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Variation in cottonmouth (*Agkistrodon piscivorus leucostoma*) resting metabolic rates

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Abstract

The study of intra- and inter-individual variation in the metabolic response to environmental variation can provide mechanistic explanations to large-scale ecological and evolutionary patterns. In a study of range-limiting factors, variation in resting metabolic rates of cottonmouths (*Agkistrodon piscivorus leucostoma*) was investigated along a latitudinal gradient in southern populations and in populations near and at the northern range limit. CO₂ production rates of 53 snakes were measured in response to body mass, temperature, time of day, latitude of origin, and sex. The within-subjects effects were similar to those reported for other pit vipers. Metabolic cold adaptation appears to exist, with cottonmouths from northern populations having higher low temperature metabolic rates. Calculations suggest that Arkansas cottonmouths allocate almost twice as much energy to resting metabolism during non-feeding periods (brumation) as Louisiana cottonmouths. While maintenance metabolism alone during brumation is more costly near the northern range limit, it is most likely not a limiting factor in geographic distribution and may be used to fuel important processes other than activity metabolism.

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1. Introduction

The examination of mechanisms that underlie ecological patterns is key to understanding organismal processes (Dunham and Beaupre, 1998). Studies of metabolism are important to physiological ecology as they can suggest potential energetic constraints that operate on individual organisms. The study of intra- and inter-individual variation in the metabolic response to environmental variation can provide mechanistic explanations to large-scale ecological and evolutionary patterns.

Temperature profiles of organismal metabolic rates can be scaled to the population level and used to predict the effects of the inevitable global climate change (Dunham, 1993; Dunham and Overall, 1994). Knowledge of the energetic effects of climate change is exceptionally important, as recent projections by the Intergovernmental Panel on Climate Change predict continued global warming of 1.4–5.8 °C during the 21st century (IPCC, 2001). In addition, species-specific metabolic relationships are important for comparative studies of metabolic adaptation (Garland and Adolph, 1994; Garland and Carter, 1994).

The quantification of metabolic demands is central to studies of ecological energetics because the requirements of maintenance metabolism must be

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met before any other allocations can be made (Congdon et al., 1982; Porter and Tracy, 1983). Resting metabolism alone can be energetically costly, typically accounting for 10–45% of a reptile's yearly energy budget (Congdon et al., 1982; Secor and Nagy, 1994; Beaupre, 1995). After the maintenance requirement is met, the competing functions of growth, storage, and reproduction can receive energetic allocations if (and only if) assimilated energy is available and the conditions are right for such an allocation. Because basal metabolism is the first allocation step, resting metabolic rates need to be quantified first before further analyses of energetic allocations begin.

Despite all that is known about allocations to resting metabolism by snakes, the effects of latitudinal variation in resting metabolic rates remain poorly addressed. In addition, the effects of mass, sex, temperature, time of day, and all higher order interactions have not been rigorously quantified in western cottonmouths (*Agkistrodon piscivorus leucostoma*). The resting metabolic rates of western cottonmouths were measured from three geographic regions, representing central (Southwestern Louisiana) and peripheral populations (Northwestern Arkansas—near the northern range limit and Southwestern Missouri—at the northern range limit), along a latitudinal gradient while controlling for longitude (93.4°W). Seasonal energetic demands were estimated using temperature and photoperiod data from the natural capture locations and comparisons were made within the family Viperidae.

2. Materials and methods

2.1. Study animals, sampling regime, and captive maintenance

Cottonmouths (*A. piscivorus*) are medium sized, semi-aquatic pit vipers whose geographic range is restricted to the Eastern US below 38°N latitude (Conant and Collins, 1991; Gloyd and Conant, 1990). *A. piscivorus* is a common and often locally abundant snake in swamps, ponds, rivers/streams, and many other aquatic habitats throughout its range (Gloyd and Conant, 1990; Conant and Collins, 1991). There are three recognized subspecies of *A. piscivorus* (*A. p. piscivorus*—the eastern cottonmouth, *A. p. conanti*—the Florida cottonmouth, and *A. p. leucostoma*—the western cottonmouth). The focal subspecies of this research is

the western subspecies, which occurs in the eastern half of Texas and Oklahoma, the southern part of Missouri, Illinois, and Indiana, the western part of Kentucky and Tennessee, most of Alabama, all of Arkansas, Louisiana and Mississippi, and on Gulf Coast islands (Gloyd and Conant, 1990).

Western cottonmouths, *A. p. leucostoma*, were sampled from the northern and southern parts of their range during May through September in 1998–2001. To control for any unforeseen longitudinal effects, sampling was restricted to along 93.4°W longitude (1 S.D.=0.69). Shortly after capture, snakes were brought back to the laboratory, weighed, and measured (using a squeeze-box; Quinn and Jones, 1974). Cottonmouths ($n=23$) were collected from the following locations in Northwestern Arkansas ($\approx 36.2^\circ\text{N}$ latitude, 94.1°W longitude, 347 m above sea level): the Middle Fork of the White River, the Illinois River, and Richland Creek (Washington County), the Little Sugar Creek (Benton County), and the Kings River and Mulberry Creek (Madison County). The Arkansas sample included 12 males (12.15–692.68 g) and 11 females (12.66–422.60 g). Cottonmouths ($n=22$) were also collected from rice field irrigation canals in Allen and Beauregard Parishes in Southwestern Louisiana ($\approx 30.6^\circ\text{N}$ latitude, 92.9°W longitude, 18 m above sea level). The Louisiana sample included 14 males (14.37–579.34 g) and 8 females (12.28–291.16 g). In addition, a smaller sample of cottonmouths ($n=8$) was collected from the Mule Shoe Conservation Area in Hickory County in Southwestern Missouri ($\approx 37.9^\circ\text{N}$ latitude, 93.1°W longitude, 312 m above sea level). The Missouri sample included five males (19.30–285.59 g) and three females (17.32–238.10 g). Throughout the text, the Arkansas, Louisiana, and Missouri samples are abbreviated as AR, LA, and MO, respectively. Climate data for 1990–2000 was obtained from the National Climatic Data Center (Asheville, NC) and used to approximate the geographic variation in thermal conditions.

