Herpetological Review, 2017, 48(4), 731–734.

Can Skin Temperature be Used as a Proxy for Body Temperature in Gartersnakes?

Body temperature (T_b) is paramount in the study of ectotherms because it impacts behavior (e.g., Bennett 1980; Halliday and Blouin-Demers 2015), physiology (Angilletta 2001), and ultimately fitness (Angilletta et al. 2004; Halliday and Blouin-Demers 2015). Because T_b is so important to the biology of ectotherms, it is imperative that measurements of T_b are accurate and represent core T_b, which is what impacts physiological performance. Unfortunately, core T_b can be difficult to measure because study animals must either undergo an invasive surgery to implant a temperature data logger or must be captured to take a measurement via the cloaca (T_{cl}). When measuring T_{cl}, handling the study animal can modify T_b due to heat exchange from the researcher's hand to the study animal, especially for small animals. Measuring T_{cl} can also increase stress (Moore et al. 1991; Langkilde and Shine 2006). For this reason, many researchers rely on implanted data loggers (such as iButtons; Seebacher et al. 2003; Dubois et al. 2008) or on automated temperature-sensitive radio-telemetry (Brown et al. 1982; Blouin-Demers and Weatherhead 2001) to measure core T_b, or on non-contact methods such as infrared thermometers (IRTs) to measure skin temperature (T_{sk}) (Shine et al. 2002; Herczeg et al. 2006). Non-contact methods are especially useful for species that are too small to receive an implant, assuming non-contact methods provide precise and accurate T_b measurements. But are measures of T_{sk} actually representative of core T_b?

Validations comparing T_{sk} (obtained with IRTs or iButtons affixed to the skin of the animal) to T_{cl} have been conducted and most revealed relatively strong correlations between T_{sk} and T_{cl} (r > 0.85) (Shine et al. 2002, 2003; Werner et al. 2005; Herczeg et al. 2006; Hare et al. 2007; Rowley and Alford 2007; Dubois et al. 2008; Besson and Cree 2010; Carretero 2012; Moreno Azócar et al. 2013; Berg et al. 2015). Only two of these validations were on snakes: one involved iButtons affixed to the dorsal surface of Red-sided Gartersnakes (*Thamnophis sirtalis parietalis*; Shine et al. 2003) and the other involved IRTs with Chinese Pit Vipers (*Gloydius shedaoensis*; Shine et al. 2002). In both cases, the authors found strong correlations (r > 0.95) between T_{sk} and T_{cl}, which suggests that T_{sk} is a good proxy for T_b in snakes. None of these studies, however, explicitly examined the relationship between T_{sk} and T_b as T_b changed, which can be important when measuring T_b in animals that are basking because T_{sk} should rise more quickly than core T_b.

In this study, we validated whether T_{sk} (measured with an IRT) reflects core T_b (measured via T_{cl}) in Common Gartersnakes (*Thamnophis sirtalis*). We also examined how the relationship between T_{sk} and T_{cl} varies as snakes cool down and warm up, investigated the effect of handling snakes on their T_{sk} and T_{cl}, and examined the effect of solar radiation on the relationship between T_{sk} and T_{cl}.

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Methods

Cooling and Warming Experiment.—We captured 12 Common Gartersnakes in fields and wetlands at Queen's University Biological Station approximately 100 km south of Ottawa, Ontario, Canada in early May 2015. We determined the sex of each snake and measured its mass and snout–vent length (SVL). We brought these snakes back to the laboratory and restrained each snake by fixing it to a plastic board with masking tape. We then inserted a thermocouple into the cloaca of the snake and monitored cloacal temperature with the data logger on our IRT (Fluke 566 infrared thermometer, Fluke Corporation, Everett, Washington, USA), which allowed us to monitor T_{sk} and T_{cl} simultaneously with the same instrument. Accuracy for both the IRT and the thermocouple was ± 1.0°C. We measured T_{sk} of the snake in the middle of its body (typically within 10–15 cm of the cloaca) with the infrared thermometer held 10 cm above the snake and recorded the T_{cl} at the same time so that all measures of T_{sk} and T_{cl} were paired.

