

Effect of temperature on metabolic rate of the mud turtle (*Kinosternon subrubrum*)

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Abstract

Temperature plays an important role in various aspects of the life history and physiology of ectotherms. We examined the effect of temperature on standard metabolic rate in the mud turtle, *Kinosternon subrubrum*. We measured O₂ consumption and CO₂ production at 20°C and 30°C using a flow through respirometry system. Standard metabolic rate was significantly higher at 30°C (9.25 ml O₂/h, 6.35 ml CO₂/h) compared to 20°C (2.10 ml O₂/h, 1.96 ml CO₂/h). The Q_{10} value for O₂ was 5.10, and for CO₂ was 3.40. Our findings generally agree with those of other studies of metabolism in vertebrate ectotherms.

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Keywords: Carbon dioxide production; *Kinosternon subrubrum*; Oxygen consumption; Q_{10} ; RQ; Respiratory quotient; Standard metabolic rate; Temperature; Turtle

1. Introduction

Temperature plays an important role in various aspects of the life history, ecology, and physiology of reptiles and other ectotherms (Angilletta et al., 2002). Growth rates (Arnold and Peterson, 1989; Avery, 1994; Litzgus and Brooks, 1998a), reproduction (Schwarzkopf and Shine, 1991; Litzgus and Brooks, 1998b; Rock and Cree, 2003), seasonal activity patterns and habitat use (Webb and Shine, 1998; Whitaker and Shine, 2002), and geographic distribution (Castonguay et al., 1999) are all influenced by environmental temperatures. Physiological processes such as metabolic rate generally increase with temperature (e.g., Gatten, 1974; Bennett and Dawson, 1976; Beaupre et al., 1993; Karasov and Anderson, 1998; McNab, 2002); however, a few reptile species are known to have plateaus of temperature-independent metabolism (Waldschmidt et al., 1987). Information on how temperature affects metabolic rates is useful for the

development of models that describe the energy budgets of organisms (Lillywhite, 1987; Secor and Nagy, 1994; Beaupre, 1995, 1996), which in turn strongly influence life histories (Congdon et al., 1982; Dunham et al., 1989).

An examination of variation in individual metabolic response to environmental variation (i.e., temperature changes) can elucidate mechanistic explanations for broad ecological and evolutionary patterns (Congdon et al., 1982). In addition, information on physiological adaptations can be gleaned from intraspecific and interspecific comparative analyses (Garland and Adolph, 1994; Zaidan, 2003). Only a handful of studies on turtles have directly tested the effect of temperature on metabolic rate. Oxygen consumption rates increased with temperature in the slider turtle (*Trachemys scripta*), box turtle (*Terrapene ornata*) (Gatten, 1974), African tortoise (*Kinixys spekii*) (Hailey and Loveridge, 1997), and in hatchling snapping turtles (*Chelydra serpentina*) (Steyermark and Spotila, 2000). Only one of these studies examined both O₂ consumption and CO₂ production (Steyermark and Spotila, 2000). The purpose of this paper was to examine the effect of temperature on

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O₂ consumption and CO₂ production rates of mud turtles (*Kinosternon subrubrum*), and to compare our calculated Q_{10} and respiratory quotient (RQ, the ratio of CO₂ produced to O₂ consumed) values to those reported for other reptile species.

2. Materials and methods

Mud turtles are relatively small freshwater turtles, typically less than 12 cm in shell length (Ernst et al., 1994). The species ranges from Long Island, New York, southward through Florida to the Gulf Coast, westward to central Texas, and northward into the Mississippi Valley (Ernst et al., 1994). Mud turtles (3 females, 4 males, mass range 97–220 g) were collected by hand between 15 and 25 February 2003, shortly after emergence from hibernation, from the Francis Beidler Forest National Audubon and Nature Conservancy Sanctuary near Harleyville, SC (33°N, 80°W). Beidler Forest is a 12,000-acre nature preserve located in Four Holes Swamp, and includes cypress-tupelo blackwater swamp, hardwood bottom swamp, and upland pine forest. Turtles were brought into the lab where they were housed in plastic tote bins (17 liters, 42 cm × 30 cm × 15 cm) with shallow water, and fasted for a minimum of 9 days to ensure post-absorptivity prior to metabolic determinations.