Cottonmouths were maintained in glass aquaria containing a newspaper substrate, cover objects and a large water dish. Cottonmouths were fed fish-scented laboratory mice every 2–3 weeks and water was provided ad libitum. Photoperiod and temperature approximated natural conditions experienced in Northwestern Arkansas. Cottonmouths did not receive a meal for 2 weeks before a metabolic trial began. The cottonmouths that were

used in the experiments were post-absorptive, not undergoing ecdysis, and resting (based on post hoc examination of the data and elimination of obvious movement data) during the metabolic trials that covered the photo cycle. In addition, any gravid females were measured after parturition. The values for metabolic rates closely approximated both resting and standard metabolic rate (definitions: resting metabolic rates are measured at any time and illumination in fasted animals that are resting, and standard metabolic rates are measured during the inactive daily time period, in the dark, in fasted and resting animals; Bennett and Dawson, 1976). Because measurements were taken throughout the day, thus violating the conditions required for standard metabolic rates, the measured metabolic rates were considered to fall under the less restrictive resting metabolic rate category throughout the text. Minimum metabolic rates were approximated as those obtained during the lowest time block. After the data were collected, all cottonmouths were returned to their point of capture.

2.2. *Respirometry*

Metabolic rates were measured via indirect calorimetry (CO_2 production rates) using a Sable Systems TR-3 open flow system. The methods used were essentially identical to those originally reported by Beaupre and Zaidan (2001). Eight chambers were available for simultaneous measurements of up to 7 cottonmouths (with one reference baseline) per block. Cottonmouths were placed in gas-tight chambers (~ 2500 ml). Air from an 80-psi line was scrubbed of carbon dioxide and water using a Whatman (model FT-IR 75-45) purge gas generator (Whatman Inc., Haverhill, MA). Clean, high-pressure air was then split into eight equal flows with a Sable Systems MF-8 airflow manifold. Flow rates in the lines were matched ($\pm 1\%$) using the Sable System sensitive mass flow meter and needle valves (that allow final CO_2 production rates to be calculated at STP) for each line on the manifold. Flow rates used varied from 300 to 700 ml min^{-1} depending on cottonmouth size and temperature. Each of the eight individual flow-matched lines was then connected to one port of each respirometry chamber and a separate line carried gas, via positive pressure, from a second port in the chamber to a syringe barrel for sub-sampling.

A Sable System eight channel multiplexer controlled sub-sampling by sequentially cycling through all eight chambers every hour. During each hour, the baseline chamber was sampled first for 3.3 min, then each of the seven occupied chambers was sampled for 6.7 min, then finally, the baseline chamber was resampled for another 3.3 min. The two baseline samples allowed for compensation for baseline drift. During each sample period, CO_2 concentration was recorded every 5 s. Sub-sampled gas was drawn by negative pressure at flow rates ranging from 150 to 250 ml min^{-1} through two 30 ml tubes of Drierite to ensure that expired water was removed. Samples were drawn next into the CO_2 sensor of a LI 6251 Li-Cor infrared gas analyzer (Li-Cor Inc., Lincoln, NE). All gas flow connections were constructed with low permeability Pharmed[®] NSF-51 tubing. Data from the CO_2 analyzer were downloaded through the Sable System Universal Interface and DATACAN v software (Sable Systems, 1991). During measurements, time (h:m:s) and CO_2 concentration (ppm) were stored to disk hourly.

Chamber temperature was maintained by immersion in a thermally stable water bath. An Omega model 3400 thermocouple controller in conjunction with a Fisher model 900 circulating unit controlled the target temperature in the water bath to ± 0.5 °C. The water bath rested on five magnetic stirrers that maintained thermal homogeneity of the water. Because the water bath was an isothermal black body and no external heat was applied to the system, water bath temperature, chamber temperature, and body temperature were assumed to be identical. The entire apparatus was housed in an isolated room with a temperature of approximately 20 °C. Diffuse light was provided at 12L:12D beginning at 07:00 CST.

2.3. *Experimental design*

CO_2 production rates ($= V_{\text{CO}_2}$ in ml h^{-1}) of all individuals were measured in response to six levels of temperature (7, 12, 17, 22, 27 and 32 °C) during 4 time periods of the photo cycle (early photophase = 07:00–10:00 CST, late photophase = 14:00–18:00 CST, early scotophase = 19:00–00:00 CST, late scotophase = 01:00–06:00 CST). The warm temperature blocks (17–32 °C) were measured during the active season (May through September) and the cold temperature blocks (7–17 °C) were measured during the brumation period

(December and January). Each measurement block of seven individuals and one baseline lasted for 3–4 days (1 day per temperature). The experimental temperatures spanned the sublethal range of mean body temperatures typically experienced by temperate pit vipers in the Southeastern US (Beaupre, unpublished data). The experimental temperature was changed at 11:00 CST and the cottonmouths habituated for 3 h before beginning the next temperature trial at 14:00 CST. Temperature order was randomized within each measurement block. Thus, each individual was measured at each temperature and time in a repeated measures design.

The data collection system sampled the seven cottonmouths and one baseline for 6.7 min h^{-1} , recording CO_2 ppm every 5 s. The values recorded for each individual during each sampling period were averaged to produce a single hourly average value for each individual under each temperature and time combination. Hourly sampling of the empty chamber provided a baseline measurement to compensate calculations for any drift in the infrared gas analyzer or input gas stream. Adjustment of data for baseline drift was accomplished with DATACAN V software (Sable Systems, 1991).