We used two environmental rooms to heat and cool snakes. The first room kept the ambient temperature between 25.6 and 27.6°C. The second room held a temperature between 12.6 and 14.6°C. At the beginning of each trial, we kept each snake in the warm room until its T_{cl} reached ca. 25°C. We then moved the snake to the cold room for ten minutes and recorded T_{sk} and T_{cl} every minute. After this, we moved the snake back to the warm

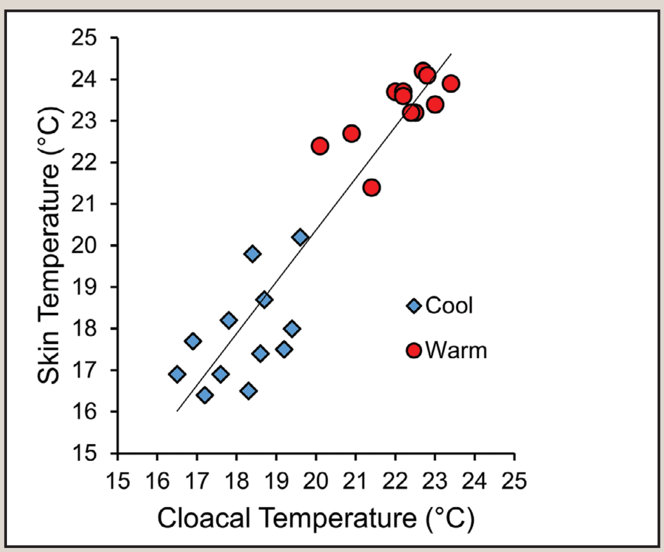

Fig. 1. The relationship between skin and cloacal temperature for Common Gartersnakes (*Thamnophis sirtalis*) in cooling and warming trials in the laboratory. Each point represents the final temperature taken during each trial for each snake.

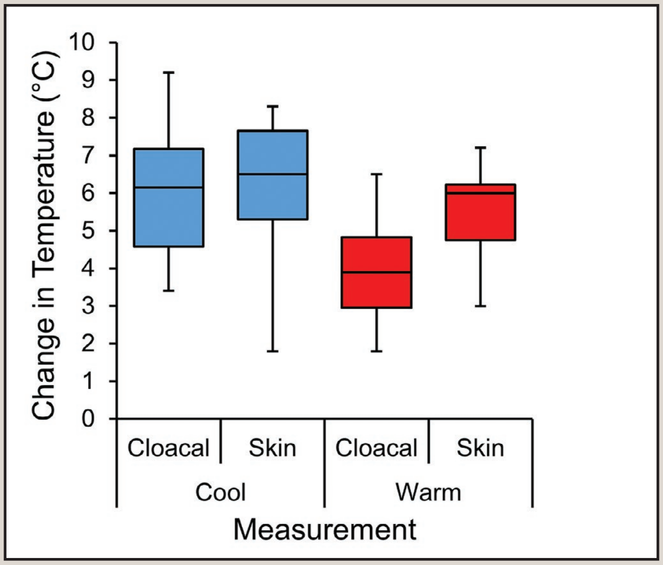

Fig. 2. Absolute change in temperature between the start and the end of a 10-minute trial examining the relationship between skin and cloacal temperature in Common Gartersnakes (*Thamnophis sirtalis*) under both cooling and warming treatments in the laboratory. Boxes represent the interquartile range, lines within the boxes are the median value, and whiskers are minima and maxima.