Oxygen consumption (VO₂) and carbon dioxide production (VCO₂) were measured using a portable, flow-through respirometer (Oxymax ER-10, Columbus Instruments, Columbus, OH) interfaced to a laptop computer. Each turtle was placed in a circular Plexiglass chamber (volume ~800 ml, diameter = 15 cm, height = 5 cm) lined with moist paper towels. Turtles were not restrained and could therefore move freely inside the chamber. Chamber volumes and leaks were measured and checked by the Oxymax ER-10 software. Each chamber was attached to an independent channel of the respirometer by tubing. The respirometer and chambers containing turtles were placed in an incubator (Percival Scientific, Boone, Iowa; 24 h dark) and metabolic rate was measured for 24 h at each of two temperatures sequentially, 30°C and 20°C. Turtles were acclimated to the treatment temperature while in respirometry chambers for 2 h prior to commencement of metabolic rate determinations. Every 1.75 h, samples of air from each turtle chamber were passed over magnesium perchlorate and rates of VO₂ and VCO₂ were simultaneously determined by the respirometer. The O₂ and CO₂ sensors were purged with outside air passed over a column containing Drierite after each measurement. Thus, at both temperatures, a total of 13 measurements of each gas were obtained for each turtle over the 24-h period. All

measurements were corrected for standard temperature and pressure by the Oxymax ER-10 software. Turtles were weighed (± 1 g) at the end of the experiment, and returned to their tote bins until release at their sites of capture.

From the VO₂ and VCO₂ data collected, we estimated standard metabolic rate (SMR), the metabolic rate of a post-absorptive animal at rest at a given temperature during the inactive part of its diel cycle (Bennett and Dawson, 1976; Waldschmidt et al., 1987). Mud turtles, like most vertebrate ectotherms, exhibited spontaneous activity during respirometry trials (Fig. 1). In an effort to exclude periods of activity from our estimates, SMR for VO₂ and VCO₂ were determined by calculating the mean of the lowest approximate 30% of the data (4 of 13 data points) for each gas for each turtle. This method of estimation eliminated obvious bouts of spontaneous activity (Fig. 1) and therefore appears to accurately represent SMR in our sample of mud turtles.

We calculated the respiratory quotient (RQ) for each temperature treatment, and the Q_{10} s (20–30°C) for VO₂ and VCO₂ using the SMR values. RQ (the ratio of CO₂ produced to O₂ consumed) allows inference about aerobic catabolism (Withers, 1992). The effect of temperature on metabolic processes can be described using the term Q_{10} , which is defined as the change in the rate of metabolism over a 10°C change in temperature; Q_{10} normally has a value between 2 and 3 (McNab, 2002).

To examine the effects of temperature on SMR, we used a repeated measures ANCOVA with body mass as the covariate (proc mixed model, SAS Institute, Cary, NC). To satisfy the assumption of linearity, both body mass and SMR were log₁₀ transformed prior to statistical analyses. Statistical significance was accepted at $P < 0.05$.

3. Results

The effect of sex on SMR was not significant for either VO₂ ($F_{1,4} = 1.49$, $P = 0.29$) or VCO₂ ($F_{1,4} = 4.67$, $P = 0.10$); therefore, data for the sexes were pooled in all further analyses. Similarly, the effect of mass on SMR was not significant for VO₂ ($F_{1,5} = 2.04$, $P = 0.21$) or VCO₂ ($F_{1,5} = 4.42$, $P = 0.09$), although a positive linear trend was detected. Mean (\pm SE) SMR and RQs for each temperature treatment are shown in Table 1. Temperature had a significant effect on SMR for both VO₂ ($F_{1,6} = 26.94$, $P < 0.01$) and VCO₂ ($F_{1,6} = 37.74$, $P < 0.001$), such that as temperature increased, metabolic rate increased. Spontaneous activity bouts were also more frequent at the higher temperature (Fig. 1). Mean (\pm SE) Q_{10} (20–30°C) for VO₂ was 5.10 ± 1.18 , and for VCO₂ was 3.40 ± 0.52 .