Activity during measurement can be a problem when attempting to estimate resting metabolic rates. Activity during sampling is characterized by high and erratic CO_2 measurement traces and has been confirmed by visual inspection of snakes in chambers. However, visual inspection is impractical for large samples (over 8100 individual hourly records were collected) and often leads to snake disturbance. In order to determine if snakes were engaged in physical activity without disturbing the snakes, individual hourly means were compared to the 24 h mean for an individual snake at a given temperature. If the individual hourly mean was two or more times greater than the 24 h mean, the outlier was removed because chamber activity will cause an inaccurate estimate of resting metabolic rates.

2.4. Data processing and statistical analysis

The methods of data processing and statistical analysis are identical to those originally published in Beaupre and Zaidan (2001), with the exception of the cold temperature and seasonal acclimation treatments (7 and 12 °C). The data were processed with a QUICKBASIC program designed to associate

values in the output data file with appropriate individual variables and calculate hourly averages of CO_2 production (ml h^{-1}) for each individual. SAS PROC MIXED was used for repeated measures analyses of variance (with \log_{10} mass as the covariate) and PROC REG for regression analyses (SAS Institute, 1985; Littell et al., 1996). Statistical significance was judged with a Type I error of 0.05.

Prior to analysis, CO_2 production ($\text{mlCO}_2 \text{ h}^{-1}$) and body mass (g) were \log_{10} transformed to linearize the relationship. The data set was adjusted for body mass by regressing \log_{10} CO_2 production rate on \log_{10} body mass to generate mass-residuals for use in data presentation only. The slopes of the relationships between \log_{10} CO_2 production rate and \log_{10} body mass was tested for heterogeneity among treatment groups before analysis proceeded. Because all statistical analyses were performed on individual response means during each time period, the means were assumed to be normally distributed.

A single repeated measures analysis that examined the within-subjects effects (temperature and time of day) the between-subjects effects (geographic location and sex), and their interactions on \log_{10} CO_2 production rates (with \log_{10} mass as a covariate) was performed. To test for seasonal acclimation effects, a *t*-test was used to compare the warm acclimated metabolic rates (at 17 °C) to the cold acclimated rates at 17 °C. Least squares regression was used to construct predictive relationships for CO_2 production rates as a function of body mass and body temperature, following Andrews and Pough (1985). An equation was fitted to each group of the following form:

$$\log_{10} V_{\text{CO}_2} = X_1 \times \log_{10} W + X_2 \times T - X_3,$$

which detransforms into:

$$V_{\text{CO}_2} = aW^b \times 10^{cT},$$

where V_{CO_2} = CO_2 production rate ($\text{mlCO}_2 \text{ h}^{-1}$), X_1 through X_3 are fitted constants, W = mass (g), T = temperature (°C), $a = X_1$, $b = X_2$, and $c = 10^{X_3}$. Metabolic rates calculated from the fitted equations were used to determine Q_{10} (defined as the ratio of resting metabolic rate at temperature T divided by the resting metabolic rate at temperature $T-10$).

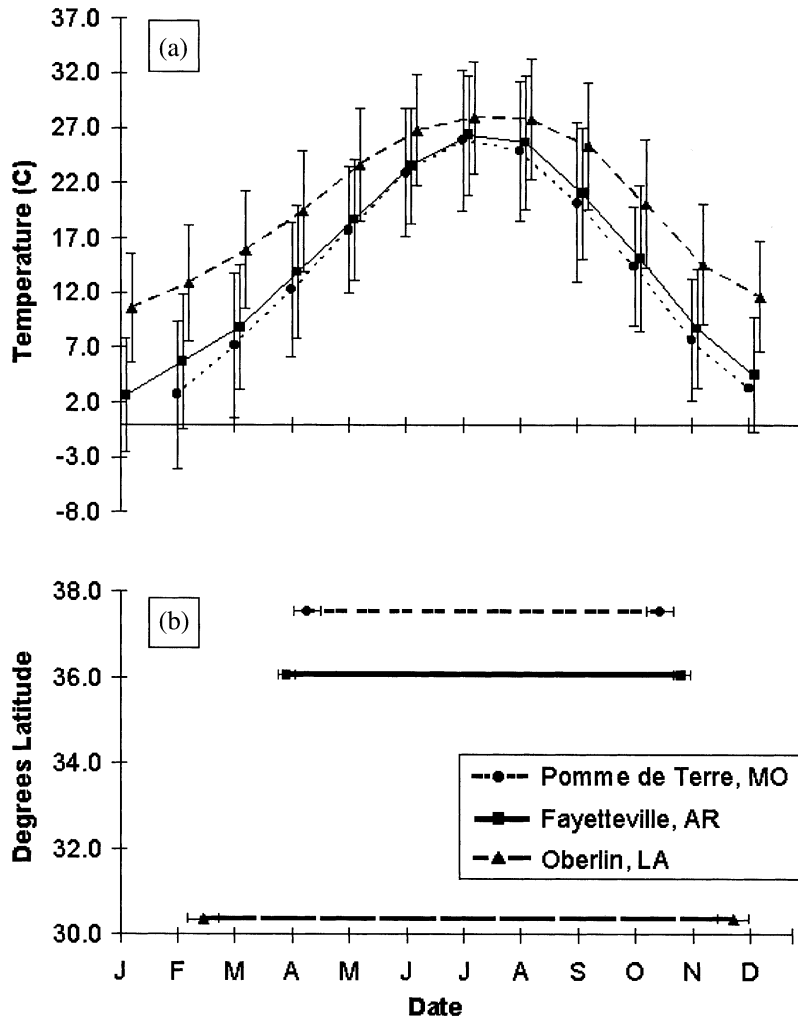


Fig. 1. Temperature and growing season length data. (a) Air temperature profiles of areas in close proximity of cottonmouth collecting sites (Pomme de Terre Dam in Missouri, Fayetteville, Arkansas, and Oberlin, Louisiana). Error bars represent the range of average maximum and minimum air temperatures. (b) Approximate growing season length for cottonmouth collecting sites in Missouri, Arkansas, and Louisiana. Error bars represent the 95% confidence interval around the mean.

