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First Evidence of Metabolic Heating in a Freshwater Turtle (*Chelydra serpentina*)

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Abstract. – **Metabolic heating caused by physiological processes during the development of oviparous embryos can raise nest temperatures above those of the surrounding substrate and may be sufficient to increase embryonic growth rates, influence sex ratios of hatchlings with temperature-dependent sex determination, and increase hatching success in seasonal environments. In sea turtles with large clutch sizes, metabolic heating can raise nest temperatures by as much as 6°C. However, no studies have directly investigated metabolic heating in any species of freshwater turtle. We investigated whether metabolic heating occurs in nests of snapping turtles (*Chelydra serpentina*) from southeastern Michigan, United States. A temperature logger was placed in the center of 8 unaltered snapping turtle nests. A second temperature logger was placed at the same depth in the surrounding substrate 5 cm from the side of the nest chamber. Metabolic heating is more pronounced in nests with larger clutches, so we artificially increased the size of 2 additional nests using donor clutches of 11 and 21 eggs, respectively. Temperatures were recorded at 2-hr intervals until after the presumptive hatch date of all nests. We found that there was a significant increase both in mean nest temperature and accumulated heat units for natural and experimental treatment nests during the last third of incubation. Further, in nests with experimentally increased clutch sizes, mean nest temperature was significantly greater than substrate temperature throughout incubation, suggesting that large nests also exhibit a thermal inertia that results in positive heat balance throughout development, at least in the soils studied.**

Key Words. – **development rate; freshwater turtles; incubation; metabolic heating; nest; sex determination**

In oviparous reptiles, rates of embryonic development and incubation temperature are tightly linked (Ewert 1985; Georges et al. 2005; Rollinson et al. 2018) and have a substantial influence on phenotype (summarized in Booth 2006). Long-term influences of incubation temperature on hatchlings can include changes in hatchling sex ratios within clutches under temperature-dependent sex determination (Bull and Vogt 1979, reviewed in Valenzuela 2004), locomotor performance (Janzen 1993; Doody 1999; Booth et al. 2004), early juvenile growth (Rhen and Lang 1995), and subsequent survival (Kingsolver 2009; Fisher et al. 2014). However, less is known about whether intrinsic organismal factors, such as metabolic heat produced by freshwater turtle embryos in nests, are sufficient to alter the thermal environment of nests.

Metabolic heating in nests is caused when the living tissues of embryos produce heat during development as a byproduct of metabolic processes, raising nest temperature beyond that of the surrounding environment (Carr and Hirth 1961; Bustard and Greenham 1968). The degree of metabolic heating in a nest is dependent on the amount of living tissue available to participate in metabolism (Broderick et al. 2001; Zbinden et al. 2006). In sea turtle nests, metabolic heating begins in the middle of the incubation period and can increase nest temperatures by 1.5°C–6.0°C above substrate temperatures (Godfrey et al. 1997; Broderick et al. 2001; Zbinden et al. 2006; DeGregorio and Williard 2011). In fact, in the green turtle (*Chelonia mydas*), metabolic heating in late development is more influential on mean nest temperature than heat caused by solar radiation (van de Merwe et al. 2006).

Metabolic heating is suspected to be a significant source of thermal energy in freshwater turtle species with large clutch and/or egg sizes (Webb et al. 1986), but there have been no studies directly investigating metabolic heating in nests of any species of freshwater turtle. Although the body sizes of freshwater turtles are typically smaller and produce substantially smaller clutch and egg

