MIAMI UNIVERSITY The Graduate School

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ABSTRACT

ACTIVITY, HEAT EXCHANGE, AND ENERGETICS DURING THERMOREGULATION by

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Thermoregulation uses behavioral and physiological adjustments to compensate for thermally unfavorable conditions, but the extent that ambient conditions affect organismal function and thermal maintenance under natural conditions remains to be explored in depth. Therefore, I investigate what environmental factors impact organismal performance and how the cardiovascular system contributes to heat exchange in the field. First, I determine the extent that ambient conditions influence activity and movement under field conditions for Eastern box turtles (Terrapene carolina carolina). In contrast to previous laboratory work investigating effects of temperature on performance, I found that box turtle activity and movement in the field was largely independent of ambient conditions. Furthermore, the activity was consistent over a wide range of temperatures indicating that box turtles are thermal generalists. Next, I develop an integrative framework that combines biophysical principles and empirical measurements to gauge the cardiovascular role in active and passive heat exchange. I demonstrate the utility of the framework on two turtle species (box turtle and painted turtle, *Chrysemys picta marginata*) that use different thermoregulatory strategies. Eastern box turtles are characterized as a thermoconformers, strictly relying on ambient conditions to regulate body temperature, while painted turtles are characterized as partial thermoregulators, utilizing behaviors such as basking to regulate body temperature. The cardiovascular heat exchange framework that I develop was able to successfully identify active and passive mechanisms of heat transfer for both species. Finally, I implement the conceptual framework to parse out the cardiovascular role in active heat exchange and energetic demands of varying thermoregulatory strategies. The thermoconformer used significantly less energy per season than the partial thermoregulator during the beginning and middle of their active seasons. Furthermore, both species utilized active mechanisms of heat exchange to mitigate heat loss at night and slow the rate of temperature increase during the day. Although many previous studies have noted 'hotter is better' for ectotherms, the consequences of upper thermal limits imply a disconnect between the ecologically relevant and physiologically

optimal temperatures. These studies provide a basis for the integration of theoretical principles and empirical data to better understand organismal function and energetics regulating temperature and heat exchange in the field.

ACTIVITY, HEAT EXCHANGE, AND ENERGETICS DURING THERMOREGULATION

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Chapter 3

3.1

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Chapter 1 General Introduction

The seminal work by Cowles and Bogert (1944) showed that ectothermic vertebrates could gain heat from the environment and maintain constant body temperatures like their endothermic counterparts, thus spurring interest in the thermal ecology of reptiles. Afterwards, studies would capture animals, record their body temperature, air temperature, and substrate temperature (e.g., Brattstrom, 1965). At the time, the relationship between air temperature and body temperature was widely used as an index of thermoregulation. However, Heath (1964) cautioned thermal biologists to reconsider this relationship by showing that water-filled cans in the sun were generally hotter, therefore deviating from the ambient temperature regulation for ectothermic vertebrates. As these questions began to develop, the approach to understanding thermoregulation diverged between theoretical (biophysical modelling, e.g., Porter, Mitchell, Beckman, & DeWitt 1973) or empirical (e.g., Grant & Durham, 1988) based studies. The work that I present in my dissertation is an attempt to bridge the gap between theoretical and empirical approaches to better understand the role of the environment in temperature regulation for ectothermic vertebrates.

Thermoregulation exploits both active and passive exchange of heat and incorporates behavioral (Glanville & Seebacher, 2006) and physiological adjustments (Seebacher & Shine, 2006; Seebacher & Franklin, 2005) to compensate for thermally unfavorable conditions. The degree to which a reptile thermoregulates varies from strict thermoconformity, solely relying on differences in ambient conditions to determine body temperature, to active thermoregulation, seeking out basking sites to increase body temperature rapidly and maintain thermal preference (Angilletta 2009). Ectothermic vertebrates are highly dependent on ambient conditions for providing suitable thermal conditions that ultimately drive heat exchange between the organism and environment. Many studies of thermoregulation only emphasize the temperature difference of the organism from the environment (Hertz, Huey, & Stevenson 1993; Blouin-Demers & Nadeau, 2005) without incorporating physiological parameters relevant in heat exchange.

For ectothermic vertebrates, persistence in any given habitat patch requires thermal conditions that are within tolerable physiological limits. Further, thermal conditions near the upper thermal limits can negatively affect species if thermal refugia are not available (Huey

1991, Belasen et al. 2017). Upper thermal limits can also potentially affect movement among habitat patches in a fragmented landscape thus creating a thermally hostile matrix. Data from free-living individuals can provide a better assessment of the ambient conditions experienced and subsequent thermoregulatory responses taken. Therefore, the goal of my first project was to determine the extent that ambient conditions influence activity and movement in the field. To do this, I monitored Eastern box turtles (*Terrapene carolina carolina*) activity and movement to compare with environmental variables pertinent to life history.

Environmental conditions often impose constraints and increased risk of mortality when temperatures reach critical limits. Physiological mechanisms, such as circulation and cardiac output, can aid in regulation of temperature by increasing or decreasing the transfer of heat between the organism and its environment (Grigg & Seebacher, 1999). For instance, an increase in heart rate during basking increases heat transfer to the organism due to redistribution of blood flow as long as the organism is cooler than the surrounding environment. Decreasing heart rate can also retain heat and allow the organism to stay warm for longer during the day. Alternatively, if the organism needs to reduce its temperature it can lose heat by shunting blood to body parts with high surface area (Bartholomew & Tucker 1963; Bartholomew 1982). Together these processes emphasize the importance of physiological mechanisms in regulating body temperature. The changes in the cardiac output increase metabolic requirements and therefore energetic demands to adjust the heat transfer rate. For my second project, I developed a conceptual framework (cardiovascular heat exchange framework; CHEF) that integrates empirical data with biophysical principles to gauge the role of cardiovascular adjustments in thermoregulation of free-living ectotherms. To do this, I incorporated data from biologging devices that measure both temperature and heart rate, and then demonstrated the utility of the framework with two species of turtle, Eastern box turtle and painted turtle (Chrysemys picta).

Temperature difference alone may not adequately represent the energetic demands of either maintaining preferred temperatures or the consequences of conforming with the environment and suboptimal conditions. Measurements of heart rate in free-living animals can be used to quantify field metabolism and provide novel insight into the energetics required to thermoregulate. Energetics of temperature selection have been quantified under laboratory conditions for feeding (Dubois, Blouin-Demers, & Thomas 2008), however, laboratory conditions do not always reflect patterns and processes observed under field conditions.

Therefore, in my final project I first compared the daily energy expenditures of two turtle species, box turtles, a terrestrial thermoconformer, and painted turtles, a semi-aquatic partial thermoregulator. Secondly, I used the CHEF with field measurements of heart rate and body temperature to assess their relevance in active and passive heat exchange during thermoregulation.

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Chapter 2

Activity and movement of free-living box turtles are largely independent of ambient and thermal conditions.

Summary

Ectotherms are assumed to be strongly influenced by the surrounding ambient and environmental conditions for daily activity and movement. As such, ecological and physiological factors contribute to stimuli influencing navigation, extent of movement, and therefore habitat use. Our study focused on the intensity of activity (from acceleration data) and extent of movement (from GPS and thread trailing data) of Eastern box turtles (Terrapene carolina carolina) in a fragmented landscape near their northern population limit. First, we quantified the thermal performance curve of box turtles using activity as a measure of performance. Second, we investigated ecological factors that could influence activity and movement and characterized the movement as extensive (exploration) and intensive (foraging). In contrast to previous lab work investigating effects of temperature on activity, we found no relationship between box turtle activity and temperature in the field. Furthermore, box turtle activity was consistent over a wide range of temperatures. Cluster analysis categorized movement recorded with GPS more as intensive than as extensive, while thread trailing had more movement categorized as extensive than intensive. Box turtle activity was higher during the morning hours and began to decrease as the day progressed. Based on the microclimate conditions tested, we found that box turtle movement was influenced by *precipitation* and *time of day*, and activity was most influenced by absolute humidity, ambient temperature, cloud cover, and time of day. Our model ectotherm in this study, the Eastern box turtle, had activity patterns characteristic of a thermal generalist. Sampling resolution altered the characterization of movement as intensive or extensive movement, possibly altering interpretation. More information on the resolution needed to definitively identify foraging and exploratory behavior in turtles is needed. Activity and movement were nearly independent of environmental conditions, which supports the overall interpretation that turtle performance is that of a broad environmental generalist. Future studies of movement of other turtle and reptile species are needed to determine the generality of these findings.

Introduction

Animals move through their environment with a suite of inputs modified by ecological and physiological factors to determine navigation, migration, dispersal, foraging, and exploration. Furthermore, the environment needs to be navigable terrain and free of any major geographic barriers. The habitat occupied by a species, whether continuous or fragmented, is patchy and selection hierarchical (Caldwell & Nams, 2006), and as a result movement patterns depend on integration of numerous ecological, physiological, and behavioral variables (Malishev, Bull, & Kearney 2018). Understanding animal navigation throughout their habitat thus requires high resolution measurements of activity, movement, and multiple aspects of ambient conditions (Sears et al., 2016), optimally with field-based studies of free-living animals.

Free-living ectotherms are also dependent on microclimate conditions being within tolerable physiological limits (Gunderson & Leal, 2012). Vegetative structure, topography, and geographic barriers are ecological factors that shape the landscape and influence the microclimate conditions available to ectotherms, thus impacting activity and movement. Additionally, the way in which an individual exploits its environment may be altered by extrinsic or intrinsic factors, potentially leading to alteration of the nature of movement in the available habitat (Nams, 2005). Within these ecological factors, microclimate conditions including temperature, humidity, and rainfall can alter activity and movement (Stickel, 1950; Price-Rees, Lindström, Brown, & Shine 2014). The relevance of microclimate on physiology depends on how each factor impacts performance. One of the most influential ambient conditions impacting ectotherms is temperature. Ambient temperature and environmental conditions must be within tolerable physiological limits for ectotherms, and laboratory observations strongly support a role for temperature on movement performance in many reptiles (Jayne, Bennett, & Lauder 1988; Rome & Bennett, 1990; Angilletta 2009) including box turtles (Adams, Claussen, & Skillings 1989). Prior work on box turtle movement and activity in the lab showed a strong thermal dependence of strides per minute, total time stopped and velocity through test temperatures between 10 and 32 °C (Adams, Claussen, & Skillings 1989). However, laboratory studies may not always reflect patterns and processes observed in nature, and thus may not represent what animals are capable of, or choose to do, under natural conditions. For example, our recent work showed that in the field, box turtle movement was not correlated with body temperature (Parlin, do Amaral, Dougherty, Stevens, Schaeffer 2017).

Further, the physiologically optimal temperatures of an organism can be decoupled from ecologically relevant temperatures (Huey & Hertz, 1984). For instance, locomotor performance tends to decrease drastically when body temperatures rise even slightly above physiologically optimal levels (Martin & Huey, 2008). As a result, body temperatures below the physiological optimum may be more ecologically relevant, especially in fluctuating environments to ensure that overheating is avoided.

Active thermoregulation potentially permits activity in otherwise sub-optimal conditions. Thermoregulation relies on heat sources and sinks within a habitat to be able to maintain temperatures for physiological processes such as locomotion, assimilation, and growth (Huey, 1991). Thermal sensitivity of performance permits us to categorize an organism as a thermal generalist or a thermal specialist. This thermal sensitivity ranges from a thermal generalist, which can perform over a broad range of temperatures, to a thermal specialist, whose performance is strongly dependent on temperature (Angilletta, 2009). The interactions between thermal sensitivity and thermoregulation ultimately allow ectotherms to regulate their body temperature in concurrence with environmentally available temperatures to grow, survive, and reproduce (Huey & Stevenson, 1979). Thus, changes in thermal conditions will potentially alter many aspects of activity and movement in ectotherms, including exploitation of available habitat (Kearney, 2013), dispersal (Kearney & Porter, 2009), and ultimately distribution (Buckley, Tewksbury, & Deutsch 2013).

The goal of the study was to analyze the ecological and physiological factors that influence daily activity and movement in box turtles throughout their active season using biologging devices carried by free-living animals. Overall dynamic body acceleration (ODBA) was used as a measure of box turtle activity, and the linear distance between consecutive GPS points or thread trailing were used as measures of movement. We analyzed the relationship between movement or accelerometer measurements with nearby weather-station data, determining the thermal sensitivity of activity, and analyzing domain and transition movement relative to activity. We hypothesized that (1) box turtle activity is thermally sensitive and thus temperature-dependent based on previous lab work, (2) box turtle movement and activity would be influenced by ambient conditions including precipitation and absolute humidity, and (3) finescale sampling methods will better reveal intensive movement while lower resolution sampling will be biased towards extensive movements.