3. Results

3.1. Environmental conditions

The temperature profiles (10 year averages of average maximum, average, and average minimum air temperatures) of Pomme de Terre Dam, Fayetteville, and Oberlin appear in Fig. 1a. The yearly mean air temperatures (± 1 S.E.) experienced by cottonmouths are warmer in Louisiana (19.73 ± 1.88 °C) than in Arkansas or Missouri (14.56 ± 2.43 °C and 14.47 ± 2.56 °C, respectively) ($F_{\text{latitude}} = 3.31$, $P = 0.0481$; LSD post hoc test indicates $LA > AR = MO$ at $\alpha = 0.05$). The growing

season length, estimated by the number of days where air temperature is above 0 °C and the first and last freeze occurrence, appears in Fig. 1b. The yearly growing season length (± 1 S.E.) experienced by cottonmouths is longer in Louisiana (286.90 ± 8.39 days) than in Arkansas or Missouri (214.00 ± 4.74 days and 193.44 ± 7.31 days, respectively) ($F_{\text{latitude}} = 49.80$, $P < 0.0001$; LSD indicates $LA > AR = MO$ at $\alpha = 0.05$).

3.2. Activity patterns and repeated measures analysis

A total of 8114 individual hourly V_{CO_2} records were recorded for all snakes at all temperatures.

Table 1

Repeated measures analysis of variance for the effects and interactions of \log_{10} mass (Lmass; covariate), latitude of origin (Lat), sex, temperature (Temp), and time of day (Time) on \log_{10} transformed CO_2 production rate

Source	d.f.	F	P
Lmass	1,125	1063.83	<0.0001
Lat	2,139	1.18	0.3098
Sex	1,140	0.34	0.5607
Temp	6,107	293.99	<0.0001
Time	3,152	23.54	<0.0001
Lat×Sex	2,142	0.99	0.3750
Lat×Temp	12,133	3.87	<0.0001
Lat×Time	6,152	2.09	0.0575
Sex×Temp	6,108	0.64	0.7003
Sex×Time	3,155	0.20	0.8943
Temp×Time	18,315	7.77	<0.0001
Lat×Sex×Temp	12,134	1.57	0.1076
Lat×Sex×Time	6,156	0.64	0.6975
Lat×Temp×Time	36,430	1.35	0.0911
Sex×Temp×Time	18,319	1.45	0.1054
Lat×Sex×Temp×Time	36,436	0.90	0.6385

Assessment for activity yielded 359 records (4.4% of all observations) that were deemed unsuitable for estimates of resting metabolic rates and were consequently removed from all analyses. The majority of activity occurred during the late photophase time period (particularly at 7 °C), which occurred shortly after temperature adjustment (Zaidan, 2001).

The CO_2 production rate of cottonmouths increased with body mass. The slopes of the relationship between $\log_{10} V_{\text{CO}_2}$ and \log_{10} mass were homogenous between latitude (\log_{10} mass by latitude interaction $F=2.13$, $P=0.1192$) and sex (\log_{10} mass by sex interaction $F=0.02$, $P=0.8779$) and therefore, the analysis of covariance proceeded. The main effects of temperature, time, and latitude significantly affected CO_2 production rates, as did the two-way interaction between temperature×time and temperature×latitude (Table 1). The main effect of sex, the remaining two-way interactions, and all higher order interactions did not significantly affect CO_2 production rates (Table 1).

Temperature positively affected CO_2 production rates and the temperature effect was also dependent on time of day and latitude of origin. Cottonmouths exhibited highest CO_2 production rates during the late photophase period of the photocycle and lowest rates during late scotophase (means of residual $\log_{10} V_{\text{CO}_2}$ in ml h^{-1} with 95% confidence interval: late photophase = 0.1083 ± 0.021 , late scotophase = -0.0733 ± 0.023). The increase in CO_2 production rates during late photophase was particularly strong at 7 °C, but dampens as temperature increases (Fig. 2). Latitude of origin affected the magnitude of the temperature effect. At the warm test temperatures cottonmouths from higher latitudes had lower CO_2 production rates, while at the cold test temperatures, the trend reversed and the slopes of the lines flattened (Fig. 3). Part of the

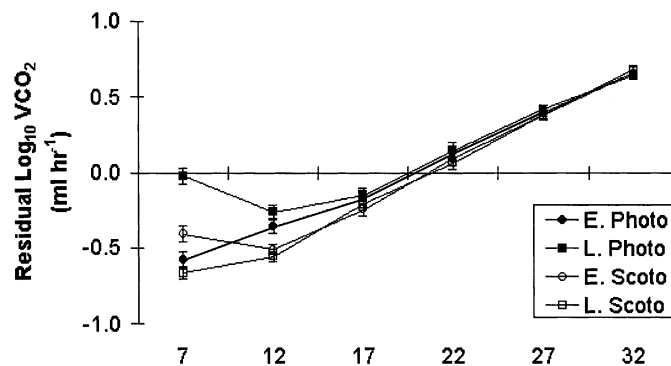


Fig. 2. Interaction of temperature and time and on cottonmouth CO_2 production (resting metabolic rates). Each symbol represents the mean value of 53 snakes. Error bars represent the 95% confidence interval around the mean. The time periods are as follows: early photophase = 07:00–10:00 CST, late photophase = 14:00–18:00 CST, early scotophase = 19:00–00:00 CST, late scotophase = 01:00–06:00 CST. The significant result is mainly due to the crossing of the lines (most evident between 7 and 12 °C).