sizes than sea turtles, there may be enough tissue mass to generate heat through metabolic processes. For example, well-studied marine turtles, such as green turtles and loggerheads (*Caretta caretta*), produce large clutches averaging > 100 eggs (Hirth 1980; Bjorndal and Carr 1989; Broderick et al. 2003). In common North American freshwater turtles such as midland painted turtles (*Chrysemys picta marginata*), clutches average 4–10 eggs each (Smith 1956). Similarly, Blanding's turtles (*Emydoidea blandingii*) have a mean clutch size of approximately 10 eggs (Congdon and van Loben Sels 1991). However, in snapping turtles (*Chelydra serpentina*), mean clutch sizes are generally large, varying from 25 to 48 eggs (Congdon et al. 1987, 2008; Ernst and Lovich 2009). Therefore, it is probable that metabolic heating influences temperatures within snapping turtle nests, as well as those of other freshwater turtle species with large clutches and/or large eggs (Webb et al. 1986). Given the considerable amount of research done on development and temperature-dependent sex determination in snapping turtles (e.g., Yntema 1968; Janzen 1992; Ewert et al. 2005), it is curious that the occurrence and influence of metabolic heating has not been studied in this species.

Metabolic heating can increase rates of developmental and other physiological processes in the embryo that result in a shorter incubation period and, thus, earlier emergence from nests (Carr and Hirth 1961; Rollinson et al. 2018). Expediting developmental rate may be particularly important in northern climates, where freshwater turtle populations experience poor recruitment as a result of embryonic failure during short growing seasons (Obbard 1983; Edge et al. 2017). For species with temperature-dependent sex determination, even a mean nest temperature increase of 1°C during the middle of incubation has been shown to affect the outcome of sex (Bustard 1972; Broderick et al. 2001; Ewert and Nelson 2003; DeGregorio and Williard 2011).

We investigated whether metabolic heating occurs in nests of the snapping turtle. We estimated the degree of metabolic heating that occurs in natural, unmanipulated snapping turtle nest cavities, as well as the effects of artificially increasing clutch size on metabolic heating, using donor eggs from other snapping turtle clutches. We present the first data collected on metabolic heating in any freshwater turtle species and discuss how physiological and ecological factors may be affected by metabolic heating.

METHODS

Over 3 decades (1975–2007), the life history and nesting ecology of snapping turtles have been studied on the University of Michigan E. S. George Reserve (ESGR) near the town of Hell in southeastern Michigan. Clutch sizes for this population average approximately 28 eggs/nest and egg wet mass averages 11.6 g (Congdon et al. 1987, 2008).

Before the nesting season began, we programmed iButton temperature loggers (Maxim Integrated, San Jose, CA) to record temperatures at 2-hr intervals. From 6 to 10 June 2017, we monitored sites for nesting snapping turtles. In this study, we included 12 snapping turtle nests of known maternity and 1 of unknown maternity (but found within 12 hr of nest construction). At the time of nesting completion, we recorded maternity where applicable, date and time, uppermost nest depth, and substrate conditions.

We grouped nests into natural and experimental treatment groups. Natural treatment nests remained in their original nest cavity, while experimental treatment nests were created by adding a donor clutch to the side of a recipient clutch's enlarged nest cavity.

For 8 natural treatment nests, we first dug a tunnel at approximate nest depth from one side of the nest until the outermost eggs were exposed. Accessing the eggs from the side of the nest allowed us to remove a few eggs, insert an iButton in the middle of the clutch, and then replace the eggs that had been removed, with minimal disturbance to the natural nest cavity. Oak (*Quercus* spp.) leaves were used to prevent the loss of air spaces between eggs while replacing the soil from the access tunnel.

We added eggs to 2 experimental treatment nests using 2 donor clutches. For these nests, we excavated 2 donor clutches of 11 and 21 eggs, respectively. We then exposed 2 recipient clutches and expanded the nest cavity on the tunnel side, while carefully maintaining the depths of the top and bottom of the original nest. An iButton was placed at midheight on the edge of the eggs in the recipient nest. Eggs from the donor nest were then stacked against the original exposed eggs to the height of the original nest, such that the iButton was in the approximate center of the resulting artificially increased nest cavity. Oak leaves were placed on the side of the added eggs to help maintain the air spaces between them, and we covered the expanded nest cavity with soil removed from the expanded nest chamber and tunnel. A second iButton was placed 5 cm from the outside of the combined egg chamber, at the same depth as the iButton located in the center of the nest. In sum, one treatment nest had an additional 11 eggs added, while the second had 21 eggs added.