Methods

Study Site

We monitored Eastern box turtles (*Terrapene carolina carolina*) in Southwest Ohio at the Miami University Natural Areas (MUNA: 39.5° N, 84.7° W). For investigation of the environmental factors influencing activity and movement, box turtles are an ideal ectotherm to study because of their ability to tolerate heavy loads relative to their mass, allowing for multiple biologging devices to be attached. Our study sites are near the northern edge of their distribution east of the Mississippi river. Forest habitat in this landscape is highly fragmented due to the dominance of agriculture in the area, with forest fragments ranging in size from 5.5 ha to 400 ha. Climate in this region is characterized as humid continental with large seasonal temperature differences including warm to hot summers with high humidity and occasional severely cold winters, with precipitation distributed throughout the year (Karl & Koss, 1984). Box turtles are listed as a species of special concern in Ohio with limited information on population demographics (van Dijk, 2017).

Movement and Activity Monitoring

We monitored two groups of box turtles in 2014 and 2015 using a combination of thread trailing devices (group one) and GPS-Accelerometer tags (e-Obs, Grünwald, Germany; hereafter 'GPS-ACC', group two) which recorded both GPS locations and overall dynamic body acceleration (ODBA) data. Devices attached to the turtles were 83 x 26 x 20 mm (L x W x H) and were placed at a downward direction. Turtles monitored with GPS-ACC devices were all male (n = 12), and thread trailed turtles were both male (n = 7) and female (n = 4). We saw no difference between the sexes in our analyses and so data from thread trailing is presented as the combined data set. All box turtles were tracked with radio-telemetry using BD-52 transmitters (Holohil, Ontario, Canada) epoxied to the top of the shell for retrieval of devices.

Turtles in group one were tracked from May to July in 2014 and from June to July in 2015 using a methodology for thread trailing similar to Claussen, Finkler, & Smith (1997). We epoxied a small plastic cylinder (height = 1.5 cm, diameter = 3 cm) to the posterior portion of the carapace that held a spool of nylon thread (228 m). Each turtle was released where it was first located and allowed a one-day acclimation period before beginning the trailing process. Each morning, individual turtles were located, and the thread tied to an anchor at the start point. We recorded the starting GPS at the beginning of each day using a Garmin 62s handheld GPS (3 -

10m resolution). Turtles were then located every 24-hours to generate maps of daily movement. Turns were determined when the thread was caught on an object and changed direction. Each turn had a flag placed at that location and the series of flags permitted us to determine the turn angle and distance between each turn to the nearest centimeter and compass bearing to the nearest degree. Each turtle was monitored for up to 5 days, and days were omitted if the thread was broken or if the turtle moved beyond the capacity of the spool (45 out of 55 days yielded usable data). We replaced the thread as needed. Thread trailing data was then converted to Universal Transverse Mercator (UTM) coordinates in Zone 16S (WGS84, Ohio, USA). We used the initial starting coordinate from each day and converted the polar coordinates (bearing and distance) measured in the field to Cartesian coordinates (x,y-coordinates) and plotted the results to verify paths. UTM coordinates were then converted back to decimal degrees for analyses as appropriate.

Box turtles in group two were monitored during the 2015 field season from May until October. Turtles with the GPS-ACC devices also had temperature data loggers implanted internally (iButton DS1922L, see methods in Parlin et al. 2017 for details) which recorded body temperature at 5-minute intervals throughout the study period. Box turtles in group two were monitored between 12 to 15 days. Loggers recorded GPS coordinates at 1-hour intervals from 0700 to 1900, and accelerometer measurements recorded every 10 minutes (a 30-second burst) also between 0700 and 1900 hours. All devices were calibrated prior to attachment and data was adjusted using device-specific calibrations. We had 1733 GPS observations for 12 turtles during the 2015 field season and 2337 thread trailing points converted to GPS coordinates for 11 turtles in 2014 and 2015 combined. All procedures followed approved MU Institutional Animal Care and Use Committee protocol (906) and complied with the Principles of Animal Care, publication no. 86-23, revised 1985, of the National Institutes of Health.

Data Analysis

All accelerometer measurements were converted into overall dynamic body acceleration (ODBA) prior to analysis. We used the average ODBA (\pm SE) at each degree Celsius of measured internal turtle body temperature to generate the data for testing non-linear splines to analyze intensity of activity as a function of body temperature. For later statistical analyses, we used the mean ODBA per hour to test across the units of measure of each relevant environmental variable. We characterized movement as extensive (larger mean turning angle and decreased step

length) and intensive (smaller mean turning angle and increased step length) using three consecutive coordinates and two distances. Cluster analysis was used to characterize the turning angle and distance into each movement type for both GPS coordinates and thread trailing using the 'ade4' and 'adehabitatLT' package in R (Calenge, 2006). Movement was defined as the distance between two consecutive GPS coordinates and the turning angle was defined as the angle produced to reach the subsequent coordinate based on prior location.

We also derived a binary classification for each 30 second burst of acceleration measurement as either active or inactive for each individual. This was achieved by power transforming ODBA (eqn. 1 $x = ODBA^{-\frac{1}{3}}$) and then fitting to a mixture distribution of two Gaussian distributions (Eqn 1). The assumption hereby is that the single ODBA measurements represent in their sum inactivity and activity resulting in a distribution consisting of two mixed Gaussian distributions each with an estimable mean (μ_a and μ_b) and variance (σ_a and σ_b in eqn. 1) (for each individual). Based on a non-linear least-squares approach (using the package nlsr in R version 3.4.3) we fitted the probability distribution function estimating both means and variances for each individual (Nash & Murdoch, 2018).

$$pdf(y) = \frac{1}{\sqrt{2\pi\sigma_a^2}} e^{\frac{-(x-\mu_a)^2}{2\sigma_a^2}} + \frac{1}{\sqrt{2\pi\sigma_b^2}} e^{\frac{-(x-\mu_b)^2}{2\sigma_b^2}}$$
(1)

Using the estimated means and variances of the two Gaussian distributions, we then estimated the probability for each burst belonging to either of the two distributions (active and inactive) based on a probability density function for single Gaussian distributions with the estimated means and variances. This classification is independent of sampling and individual differences, which allows cross comparability among all individuals in the study (for the complete R code used see supplemental material).

After defining ODBA values as active or inactive, we determined the proportion of activity (%) by counting the total number of active ODBA values per hour and dividing by the number of recordings taken during that hour. We analyzed the proportion of activity using a logistic regression test for differences between percentage groupings with turtle ID as a random effect, and analyzed comparisons using least-squares means.

Statistical Analysis

We then generated multiple non-linear splines and compared the equations using AIC to determine the best model fit. We then used the equation with the lowest AIC score for the thermal performance curves (TPC). We analyzed distance between GPS coordinates in relation to ecological factors from a nearby weather station (Butler County Regional Airport-Hogan Field weather station, Hamilton, OH, USA). We used stepwise regression (backward deletion) analysis to compare distance moved by box turtles and hourly ODBA with our predictor variables: ambient temperature (°C), absolute humidity (g/m^3) , precipitation, cloud cover, time of day, and their interactions. Predictor variables were based on previous natural history studies on box turtles and were consolidated to the most pertinent variables that could have an impact on box turtle activity and movement. Mixed-effect models of predictor variables as fixed effects and turtle ID as a random effect were tested against a null model using a likelihood ratio test (Bates, Mächler, Bolker, & Walker 2015). We also compared the effect of slope on distance moved and intensity of activity with mixed effect models using turtle ID as a random effect and slope as a fixed effect. We did not use the distance between GPS coordinates from 1900 and 0700 hours the next day as box turtles were sometimes active before our devices began recording. Distance data were natural log-transformed to meet assumptions of normality. Analysis of distance moved and percentage of activity, based on a gaussian distribution to define active and inactive ODBA values, was done using a least-squares regression analysis. For cluster analysis using GPS coordinates, we compared ODBA values associated with either extensive or intensive with a student's t-test. We also used student's t-test to compare distance moved and activity in the presence of precipitation. Data were analyzed in R version 3.0.2 (Team R, 2013), and in all cases α was set at 0.05.

Results

Thermal Performance Analysis

We determined the relationship between the intensity of activity, measured as ODBA, as a function of the body temperatures experienced by the turtles in the field. Body temperature measurements for box turtles ranged between 11.0 and 36.0 °C, and very few temperatures were measured below 12 or above 30 °C. The thermal performance curve (TPC) based on field data showed a broad, nearly uniform, performance between 14 and 23 °C (Fig. 1). The wide relationship between intensity of activity and body temperature indicates that box turtles are

thermal generalists, and thus the performance of free-living box turtles is not dependent on temperature.

Environment and Activity Analysis

Linear mixed effect model analysis of microclimate variables including *precipitation*, *ambient temperature*, *cloud cover*, *time of day*, and *humidity*, indicated that *precipitation* (estimate = -0.197; CI = - 0.279 – -0.115) and *time of day* (estimate = 0.00293 ; CI = -0.0266 – -0.0135) had the most influence on turtle movement ($\chi^2_2 = 8.7552$, p = 0.01256) with a marginal r² of only 0.008 based on the fixed effects of *precipitation* and *time of day* and a conditional r² of 0.076 when also incorporating the random effect of turtle ID. We found that box turtles move 13 m each hour after it rains compared to the average of 17 m each hour when not raining (Fig.2, $t_{171} = 3.02$, p = 0.002). We had previously reported that box turtles move more during the morning than in the evening (Parlin, do Amaral, Dougherty, Stevens, Schaeffer 2017), although the differences were small. As box turtles are influenced by geotaxis, we tested for an effect of slope on movement, but found no significant influence on distance moved ($\chi^2_1 = 2.63$, p = 0.104). The regression analysis shows that more than 90% of the variation in the distance moved is unexplained by the model.

Box turtle activity was most influenced by *ambient temperature* (estimate = 0.000135; CI = 0.0009376 – 0.0017784), *absolute humidity* (estimate = 0.00291; CI = 0.00238 – 0.00345), *cloud cover* (estimate = 0.00229; CI = 0.00138 – 0.0032), and *time of day* (estimate = -0.00373; CI = -0.00419 – -0.00326) as significant (χ^2_4 = 181.02, p = 2.2e-16) with a marginal r² of 0.147 based on fixed effects and a conditional r² of 0.198 when also incorporating the random effect of the turtle (Fig. 3). We also found no significant difference in turtle activity in the presence or absence of *precipitation* (t_{124} = -0.76004, p = 0.44) or for an effect of slope (χ^2_1 = 2.63, p = 0.90). However, *precipitation* was only observed during 135 of the 1596 one-hour intervals when turtles were monitored. We additionally analyzed box turtle activity state as a percentage likelihood of activity during each hour interval and found that *ambient temperature*, *absolute humidity*, *cloud cover*, and *time of day* (χ^2_4 = 48.962, p = 5.9e-10) had similar significant effects although the marginal r² was 0.076 and the conditional r² was 0.108. Thus, around 80% of the variation in the intensity of activity and about 90% of the variation in the likelihood for being active remains unexplained by either model.

Movement and Activity Analysis

We categorized coordinates for GPS and thread trailing into extensive and intensive movement to compare continuous micro-scale and hour interval macro-scale resolution. Comparison of sampling resolutions using thread trailing (micro-scale) and GPS-coordinates (macro-scale) yielded opposite characterizations of extensive and intensive movement (Table 1). Cluster analysis using GPS locations assigned 36% of the movement as extensive, and 63% of the movement as intensive. For the thread trailing data, 79% of the values were characterized as extensive movement and 21% of the values as intensive. Mean turning angle for both movement modes were higher for the thread trailing data than the GPS coordinates (Table 1).

We then separated all the ODBA values into two distributions as active (0.0110 - 1.387g) and inactive (0.0011 - 0.01085g). Using this distribution, we analyzed the intensity of activity for each category of movement and found that intensity of activity for either movement mode had no correlation with the distance moved (Fig. 4). Although maximal values recorded for extensive and intensive movement were similar, we found the mean intensity of activity to be significantly higher for movements characterized as extensive (Fig. 4a, 0.046g) than for those characterized as intensive (Fig. 4b, 0.026g, t₇₆₇ = 6.1192, p < 0.05), mainly due to a decreased likelihood of activity for intensive movement characterization. Although there was no relationship between the intensity of activity and movements, we found a significant relationship between the likelihood of activity in each hour and distance moved (F₆ = 5.5994, p < 0.05, Additional File 1), such that the mean distance moved varied from 16.1 to 24.6m as the likelihood of activity increased.