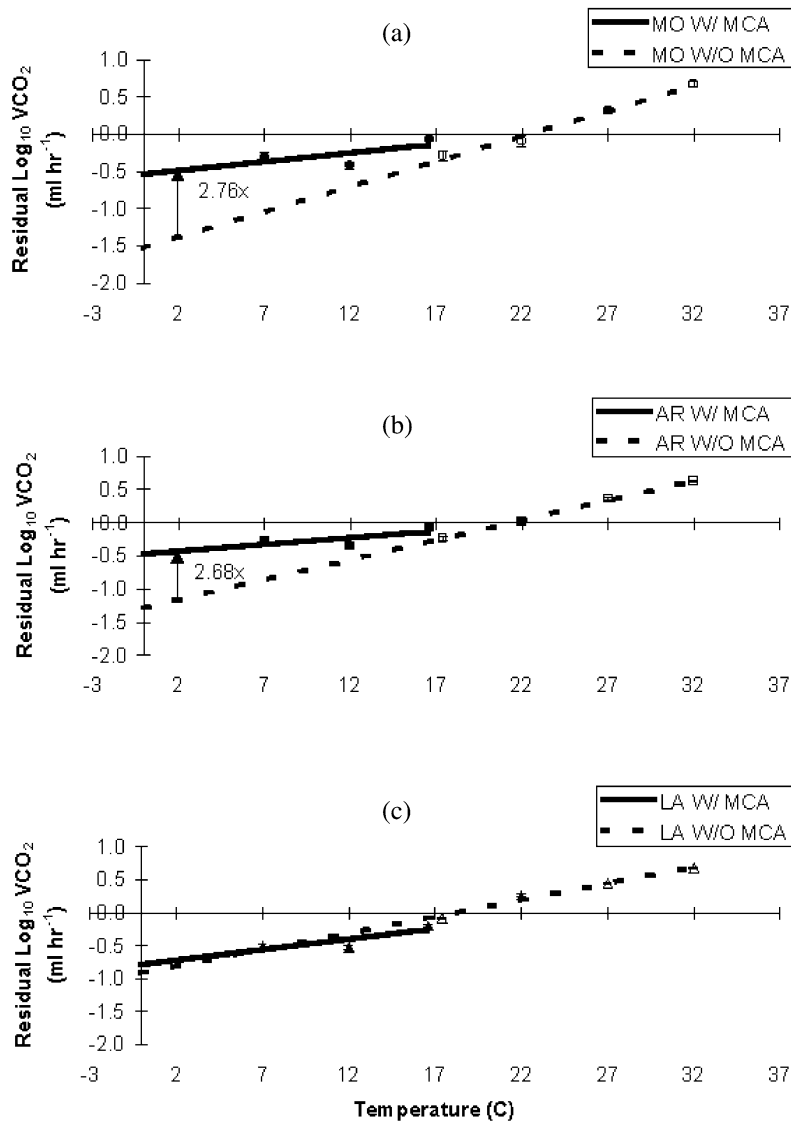


Fig. 3. Interaction of temperature and latitude of origin on cottonmouth CO_2 production (resting metabolic rates) and the increase in cottonmouth resting metabolic rates associated with MCA. Each point represents the mean for (a) Missouri ($n=8$); (b) Arkansas ($n=23$); and (c) Louisiana ($n=22$) snakes. Error bars represent the 95% confidence interval around the mean. The significant interaction is due to the increase of metabolic rates at low temperatures (MCA). The value represents the increase at 2°C over what is predicted by extending the warm temperature regression line through 0.

observed trend may be due to seasonal acclimatization effects. Paired t -tests on the individual warm and cold acclimated CO_2 production rates at 17°C indicated the following within each population: The CO_2 production rates were significantly higher in the cold acclimated AR ($t=7.226$, $P<0.001$) and MO ($t=9.631$, $P<0.001$) cottonmouths, but significantly lower in the cold acclimated LA cottonmouths ($t=-7.096$, $P<0.001$)

3.3. Regression analyses

Predictive equations, and their associated parameters, for estimating CO_2 production rates (ml h^{-1}) based on mass (g) and temperature ($^\circ\text{C}$) were calculated for all three latitudes during all four time blocks (Table 2). The mass-scaling exponents ranged from 0.542 to 0.715 across the time blocks and geographic locations (Table 2).

Table 2

The results generated by regressing \log_{10} CO_2 production (ml h^{-1}) on \log_{10} mass (g) and temperature ($^{\circ}\text{C}$) for Missouri, Arkansas and Louisiana cottonmouths