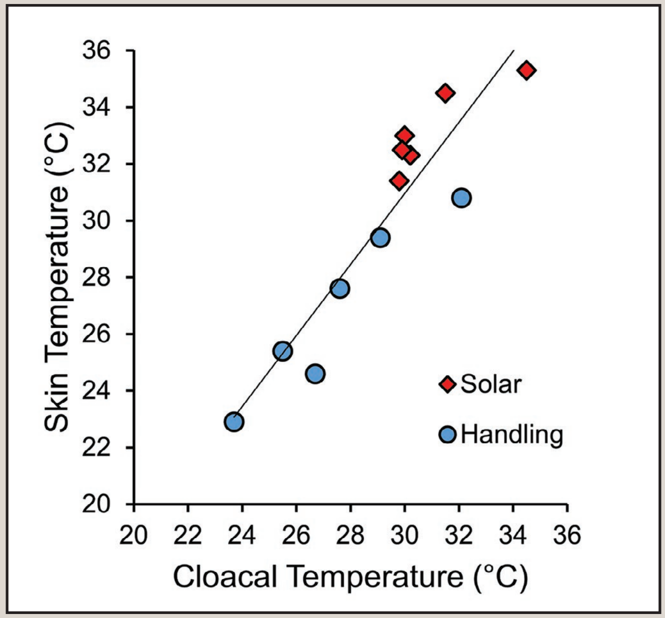

Fig. 3. The relationship between skin and cloacal temperature for Common Gartersnakes (*Thamnophis sirtalis*) in handling and solar radiation trials in the field. Each point represents the final temperature taken during each trial for each snake.

room for an additional ten minutes and continued recording T_{sk} and T_{cl} every minute. We thus obtained paired T_{sk} and T_{cl} measurements for each snake while it cooled down and warmed up. We returned each snake to its point of capture following this experiment.

Handling and Solar Radiation Experiments.—We captured six Common Gartersnakes in Pontiac County, Québec, Canada in June 2015. We kept these snakes in outdoor enclosures as part of another experiment (Halliday and Blouin-Demers 2017). We returned each snake to its point of capture following these experiments.

On clear sunny days between 1030 h and 1130 h, we fixed snakes to plastic boards as in the previous experiment and moved the snake to the forest shade. We then conducted two trials on each snake. First, we examined the effect of handling on their T_{sk} and T_{cl}. Second, we examined the effect of solar radiation on T_{sk} and T_{cl}. For the handling trial, we placed a hand on the middle of each snake to mimic handling and simultaneously monitored T_{sk} and T_{cl} every ten seconds for sixty seconds, which is representative of typical handling time. For the solar radiation trial, we moved the snakes into full sun and recorded T_{sk} and T_{cl} every 30 seconds for five minutes.

Analyses.—We conducted three analyses for each experiment. First, we examined the correlation coefficient for the relationship between T_{sk} and T_{cl}, which is a commonly reported metric of the relationship between T_{sk} and T_{cl}. We conducted Spearman's rank correlations in R (package: stats; function: cor.test; method: spearman; R Core Team 2015) comparing T_{sk} and T_{cl} based on the final pair of measurements taken in each trial. For these correlations, we combined data from the cooling and warming experiment for one correlation, and combined the data for the handling and solar experiment for the second correlation to examine a wider range of temperatures.

For our second analysis, we calculated the change in temperature (final temperature – initial temperature) for both T_{sk} and T_{cl} during each trial to examine whether T_{sk} and T_{cl} change at similar rates. We compared these changes by measurement type using linear mixed effects models in R (package: nlme; function: lme; Pinheiro et al. 2015). We included individual ID as a random effect and controlled for body size by including SVL as a covariate.

Finally, we examined the difference between T_{sk} and T_{cl} through time to determine whether the differences between T_{sk} and T_{cl} are consistent through time. We analyzed the difference between T_{sk} and T_{cl} in each trial with linear mixed effects models with time into the trial and SVL as fixed effects and ID as a random effect.

Results

Cooling and Warming Experiment.—T_{sk} and T_{cl} were strongly correlated at the end of the cooling and warming experiment (rho = 0.91, $p < 0.01$; Fig. 1). The change in temperature was greater for the cooling treatment than for the warming treatment (mean difference = 1.5 ± 0.3°C, $t_{34} = 4.61$, $p < 0.01$) and T_{sk} changed more than T_{cl} (mean difference = 0.9 ± 0.3°C, $t_{34} = 2.66$, $p = 0.01$; Fig. 2). Change in temperature also decreased as SVL increased (slope = -0.02 ± 0.01°C/mm, $t_{10} = 3.33$, $p < 0.01$). T_{cl} was 0.6 ± 0.1°C ($t_{239} = 4.26$, $p < 0.01$) warmer than T_{sk} during the cooling treatment, but T_{sk} was 1.3 ± 0.1°C ($t_{239} = 17.87$, $p < 0.01$) warmer than T_{cl} during the warming treatment. The difference between T_{cl} and T_{sk} did not change as SVL changed ($t_{10} = 0.10$, $p = 0.92$) or as time into the experiment changed ($t_{238} = 0.21$, $p = 0.83$).

