in thermal physiology and behavior have been documented (Cecchetto and Naretto, 2015; Bodensteiner et al., 2019; Logan et al., 2021), and reproduction might exacerbate any existing sex differences (Bodensteiner et al., 2019). We previously found that most snakes were gravid earlier in the year (Cox et al., 2018), while relatively few snakes were gravid later in the year (current study). Gravid snakes show aggregating behavior and maintain body temperature above their thermal preference when aggregated. We also found substantially fewer snakes in the current study than in surveys conducted earlier in the year, which suggests that individuals may have dispersed away from this likely hibernaculum and oviposition site by the time of this study. Seasonal variation in thermal physiology and behavior is relatively well established in thermoregulating ectotherms (Tu and Hutchison, 1995; Neel et al., 2020; Clifton and Refsnider, 2022), and it is possible that the behavior of ring-necked snakes, with respect to local thermal environments, changes along with life-history phenology as the active season progresses.

Regional heterothermy has been documented in only a few snake species (e.g. Australian pythons of the genera *Liasis*, *Aspidites* and

Morelia: Johnson, 1973; *Masticophis flagellum*: Hammerson, 1977; *Coluber constrictor*: Hammerson, 1987; *Thamnophis elegans*: Peterson, 1987; *Thamnophis sirtalis*: Gregory, 1990; *Charina bottae*: Dorcas and Peterson, 1997; *Pituophis melanoleucus*: Ashton, 1998; *Pantherophis guttatus*: Roark and Dorcas, 2000), and our research confirms regional heterothermy in the smallest snake species in which it has been investigated. Small and elongate ectotherms should rapidly equilibrate to environmental temperatures because of their high surface area to volume ratio and low thermal mass, both of which facilitate thermal exchange (Stevenson, 1985; Porter and Kearney, 2009; Cox et al., 2020). Regional heterothermy has been documented in small-bodied arthropods such as kissing bugs (Lahondere et al., 2017) and dung beetles (Chown et al., 1995), and was associated with blood feeding on endotherms (kissing bugs: Lahondere et al., 2017) or powered flight (dung beetles: Chown et al., 1995). It is likely that aspects of invertebrate physiology, such as open circulation and tracheal respiration, play a role in regional heterogeneity in arthropods. Regardless, our results demonstrate that regional heterothermy is possible in ectothermic vertebrates that represent extreme examples of elongated body structure, which suggests that regional heterothermy is worth examining in other small-bodied and elongate ectotherms.

Previous research in snakes has found that head and body temperature can differ from cloacal body temperature (Peterson, 1987), but the direction of this difference varies among species. We
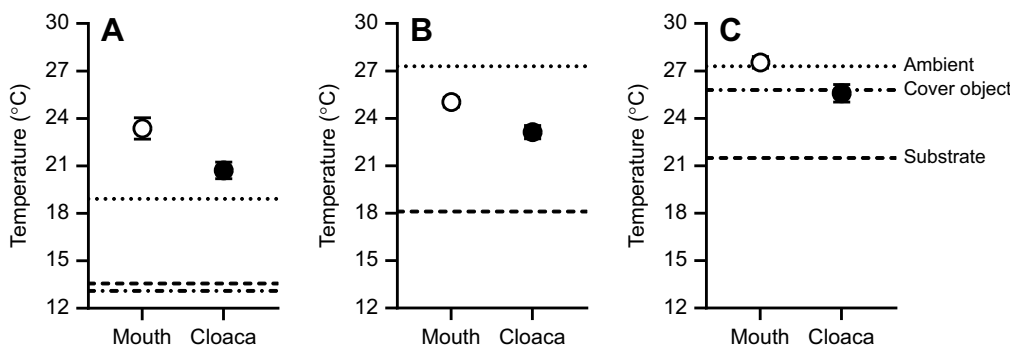
Fig. 5. Regional heterothermy of ring-necked snakes. Snakes maintained consistent regional heterothermy in the field (A), in an ambient (relatively thermally homogeneous) laboratory environment (B), and in a laboratory thermal gradient (C). Symbols are means±s.e.m. Dotted/dashed lines indicate mean ambient, tile and substrate temperatures.