Geographic origin	Time block	X_1 (S.E.)	X_2 (S.E.)	X_3 (S.E.)	Adj. R^2	V_{CO_2} equation	Q_{10}
MO	Early photophase	0.5416 (0.0456)	0.0518 (0.0031)	-2.2081 (0.1062)	0.6692	$0.006W^{0.542} \times 10^{0.052T}$	3.062
MO	Late photophase	0.5966 (0.0453)	0.0444 (0.0030)	-2.1683 (0.1048)	0.6001	$0.007W^{0.597} \times 10^{0.044T}$	2.754
MO	Early scotophase	0.5771 (0.0381)	0.0510 (0.0025)	-2.3307 (0.0866)	0.6536	$0.005W^{0.577} \times 10^{0.051T}$	3.236
MO	Late scotophase	0.5802 (0.0335)	0.0542 (0.0023)	-2.3828 (0.0768)	0.7239	$0.004W^{0.580} \times 10^{0.054T}$	3.467
AR	Early photophase	0.7099 (0.0237)	0.0468 (0.0017)	-2.4161 (0.0622)	0.7256	$0.004W^{0.710} \times 10^{0.047T}$	2.951
AR	Late photophase	0.6973 (0.0236)	0.0352 (0.0018)	-2.0630 (0.0633)	0.6455	$0.009W^{0.697} \times 10^{0.035T}$	2.239
AR	Early scotophase	0.7066 (0.0208)	0.0399 (0.0015)	-2.2521 (0.0545)	0.6591	$0.006W^{0.707} \times 10^{0.040T}$	2.512
AR	Late scotophase	0.6937 (0.0190)	0.0488 (0.0014)	-2.4472 (0.0496)	0.7288	$0.004W^{0.694} \times 10^{0.049T}$	3.090
LA	Early photophase	0.6954 (0.0178)	0.0577 (0.0012)	-2.5761 (0.0435)	0.8683	$0.003W^{0.695} \times 10^{0.058T}$	3.802
LA	Late photophase	0.6736 (0.0185)	0.0514 (0.0013)	-2.3413 (0.0468)	0.8144	$0.005W^{0.674} \times 10^{0.051T}$	3.236
LA	Early scotophase	0.7155 (0.0156)	0.0557 (0.0011)	-2.5709 (0.0385)	0.8429	$0.003W^{0.715} \times 10^{0.056T}$	3.631
LA	Late scotophase	0.6890 (0.0134)	0.0629 (0.0009)	-2.7080 (0.0328)	0.8910	$0.002W^{0.689} \times 10^{0.063T}$	4.266

The equation generated by PROC REG is $\log_{10} V_{\text{CO}_2} = X_1 \times \log_{10} W + X_2 \times T - X_3$, where $X_1 - X_3$ are fitted constants, W = mass (g), and T = temperature ($^{\circ}\text{C}$). Q_{10} is the ratio of V_{CO_2} at 32–22 $^{\circ}\text{C}$, based on a 400-g cottonmouth.

The Q_{10} values (calculated by the resting metabolic rates at 22 and 32 $^{\circ}\text{C}$) varied from 2.239 to 4.266 across the time blocks and geographic locations (Table 2). For each time block, the LA cottonmouths had consistently higher Q_{10} values than the MO or AR cottonmouths (Table 2).

4. Discussion

4.1. Effects of body mass and sex on RMR

The mass-scaling exponents ranged from 0.54 to 0.72. Mass effects on metabolic rates have been reviewed by Bennett and Dawson (1976), Andrews and Pough (1985) and a range of intraspecific mass-scaling exponents (0.51–0.80) has been reported. Based on the high variability of reported mass-scaling exponents in different species, a universal mass-scaling exponent that adequately describes the effects of body mass on metabolic rates may not exist. Rather, a wide range of values is possible, depending on the species being measured and the amount of actively metabolizing tissue in the individual, and models should include species-specific data (as recommended by Andrews and Pough, 1985).

Male and non-gravid female *A. piscivorus* did not differ in their CO_2 production rates once adjusted for body mass. A lack of intersexual variation in metabolic rates appears to be typical in most measured snake species (reviewed by Zaidan, 2001). While the effects of sex on meta-

bolic rates needs to be considered on an individual basis (Bennett and Dawson, 1976; Garland and Adolph, 1991), the bulk of evidence seems to indicate that male and non-gravid female snakes do not possess intrinsic differences in metabolic rates.

4.2. Effects of time of day and temperature on RMR

Circadian rhythms in metabolic rates may vary among species (reviewed by Bennett and Dawson, 1976). However, few recent studies have addressed the possibility of temporal variation in metabolism. Blem and Killeen (1993) reported that *A. piscivorus* and *Nerodia taxispilota* exhibited higher scotophase metabolic rates in snakes that were acclimated to a reverse photoperiod and tested in complete darkness. Blem and Killeen (1993) believed that movement in the chambers was partially responsible for the observed temporal variation. In rattlesnakes (*Crotalus lepidus*, *C. molossus*, *C. atrox*, and *C. horridus*), the lowest oxygen consumption or carbon dioxide production rates typically occurred between 08:00 and 11:00 CST/MST (Beaupre, 1993; Beaupre and Duvall, 1998; Beaupre and Zaidan, 2001), which closely matched my early photophase period (07:00–10:00 CST). In contrast, the lowest V_{CO_2} in *A. piscivorus* occurred earlier during the late scotophase period (01:00–06:00 CST). The highest V_{CO_2} in *A. piscivorus* occurred during the late

photophase period (13:00–18:00 CST). Because all temperature adjustments occurred approximately 11:00 CST, the possibility exists that the disturbance increased alertness of the experimental animals (thus leading to increased gas exchange rates). However, attempts to time-shift the maximum and minimum CO₂ production rates by making the temperature adjustments during a different time block failed. Instead of making the temperature adjustments approximately 11:00 CST, the temperature was changed at 18:00 CST in a single block of seven snakes. The existing pattern remained unchanged. The maximum during late photophase and the minimum during the late scotophase likely represent real circadian variation in *A. piscivorus* metabolism.

Temperature effects on snake ecology and physiology have been well documented (Huey, 1982; Andrews and Pough, 1985; Lillywhite, 1987; Peterson et al., 1993). Metabolic rates of ectotherms typically increase with increasing temperature (Bennett and Dawson, 1976). However, exceptions do exist, primarily with species that exhibit plateaus (a range of temperatures where a biological rate function is insensitive to temperature change; Pough and Gans, 1982) in their temperature response curves (Buikema and Armitage, 1969; Aleksiuik, 1971; Abe and Mendes, 1980; Stevenson et al., 1985; Ellis and Chappell, 1987). Over a temperature range of 12–32 °C, *A. piscivorus* exhibited a linear (once V_{CO_2} was log₁₀ transformed) metabolic response (Residual log₁₀ V_{CO_2} = 0.0545(Temp) – 1.0882; R^2 = 0.9919). However, at 7 °C the observed resting metabolic rate was approximately 168% higher than predicted. Metabolic cold adaptation (MCA) (a within-species process by which ectotherms in cold environments exhibit an increase in metabolic rates, at a given temperature, when compared to ectotherms in warm environments—Spicer and Gaston, 1999), may be responsible for the elevated response at 7 °C.