To prevent predators from disturbing the nests, we placed a nest cage made of wire fencing over each nest and securely staked it. With the exception of donor clutches, all other clutches remained at the natural sites selected by the female turtles until September of 2017, when we removed the iButtons after hatchlings had emerged.

We downloaded temperature data from the iButtons after hatching had occurred in fall 2017. We did not monitor hatching in the field; therefore, we selected 90 d from laying as the hatch date based on the typical incubation time for this population (Congdon et al. 1987). The iButtons have a 0.5°C precision and are accurate to ± 1°C, and previous experience with these loggers suggests the existence of small but systematic differences in temperature readings between iButtons, even when held

under identical temperatures. We therefore corrected for potential systematic iButton error within pairs of iButtons retrieved from inside and outside each nest. In winter 2018, we placed iButton pairs adjacent to one another within a small container in a Reptibator incubator (ZooMed Laboratories Inc, San Luis Obispo, CA), at constant temperature for 12 hrs. The average difference between iButton pairs over 12 hrs was taken and the difference between temperature inside and outside the nest was subsequently adjusted for each nest.

For each nest, we calculated the average daily difference in temperature between the iButton located in the center of the nest and the iButton located 5 cm from the edge of the nest cavity. Positive differences are a measure of warmer temperatures within the nest. We also calculated the amount of heat accumulated inside and outside each nest using the classic degree-day approach. Degree-days (°D) are calculated as cumulative exposure to heat above a lower threshold temperature over a certain length of time (Pedigo and Rice 2009). For example, the °D accumulation for an embryo incubated at a constant temperature of 22°C with a threshold of 15°C would be 7°D/d. We calculated °D accumulation using a threshold temperature of 15°C, given that the development of embryos incubated at a constant temperature of 15°C is trivial (Rollinson et al. 2018). We calculated the cumulative °D inside and outside each nest and calculated the cumulative difference in °D inside vs. outside each nest over the incubation period.

Finally, we performed a broken line regression in order to estimate the time point at which metabolic heating begins (Knowles et al. 1991). The broken-line regression method estimates a single change point in a linear regression. In the present study, we expected that the relationship between the difference in °D accumulation (inside vs. outside the nest) and day of incubation would become stronger (i.e., a steeper slope of °D accumulation over time) once metabolic heating began. Thus, we expected a single change point would occur sometime after day 30 (i.e., after the first third of incubation), consistent with previous studies on metabolic heating (van de Merwe et al. 2006; Zbinden et al. 2006).

RESULTS

In total, we sampled 10 nests, 8 with single clutches in the "natural" treatment group and 2 in the "experimental" treatment group. We were unable to retrieve data-loggers from one natural nest, so in total, we analyzed data from 7 natural nests and 2 experimental nests. Five of the 9 iButton pairs were corrected for temperature after calibration; all iButton temperature corrections were less than ± 0.5°C and results were qualitatively unchanged when uncorrected values are used (data not shown).

For the natural treatment, the greatest mean difference we observed was 0.276°C (± 0.064°C SE), the minimum difference was –0.076°C (± 0.102°C SE), and the average difference became consistently positive after day 49 of incubation (Fig. 1). For the experimental treatment, the greatest mean difference between the inside and outside of the nest on a given day was 0.915°C (± 0.024°C SE), the minimum mean difference was 0.311°C (± 0.045°C SE), and the average difference was consistently positive (Fig. 1).

By day 90, the total average °D for the natural treatment were 621.4°D (± 30.2°D SE) and 717.8°D (± 29.1°D SE) for the experimental treatment (Fig. 2). Broken-line regression revealed a significant breakpoint in both treatments, with the rate of accumulation of °D becoming more rapid on average at day 55.2 (lower confidence interval [LCI] = 54.6; upper confidence interval [UCI] = 55.8) for natural treatments and at day 48.8 (LCI = 48.0; UCI = 49.8) in experimental treatments (Fig. 2). On day 90, the mean difference in °D from the inside of the nest to the outside of the nest was 10.5°D (± 7.23°D SE) for the natural treatment and 38.3°D (± 19.5°D SE) for the experimental treatment (Fig. 2). In the natural treatment, the value of the mean accumulated °D of the nest (relative to the soil) was significantly larger (paired *t*-test, $t = 3.20$, df = 6, $p = 0.019$), whereas in the experimental treatment, the value of the mean accumulated °D was not significantly different (paired *t*-test, $t = 1.97$, df = 1, $p = 0.30$).