Discussion

Eastern box turtles are a species of special concern across most of their range with worrying population declines reported. One of the reasons for this decline is linked in part to habitat loss (van Dijk, 2017). As habitats for box turtles are lost and other human impacts increase, we may expect turtle movement to decrease similarly to that of mammals as recently reported (Tucker et al. 2018). We had previously reported that the body temperature of box turtles was unrelated to the distances moved. We thus sought to expand this observation to determine what, if any, environmental factors may dictate turtle movement, based on prior work done on box turtles. Further, the determinants of the intensity of activity, as measured by 3-dimensional accelerometry, may also give insight into the movement ecology of free-living

turtles as they interact with their environment. Previous work indicated that box turtles exist within a narrow range of microclimate variables (Reagan, 1974), become more active if the temperature drops and rain begins to fall due to thunderstorms (Webb, Minckley, & Craddock 1963), and have their peak activity during the morning and become relatively inactive during the evening (Dodd, 2002). We found that (1) box turtles movement was not temperature-dependent and were thermal generalists, (2) box turtle movement and activity were influenced by some microclimate conditions and not others, and (3) fine-scale sampling methods revealed intensive movement while lower sampling resolution was biased towards extensive movement.

The recent advancement of biologging technology, allowing for continuous monitoring for extended periods of time, permits a more in depth understanding of box turtle life history. Our analysis based on these relevant environmental parameters showed no relationship between movement and *ambient temperature*, *absolute humidity* or *cloud cover*, while revealing significant impacts of both time of day and precipitation on movement. However, these only explained about 10% of the variation in distance moved. Thus in several important ways, our data did not agree with these previous studies as we found that the factors measured did have an impact on box turtle movement but often in an opposite direction. For example, similar to our previous work (Parlin, do Amaral, Dougherty, Stevens, Schaeffer 2017), we found no effect of temperature, and we found that precipitation decreased movement in our population of box turtles, unlike Webb, Minckley, & Craddock (1963).

Intensity of activity, measured as ODBA, was best explained by *ambient temperature*, *absolute humidity*, *cloud cover*, and *time of day*, while *precipitation* was not significant. Similar to the extent of movement, these significant environmental factors only explained around 20% of the variation in activity. The environmental factors influencing the likelihood of activity, measured as a percentage of recording categorized as active per hour, also only explained around 10% of the variation in hourly activity. This fits well with our observation that the thermal reaction norm showed that the intensity of activity was nearly unchanged across a very broad range of body temperatures. As we previously showed that box turtles are thermal generalists, with body temperatures similar to ambient (Parlin, do Amaral, Dougherty, Stevens, Schaeffer 2017), it comes a no surprise that the ambient temperature also had little effect on activity. This work further supports the conclusion that box turtle movement and daily activity are highly resilient in response to microclimate conditions.

It is also plausible that activity is driven by prior exposure to the landscape. Box turtles have an incredible ability to navigate back towards their home range when displaced and are even capable of moving at night (Metcalf & Metcalf, 1970). Further research monitoring the activity and movement of displaced turtles using biologging technology would provide added insight into the capability of these ectotherms to navigate their habitat, especially in fragmented landscapes.

Unlike previous laboratory work of temperature-dependent movement on reptiles (Jayne, Bennett, & Lauder 1988; Rome & Bennett, 1990; Angilletta 2009) including box turtles (Adams, Claussen, & Skillings 1989), we found that ambient and body temperature were generally irrelevant as determinants of both activity and movement. This suggests that laboratory measures of maximal locomotor capacity may be erroneous for most movement in nature, at least for turtles. Laboratory conditions may not represent patterns and processes observed in the field, thus our data point to the importance of studies of free-living animals to best delineate the factors that determine the realized performance. As box turtles were able to be active at all temperatures observed in the field, other factors presumably act to determine the extent moved. However, temperature is a driving force for ectotherms (Angilletta, 2009), dictating many physiological functions, one of which may supersede locomotion.

To better understand the nature of box turtle movement within a habitat patch, we categorized each step as intensive (foraging) or extensive (exploratory). As resolution of the data collection method could influence this analysis (Benhamou, 2004; Nams, 2006), we compared movement data collected with animal borne GPS loggers (with 1-hour intervals) to data from continuous thread trailing. The outcomes from this analysis returned nearly opposite interpretations, suggesting that when data were sampled at low resolution that turtles are primarily foraging, while at when sampled at high resolution that turtles are primarily engaged in exploratory movement. However, given that we did not simultaneously monitor GPS location and thread trailing, direct comparison between movement measurements in foraging sites remains unclear and interpretation should be cautioned.

This data support and extend our earlier observation that Eastern box turtles are a thermoconforming ectotherm, with movement that is nearly identical across an impressively wide range of body temperatures (Parlin, do Amaral, Dougherty, Stevens, Schaeffer 2017). Although the extent of movement and intensity of activity are significantly affected by several

environmental factors, none do so with great explanatory power. Thus we conclude that in addition to being thermal generalists, the movement ecology of Eastern box turtles is largely independent of the habitat characteristics examined. Movement patterns in Galapagos tortoises are driven by changes in vegetation (Yackulic, Blake, & Bastille-Rosseau 2017) and similar constraints may be relevant for box turtles. Although we frequently observed turtles feeding and they tended to congregate in a region of the study area with a high density of mulberry trees (*Morus* spp.), we lack detailed data on feeding habits. Generally, box turtle movement appeared to be haphazard within each forest patch and we documented no box turtles traversing from one habitat fragment to another. Finally, given the marked difference between the categorization of movements as intensive (foraging) or extensive (exploratory) depending on the method used to record movements, further comparisons of fine-scale and coarse-scale movement, undertaken with a more uniform methodology, may be important for future studies.

This study contributes to understanding the interaction between physiology and movement, and the effects of climate conditions on activity in the field. Our results also show the importance of difference in fine-scale sampling resolution compared to coarse-scale resolution for characterizing and analyzing movement. Although laboratory studies have shown a strong thermal dependence of physiological performance, field monitoring can decouple the ecologically relevant temperatures from the physiologically optimal. This provides further insight into the patterns and processes observed in the field for free-living individuals and can further our understanding of how changes in climate conditions can impact a species.

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Tables

Table 1. Total coordinates recorded for GPS and thread trailing monitoring techniques and subsequent classification of extensive and intensive movement counts using cluster analysis. Cluster analysis for GPS locations had 36% of the recordings characterized as extensive, and 63% of the recordings as intensive. For the thread trailing data, extensive movement was characterized as 79% of the values and intensive as 21% of the values. Mean turning angle was larger in the thread trailing for both the extensive and intensive movements.

Technique	Total	Extensive	Intensive	Mean Turn Angle	Mean Turn
	Coordinates			Extensive	Angle Intensive
GPS	1733	581	1002	128.2 ± 8.1	31.8 ± 6.7
Thread Trailing	2337	1837	474	150.8 ± 4.8	81.2 ± 9.7

Figure Legends

Figure 1. Thermal performance curve (TPC) of box turtle turtles (n = 12) monitored in 2015. Non-linear equation was fit to the mean overall dynamic body acceleration (ODBA) values. The black line represents the best fit regression. Box turtles had a relatively constant performance from 14 to 23 °C. Performance as a function of body temperature was not thermally dependent across this range giving box turtles a wide performance breadth.

Figure 2. Distance moved as a function of precipitation measured by a nearby weather station. Mixed-effect model indicated precipitation and time of day as the best predictors of distance moved with a marginal r^2 of only 0.008 based on the fixed effects of precipitation and time of day and a conditional r^2 of 0.076. Although including the turtle ID as a random effect increased the r^2 , more than 90% of the data remained unexplained by the mixed-effect model. Precipitation was only recorded during 135 of the hour-intervals where movement occurred in box turtles.

Figure 3. Climatic variables from the best model that had a significant influence on mean overall dynamic body acceleration (ODBA) including (a) absolute humidity, (b) weather condition, (c) temperature, and (d) time of day. Note that weather condition, classified by cloud cover, was split into five categories as follows: 0 = clear, 1 = scattered clouds, 2 = partly cloudy, 3 = mostly cloudy, 4 = overcast/haze, and 5 = light rain/thunderstorms. Intensity of activity as a function of climatic variable had a slight positive correlation with humidity and ambient temperature recorded. Mean ODBA decreased as time of day progressed from morning until the evening. The effect of clouds and rain did not follow a clear pattern.

Figure 4. Mean hourly distance moved as a function of mean overall dynamic body acceleration (ODBA) for (a) extensive movement and (b) intensive movement categorizations. There was no correlation between distance moved and activity for either extensive or intensive movements. However, mean ODBA was significantly higher during extensive movement (0.049g) than intensive (0.026g), mainly due to a decreased likelihood of activity in the latter category (see additional file 1).








Chapter 3

Cardiovascular contributions to reptile thermoregulation: an integrative heat exchange framework.

Summary

A critical question in reptile thermoregulation is the role of the environment in temperature regulation and heat transfer. Heat transfer is a result of the temperature difference between an organism and the environment. Ectotherms use physiological and behavioral mechanisms to maintain relative internal thermal stability and compensate for thermally unsuitable conditions, resulting in varying degrees of thermoregulation. A less appreciated aspect of thermoregulation are the cardiovascular contributions to heat exchange under field conditions. The goal of our study was to introduce an integrative heat exchange framework to determine the cardiovascular contributions to thermoregulation in free-living ectothermic vertebrates: the cardiovascular heat exchange framework (CHEF). We start by identifying the fundamental components of heat transfer and the cardiovascular mechanisms for modulation of active and passive heat exchange and then integrate these components of heat transfer and cardiovascular response into a single framework. To demonstrate the utility of our framework, we test the CHEF using data from two species of turtles, the Eastern box turtle (*Terrapene carolina carolina*), and the painted turtle (Chrysemys picta marginata). Specifically, we test (1) to what extent does the model identify scenarios of heat transfer during heating and cooling and (2) how do ambient and body temperature contribute to the cardiac adjustments for heat exchange and driving rates of heat transfer in the field. We found that the CHEF effectively integrated biophysical principles with field data for gauging the cardiovascular contributions to thermoregulation and heat exchange. Both turtle species had important cardiovascular contributions to thermoregulation, and we identified increased and decreased rates of heat transfer in our representative day. Further, we found that body temperature was influencing heart rate for the box turtle and ambient temperature was influencing heart rate for the painted turtle in the field. Merging theoretical concepts of biophysical modelling with empirically collected field data allow us to tease apart physiological, behavioral, and passive mechanisms of heat exchange between the animal and the environment by measuring three parameters: ambient temperature, body temperature, and heart rate. Future iterations of this integrative framework can be applied to other species and can include additional parameters (e.g., activity, movement, or behavioral observations) to help us further understand mechanisms contributing to thermoregulation in ectothermic vertebrates.

Introduction

Since the seminal work by Cowles and Bogert (1944) there has been an increasing interest in the thermal ecology of reptiles. Of particular interest is the role of the environment in heat transfer and regulation of body temperature. Reptiles use physiological and behavioral mechanisms to maintain relative internal thermal stability and compensate for thermally unsuitable conditions, resulting in varying degrees of thermoregulation (Angilletta, 2009). However, a less appreciated aspect of thermoregulation are the cardiovascular contributions to heat transfer.

Temperature influences rates of biochemical reactions within an organism, determining performance and maintaining physiological functions (Angilletta, 2009). Equally, temperature differences determine the rates of heat flow across the physical environment (Clarke, 2017). Heat exchange results from the temperature difference between an organism (i.e., the system) and the environment (i.e., its surroundings). Ectothermic vertebrates that rely on external heat sources to regulate body temperature are in a near constant state of thermal flux with ambient conditions, using conductive, convective, and radiative pathways of heat transfer. As a result, variation in the contributions these pathways will alter the rates that an individual heats and cools (Gates, 2012).