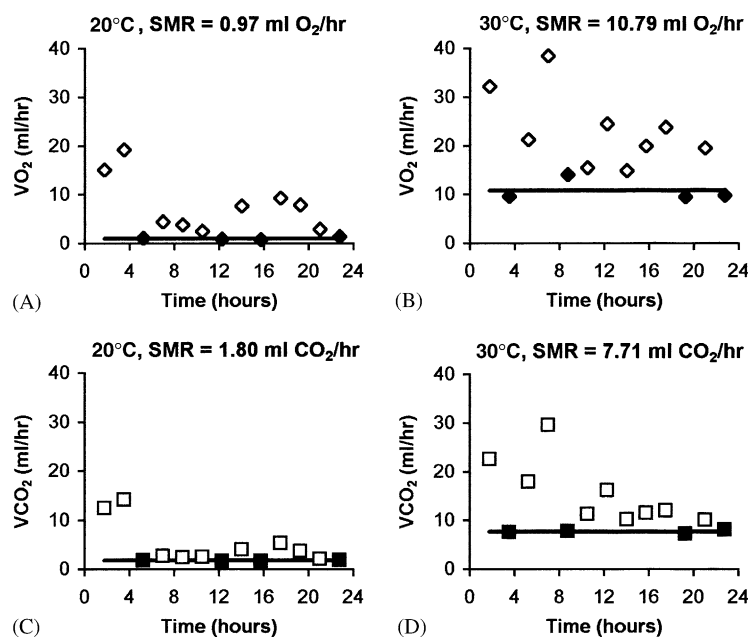


Fig. 1. Oxygen consumption (VO_2 , diamonds, A and B) and carbon dioxide production (VCO_2 , squares, C and D) of a mud turtle (*Kinosternon subrubrum*) (mass = 220 g) at 20°C (A and C) compared to the same turtle at 30°C (B and D). The horizontal lines represent the standard metabolic rates (SMR) calculated as the mean of the lowest 30% of the data for each gas. The lowest 30% of the data are shown as solid symbols, and the highest 70% of the data (not used in the calculation of SMR) are represented as hollow symbols. This method of estimation reduces the influence of spontaneous activity on the calculation of SMR.

Table 1

Mean \pm SE standard metabolic rate (SMR) measured indirectly as oxygen consumption (VO_2) and carbon dioxide production (VCO_2), and respiratory quotients (RQ) of mud turtles, *Kinosternon subrubrum* ($N=7$, mean mass \pm SE = 170 \pm 18 g) at two temperatures

Parameter	20°C	30°C
SMR VO_2 (ml/h)	2.10 \pm 0.35	9.25 \pm 1.70
SMR VCO_2 (ml/h)	1.96 \pm 0.22	6.35 \pm 0.99
RQ	1.06 \pm 0.17	0.73 \pm 0.06

4. Discussion

As expected, temperature had a significant effect on metabolic rate in mud turtles. Turtles were more active and had significantly higher rates of VO_2 and VCO_2 at 30°C compared to 20°C. Other studies that directly tested the relationship between temperature and metabolic rate in turtles also found a strong positive relationship (e.g., Gatten, 1974; Hailey and Loveridge, 1997; Steyermark and Spotila, 2000; Table 2). This is in agreement with the finding that, in vertebrate ectotherms, metabolic rate generally increases with temperature (e.g., Gatten, 1974; Bennett and Dawson, 1976; Beaupre et al., 1993; Karasov and Anderson, 1998; McNab, 2002). Unfortunately, most studies that report

metabolic rates of turtles did not test the effect of temperature, making broad comparisons of SMR values at various temperatures difficult.

In our mud turtles, the Q_{10} value (20–30°C) for VO_2 was 5.10, and for VCO_2 was 3.40. These values are somewhat higher than those calculated for other turtle species at the same temperature interval (Table 2), but fall well within the range of Q_{10} values reported for turtles at other temperature intervals. Some evidence suggests that, in turtles, Q_{10} values are higher at cooler temperatures than at warmer temperatures, indicating that the rate of metabolic change is not isometric with temperature. For example, the Q_{10} for box turtles (*Terrapene ornata*) between 10°C and 15°C was 54.38 compared to 1.60 between 20°C and 30°C (Gatten, 1974). Similarly, the Q_{10} for painted turtles (*Chrysemys picta bellii*) between 3°C and 10°C was 8.5 compared to 2.9 between 15°C and 20°C (Herbert and Jackson, 1985). A higher Q_{10} at cooler temperatures may reflect a transition from aerobic to anaerobic metabolism. Future work examining the effect of temperature on metabolic rate in turtles should include a wide range of experimental temperatures and an examination of lactic acid accumulation to further test this hypothesis.