In both treatments, the greatest significant difference in average temperature from the inside of the nest to outside of the nest occurred during the last third of incubation, and the greatest °D accumulation occurred during the last third of incubation (Table 1). Both results are consistent with the breakpoint estimates from the broken-line regression.

DISCUSSION

We found that snapping turtle nests were warmer than their surrounding substrate and that warming begins to increase rapidly approximately halfway through incubation. We attribute some of this heating to metabolic heating itself and some to thermal inertia caused by the high specific heat capacity of eggs. Our experimental enlargement of clutch size appeared to result in an increase in °D heat production, although the difference in heat accumulation between the inside and outside of experimental treatment nests was not significant at the end of incubation, perhaps owing to small sample size ($n = 2$). Estimating the realized effect of metabolic heating on development time is not possible in the present study because ontogeny of the embryo development in nests from the ESGR has not yet been mapped onto °D accumulation. However, using values from the snapping turtle development model of Rollinson et al. (2018), the data suggest that, by day 90, natural and experimental treatment nests experienced the equivalent of an additional 12.5 d (± 10.8 d SE) and 27.1 d (± 17.2 d SE), respectively, of development at 20°C above soil temperature alone. Although these values are approximations, having been estimated for a different threshold tempera-

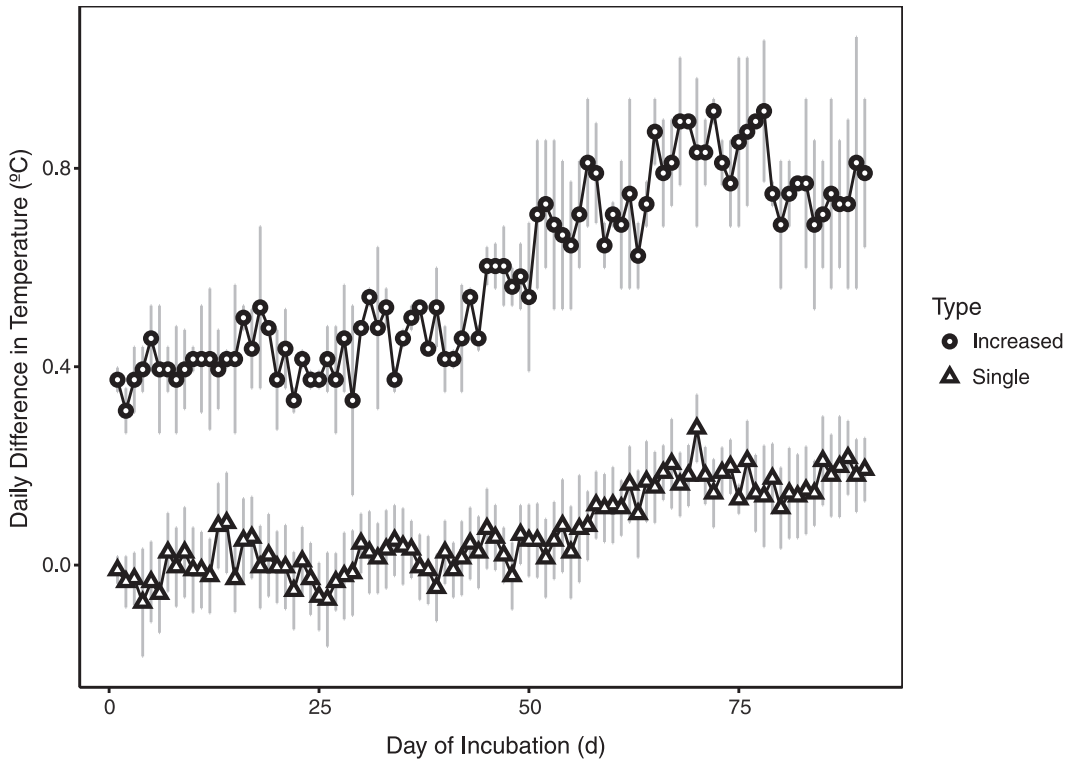

Figure 1. Mean daily difference in temperature between the inside and outside of snapping turtle (*Chelydra serpentina*) nests, for natural treatment (triangle) and experimental treatment (circle) nests. Positive differences indicate the nest is warmer than the surrounding substrate; black horizontal line is at 0. Standard error is represented by gray bars.

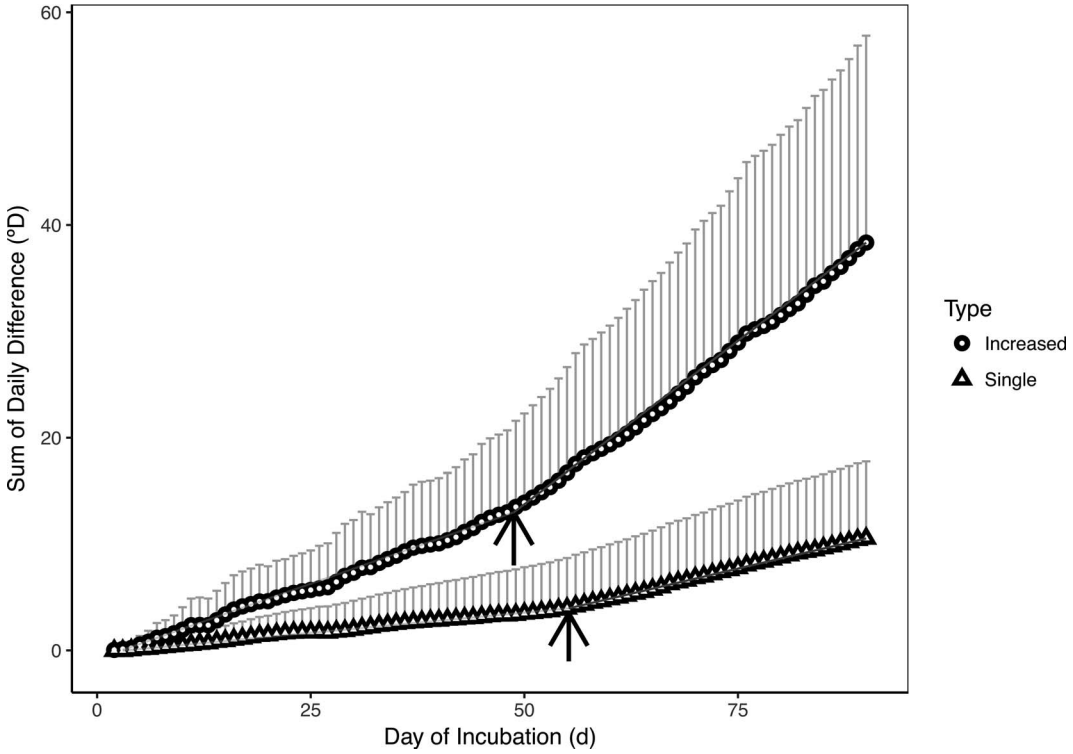

Figure 2. Average accumulated difference in degree-days (°D) over the incubation period for natural treatment (triangle) and experimental treatment (circle) of snapping turtle (*Chelydra serpentina*) nests. The accumulated differences were calculated by summing the difference in °D over time. Standard errors become larger as time progresses because small differences in °D accumulation among nests within treatments are compounded over time. Arrows represent breakpoints where °D accumulation becomes more rapid as a function of time, and linear trend lines represent accumulation trends before (left of the arrow) and after (right of the arrow) the breakpoint.