Q_{10} values in snakes typically range from 1.5 to 3.0 (Lillywhite, 1987). The Q_{10} values of 2.24–4.27 for *A. p. leucostoma* mostly fall above the squamate mean of 2.4 (Andrews and Pough, 1985) and the boid mean of 2.6 (Chappell and Ellis, 1987). However, the Q_{10} values for *A. p. leucostoma* compare well to those for other crotaline snakes (1.8–3.8 for *A. p. conanti*—McCue and Lillywhite, 2002; 1.7–3.1 for *C. atrox*—Beaupre and Duvall, 1998; 3.7–4.8 for *C. horridus* and 3.4

for *C. lepidus* and *C. molossus*—Beaupre and Zaidan, 2001). Cold adaptation has been previously implicated in high Q_{10} values (Davies and Bennett, 1981; Nielsen et al., 1999). While reasons for high Q_{10} values are difficult to explain, it is likely that differential selection on the temperature—metabolic rate relationship may be based on different operative temperature availability (Bakken, 1992; Beaupre and Zaidan, 2001).

4.3. Geographic variation in RMR

Both the presence and absence of geographic variation in metabolic rates has been documented over both small and large spatial scales. Along a very short geographic scale (\approx 30 km between the two sites), Beaupre (1993) detected significant differences (due to elevation differences) in metabolic rates between the populations of *C. lepidus* and *C. molossus*. In contrast, Beaupre and Zaidan (2001) detected no difference in metabolism between *C. horridus* that were collected in Northwestern Arkansas and Southeastern Virginia (approximate straight-line distance between the two sites is over 1500 km). Distance alone has little to do with intrinsic differences in metabolic rates between populations. Rather, local climatic factors provide different selective pressures on populations. The *C. lepidus* and *C. molossus* populations varied in elevation and consequently, in temperature profiles, rainfall, and food availability (Beaupre, 1993), whereas the *C. horridus* populations likely received similar thermal inputs due to both occurring in a buffered closed canopy deciduous forest lying on roughly the same latitude (Beaupre and Zaidan, 2001).

Approximately 823 km separated the northernmost and southernmost populations of *A. piscivorus* that were sampled, which represents roughly 95% of the total north–south width (867 km) of the cottonmouth's range along the sampled longitudinal transect. Not surprisingly, *A. piscivorus* receives higher mean yearly temperatures (19.7 °C) and a longer active season (351 days) in the southern part of its range when compared to the northern part (14.5 °C and 285 days). Thus, *A. piscivorus* experiences different thermal selective pressures across its range. Whether this is due to latitude alone or also to elevation is unclear, as elevation is confounded with latitude through much of the species' range. However, because higher elevations are typically encountered by

cottonmouths at higher latitudes, the two effects are not opposing and the end result would be colder temperatures and shorter active seasons in the northern part of the range.

Do the differences in thermal environments translate into different metabolic responses across the range of *A. piscivorus*? In the case of metabolic performance at low temperatures, one can argue that different selective pressures have shaped the organismal metabolic rates accordingly. However, it is unknown whether the differences are genetically fixed or the result of a genotype–environment interaction. The interesting information lies in the significant temperature interaction with latitude of origin. In the northern part of their geographic range, the AR and MO *A. piscivorus* show an elevated resting metabolic rate at low temperatures. In contrast, the LA snakes showed practically no increase in resting metabolic rate at low temperatures. Extending the regression lines to freezing and comparing the values at 2 °C in Fig. 3a–c shows the magnitude of the effect. While the mean overwintering body temperatures of snakes in Northwestern Arkansas are approximately 7 °C, individual body temperature records can drop to just above 0 °C (Beaupre, Unpublished data); therefore, 2 °C serves as a reasonable comparison point. The MO *A. piscivorus* exhibited a 2.76 times increase in low temperature metabolic rates over expected (based on scaling at higher temperatures). The AR *A. piscivorus* exhibited a 2.68 times increase in low temperature metabolic rates. Based on the data presented in Fig. 3a–c, MCA likely exists in northern populations of *A. piscivorus* where brumation periods are typically longer than 5 months. In contrast to the northern cottonmouths, the LA *A. piscivorus* exhibited a negligible (0.13 times) increase in low temperature metabolic rates and spend much less time (estimated to be \approx 3 months) confined to a den site.

MCA has been observed in a variety of taxa and researchers have suggested that MCA allows cold-environment ectotherms to be active at low environmental temperatures by preventing metabolic rates from reaching 0 at temperatures that would cause warm-environment ectotherms to bottom out their metabolism (Spicer and Gaston, 1999 and cites therein). The concept of MCA is not universally accepted and has received criticism by several authors due to the absence in many taxa and lack of general applicability (Clark, 1993; Johnson et al., 1998; Chown and Gaston, 1999).

What causes the supposed MCA? Previous work has suggested that selection for activity at low temperatures is responsible for the effect (reviewed by Spicer and Gaston, 1999). The increase in metabolic rates would occur when the snakes are most likely either in or very near the overwintering sites and when activity is minimal. In addition, the increase in metabolism can be costly, particularly when the snakes are surviving by using stored energy. An explanation may lie in overwinter survival, as Blem and Blem (1995) reported that overwinter mortality may be an important northern range-limiting mechanism in *A. piscivorus*. Rather than activity alone, I suggest that the increase in metabolism is used to fuel some other physiological function that is important for brumation survival.