Heating and cooling rates in ectotherms are determined in part by the thermal mass of the individual and the conductance of tissues (Grigg, Drane, & Courtice 1979). Heat is transferred between the core and surface of the organism to be exchanged with the surrounding environment, thus the transfer rate can vary depending on the thickness and conductivities of these tissues (Gates, 2012). Smaller organisms have a larger surface area to volume ratio thus permitting faster rates of heat transfer through passive exchange (Stevenson, 1985a; Stevenson, 1985b). However, heat transfer is not just limited to passive exchange. Many organisms exhibit a hysteresis between heating and cooling, with differing heat transfer rates that impact body temperature and physiological processes such as heart rate (Franklin & Seebacher, 2003; Zaar, Larsen, & Wang 2004).

Ectotherms have some degree of control over blood flow that allows them to modulate their rates of heating and cooling (Seebacher & Franklin, 2005). Individuals are able to alter the distribution of blood flow and total cardiac output and thus thermal conductivity (Smith, 1975b; Lillywhite, Zippel, & Frappell 1999), suggesting that cardiovascular mechanisms are important for thermoregulation. In addition to passive thermoregulatory behaviors (e.g., basking; Gates,

2012), active thermoregulation (e.g., altered cardiac output; Seebacher & Grigg 2001) likely plays an integral role in the daily maintenance of body temperature. For instance, heart rate during heating is higher than heart rate during cooling under laboratory conditions (Bethea, 1972; Smith, 1979; Franklin & Seebacher, 2003). During basking, increases in heart rate indicate increases in heat transfer and thus reduction in time spent basking. When there is the risk of overheating, reptiles are able to reduce their heart rate to avoid increasing heat transfer in a thermally hostile environment (Grigg & Seebacher, 1999). As a response to heat stress, moving to a cooler location and increasing heart rate increases the rate of heat transfer from the body (reverse hysteresis; Grigg and Seebacher 1999, Zaar, Larsen, & Wang 2004).

Another important component to thermoregulation is the surrounding environment, terrestrial or aquatic, in providing suitable thermal conditions (Sears & Angilletta, 2015). In terrestrial environments, thermal variation results from changes in radiative heat transfer (e.g., cloud cover, photoperiod, etc.) and therefore presents moderate to extreme variation in the thermal landscape. Aquatic environments have less thermal variation due to the high specific heat capacity of water thus providing stable thermal conditions compared to terrestrial environments. Furthermore, shifting from terrestrial to aquatic environments can impact cardiovascular function because of increasing or decreasing heat load, resulting in a rapid cardiac response where heart rate is controlled independently of body temperature (Franklin & Seebacher, 2003).

Understanding the cardiovascular contributions to daily temperature regulation is difficult, but the advent of novel biologging technologies has alleviated the challenge of obtaining high-resolution field measurements. Extensive theoretical and empirical work that laid the foundation for organismal heat exchange only describes the physical pathways (i.e., conduction, convection, radiation, etc.) of the organism-environment interaction (Bakken & Gates, 1975; Gates, 2012). These models calculate the operative temperatures, values that represent the temperature of an organism in the absence of metabolic heat production or evaporative cooling, and focus primarily on the physical pathways of heat exchange, excluding physiological responses due to technological limitations in obtaining measurements at the time. As a result, in organismal measurements deviations from the operative temperature indicate active thermoregulation (Bakken, 1992; Bakken & Angilletta, 2014). While temperature differences are informative (Fitzgerald & Nelson, 2011), the combination of physiological mechanisms with organism-environment temperature differences remains largely unexplored in

free-living animals. Equally, laboratory experiments have aimed to explain the physiological functions controlling heating and cooling rates (e.g., Smith, 1975a; Smith, 1976) but do not incorporate in situ field measurements. Biologging devices provide the opportunity to monitor physiological mechanisms of free-living organisms (Wilmers et al. 2015) that may not be reflected under laboratory conditions and allows for development of new frameworks that join empirical and theoretical work to elucidate patterns and processes observed in the field.

Here, we propose an integrative framework that allows us to tease apart physiological, behavioral, and passive mechanisms of heat exchange between the animal and the environment. By incorporating biologging devices that measure both temperature and heart rate, we outline and show how our proposed cardiovascular heat exchange framework (CHEF) integrates empirical data with biophysical principles to gauge the role of heart rate modulations in thermoregulation of free-living ectotherms. The framework has numerous potential applications in ecophysiology, thermal biology, and forecasting environmental consequences from the climate crisis. We start by identifying the fundamental components of heat transfer and the cardiovascular mechanisms for active and passive exchange. Next, we integrate these components of heat transfer and cardiovascular response into a single framework while identifying active and passive mechanisms during heat exchange. To demonstrate the utility of our framework, we test the CHEF using data from two species of turtles, the Eastern box turtle (Terrapene carolina carolina), and the painted turtle (Chrysemys picta marginata). Specifically, we investigate the capability of the framework to identify different heating and cooling scenarios and the cardiovascular contributions to thermoregulation of free-living ectotherms to address the following questions: (1) to what extent does our proposed model identify scenarios of heat transfer during heating and cooling, and (2) how do ambient and body temperature contribute to the cardiac adjustments for heat exchange and drive rates of heat transfer in the field.

Methods

Overview of the Cardiovascular Heat-Exchange Framework (CHEF)

Our framework integrates the fundamental components of heat exchange with cardiovascular thermoregulation of ectotherms to achieve a greater understanding of how these processes interact to maximize thermoregulation. Data from temperature loggers can be used to quantify thermal changes between the organism (i.e., system, measured as body temperature

using implanted loggers) and the immediate environment (i.e., surrounding, ambient temperature measured using an animal borne temperature logger). The integrative framework we develop here is based on two *system-surrounding* relationships: *organism-environment* and *cardiovascular-organism*. We use measurements of body and ambient temperature as well as heart rate to differentiate between passive and active rates of heat exchange and cardiovascular function. Below, we outline the two major components and describe how they are conceptually linked.

First Component of the Cardiovascular Heat-Exchange Framework (CHEF)

In thermodynamics, heat flow can be simplified into understanding the relationship between the system (i.e., object of interest) and its surroundings (i.e., everything external to the system). The first *system-surrounding* relationship involves the organism (i.e., system, measured as body temperature) and the environment (i.e., surrounding, measured as ambient temperature). In the simplest case, the recorded temperatures are either a scenario of heat gain, where there is an expectation of heat flow leading to an increase in body temperature because the ambient temperature is greater than the organism, or heat loss, where there is an expectation of decrease in the body temperature because the organism's temperature is greater than the recorded environment.

For this first *system-surrounding* relationship, the rate of heat gain or loss in a free-living animal is compared to heat exchange between a corpse and ambient temperature, which is taken to represent passive heat flow. Based on these data, in scenarios of heat gain in the living organism, the increasing body temperature can be one of three results: (1) within expected (heating no differently than a corpse), (2) higher than expected (increased heating), or (3) lower than expected (reduced heating). Similarly, for heat loss in the living organism the decreasing body temperature can be one of three results: (1) within expected (cooling no differently than a corpse), (2) higher than expected (cooling no differently than a corpse), (2) higher than expected (cooling no differently than a corpse), (2) higher than expected (cooling no differently than a corpse), (2) higher than expected (cooling no differently than a corpse), (2) higher than expected (cooling no differently than a corpse), (2) higher than expected (cooling no differently than a corpse), (2) higher than expected (cooling no differently than a corpse), (2) higher than expected (cooling no differently than a corpse), (2) higher than expected (reduced cooling), or (3) lower than expected (increased cooling).

Organism-Environment Mechanism

We predict body temperature at each measurement interval using the previously recorded body temperature as the starting core body temperature and the subsequent external ambient temperature as the new step response. We used Newton's second law of cooling to predict the body temperature using the heating and cooling constant (tau, τ) of a deceased turtle, which

represents passive exchange of heat between the organism and the environment. Although Newton's second law of cooling is a theoretical oversimplification of heat exchange between the organism and the environment, we argue that the data loggers implanted inside the animal and on top of the shell represent the combined effects of conduction, convection, radiation, and other pathways of heat exchange, thus allowing us to apply the CHEF empirically on field tested animals. We use the modified equation based on Sears & Angilletta (2015):

$$T_b(t) = T_{a+1} + (T_b - T_{a+1})e^{(-\frac{t}{\tau})}$$
(1)

where $T_b(t)$ is the predicted body temperature at time t, T_b is the body temperature at t = 0, T_{a+1} is the ambient temperature at the subsequent sampling time, τ is the thermal time constant described by Smith (1976), and t is the time in minutes between temperature step change (equal to the sampling interval of the devices). To determine if the recorded body temperatures were above, below, or within the predicted temperature, we compare recorded to calculated values and use the measuring accuracy of the data logger ($\pm 0.0625^{\circ}$ C in our case) as the confidence interval.

Second Component of the Cardiovascular Heat-Exchange Framework (CHEF)

The rates of biochemical reactions depend on the organism's body temperature. Thus, the thermal effects on heart rate can be separated into understanding the relationship between the heart (i.e., system, measured as heart rate) and the organism's body temperature (i.e., surrounding, measured as body temperature) using simultaneously recording biologging devices. Independent of intrinsic factors, cardiac contraction rates should increase as temperature increases. In ectothermic vertebrates, tachycardia, a higher than normal heart rate, and bradycardia, a lower than normal heart rate, have the potential to occur at any recorded body temperature. In many ectothermic vertebrates, tachycardia is observed because of periodic lung ventilation or avoidance of hypotension to keep tissues oxygenated while bradycardia is commonly observed during short-term diving or during disturbance events (Lillywhite, Zippel, & Farrell 1999).

The second *system-surrounding* relationship is between the heart and the whole organism (*cardiovascular-organism*). The recorded heart rate represents an internal response to extrinsic factors potentially contributing to the higher or lower than expected body temperatures. Just as with body temperature, heart rate can be higher (tachycardia), lower (bradycardia), or within a expected range based on an established relationship between heart rate and temperature.

Tachycardia and bradycardia can be observed during heating or cooling scenarios, identified using the first system-surrounding relationship, and represent an active physiological mechanism for adjusting the rates of heat transfer between the organism and the environment. *Cardiovascular-Organism Mechanism*

We base the equation for estimating heart rate from body temperature on data from previously established studies. In our test case we assumed that heart rate at 0° C was 0 BPM, and that maximal heart rate was 100 BPM (Lillywhite, Zippel, & Frappell 1999) at 40° C the temperature at which myocardium from excised turtles was unable to function properly (Barnes & Warren, 1937). We used mean heart rates at three different temperatures (10, 15, and 20 °C) based on whole excised *Trachemys scripta* hearts from Barnes & Warren (1937) to fill in the mid-temperatures and used non-linear splines to estimate the slope coefficient (*B*) and the body temperature at mid-point (*C*). We incorporate the maximum heart rate and body temperature recorded, and minimum heart rate and body temperature from each species to improve the fit of a sigmoidal curve at the extreme temperatures where measurements were lacking. We based the sigmoidal curve on the equation from Reid (1996) and used the following equation for calculation of heart rate at a given body temperature:

$$HR = \frac{HR_{max} - HR_{min}}{1 + (\frac{T_b}{C})^B} + HR_{min}$$
(2)

where *HR* represents the expected heart rate as a function of body temperature, HR_{max} is maximum possible heart rate, HR_{min} is the minimum possible heart rate, T_b is the body temperature, *C* is the body temperature at mid-point of the heart rate range, and *B* is a slope coefficient. We generated 95% confidence intervals around the mean heart rates measured in Barnes and Warren (1937) for the fitted non-linear regression with error propagation using Monte Carlo simulations. The 95% confidence interval at a given temperature represents the range of beats per minute that a heart would experience in the absence of intrinsic mechanisms. Therefore, values that are above the 95% confidence interval would be indicative of tachycardia while values below the 95% confidence interval would be indicative of bradycardia. *Identifying Scenarios of Heat Gain*

Measuring the body temperature and ambient temperature simultaneously allows us to determine the heat flow from the warmer entity to the cooler entity. The null hypothesis is that heat transfer is passive if the heat exchanged is the same as measured with a corpse based on the modified Newton's second law of cooling. Alternatively, organisms can have a higher rate of

heat transfer to the body resulting in increased heating, where the body temperature is greater than the predicted (denoted with North-East arrow, Table 1), or a lower rate of heat transfer, where, although heating, body temperature is less than the predicted, indicating reduced heating (denoted with South-East arrow, Table 1). A cardiac contribution to heat transfer is identified by tachycardia (higher than predicted heart rate) or bradycardia (lower than predicted heart rate) and represents an active role in modulating rates of heat transfer. When the organism is heating at a faster rate than a null model (increased heating scenario), we define three categories: Tachycardic Heating, Bradycardic Heating, and Increased Heat Gain based on the cardiovascular response. In Tachycardic Heating, there is the presence of tachycardia and a higher than expected body temperature during increased heating. We are inferring that Tachycardic Heating is the organism increasing its rate of heat transfer in order to spend less time at lower body temperatures. In *Bradycardic Heating*, there is the presence of bradycardia and a lower than expected body temperature during heating. We infer that the organism is decreasing the heat transfer to the body by reduced circulation. When the heart rate is normal, the likely cause for higher than predicted body temperature would be *Increased Heat Gain* due to exploitation of radiative heat transfer.