Table 1. The mean difference in daily temperature (°C) and degree-days (°D) between the inside and the outside of snapping turtle (*Chelydra serpentina*) nests for clutches in each third of incubation: first (days 1–30), middle (days 31–60), and last (days 61–90). °D were calculated cumulatively, such that for each third of development, each day's difference in °D was added to the previous day's heat unit difference. Standard errors were similarly compounded. Values where 95% confidence intervals (CI) do not overlap zero are denoted with an asterisk.

Treatment	Metric	First	Lower CI	Upper CI	Middle	Lower CI	Upper CI	Last	Lower CI	Upper CI
Natural	Mean temperature (°C)	−0.007	−0.158	1.43	0.040	−0.103	0.183	0.170*	0.026	0.314
Natural	Degree-days (°D)	1.97	−3.34	7.28	2.88	−1.88	7.64	5.70*	1.61	9.79
Experimental	Mean temperature (°C)	0.408*	0.268	0.547	0.573*	0.433	0.714	0.789*	0.587	1.00
Experimental	Degree-days (°D)	3.52	−1.86	8.9	9.20	−0.407	18.8	16.21*	0.905	31.5

ture and for a different population of embryos (Rollinson et al. 2018), they lead to the suggestion that metabolic heating may have a nontrivial effect on incubation time in snapping turtles. Whereas the maximum heating ranged from approximately 1.5°C to 2.5°C in nests of loggerhead turtles (Zbinden et al. 2006; DeGregorio and Williard 2011) and from 2.6°C to 5.9°C in nests of green sea turtles (Carr and Hirth 1961; Broderick et al. 2001), we found that these values are much lower in snapping turtles. In natural snapping turtle nests, maximum heating was approximately 0.3°C, while in experimentally enlarged nests, maximum heating was approximately 0.9°C. These relatively low values for heating in snapping turtle nests were expected because they have considerably less embryonic tissue mass than do sea turtles (Broderick et al. 2001; Zbinden et al. 2006).

The effects of metabolic heating on developmental rate in embryos have not been well explored, beyond the suggestion that high levels of metabolic heat may contribute to increased mortality when embryos are already near their upper thermal limit for development (van de Merwe et al. 2006). The effect of metabolic heating on development rate, however, becomes apparent in freshwater species whose periods for embryonic development and hatchling growth are seasonally constrained and may influence winter survival. For instance, limited exposure to suitable temperatures for development can result in high embryo mortality and poor juvenile phenotypes, a phenomenon particularly associated with northern environments (Ewert 1985; Bobyn and Brooks 1994a, 1994b; Parker and Andrews 2007; Edge et al. 2017). Both female size and clutch size tend to increase with latitude in oviparous reptiles (Galbraith and Brooks 1987; Iverson et al. 1993; Santilli and Rollinson 2018); therefore, the larger clutch sizes that typify more thermally constrained environments should exhibit a greater degree of metabolic heating if egg size stays the same or also increases. For instance, the mean clutch size of Michigan snapping turtles is 28 eggs, whereas mean clutch size in Algonquin Park is 36.1 ± 7.94 eggs SD (range = 12–64 eggs; N. Rollinson, J. Litzgus, and R.J. Brooks, unpubl. data, 2018), which is likely close to the clutch size of the experimental treatment nests in this study. For freshwater turtles, metabolic heating may therefore become increasingly ecologically relevant to embryo development and overwintering survival as latitude increases, particularly for species with large clutch sizes.

Sex ratios in green sea turtles (Broderick et al. 2001; van de Merwe et al. 2006), loggerhead sea turtles (DeGregorio and Williard 2011), and American alligators (*Alligator mississippiensis*; Ewert and Nelson 2003) have previously been found to be influenced by metabolic heating, although this finding is not ubiquitous: in some populations, metabolic heating is negligible (Zbinden et al. 2006). For metabolic heating to influence sex ratios, a nontrivial amount of heating must occur during the period in which sexual differentiation occurs; in turtles, this period is generally around the middle third of development (Yntema 1979; Bull and Vogt 1981). Interestingly, we found that the average increase in temperature during the middle third of incubation was 0.573°C (± 0.072°C SE) in experimental treatment nests, but was negligible in natural treatment nests (Table 1). For snapping turtles, the combined effect of metabolic heating and nest thermal inertia may have the potential to significantly influence sexual differentiation when clutches are large, because the temperature-sex reaction norm can curve sharply across temperatures (Ewert et al. 2005).