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found that while cloacal temperature was significantly correlated with rock, substrate and air temperature in the field, head temperature was decoupled from environmental temperature. For species that are found in temperate environments, body temperature of the head or the anterior portion of the body is often maintained higher than that of the midbody and posterior regions (*Thamnophis elegans* and *Thamnophis sirtalis*: Gregory, 1990; *Pantherophis guttatus*: Dorcas and Peterson, 1997). However, for snake species that are found in tropical environments, the head is often maintained at a cooler temperature than the body. Many of these studies also found that head temperature was less variable than body temperature (*Liasis childreni*, *Liasis fuscus*, *Aspidites melanocephalus* and *Morelia spilotes*: Johnson, 1973; *Candoia aspera*, *Candoia carinata* and *Boiga irregularis*: Johnson, 1975b; *Oxyuranus scutellatus*: Johnson, 1975a). Detailed research in rubber boas (*Charina bottae*) suggests one potential factor that might be important for these patterns (Dorcas and Peterson, 1997). Rubber boas maintain the head at warmer temperatures than the body when the body is below their preferred temperature, but maintain lower temperatures in the head than in the body when the body is above their preferred temperature (Gregory, 1990; Dorcas and Peterson, 1997). Hence, in suboptimal environmental temperatures, snakes might prioritize precise thermoregulation of the head, rather than the body, because it is a particularly crucial body region. Previous authors have suggested that maintaining proper function of the brain is likely tightly linked to fitness (Hammerson, 1977; Gregory, 1990; Dorcas and Peterson, 1997). While our work generally agrees with this notion, more research is necessary to determine the functional significance of regional heterothermy in snakes.

We found only weak evidence that ring-necked snakes were behaviorally thermoregulating to create differences in body temperature between the head and the body. Regional heterothermy persisted even in ambient temperatures in the laboratory, which had minimal thermal heterogeneity that could be exploited. However, the difference between head and body temperature was the least in the ambient laboratory environment, suggesting that environmental variation may still play some role in maintaining regional heterothermy. In the absence of behavioral thermoregulation, physiological or morphological mechanisms, such as patterns of circulation, or differing physical (and thus thermal) properties of the head and body, are the most likely proximate mechanisms that can explain regional heterothermy in ring-necked snakes. For example, squamates possess an intracardiac shunt that can divert blood towards or away from the systemic circulation (Burggren, 1987). This shunt or other mechanisms of diverting circulation could explain differences in head and body temperatures (Dzialowski and O'Connor, 2001). Regardless of the precise mechanisms generating regional heterothermy in this species, an interesting question that remains unanswered is whether these mechanisms evolved because regional heterothermy is adaptive, or whether they are incidental biproducts of other aspects of ring-necked snake biology.

Our results have implications for understanding the evolution of traits that might be related to regional heterothermy. For example, many snake species have head coloration that is distinct from the body color, and some have contrastingly black heads. While some authors have speculated that black heads in snakes are for prioritizing thermoregulation of the brain, other explanations such as protection from UV damage are also plausible, and there have been no rigorous tests of these various hypotheses (Pough et al., 1978; Crowell et al., 2021). Similarly, many snakes have either contrastingly dark or light rings around the neck, including many small-bodied species, and the

function of this color trait is unknown. It is possible that a neck ring might disrupt the outline of the head in a snake with an exposed head for thermoregulation, which could reduce detection by predators. While our work cannot clarify the function of head and neck coloration in light of regional heterothermy, our data demonstrate that regional heterothermy can be present in very small ectotherms, which implies that future research should consider morphological and physiological mechanisms that might generate and maintain regional heterothermy, as well as its potential adaptive function.

Author contributions
Conceptualization: C.L.C., A.K.C., M.E.D., S.A.D., S.T.G., T.K.L., K.M., P.T.N., D.R.N., P.F.R., B.J.W., M.L.L.; Methodology: C.L.C., A.K.C., M.E.D., S.A.D., S.T.G., T.K.L., K.M., P.T.N., D.R.N., P.F.R., B.J.W., M.L.L.; Formal analysis: C.L.C.; Investigation: C.L.C., A.K.C., M.E.D., S.A.D., S.T.G., T.K.L., K.M., P.T.N., D.R.N., P.F.R., B.J.W.; Writing - original draft: C.L.C.; Writing - review & editing: C.L.C., A.K.C., M.E.D., S.A.D., S.T.G., T.K.L., K.M., P.T.N., D.R.N., P.F.R., B.J.W., M.L.L.; Visualization: C.L.C.; Supervision: C.L.C., A.K.C.; Project administration: C.L.C., A.K.C.

Data availability
Data are available from figshare.com: https://doi.org/10.6084/m9.figshare.22179973.v1.

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