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Internal Body Temperatures of an Overwintering Adult *Terrapene carolina* (Eastern Box Turtle)

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Abstract - *Terrapene carolina* (Eastern Box Turtle) is the only turtle species in which adults are known to be tolerant of freezing. We report the first systematically collected data on internal body temperatures of an overwintering Eastern Box Turtle. Despite nearby air temperatures as low as -21.8 °C, this turtle probably supercooled rather than froze. Snow cover, thermal inertia, and the insulating effects of its refugium's substrate may have protected this turtle from the very cold conditions.

Introduction

Turtles living in regions where winter temperatures reach below 0.0 °C must either avoid or tolerate freezing. Many turtles accomplish the former by overwintering in either aquatic habitats that do not freeze or in burrows below the frost line (Ultsch 2006). *Terrapene carolina* L. (Eastern Box Turtle) is the only turtle known to be freeze-tolerant as adults, withstanding freezing of as much as 58% of their body water in a laboratory setting (Costanzo and Claussen 1990). This adaptation explains these turtles' ability to regularly overwinter in shallow refugia on land at latitudes as high as 44 °N; however, their freezing has never been tested systematically under field conditions. Claussen et al. (1991) occasionally recorded internal body-temperatures (T_b) in Eastern Box Turtles over 3 winters in Ohio, and obtained measurements slightly below 0.0 °C at least once each winter. However, they could not determine the duration of sub-zero events. Costanzo and Claussen's (1990) laboratory experiments demonstrated that cooling Eastern Box Turtles had significant thermal gradients, and thus, T_b measurements may lag behind external body measurements (T_e) that are typically recorded with temperature loggers attached to the shell (e.g., Savva et al. 2010). We measured T_b to determine whether Eastern Box Turtles actually freeze in the field as indicated by Costanzo and Claussen (1990).

Methods

On 24 September 2014, under general anesthesia induced with 4.8 mg/kg IV propofol (PropoFol 28, Zoetis Inc., Kalamazoo, MI) and regional analgesia induced with subcutaneous 1.2 mg/kg bupivacaine and 0.25% Marcaine (Hospira, Inc., Lake Forest, IL), we surgically implanted a heart-rate and temperature logger (DST micro-HRT, Star Oddi, Skeidaras 12, 210 Gardabaer, Iceland) in a

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subcutaneous location in the anterior cervical region at the thoracic inlet of a wild adult male Eastern Box Turtle that was already part of a long-term radio-telemetry project near Brookhaven, NY. The 8.3 mm x 25.4 mm logger weighed 3.3 g and was accurate to ± 0.2 °C. We programmed the logger to begin collecting heart-rate and temperature readings every 15 min starting 1 November 2014 and continuing until its battery died, ~3 months later. The turtle made an uneventful recovery, and we released it near its capture location, where this individual normally overwintered. It exhibited normal overwintering behavior (e.g., Clauseen et al. 1991), mostly burying itself in superficial leaf litter and soil. We removed the logger on 18 June 2015 using similar anesthetic protocols; the turtle recovered without incident and was released.

Results and Discussion

Unfortunately, the heart-rate monitor did not work. T_b varied from -0.8 °C to 13.7 °C. The logger detected only a single sub-zero event, which lasted from 17:45 on 17 January to 02:15 on 19 January 2015 (duration = 31.5 hrs); partway through this period, T_b was at -0.8 °C for 2.5 hrs. We note that this minimum field-collected T_b is remarkably close to the average supercooling T_b limit for Eastern Box Turtles, -0.83 °C as determined by Costanzo and Claussen (1990), below which freezing would usually be initiated within 2.0 hrs. Thus, we conclude that this turtle probably did not freeze, but may have supercooled very close to its limit. Snow cover, thermal inertia, and the insulating effects of the substrate may preclude the necessity of freeze tolerance for this species at this latitude.

Claussen et al. (1991) detected T_b slightly below 0.0 °C at least once each winter in some of the Eastern Box Turtles they monitored, but did not report the duration of the sub-zero event; thus, it is not possible to say whether freezing actually occurred. Bernstein and Black (2005) recorded T_e of *T. ornata* Agassiz (Ornate Box Turtle) and detected extended periods (54 d) of sub-zero T_e , as low as -8 °C for 2 individuals. It is likely these individuals actually froze, though the T_b was not measured and freezing was not confirmed, and it is important to note they survived.

We recognize that temperature data from standard weather stations are commonly used to investigate the relationships between species distributions and environmental variables (e.g., Ihlow et al. 2012), so we further explored the relationship between T_b and these data. We examined temperature data from the same time period from a National Oceanic and Atmospheric Administration weather station 2 m above ground and 2.85 km W of the overwintering turtle we monitored. These data indicated 73 freezing events of greater duration (mean duration = 21.2 hrs), and with a much colder minimum of -21.8 °C than the event we documented. We conclude that standard weather-station data are insufficient for depicting conditions experienced by a partially covered, overwintering Eastern Box Turtle.

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