Metabolic processes of the embryos are only one of many factors that may affect temperature differences between the nest and surrounding substrate. For example, during the first third of development of experimental treatment nests, we noted (as others have; Zbinden et al. 2006) a positive mean temperature difference between the outside and inside of the nest, which cannot result from metabolic heating alone because embryo mass and metabolism are minimal in early development. Nest temperatures during this period should therefore be determined predominantly by physical and environmental factors surrounding the nest or the physical properties of the nest itself (Ackerman et al. 1985; Maloney et al. 1990). We suggest that the positive heat balance may be due to high specific heat capacity of eggs. The observed "thermal buffering" occurs because of extreme diel fluctuations in nesting substrate, which are more apparent when nests are shallow (Kaska et al. 1998; Chu et al. 2008; DeGregorio and Williard 2011). Furthermore, the effect of clutch size on thermal buffering should be dependent on the nesting substrate because thermal conductivity will vary with physical characteristics of the medium (Milton et al. 1997).

Indeed, the majority of variation in metabolic heating has been attributed to the physical properties of sand itself (Broderick et al. 2001), such that it is difficult to disentangle the thermal effects of the surrounding substrate from the heat produced by embryos themselves. Nevertheless, the values we report represent the actual thermal differences experienced by in situ snapping turtle nests on the ESGR, even if the sum of all differences in temperature cannot be entirely attributed to metabolic heating per se. Furthermore, our breakpoint analyses showed a considerable increase in the rate of accumulation of heat units beginning approximately halfway through incubation for all nests, which is unlikely to be due to thermal inertia alone.

We present the first evidence of metabolic heating in the nest of any freshwater turtle and suggest heating may be sufficient to hasten development and influence hatchling sex ratios. However, there are several areas in which our experimental protocol could be improved in future work. First, in keeping with our long-term study protocol aimed at reducing nest disturbance, we did not count clutch size in nests. We would expect clutch size to explain some of the variation in metabolic heating we observed because previous studies have found that variation in clutch size explains a significant amount of variation in nest metabolic heating (Broderick et al. 2001; van de Merwe et al. 2006; Zbinden et al. 2006). Similarly, we did not assess embryo survival, which represents an effective clutch size, as a source of variation in metabolic heating. Future studies should take initial clutch size, number of fertile embryos, and full-term embryo survival into account because these factors contribute toward thermal buffering and/or metabolic heating. Further, future metabolic heating studies that include clutch size data from different populations of snapping turtles may yield insight into the possible adaptive value of larger clutch sizes through warming of the nest. Additional heat that allows embryos to grow faster may relax seasonal constraints on development rates in seasonal environments at high latitudes (Bobyn and Brooks 1994a).

Enhanced understanding of the thermal characteristics of nests will benefit future studies. Temperatures vary from the center to the outside of the clutch in three dimensions (Booth and Astill 2001); however, we consistently placed temperature loggers in the center of the nest only. Therefore, we believe that our point estimation of the temperature difference at the center of the nest specifically estimates the maximum degree of additional heat experienced by embryos. Furthermore, to isolate the effect of thermal buffering in nests due to the physical properties of eggs, we recommend that future experiments include false nests using egg replicas of a high specific heat capacity (e.g., spheroids filled with water). An experimental design using false nests has not yet been attempted in the study of metabolic heating.

Metabolic heating in freshwater turtle nests is a largely unexplored field and our study suggests it may be a promising one. Further studies on other large-bodied species of freshwater turtles (e.g., *Apalone spinifera, Carettochelys insculpta, Macrochelys temmincki, Chelus fimbriata*) may yield insight into how the thermal environments of their nests are determined and how they impact important aspects of their development.

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