When the organism is heating at a slower rate than a null model (reduced heating scenario), we define three categories: *Heating Rapid Response*, *Active Heat Gain Reduction*, and *Decreased Heat Gain* based on the cardiovascular response. In *Heating Rapid Response*, there is a presence of tachycardia with a lower than expected body temperature during heat gain. We are inferring that a shift in microclimate conditions occurred, resulting in a decreased rate of heat transfer to the organism, possibly to dump excess heat and prevent overheating or due to increased heat load causing a cardiac reflex. In *Active Heat Gain Reduction*, there is the presence of bradycardia and heating is at a lower than expected rate indicating a decrease in the rate of heat transfer to the body based on a reduced cardiac output, likely to prevent the organism from potentially overheating. Lastly, when heart rate is within expected and temperature increase is slower than expected, the organism would have *Decreased Heat Gain* resulting from presence in cooler microclimates.

When the body temperature is within the predicted range during heating, all forms of heat exchange are passive, and the adjustments of heart rate are related to movement or inactivity. *Identifying Scenarios of Heat Loss*

The same body and ambient temperature measurements that allowed us to identify heat gain to the organism also allow us to identify heat loss from the organism to the surrounding environment. Again, the null hypothesis is that the heat exchange is passive if the heat loss is no different than a corpse based on the modified Newton's second law of cooling. Organisms can also have a higher rate of heat transfer, where body temperature is less than the predicted, indicating increased cooling (denoted with South-West arrow, Table 1), or lower rate of heat transfer from the body resulting in decreased cooling, where the body temperature is greater than the predicted (denoted with North-West arrow, Table 1). When an organism is cooling at a faster rate than a null model (increased cooling scenario), we define *Tachycardic Cooling*, *Bradycardic Cooling*, and *Increased Heat Loss*. In *Tachycardic Cooling*, where tachycardia is present, there would be an increasing rate of heat transfer from the organism to the environment resulting in a lower than expected body temperature while cooling. In Bradycardic Cooling, the presence of bradycardia and a lower than expected body temperature indicate a reduction in cardiac output that limits energy expenditure while still maintaining an increased cooling rate during the day. In *Increased Heat Loss*, cardiac output would be within the expected rate despite body temperature being lower than expected, suggesting passive heat exchange between the organism and a cooler microclimate.

When an organism is cooling at a slower rate than a null model (decreased cooling scenario), we define *Cooling Rapid Response*, *Active Heat Loss Reduction*, and *Decreased Heat Loss*. In *Cooling Rapid Response*, the presence of tachycardia and a higher than expected body temperature likely indicate a presence in a warmer microclimate where the heat load causes a cardiac reflex. It could also be an initial attempt at increasing heat transfer from the body when the organism is above its upper thermal limit. In *Active Heat Loss Reduction*, the presence of bradycardia and a higher than expected body temperature indicate a decrease in heat exchange from the body, likely in an attempt to reduce decreasing temperatures. In *Decreased Heat Loss*, the cardiac output being at the expected rate and a higher than predicted body temperature would be the result of exploiting a microclimate that retains heat despite ambient conditions being cooler than the organism.

When the body temperature is within the predicted range during cooling, all forms of heat exchange are passive, and the adjustments of heart rate are related to movement or inactivity. *Active and Passive Forms of Heat Exchange*

Organisms in the field are constantly exchanging heat with their environment as a result of temperature differences between the individual and their immediate surroundings. In a representative day, we would expect that organisms would follow the proposed pattern of active heat exchange separated into four phases based on photoperiod and maximum ambient temperature (Fig. 1). At night, from midnight to sunrise (Phase 1), individuals are likely to have a higher body temperature than ambient due to thermal mass and therefore rely on either passive exchange or use active mechanisms to reduce heat loss (denoted by the North-West arrow). As temperatures drastically increases from sunrise until the peak ambient temperature for the day (Phase 2), we would expect rapid increases in body temperature concurrent with ambient likely to allocate more time towards activity (North-East arrow). As temperatures begin to decrease from peak ambient to before sunset (Phase 3), we would expect ectotherms to either reduce heat loss (North-West arrow) or reduce heat gain (South-East arrow) possibly to conserve energy. If ambient temperatures continue to rise beyond the upper thermal tolerance (UTT) during the second or third phases of the day, we expect a reduction in heat gain to offset thermally hostile environments (South-East arrow). It is also plausible for ectotherms to actively dump excess heat through exploitation of microclimates in order to decrease body temperatures (South-West arrow). After sunset until midnight (Phase 4), we would expect ectotherms to either passively exchange heat or rely on decreased cooling to reduce heat loss (North-West arrow).

During heat gain to the organism, *Tachycardic Heating, Bradycardic Heating, Heating Rapid Response*, and *Active Heat Gain Reduction* represent forms of active heat exchange involving control of cardiovascular function to increase or decrease the rates of heat transfer while *Increased Heat Gain* and *Decreased Heat Gain* are modes of passive heat exchange that rely on temperature gradients. Equally, during heat loss from the organism, *Tachycardic Cooling, Bradycardic Cooling, Cooling Rapid Response*, and *Active Heat Loss Reduction* are forms of active heat exchange because of the reliance on the cardiovascular output (i.e., tachycardia or bradycardia) while *Increased Heat loss* and *Decreased Heat Loss* are passive forms of heat exchange. When the body temperature is within the predicted range, all forms of heat exchange are passive, and the adjustments of heart rate are related to movement or inactivity. A detailed description of the scenarios can be found in Table 1. *Empirical Case Study: Study Animals and Sites*

We now demonstrate the utility of the framework from data collected on individuals from two turtle species in the field. We hypothesize that (1) active heat exchange will be more prevalent during heating than cooling and (2) heat transfer rates and cardiac function will be dependent on body temperature, specifically, increased or decreased heat transfer rates will be dependent on whether body temperature is above or below the upper thermal limit, and cardiac function will be most influenced by body temperature, not ambient, in the field.

We applied our CHEF to data collected from a box turtle and a painted turtle in which all relevant parameters were recorded (see Parlin, do Amaral, Dougherty, Stevens, & Schaeffer 2017 for implantation details) at 5-minute sampling intervals. Biologging devices included DS1922L thermochron iButtons for body temperature (Maxim Integrated, San Jose, CA) and an ECG logger (HR1AA2, e-Obs, Grunwald Germany) for ambient temperature and heart rate measurements. The box turtle was monitored at the Miami University Natural Area (MUNA) with habitat being characterized as mid- to late-successional forests containing sugar maple (*Acer saccharum*), oak (*Quercus rubra* and *Q. alba*), ash (*Fraxinus spp.*), beech (*Fagus grandiflora*), and black walnut (*Juglans nigra*). For the painted turtle, we constructed aquatic mesocosms using aquaculture tanks (1m x 2m; Frigid Units Inc, Toledo, OH, USA) placed in a deciduous forested area next to each other at the Ecology Research Center (ERC) at Miami University. Each tank had a basking area in the center and the depth of the mesocosms was representative of pond depths in the surrounding area where painted turtles have been found at the MUNA.

Data Analysis

We developed our cardiovascular heat exchange framework (CHEF) to identify cardiovascular contributions to temperature regulation during heating and cooling. After identifying the scenarios of increased or decreased heat transfer, we then visualize the physiological data of body temperature, heart rate, and ambient temperature to show the respective changes in parameters throughout a representative day. Our framework is comprised of two *system-surrounding* relationships that require ambient and body temperature measurements. To determine which temperature is likely to drive cardiac adjustments for heat exchange, we determine the response of cardiovascular function to each temperature in the field for both species.

We applied our CHEF to this representative day to test how well the model identifies the different mechanisms of heat exchange. Next, we compare the active heating-cooling rates above and below the upper level of thermal tolerance to passive rates and then test the effects of ambient and body temperature on heart rate for each species. We based the upper level of thermal tolerance on the 75th quantile of thermal preference for each species, which was 30.5° C for the painted turtle (Rowe, Converse, & Clark) and 31.3° C for the box turtle (Parlin, do Amaral, Dougherty, Stevens, & Schaeffer 2017), and compared the increased, decreased, and passive heat transfer rates to the conceptual framework (Fig. 1) using a test for difference between two Poisson rates.

We use generalized linear mixed-effect model analysis (GLMM) from the 'glmmTMB' package (Brooks et al. 2017). We assessed the significance of body temperature ambient temperature, and their interactive effect on heart rate. Body temperature and ambient temperatures were fixed effects in their respective model comparisons. For random effects we used *turtle ID* for all models and incorporated time-of-day as an autoregressive term to correct for temporal autocorrelation in all models. We use stepwise regression (forward selection and backward deletion) to compare heart rate with our predictor variables ambient temperature, body temperature, and their interaction. Subsequently, we compare the resulting model against a null model using a likelihood ratio test for each species. All model output data were analyzed using the R language and environment v. 3.4.4 (R Core Team, 2017) and all statistical analyses were tested for significance at the $\alpha = 0.05$ level except for multiple comparisons. We adjusted P-values for multiple comparisons using the false discover rate correction.

Results

Cardiovascular Heat Exchange Framework (CHEF) Scenarios

Our integrative framework associated four active cardiovascular roles in heat exchange for the painted turtle (51.55% of data) and three for the box turtle (28.74% of data) while the remaining data were associated with passive mechanisms of heat exchange (Table 2). Both species had *Bradycardic Heating* and *Active Heat Gain Reduction* observed during heat gain scenario, but the box turtle had proportionally more *Active Heat Gain Reduction* (17.78%) compared to the painted turtle (5.57%). During heat loss, the box turtle had 1.18% of the data as *Bradycardic Cooling* while the painted turtle had higher association of data as *Active Heat Loss*

Reduction (20.21%) and *Bradycardic Cooling* (16.02%). For both species, the remaining data were passive forms of heat exchange that included *Increased Heat Gain, Decreased Heat Gain, Increased Heat Loss*, and *Decreased Heat Loss* where heart rates were within the expected range at a given body temperature. The box turtle relied more on passive forms of heat transfer while the painted turtle utilized cardiac output to regulate body temperature in addition to using passive forms of heat transfer, similar to the thermoregulatory strategy of each species.

We found that the number of passive heat exchange occurrences at 5-minute intervals for the box turtle were greatest from midnight to sunrise (Phase 1) and significantly greater than passive exchange from sunrise to peak ambient (Fig. 2a, Phase 2; P = 0.00058) and from peak ambient to sunset (Fig. 2a, Phase 3; P = 0.036) indicating active mechanisms for heat exchange during these times. Occurrence of increased heating rates were significantly greater during the second phase compared to the third ($P = 1.3 e^{-07}$) when body temperatures were below the upper thermal limit and ambient temperatures were greater than body temperatures. The occurrence of decreased heating rates was greater during the third phase than during the second phase (P =0.012) likely due to the overall decreasing ambient temperature as the sun began to set. When body temperatures were above the upper thermal limit, occurrence of passive heat exchange was no different than the first phase of the day (Phase 2 - Above UTT: P = 1.0; Phase 3 - Above UTT: P = 1.0) implying a lack of active mechanisms for heat exchange.