Handling and Solar Radiation Experiments.—T_{sk} and T_{cl} were strongly correlated during the handling and solar radiation experiments (rho = 0.87, $p < 0.01$; Fig. 3). The change in temperature during the handling experiment was higher for T_{sk} than for T_{cl} (mean difference = 1.1 ± 0.2°C, $t_5 = 3.30$, $p = 0.02$; Fig. 4) and was unaffected by SVL ($t_4 = 1.06$, $p = 0.35$). In fact, the change in T_{cl} was not different from zero (one sample t test; $t_5 = 1.96$; $p = 0.11$), which indicates that T_{cl} was unaffected by handling, whereas T_{sk} was affected by handling. T_{cl} was 1.7 ± 0.5°C ($t_{35} = 3.77$, $p < 0.01$) warmer than T_{sk} at the start of the handling

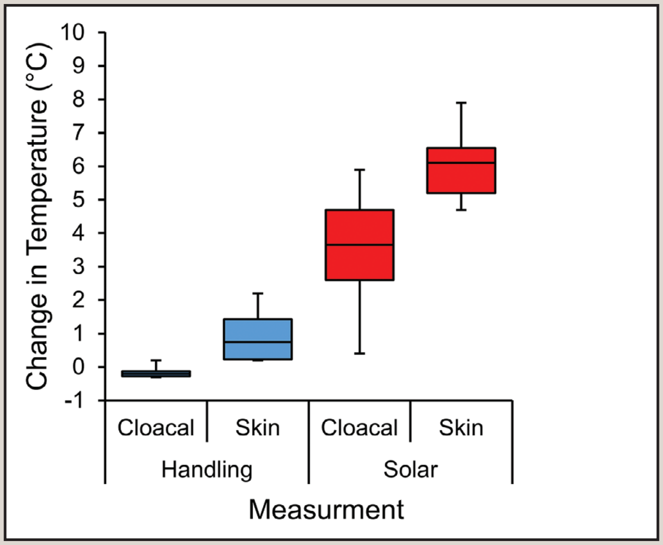

Fig. 4. Absolute change in temperature between the start and the end of trials examining the effect of handling and solar radiation on skin and cloacal temperature in Common Gartersnakes (*Thamnophis sirtalis*). Handling trials lasted 60 seconds and solar radiation trials lasted five minutes. Boxes represent the interquartile range, lines within the boxes are the median value, and whiskers are minima and maxima.

trial, but this difference decreased as time progressed (slope = 0.02 ± 0.002°C/sec; $t_{35} = 7.65$, $p < 0.01$) because T_{cl} did not change while T_{sk} increased.

Change in temperature was significantly greater for T_{sk} than for T_{cl} during the solar radiation experiment (mean difference = 2.6 ± 0.5°C, $t_5 = 4.90$, $p < 0.01$; Fig. 4) and was unaffected by SVL ($t_4 = 1.27$, $p = 0.27$). T_{sk} was not significantly different from T_{cl} at the beginning of the solar trial (intercept = 0.8 ± 0.5°C; $t_{57} = 1.78$, $p = 0.08$), but T_{sk} became significantly warmer than T_{cl} as time elapsed (slope = 0.007 ± 0.001°C/sec; $t_{57} = 6.41$, $p < 0.01$).

Discussion

T_{sk} and T_{cl} were strongly correlated in our experiments, which corroborates results from other studies with snakes (Shine et al. 2002, 2003). These results suggest that, in general, T_{sk} is a useful proxy for core T_b in small to medium snakes (snakes in our experiment ranged in SVL from 317 to 626 mm). Despite this strong correlation between T_{sk} and T_{cl}, our results also demonstrate significant differences between T_{sk} and T_{cl}. In our cooling trial, T_{sk} was colder than T_{cl} and, in the warming and solar radiation trials, T_{sk} was warmer than T_{cl}. However, the largest mean difference between T_{sk} and T_{cl} was 2°C and this was at the end of the solar radiation trial. This suggests that the difference between T_{sk} and T_{cl} is generally small, but researchers should be aware that T_{sk} changes more rapidly than T_{cl}.