4.4. Comparative patterns

While ectotherms in general have low metabolic rates, snakes in the Family Viperidae and Boidae appear to have particularly low metabolic rates when compared to other squamates (text and original Fig. 5 in Beaupre and Zaidan, 2001) and may be considered low energy specialists. A comparison of viperid resting metabolic rates appears in Table 3. Among species comparisons are enigmatic due not only to phylogeny, but also to methodology. However, the data on *A. piscivorus* contribute to the body of evidence that crotaline snakes are low energy systems.

Within *A. piscivorus*, my data on the subspecies *leucostoma* compare well with the values for the subspecies *conanti* (McCue and Lillywhite, 2002). By combining the two data sets, resting metabolic rates in *A. piscivorus* appear to increase with decreasing latitude. The values for *A. p. piscivorus* (Blem and Blem, 1990) were more than 4.7 times greater than the highest *A. p. conanti* (McCue and Lillywhite, 2002). Whether the higher metabolic rates of *A. p. piscivorus* reflect a genuine geographic/subspecies effect or are simply due to measurement errors remain unknown.

4.5. Implications for northern range limits

How do resting metabolic rates influence the latitudinal distribution of *A. piscivorus*? Previous work appears to indicate that the presence of elevated metabolic rates during cold seasons allow

Table 3
Comparison between published Viperid resting metabolic rates

Species	Open/closed system (original units)	Metabolic rate (J h ⁻¹)	Citation
<i>A. piscivorus (leucostoma)</i>	Open (mlCO ₂ h ⁻¹)	35.5 ± 20.8 (MO) 45.0 ± 22.5 (AR) 49.2 ± 13.5 (LA)	Present study
<i>A. p. conanti</i>	Open (mlO ₂ h ⁻¹)	62.6 (FL-mainland) 69.5 (FL-insular)	McCue and Lillywhite (2002)
<i>A. p. piscivorus</i>	Open, O ₂ (kJ h ⁻¹)	336.6 (22 °C acc.) 509.2 (32 °C acc.)	Blem and Blem (1990)
<i>Bothrops moojeni</i>	Closed (mlO ₂ h ⁻¹)	282.7 ± 142.7 ^a	Cruz-Neto and Abe (1994)
<i>Cerastes cerastes</i>	Open (mlO ₂ g ⁻¹ h ⁻¹)	73.8 ^b	Dmi'el (1972)
	Closed (mlO ₂ g ⁻¹ h ⁻¹)	93.5 ^a	Al-Sadoon (1991)
<i>Crotalus atrox</i>	Open (mlO ₂ h ⁻¹)	56.1 ± 69.8	Beaupre and Duvall (1998)
<i>C. atrox/molossus/tigris</i>	Open (mlO ₂ h ⁻¹)	1866.6	Beck (1995)
<i>C. cerastes</i>	Closed (mlO ₂ g ⁻¹ h ⁻¹)	37.1	Secor and Nagy (1994)
<i>C. horridus</i>	Open (mlCO ₂ h ⁻¹)	29.3 ± 71.9	Beaupre and Zaidan (2001)
<i>C. lepidus/molossus</i>	Closed (mlO ₂ h ⁻¹)	24.4 ± 53.7	Beaupre (1993)
<i>C. viridis</i>	Closed (mlO ₂ g ⁻¹ h ⁻¹)	157.4 ^c	Ruben (1976)
<i>Vipera berus</i>	Open (mlO ₂ g ⁻¹ h ⁻¹)	182.0 ^b	Johansen and Lykkeboe (1979)
	Closed (mlO ₂ g ⁻¹ h ⁻¹)	195.8 ^a	Al-Sadoon (1991)
<i>V. palaestinae</i>	Open (mlO ₂ g ⁻¹ h ⁻¹)	68.9 ^b	Dmi'el (1972)

The values shown were calculated from the literature for a 100 g animal at 25 °C. When necessary, an R.Q. of 0.72 and 1 mlO₂ = 19.68 J or 1 mlCO₂ = 27.42 J was assumed (following Brody, 1945; Gessaman and Nagy, 1988) in order to convert to a common unit of J h⁻¹. When possible, the 95% confidence interval was calculated around each mean value.

^a Average of values at 20 and 30 °C.

^b Estimated from graph.

^c Measured at 35 °C.

for larger northern range distributions (Ayres and Scriber, 1994) and its absence may be limiting (Rogowitz, 1996). A 400 g AR *A. piscivorus* requires approximately 11.3 kJ mol⁻¹ for resting metabolism at 7 °C, compared to the 6.9 kJ mol⁻¹ required by a LA *A. piscivorus* of the same size and temperature (assuming that lipids are the primary metabolic substrate and a conversion factor of 27.8 J mlCO₂⁻¹; Withers, 1992). In the case of *A. piscivorus*, the presence of MCA may not allow for a more northerly geographic range limit, in the sense of preserving the potential for low temperature activity. The elevation in metabolic rates may be adaptive for increasing some other unknown function during the colder temperatures and longer amount of time spent in the hibernacula at higher latitudes, thus increasing overwinter survivorship. During shorter brumation periods, it may not be necessary to pay the costs associated with elevated metabolic rates. The results appear to indicate that a lack of low

temperature metabolic elevation (as was observed in the LA *A. piscivorus*) may be energetically advantageous under environmental conditions that allow for longer active seasons and when temperatures do not fall to levels that endanger other processes.

One important question remains: Does enough plasticity (i.e. acclimatization or the genotype–environment interaction) exist in metabolic rates to allow for the development of MCA? The implications for northern range limits are important. If the maximum allowable increase in low temperature resting metabolic rates is already present in the northern populations of *A. piscivorus*, the ability to expand the range northward is limited. If a further increase is possible the northern range limit may be able to be expanded. Further research is needed to address the possibility. However, the observed geographic variation in *A. piscivorus* resting metabolic rates may be important to shaping the species' distribution.

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