The number of passive heat exchange occurrences at 5-minute intervals for the painted turtle was significantly greater than sunrise to peak ambient temperature (Phase 2- Below UTT: P = 0.034; Phase2 - Above UTT: P = 0.029) and peak ambient temperature to sunset (Phase 3 - Below UTT; P = 0.00028) indicating active mechanisms of heat exchange during these times. The occurrence of passive exchange was similar (Phase 4; P = 0.72) and the third phase above the upper thermal limit (P = 0.06) implying a lack of active mechanisms for heat exchange during these phases. The occurrence of increased heating was significantly greater during the second phase than the third phase (P = 0.0055) when we expected, primarily due to the ambient temperatures being greater than body temperatures. However, the number of occurrences for decreased cooling (P = 0.055) and decreased heating (P = 0.29) were similar between the second and third phases of the day likely due to shifts from terrestrial to aquatic environments. *Influence of Temperature on Cardiovascular Function*

Given that the CHEF is composed of two system-surrounding components involving multiple measurements, we test the effect of body and ambient temperature on heart rate for each species to determine which was more likely to drive cardiac function for heat exchange in the field. We test the predictors of ambient temperature, body temperature, and the interaction on heart rate for each species. Linear mixed effect model analysis for the painted turtle indicated ambient temperature as a significant (estimate = 1.8832; CI = 1.7008 – 2.0656; χ^2_1 = 90.82, p = 2.2e-16) influence on heart rate. However, for the box turtle the linear mixed effect model analysis indicated body temperature as a significant (estimate = 2.5635; CI = 2.2626 - 2.8644; $\chi^2_1 = 39.117$, p = 3.991e-10) influence on heart rate. When comparing heart rate and body temperature, we found that the box turtle exhibited a trend for increasing heart rate as body temperature increased while the painted turtle had no apparent pattern as body temperature could rapidly change when shifting from basking platform to water and vice versa (Fig. 2). Interestingly, heart rate for the painted turtle exhibited a tendency to both increase and decrease with ambient temperature. The relationship between ambient and body temperature for the box turtle was characteristic of their thermoconformity while the same relationship for the painted turtle was characteristic of a partial thermoregulator. There did appear to be a temporal trend during the night hours where body temperature would closely follow ambient temperature, likely due to the painted turtle being at the bottom of the mesocosm in the water and the box turtle creating a form or shallow depression in the soil.

Discussion

The cardiovascular heat exchange framework (CHEF) we develop shows the utility of integrating biologging devices with biophysical principles to gauge the role of cardiovascular adjustments in heat exchange and thus thermoregulation for ectotherms in the field. With our integrative framework we identified scenarios of increased or decreased heat transfer rates that corresponded with heart rate in free living individuals based on the conditions of body and ambient temperature. Cardiovascular function was important for heat exchange in both species but in different ways. We found that when we applied the CHEF to data we collected, the first hypothesis that active heat exchange would be more prevalent during heating than cooling was supported by the box turtle (thermoconformer) and refuted by the painted turtle (partial thermoregulator). The difference in how each species modulated heart rate to adjust heat transfer

rates does bring into question the role of heart rate under varying thermoregulatory strategies. The occurrence of increased or decreased heat transfer rates below the upper thermal limit did match our conceptual framework for when we expected to observe them, but it did not match expectations above the thermal limit for both species. Below the upper thermal limit increased heating rates were greater from sunrise to peak ambient (Phase 2) than peak ambient to sunset (Phase 3), and decreased heating rates were greater during the third phase than the second phase for box turtles. Painted turtles had similar occurrences of decreased heating during the second and third phases of the day. Above the upper thermal limit rates of heat transfer were no different than passive for both species. When comparing the effects of temperature on cardiac function, we found that the box turtle was most influenced by body temperature alone, where heart rate increased and decreased with body temperature. However, cardiovascular function was most influenced by ambient temperature alone for the painted turtle, where the peaks of ambient temperature in the representative day correspond with peaks in heart rate. Overall, the framework we developed shows the potential influence that cardiovascular function has in thermoregulation for free-living individuals that may not have been observed under laboratory conditions.

The scenarios identified by the CHEF for the terrestrial and semi-aquatic species provided insight into the interpretation and utility of cardiovascular function in heat exchange and thus thermoregulation. Box turtles are characterized as thermoconformers (Parlin, do Amaral, Dougherty, Stevens, & Schaeffer 2017) while painted turtles are characterized as partial thermoregulators (Rowe, Converse, & Clark 2014). Although most of the active cardiovascular role for the box turtle was during heating, the majority was Active Heat Gain Reduction where there was reduced heating and presence of bradycardia. Emphasis on decreasing heat gain is likely a mechanism to prevent body temperatures from becoming exceedingly high, which can lead to fatality. Aldabra tortoises (Aldabrachelys gigantea) have been shown to continue increasing in body temperature despite locating a cool microclimate after foraging in an open area for extended periods of time, thus leading to overheating related death in some instances (Swingland & Fraiser, 1979). The cardiovascular role for the painted turtle was more prevalent during cooling, specifically Active Heat Loss Retention and Bradycardic Cooling, rather than during heating. We did not find any instances of *Tachycardic Heating* whereby presence of tachycardia increases body temperature higher than expected, which we would have anticipated based on the life history in the case of the painted turtle (Rowe, Converse, & Clark 2014).

Further, when both turtle species exceeded their upper thermal tolerance the rates of heat transfer were no different than passive heat exchange when we expected either reduced heating or increased cooling.

The foundation of the CHEF is comprised of two system-surrounding components whereby ambient or body temperature can drive cardiac function for heat exchange. Cardiac output is tightly linked with the internal temperature of the organism and is influenced by a suite of extrinsic and intrinsic factors (Gleeson & Bennett, 1985). Under laboratory conditions both species have been found to have close associations between heart rate and body temperature (Gatten 1974a; Stockhard & Gatten, 1983). While the cardiovascular function for the box turtle followed this relationship in the field, where body temperature significantly influenced heart rate and likely heat exchange, the painted turtle did not follow this relationship. There are two possible reasons why we did not observe this tendency in freely active painted turtles. First, painted turtles rely heavily on anaerobic metabolism during submergence. The percentage contribution of increased heart rate to increased oxygen transportation is not sufficient with regular ventilation, thus painted turtles must hyperventilate in order to allow bicarbonate buffering that in turn may cause rapid heart rate upon reaching the surface (Gatten, 1975). Second, large positive and negative heat loads have been shown to cause large increases and decreases in heart rate in other semi-aquatic species such as Crocodylus porous (Franklin & Seebacher, 2003). In this representative day the painted turtle was subjected to large heat loads during shifts from terrestrial to aquatic environments and vice-versa that could potentially lead to tachycardia. However, the presence of tachycardia was never observed with the painted turtle or box turtle in the data tested with the CHEF. In contrast, the presence of bradycardia was found to occur at night for both species, associated with body temperatures that were higher than ambient. It is plausible that the bradycardia measured was associated with both minimizing energetic demand and minimizing the heat transfer from the organism to the environment in order to become active sooner in the day.

Temperature differences between the organism and the environment inevitably drive heat exchange. Heat exchange differs significantly during the day and at night due to differences in direct solar radiation (Gates, 2012). Ectotherms at night lose heat as long as their body temperature is greater than the surrounding ambient temperature. Behavioral and physiological adaptations can minimize heat loss and allow organisms to reduce decreases in body temperature

thus allowing earlier onset of activity. The CHEF was able to successfully determine increased and decreased rates of heat exchange that permit insight into daily dynamics of heat transfer. Terrestrial and aquatic environments differ in the thermal properties of the surrounding physical attributes, such as the high specific heat capacity of soil and water (Geiger, Aron, & Todhunter 2009). In aquatic environments, if temperatures in water are warmer than air at night, then occupancy in water can slow heat transfer rates and allow organisms to maintain a more consistent body temperature (Smith, 1975; Seebacher, Elsey, & Trosclair 2003). Combined with basking after sunrise, high body temperatures can be achieved quickly earlier in the day. In terrestrial environments, burrowing in soil acts as an insulator allowing for decreased heat transfer rates. Similar to aquatic environments, soil has a higher specific heat capacity than air and thus is more resistant to changes in temperature (Geiger, Aron, & Todhunter 2009). However, passive heat exchange was found to have the greatest occurrence during the nighttime for both species, regardless of thermoregulatory strategy. For increased and decreased heat transfer during the daytime hours the variation in occurrence was likely the result of variable environmental conditions. Although our comparison in occurrence of rates is limited to one day per species, the utility of the CHEF to provide behavioral and physiological inferences that can inform temporal habitat associations is crucial for future implementation.

Other physiological mechanisms that we did not measure with our dataset could contribute to the adjustments in rates of heat exchange. Heat exchange rates can be altered by a combination of vasomotor responses including total peripheral resistance, blood flow and blood viscosity (Dzialowski & O'Connor 1999; Seebacher & Franklin, 2007). However, the dynamics of these minute physiological responses cannot yet be measured easily with biologging devices. It is plausible that behavioral adjustments, such as responses to predator presence, could influence heart rate resulting in values being outside predicted. Variability in heart rate is another powerful tool for determining the magnitude of cardiovascular adjustments in heat exchange as the precise frequency can be obtained. A current limitation of measuring heart rate variability (HRV) is the long recording intervals required. Our devices only recorded for about 24 seconds per measurement, which is too short to accurately obtain HRV measurements with full confidence. Incorporation of these parameters on larger ectothermic animals may provide additional information on concurrent mechanisms influencing heat exchange in free-living individuals and the extent to which the CHEF can be applied.

Considerations and Critiques of the CHEF

Merging theoretical concepts of biophysical modelling with empirical field measurements allow us to tease apart physiological, behavioral, and passive mechanisms of heat transfer between the animal and the environment. However, there are caveats to the framework we developed that can limit the extent of interpretation. The first is cause-and-effect for each of the proposed scenario combinations from the two *system-surrounding* relationships, and the second are the measurements and parameters used by the CHEF.

Each proposed scenario does not work in isolation; thus cause-and-effect of body temperature from heart rate can depend on parameters that we did not measure with our devices. In the case of *Bradycardic Cooling*, it is not clear at first how a reduction in heart rate can lead to a lower than expected body temperature (increased cooling) compared to a corpse that represents the lack of physiological control. Although plausible explanations include increased evaporation that can hasten cooling, radiative heat loss at night, and exploitation of microclimates, we were unable to account for all physiological parameters in the field. We do assume that blood flow and cardiac output are proportional to heart rate, but physiological responses such as vasodilation, blood flow, shunting, and blood viscosity likely contribute to the proposed scenarios. The fact that there was presence of *Bradycardic Cooling* indicates one of two possible outcomes. The first is that the unaccounted parameters are contributing to heat transfer in the field and warrant further investigation, and the second is the number of measurements and placement of the biologging devices.

The data logger that sits on top of the shell for the turtles represents the surrounding ambient temperature, primarily through convection and radiation. There were instances where ambient temperature decreased but body temperature continued to rise for the painted turtle. We did note in our field observations that painted turtles would sometimes be partially submerged with the recording device under water and the rest of the body in the sun (Parlin, pers. obs.). Posture and position are undoubtably going to influence heat transfer in the field, and whether incorporating more data loggers would change the outcome significantly remains to be tested. A current limitation of the framework is the small number of parameters measured: body temperature, ambient temperature, and heart rate. Many behavioral mechanisms (e.g., basking, locating cooler microclimates, etc.) inferred by the CHEF are based on the properties of heat transfer and heart rate. Inclusion of movement, activity, hormonal measurements, and

observations in enclosures would not only validate these proposed scenarios but also provide additional insight on the dynamic interactions between behavior and physiology as it relates to heat transfer.

Even with a limited set of parameters and using one representative day, we successfully developed a framework that parsed out the cardiovascular contributions to thermoregulation for free-living ectothermic vertebrates. Although our CHEF required an immense amount of recorded data, the ease of access to biologging devices and creativity with attachment will allow for these types of studies to become more frequent and promote novel inquiries moving forward, especially important under the threat of the climate crisis.

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Tables

Table 1. Identification and description of heat exchange based on the combination of *organismenvironment* and *cardiovascular-organism* mechanisms. The rate of body temperature change (dT_b/dt) and heart rate (HR) columns are denoted with higher (\uparrow) or lower (\downarrow) than predicted rate of body temperature change, or predicted heart rate, respectively. Right-facing red arrows indicate heat gain while left-facing arrows indicate heat loss. Instances where T_b or HR are within expected ranges (=) are defined as passive heat exchange while active exchange involves the presence of tachycardia or bradycardia during increased or decreased rate of heat exchange.

Description	dT _b /dt	HR	HR Exchange Type	
<u>Heat Gain</u>				
Tachycardic Heating is the presence of tachycardia and	7	↑	Active Exchange	
an increased heating rate resulting in a more rapid heat				
gain to the organism during a basking event through				
active cardiovascular control.				
Bradycardic Heating is the presence of bradycardia and	↗	\downarrow	Active Exchange	
an increased heating rate during a basking event. The				
lower heart rate is likely to reduce energy expenditure on				
heat gain.				
Increased Heat Gain is the expected heart rate and an	7	=	Passive Exchange	
increased heating rate. Exposure to a warmer				
microclimate or direct solar radiation could cause faster				
heat transfer rates.				
Heating Rapid Response is the presence of tachycardia	Ы	↑	Active Exchange	
and a reduced heating rate. The observed tachycardia				
would be a cardiac reflex to a positive heat load.				
Active Heat Gain Reduction is the presence of	Ы	\downarrow	Active Exchange	
tachycardia during a reduced heating rate whereby				
decrease in heart rate and thus circulation. This may				
allow an organism to remain in an exposed, sunny area				
for longer.				