The RQ allows inference about the substrate used to fuel aerobic metabolism (Withers, 1992). An RQ value of 1.0 suggests oxidation of carbohydrate, an RQ of 0.84

Table 2

Q_{10} values based on metabolic rates (oxygen consumption, VO_2) of turtles between 20°C and 30°C. The studies included here reported standard metabolic rates or resting metabolic rates of fasted turtles as mass-specific rates. Note that Q_{10} s from previous studies were calculated using the reported SMR values in those studies

Species	Temp (°C)	VO_2 (ml/h)	VO_2 (ml/g/h)	Range of body mass (g)	Q_{10}	Reference
<i>Kinosternon subrubrum</i>	20	2.10	0.0127	97–220	5.10	This study
<i>Kinosternon subrubrum</i>	30	9.25	0.0557			
<i>Chelydra serpentina</i>	20	—	0.0209	57–73 (juv)	2.49	Steyermark and Spotila (2000)
<i>Chelydra serpentina</i>	30	—	0.0521			
<i>Trachemys scripta</i>	20	—	0.0109	257–353	2.87	Gatten (1974)
<i>Trachemys scripta</i>	30	—	0.0313			
<i>Terrapene ornata</i>	20	—	0.0093	180–538	1.60	Gatten (1974)
<i>Terrapene ornata</i>	30	—	0.0149			
<i>Kinixys spekii</i>	20	—	0.0116	244–1383	3.35	Hailey and Loveridge (1997)
<i>Kinixys spekii</i>	30	—	0.0389			

indicates catabolism of protein, and a value of 0.7 suggests oxidation of lipid (Seidel, 1978; Withers, 1992). RQ values of mud turtles suggested that at 20°C, carbohydrate is the primary energy source utilized, and that at 30°C, mainly lipids are oxidized to sustain metabolism. Similarly, in the congeneric yellow mud turtle (*K. flavescens*), the RQ was 0.7 at 28°C, implying metabolism of lipids (Seidel, 1978). In snapping turtle hatchlings (*Chelydra serpentina*), RQ values were lower at about 0.64 at 15°C and 0.63 at 25°C (calculated from Table 3 in Steyermark and Spotila, 2000); however, these values also implicate lipid as the primary metabolic substrate. In addition, RQs between 20°C and 25°C for the freshwater turtles *Chelydra serpentina* and *Chrysemys picta* suggested lipid catabolism as the values were generally around 0.7 (Bennett and Dawson, 1976). In the tortoises *Testudo graeca* and *T. hermanni*, RQ values for body temperatures between 20°C and 25°C ranged from 0.7 to 0.91 (Bennett and Dawson, 1976), implying that both lipid and protein serve as metabolic substrates. Similarly, others have noted that resting and fasting reptiles exposed to a range of body temperatures generally have RQs near 0.7, implying catabolism of lipids (see Table XII in Bennett and Dawson, 1976).

Body mass and sex are important sources of variance in metabolic parameters. Despite a two-fold range in body size among individual mud turtles in our study (mass range 97–220 g), we did not find a statistically significant effect of body size on metabolic rate, although a weak positive trend was detected. The

relationship between body size and metabolic rate is well documented among ectothermic animals (e.g. Bennett and Dawson, 1976; Andrews and Pough, 1985; Feder and Burggren, 1992; McNab, 2002), and with a larger sample size, we would have likely detected such a trend. Some studies of ectothermic vertebrates have also found significant effects of sex on metabolism (e.g., Taigen et al., 1985; Pough et al., 1992; Ryan and Hopkins, 2000), whereas other studies have found no effect of sex on metabolic rate (Beaupre et al., 1993; Zaidan, 2003). In snakes as a group, it has been suggested that males and non-gravid females do not possess intrinsic differences in metabolic rates (Zaidan, 2003). We did not find a significant difference between the sexes in metabolic rate; however, with respect to VCO_2 , males tended to have a higher metabolic rate than females. Our sample size for each gender was small ($N=3$ females, $N=4$ males), so the lack of significance may simply reflect a lack of statistical power, and thus additional work is needed to examine potential sex effects on metabolism in turtles.

Information on how metabolic rate varies with body temperature can provide insight into individual variation in growth, reproduction, survival, and therefore, fitness. Our study adds to the limited data concerning the effect of temperature on metabolism in turtles. Future work assessing metabolic rates in turtles in response to temperature variation is an important step towards the development of a comprehensive picture of intra- and inter-individual metabolic variation and

adaptation, and the comparison of energy budgets among related ectotherms.

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