Our analysis of change in temperature demonstrated that T_{sk} always changed more than T_{cl} during a trial. This is because T_{sk} is a measure of the external surface of the snake, which is the first part of the snake to be exposed to changes in ambient temperature or radiation. T_{cl} measures core temperature, which reacts more slowly to changes in ambient temperature or radiation. The exception to this would be if heat was radiating from below and not from the sun, which could be the case if snakes were staying warm on rocks after sunset. In this case, T_{cl} may be warmer than T_{sk} due to its proximity to the heat source.

Handling snakes caused significant increases in T_{sk} through time, but had no effect on T_{cl}, at least over the 60 seconds when we measured changes in T_{sk} and T_{cl}. Researchers that must handle snakes to obtain measures of T_{sk} should therefore minimize handling time before taking their measurements. However, T_{cl} is less affected by handling, so researchers measuring T_{cl} on snakes may have more time to take this measurement.

There can be a variety of reasons for using one method of measuring T_b over another. Handling snakes to insert a thermocouple can increase stress levels and modifies body temperature via heat transfer from the researcher's hand; the snake may also defecate when the thermocouple is inserted into the cloaca, which may affect the temperature measured. IRTs are also imperfect. The accuracy of IRTs diminishes as the distance between the device and the snake increases. T_{sk} may also vary depending on the location where it is measured: snakes basking in the sun likely have higher T_{sk} on their dorsal than on their abdominal surface, and the middle of the body may have different T_{sk} than either the head or tail. It may be very difficult to obtain accurate measurements of T_{sk} with an IRT when a snake is moving quickly in the field. The best T_b measurement method will therefore vary depending on the particulars of a given study.

In summary, IRTs are useful for obtaining measures of T_{sk} that are highly correlated with core T_b in small to medium-bodied snakes. The behavior of the snake and the local environment, however, will affect the strength of the correlation between T_{sk} and T_b. Documenting the relationship between T_{sk} and T_b under various scenarios may be important, especially if an accurate measure of core T_b is crucial.

Acknowledgments.—We are grateful to F. Lanoix and M. Routh for assistance in the field. This project was supported by the University of Ottawa and funded by the Natural Sciences and Engineering Research Council of Canada through a post-graduate scholarship to WDH and a Discovery Grant to GBD. All methods in this study were approved by the University of Ottawa Animal Care Committee under protocol BL-278, and all snakes were used under Ontario Ministry of Natural Resources Wildlife Scientific Collector Permit 1079774 and Québec Permis Scientifique 20150307001SF.

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Herpetological Review, 2017, 48(4), 734–739.

Distribution and Activity Season of the Introduced Red-Eared Slider (*Trachemys scripta elegans*) in Colorado, USA

Red-eared Sliders (*Trachemys scripta elegans*) are native to the central and southeastern United States (Powell et al. 2016). To the west, the natural range of this species extends into New Mexico, Oklahoma, Kansas, and extreme southeastern Nebraska, especially along major river systems (Powell et al. 2016). A locality in Wallace County, Kansas, is the most northwesterly natural occurrence of Red-eared Sliders, and is only 2.5 km E of the Colorado border (Collins 2007). However, many extra-limital populations of this species have become established, not only in the United States but around the world, earning this species a ranking of among the World's 100 Worst Invasive Alien Species (Lowe et al. 2004; Powell et al. 2016). No native occurrences have been reported from Colorado. The first record in the state, a specimen with a label stating "Denver, Colo." was included on a list compiled by Ellis and Henderson (1913). Since that time, additional specimens have been reported from Boulder, Denver, Mesa, and Rio Blanco counties (Livo et al. 1998). Despite its proven ability to establish extra-limital populations, this species continues to be sold at pet stores in Colorado and is considered an unregulated species by the Colorado Department of Natural Resources, so it "may be imported, sold, bartered, traded, transferred, possessed, propagated and transported in Colorado provided that all importation, disease requirements and any other state, local or federal requirements are met" (Colorado Parks and Wildlife 2016). The present report provides updated information about the distribution of *T. s. elegans* in Colorado and documents its activity season in the state. State agencies can use this information to determine whether the import and sale of Red-eared Sliders should be prohibited in an effort to eliminate the release of unwanted pets and reduce the establishment of extra-limital populations in Colorado.

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Materials and Methods

We made observations of turtles opportunistically in publically accessible sites, usually using binoculars and cameras equipped with telephoto capabilities; we did not deploy turtle