Decreased Heat Gain is the expected heart rate and	N	=	Passive Exchange
reduced heating rate resulting from possible presence in			
a cooler microclimate or substrate.			
Movement is the presence of tachycardia and an	=	1	Passive Exchange
expected heating rate.			
Rest is the presence of bradycardia and an expected	=	\downarrow	Passive Exchange
heating rate.			
Inactive is the expected heart rate and an expected	=	=	Passive Exchange
heating rate.			
<u>Heat Loss</u>			
Tachycardic Cooling is the presence of tachycardia and	Ľ	1	Active Exchange
an increased cooling rate resulting in a greater amount of			
heat loss and thus lower body temperature through active			
cardiovascular control.			
Bradycardic Cooling is the presence of bradycardia and	Ľ	\downarrow	Active Exchange
an increased cooling rate. The reduction in cardiac output			
limits energy expenditure while still maintaining an			
increased cooling rate.			
Increased Heat Loss is the predicted heart rate and an	Ľ	=	Passive Exchange
increased cooling rate possibly due to exposure to a			
cooler microclimate.			
Cooling Rapid Response is the presence of tachycardia	R	Ţ	Active Exchange

Cooling Rapid Response is the presence of tachycardia and reduced cooling rate. The observed tachycardia would be a cardiac reflex to a large negative heat load.

Active Heat Loss Reduction is the presence of bradycardia and reduced cooling rate where a lower heart rate reduces circulation and allows the organism to mitigate heat loss.

53

↓

Γ

Active Exchange

Decreased heat loss the expected heart rate and reduced	R	=	Passive Exchange
cooling rate resulting from possible presence in a warmer			
microclimate or substrate.			
Movement is the presence of tachycardia and an	=	↑	Passive Exchange
expected heating rate.			
Rest is the presence of bradycardia and an expected	=	\downarrow	Passive Exchange
heating rate.			
Inactive is the expected heart rate and an expected	=	=	Passive Exchange
heating rate.			

Table 2. Classifications of active mechanisms for the cardiovascular heat-exchange framework (CHEF) where the cardiac output has a role in altering rates of heat transfer for Eastern box turtles (*Terrapene carolina carolina*) and painted turtles (*Chrysemys picta marginata*). In box turtles only *bradycardic basking, active heat-gain reduction,* and *bradycardic cooling* were classified by the CHEF while for painted turtles there was the additional *active heat loss reduction* classified.

Exchange Type – (Species)	dT _b /dt	HR	Percentage
Heat Gain – (Terrapene carolina)			
Increased Heating (Tachycardic Heating)	₹	ſ	0.00%
Increased Heating (Bradycardic Heating)	7	\downarrow	9.78%
Reduced Heating (Heating Rapid Response)	Ы	ſ	0.00%
Reduced Heating (Active Heat Gain Reduction)	И	\downarrow	17.78%
Heat Loss – (Terrapene carolina)			
Reduced Cooling (Cooling Rapid Response)	R	ſ	0.00%
Reduced Cooling (Active Heat Loss Reduction)	R	\downarrow	0.00%
Increased Cooling (Tachycardic Cooling)	Ľ	ſ	0.00%
Increased Cooling (Bradycardic Cooling)	Ľ	Ļ	1.18%
Heat Gain – (Chrysemys picta)			
Increased Heating (Tachycardic Heating)	7	ſ	0.00%
Increased Heating (Bradycardic Heating)	7	\downarrow	9.75%
Reduced Heating (Heating Rapid response)	Ы	ſ	0.00%
Reduced Heating (Active Heat Gain Reduction)	И	\downarrow	5.57%
Heat Loss – (Chrysemys picta)			
Reduced Cooling (Cooling Rapid Response)	R	ſ	0.00%
Reduced Cooling (Active Heat Loss Reduction)	R	\downarrow	20.21%

Increased Cooling (Tachycardic Cooling)	Ľ	1	0.00%
Increased Cooling (Bradycardic Cooling)	Ľ	\downarrow	16.02%

Figure Legends

Figure 1. A conceptual framework for when active heat exchange of an ectotherm would occur in a representative day. The presence of black bars represents night, the black solid line is body temperature (° C), the dashed line is ambient temperature (° C), and horizontal lines are the upper thermal tolerance (black) and critical thermal maximum (red). Arrows represent increase (upper angle) or decreasing (lower angle) heat exchange rate during heating (red) and cooling (blue). The representative day is separated into four phases (1-4) where phase 1 is midnight to sunrise pre-sunrise, phase 2 is sunrise to peak ambient temperature, phase 3 is the peak ambient temperature until sunset, and phase 4 is after sunset until midnight. The heating and cooling arrows represent potential scenarios based on whether ambient or body temperature of the animal is higher.

Figure 2. Empirical data from the (A) box turtle and (B) painted turtle in a representative day. Top portions of the graph are the ambient (dashed line) and body (colored line) with photoperiod (black = night) while the bottom is corresponding heart rate. Gaps in the data indicate heart rates that were unable to be determined. For body temperature warm colors represent heating (red = increased, orange = decreased), cool colors represent cooling (light blue = decreased, dark blue = increased), and grey represents passive heat exchange based on the CHEF. Heart rate for the painted turtle peaked with corresponding peaks in ambient temperature. However, the box turtle heart rate increased and decreased with body temperature.








Chapter 4

Comparative field energetics: the role of heart rate in thermoconformity and partial thermoregulation

Summary

Ectothermic vertebrates use a suite of physiological and behavioral mechanisms to thermoregulate, which results in a variety of different thermoregulatory strategies from thermoconformity to active thermoregulation. Active regulation of body temperature incurs a greater energy demand than relying on passive exchange of heat bringing into question the energetic demands of thermoregulation. The goal of the study was to tease apart the role of heart rate modulation in active and passive heat exchange as these measurements provide a strong link to the energetic demands in thermoregulation. We quantified the seasonal energetics of two turtle species, a thermoconformer (Eastern box turtle, Terrapene carolina carolina) and a partial thermoregulator (Painted turtle, Chrysemys picta marginata) and characterize the cardiovascular contributions to active and passive mechanisms of heat exchange. We hypothesized that (1) thermoconformers would have a lower field metabolic rate when compared with partial thermoregulators, and (2) the cardiovascular contribution in the thermoconformers would be associated with passive forms of heat exchange while in the partial thermoregulators it would be associated with active forms of heat exchange. We measured field metabolic rate (FMR) in freeliving individuals and use the recently developed cardiovascular heat exchange framework (CHEF) to determine the cardiovascular role in active heat exchange for thermoconforming box turtles and partial thermoregulating painted turtles. We support both of our initial hypotheses. Box turtles, used less energy than painted turtles based on our estimates from body temperature and heart rate measurements, and box turtles relied more on passive mechanisms for heat exchange whereas the painted turtle relied more on active mechanisms. We did find that there was a less active cardiovascular role during heat gain and a more active role during heat loss for both species. Our study quantifies the associated energetic costs with thermoregulation and broadens our interpretation of heart rate modulation during heat transfer under natural conditions. Although for some species 'hotter is better,' the consequences of reaching upper thermal limits and possibly overheating may outweigh the benefits of higher body temperatures in temperate regions.

Introduction

Ectotherms gain and lose heat through interactions with the physical environment and possess many adaptations that permit the modulation of heat transfer rates. The first step to understanding the resiliency of these adaptations to extreme conditions is gauging the energy demands of thermoregulation. Many ectotherms heat twice as fast as they cool (Smith, 1975; Seebacher & Franklin, 2005), indicating the importance of increasing body temperature quickly when temperatures are low and therefore extending activity when conditions are thermally limiting (Grigg & Seebacher 1999; Seebacher, 2000). While ectotherms often respond passively to thermal variation (Gates, 2012), many physiological processes perform best over limited ranges of body temperature (i.e., within thermal preference; Hertz, Huey & Stevenson 1993). Physiological and behavioral mechanisms permit ectotherms to achieve and maintain their thermal preference, the range of temperatures that maximize various performance traits (Dillion, Wang, Garrity & Huey 2009). While behavioral mechanisms allow for passive heat exchange through radiation, conduction, and convection (Gates 2012), physiological mechanisms can increase or decrease the rate of heat transfer. Therefore, active behavioral and physiological mechanisms used for thermoregulation have energetic requirements that vary depending on the surrounding physical environment, including costs of movement to select favorable microclimates (Sears et al. 2016) or cardiovascular adjustments to alter blood flow (Dzialowski & O'Connor, 1999; Seebacher & Franklin, 2007).

Thermoregulation encompasses strategies ranging from strict thermoconformity, solely relying on differences in ambient conditions to determine body temperature, to active thermoregulation, seeking out basking sites to increase body temperature rapidly and maintain thermal preference (Angilletta, 2009). Thermoregulation is also dependent on the thermal landscape of the environment, sometimes leading to increased difficulty in maintenance of preferred body temperatures (Sears & Angilletta, 2015). Maintaining high body temperatures has benefits for increased growth (Autumn & DeNardo, 1995) and reproductive output (Congdon and Tinkle 1982; Krawchuk & Brooks 1998). However, the degree to which ectotherms thermoregulate varies and not all reptiles use the same thermoregulatory strategy. As the thermal quality of the habitat starts to deviate farther from the thermal preference to thermally limiting conditions, the energetic costs of active thermoregulation begin to increase (Huey & Slatkin, 1976; Blouin-Demers & Nadeau, 2005; Brewster, Sikes, & Gifford 2013). Some species can

modify their thermoregulatory behavior to switch between thermoconformity and thermoregulation (Randriamahazo & Mori, 2004) while others utilize facultative endothermy to increase activity at lower temperatures (Tattersall et al. 2016).

While the importance of behavioral thermoregulation has been well documented (Angilletta, 2009; Bels & Russell, 2019), less appreciated is the cardiovascular contribution to thermoregulation. Cardiac output is tightly linked with oxygen consumption in reptiles and thus serves as a proxy for field energetics (Green, 2011). Energetic costs of thermoregulation are dependent on the thermal quality of the habitat and ability of the ectotherm to effectively exchange or retain heat (Huey & Slatkin, 1976; Blouin-Demers & Nadeau, 2005). Physiological regulation is likely engaged to assist behavioral mechanisms to meet regulatory demands. The extent of cardiovascular contribution to thermoregulation will therefore be dependent on the interplay of multiple environmental and physiological inputs during heating and cooling. The combination of cardiac output and distribution of blood flow have a significant role in altering rates of heat exchange during heating and cooling phases (Lillywhite, Zippel, & Frappell 1999). For reptiles, heart rate at a given temperature during a heating phase is usually higher than heart rate during a cooling phase, a phenomenon referred to as heart rate hysteresis (Zaar, Larsen, & Wang 2004; Seebacher & Franklin, 2005). Increased cardiac output during the heating phase can lead to increased cutaneous perfusion that is crucial for heat exchange and thus thermoregulation (Smith, 1975; Hochscheid, Bentivegna, & Speakman 2002; Dzialowski & O'Connor, 2004). Reduced cardiac output during the cooling phase with limited peripheral blood flow can reduce the rate of heat exchange and allow for retention of heat (Zaar, Larsen, & Wang 2004). However, when body temperatures reach upper lethal limits individuals must decrease their body temperature because of the risk of impairment to physiological functions and thus survival. Active mechanisms can allow overheating reptiles to dump excess heat by increasing cardiac output, and thus circulation, to increase the rate of heat transfer from their body to a cooler microclimate (reverse hysteresis; Grigg & Seebacher, 1999). Similarly, it is possible for active mechanisms to reduce heart rate during a heating phase in thermally hostile conditions to permit prolonged activity and reduce the rate at which body temperature increases. Thus, cardiovascular adjustments have the ability to influence heat exchange between the reptile and the environment. Currently, the energetic demands of thermoregulation and the cardiac contribution to active heat exchange in free-living reptiles remains largely unexplored.

Biologging devices have the capability to monitor physiological parameters at high resolution in the field and can be used to estimate field metabolic rate using body temperature and heart rate as proxies (Green & Frappell, 2007; Green, 2011). Not only are we able to estimate the field metabolic rate of individuals with different thermoregulatory strategies, but we can use heart rate measurements to separate active (i.e., physiological) and passive (i.e., behavioral) mechanisms of heat exchange during thermoregulation. Temperature differences between the organism and the environment define scenarios of heat gain or heat loss, and deviations from passive exchange with the environment indicate active behavioral (i.e., basking) or physiological mechanisms (i.e., heart rate) that alter the heating or cooling rate. Therefore, fine scale measurements of ambient and body temperature permit us to quantify the heat flow between the organism and the surrounding physical environment. The ability to monitor multiple parameters simultaneously with biologging technology allows for novel inquiry into the role of energetics in thermoregulation and physiological mechanisms utilized in the field by reptiles.

Disentangling the role of cardiovascular adjustments in active and passive heat exchange provides a strong link to the energetic demands in thermoregulation. Although previous laboratory experiments have shown the importance of discrete mechanisms such as rapid changes in heart rate in response to heat load and cardiac hysteresis during heating and cooling (Franklin & Seebacher, 2003), observations under field conditions reflect the ecologically relevant energetic costs associated with thermoregulation. Turtles are excellent study species to pursue problems regarding the energetics of thermoregulation for several reasons, including morphological adaptations (i.e., shell) and occupancy of both terrestrial and aquatic habitats, all of which strongly influence pathways of heat exchange (Gates, 2012). As such, we use the cardiovascular heat exchange framework (Chapter 3) to understand the cardiovascular contribution to thermoregulation. The framework integrates empirical data with biophysical principles to gauge the role of cardiovascular adjustments in thermoregulation in free-living individuals using biologging devices. We aim to show the utility of combining biologging devices with biophysical principles to bridge the gap between theoretical and empirical work. We apply the CHEF to data from two different turtle species collected across their active seasons to clarify the role of energetics and cardiovascular contributions to thermoregulation in freeliving individuals.

The purpose of our study was first, to compare the daily energy use of two turtle species that occupy a similar climatic region, a terrestrial thermoconformer and a semi-aquatic partial thermoregulator and, second, to use the CHEF with field measurements of heart rate and body temperature to assess the relevance in active and passive heat exchange during thermoregulation. With our approach, we take a first look at the costs of thermoregulation for ectothermic vertebrates in a natural context by comparing the field metabolic rate of the thermoconforming Eastern box turtle (*Terrapene carolina carolina*) and the partial thermoregulating painted turtle (*Chrysemys picta marginata*) in the same region of Ohio, and by characterizing active and passive mechanisms of heat exchange. We hypothesized that (1) thermoconformers would have a lower field metabolic rate when compared with partial thermoregulators, and (2) the cardiovascular contribution in the thermoconformers would be associated with passive forms of heat exchange. To test these hypotheses, we (1) measured field metabolic rate (FMR) in free-living individuals, and (2) implemented the CHEF to determine the cardiovascular role in active heat exchange for thermoconforming box turtles and partial thermoregulating painted turtles.

Methods

Study Area

We monitored both turtle species in Southwest Ohio near Miami University (39.5° N, 84.7° W). For box turtles, we followed free-living individuals at the Miami University Natural Areas (MUNA), Ecology Research Center (ERC), and Hueston Woods State Preserve (HWSP) in 2014. Habitat in the area is highly fragmented from agriculture and is comprised of mid- to late-successional forests while the ERC also included agricultural fields. Box turtles freely moved throughout the ERC, HWSP, and MUNA from late May until October. Painted turtles were held in mesocosms at the ERC for two-week periods during (1) late May and early June, and (2) August. The mesocosms were located on the edge of a forest fragment and were exposed to direct solar radiation for more than 6 hours a day, similar to other ponds in the area (see description below).

Field Monitoring

For this study, Eastern box turtles (n = 9) were monitored during their active season from April until August in 2014 for two-week periods during the beginning (late May – early June),

and middle (late July – early August) of their active season. We did not calibrate heart rate and body temperature with respirometry measurements for box turtles in 2014 so we used measurements from a second group of box turtles in 2015 (n = 11) to generate a group calibration curve to apply to the 2014 field data. (see Heart Rate and Respirometry Calibration section). Box turtles were located by hand and GPS coordinate recorded once a day (62s Garmin GPS, \pm 3-10m resolution; Ontario, Canada). We tracked individuals with BD-2 transmitters (Holohil; Ontario, Canada) for 12 days each (range: 11 - 14 days). Painted turtles were purchased from Niles Biological (Sacramento, CA, USA) and were monitored during the beginning (late May – early June) and middle (mid-August) of their active season in 2017. We monitored painted turtles (n = 8) at the ERC in two mesocosms made using aquaculture tanks (Frigid Units Inc, Toledo, OH, USA) that were one meter in depth and two meters in diameter, and that were placed next to each other at the edge of a deciduous forested area. Each mesocosm held only two turtles during either the beginning or the middle of the active season. Each tank had a basking area in the center constructed from wood with a ramp and rocks in the center. We obtained sediment and dirt from a nearby pond at the ERC to fill the bottom of the mesocosm with approximately 5cm of organic material and placed several rocks and leaf litter. Each tank was filled with pond water from the ERC. The depth of the mesocosms was representative of pond depths in the surrounding area where painted turtles have been found at the MUNA (Parlin, pers. obs.). Both tanks were created and filled with water during late April and remained operational for the duration of the experiment.

Biologging Surgery and Attachment

We implanted biologging devices to record internal body temperature (T_b), ambient temperature (T_a), and heart rate (HR). We implanted a DS1922L iButton (Maxim Integrated, San Jose, CA, USA) into the left posterior region of the coelomic cavity, anterior to the hind leg in both species through a 2cm incision. We epoxied a HR-1AA2-ID ECG-logger (e-obs, Grünwald, Germany) to the top of the carapace and inserted the electrodes through two holes drilled in the carapace (4.5 mm diameter) at the R4 and L8 marginal scutes in both species. We filled openings in the carapace with sterile bone wax to prevent secondary infection and covered the electrode leads with epoxy to prevent damage from field conditions and to hold them in place. ECGloggers recorded HR (in bpm) at 56.32Hz (recording duration: 15.2 seconds; box turtle) and 31.62 Hz (recording duration: 24.8 seconds; painted turtle) as well as ambient temperature (T_a). All biologging devices were programmed to record simultaneously at 5-minute intervals for the duration of monitoring. Both species underwent similar surgical procedures for attachment of biologging devices. For a more detailed description of surgery procedure with device settings see Parlin, do Amaral, Dougherty, Stevens, & Schaeffer (2017). Surgery procedure followed approved Miami University Institutional Animal Care and Use Committee protocols #906 and 952.

Respirometry Measurements

Turtles used for measurements of oxygen consumption rates were either field acclimated (2015 box turtles, n = 11) or held in mesocosms for two weeks (painted turtles, n = 12) prior to measurements. We used indirect calorimetry to measure the oxygen consumption rates (\dot{V}_{O_2}) of each turtle species and simultaneously measured heart rate and body temperatures for correlation with metabolic rate (Green, 2011). Metabolic rates were measured at four different temperatures for calibration curves in resting and fasted (48-hours) individuals. We tested oxygen consumption of box turtles at 15, 20, 25, and 30° C and of painted turtles at 20, 25, 30, and 35 °C. We selected these temperatures because of the environmental relevance (i.e., maximum body temperature recorded in the field) and due to the lack of a discernable rate of O₂ consumption below the minimum tested temperature in both species.

Individuals were measured in a temperature controlled environmental chamber (model 141VL Percival unit; Perry, IA, USA) and allowed 1-hour to equilibrate prior to the start of each temperature after allowing 20 minutes for the environmental chamber to reach the target test temperature. All testing temperatures were randomized and never greater than 10° C in magnitude between trials. After we pumped dry air for 24-hours through the chamber, we placed turtles in a plexiglass chamber (7080 mL) inside the temperature-controlled Percival unit with tubing exiting the chamber through a side port that was sealed to limit temperature change. The air pulled from the chamber passed through a desiccant (Drierite, 10-20 Mesh; Acros Organics, New Jersey, USA), then entered the oxygen analyzer (Foxbox Respirometry System; Sable Systems, Las Vegas, NV, USA). CO₂ was then scrubbed (Ascarite; Thomas Scientific, New Jersey, USA) before the air reentered the chamber. Flow rate was set to 250 mL/min. The turtles were tested with no lights, at rest, and with the only audible sound being from the Percival chamber. For calculating rate of oxygen consumption, we used the following equation from Lighton (2008):

$$M_{s} O_{2} = \frac{FR(F''iO_{2} - F''eO_{2})}{1 - F''iO_{2}}$$
(1)

where M_sO_2 is the measured rate of O₂ consumption, FR is the STP-corrected mass flow rate, F"_iO₂ is the initial fractional concentration of water-free, CO₂-free air that provides the baseline O₂ concentration for the system, and F"_eO₂ is the final fractional concentration of water-free, CO₂-free air. All \dot{V}_{O_2} data were analyzed in Expedata (Sable Systems, North Las Vegas, NV, USA) and the R-package 'SableBase' 1.0.0 (Foerster 2013).

Heart Rate Calculation

We hand calculated heart rate for box turtles and used a quality-controlled algorithm for painted turtles. The quality-controlled algorithm was a non-commercial stand-alone variation of Star-Oddi's heart rate algorithm that estimates the quality of each ECG recording and outputs heart rate and an associated quality index written in Python. The quality index is calculated from a set of rules described in Star-Oddi user manual and grades each recording from 0 (best) to 3 (worst), the algorithm has been validated in rainbow trout (*Oncorhynchus mykiss*) against a reference system (Brijs et al. 2019). Hand calculations were done in excel to visualize the ECG trace and we measured multiple R-R intervals. We tested the algorithm against hand-calculations and found similar values for heart rate based on the quality index score.

Heart Rate and Respirometry Calibration

We compare the field energetics for each species using group calibration curves generated from respirometry measurements. To ensure the accuracy of the group calibration curve, we generated individual calibration curves to compare with the group curves for box turtles and painted turtles. Given the nearly identical results between individual and group equations for both species, we applied the respective group calibration curves to the field collected data (box turtles in 2014 and painted turtles in 2017).

Cardiovascular Heat Exchange Framework

The purpose of the framework was to characterize cardiac contributions to thermoregulation by identify heat exchange between the organism (i.e., the system) and the environment (i.e., the surrounding) and then determining the cardiovascular activity (i.e., system) in relation to the body temperature (i.e., surrounding). The framework is grounded in the passive heat exchange between ambient and animal body temperatures, and the predicted heart rate as a function of body temperatures independent of intrinsic factors. We used the data from the biologging devices to first measure the difference in temperatures between the ambient conditions and body temperature for the turtles (i.e., *organism-environment*). With these data, we used a modified version of Newton's second law of cooling (see Sears & Angilletta 2015) to predict the passive change in body temperature for each 5 minute interval, with the null hypothesis that the turtle heated and cooled at the same rate (i.e., passive exchange). This permitted identification of instances of slower or faster than expected rates of heating or cooling as ambient temperature changes. Second, we made a comparison of the body temperature and corresponding heart rate (i.e., *cardiovascular-organism*). With these data, we use a modified sigmoidal equation to predict the current heart rate based on what the heart rate should be at that temperature and identify instances of brady- or tachy-cardia. With the simultaneous sampling at 5-minute intervals, we capture discrete events in which temperature and heart rate changes can be determined against time.

We focus on the extent to which individuals use active heat exchange during thermoregulation, where body temperature and heart rate are higher or lower than predicted to determine the extent to which active heat exchange occurs. Specifically, we identify the following scenarios for heat gain: *Tachycardic Heating* (increased heating and tachycardia), *Bradycardic Heating* (increased heating rate and bradycardia), *Heating Rapid Response* (reduced heating rate and tachycardia), and *Active Heat Gain Reduction* (decreased heating rate and bradycardia). Similarly, we emphasize the following scenarios for heat loss: *Tachycardic Cooling* (increased cooling rate and tachycardia), *Bradycardic Cooling* (increased cooling rate and tachycardia), *Response* (reduced cooling rate and tachycardia), and *Active Heat Loss Reduction* (reduced cooling and bradycardia). When heart rate is within the predicted range, we are inferring that heat exchange with the environment is passive. A detailed description of the CHEF can be found in Chapter 3.

Data Visualization and Analyses

We plotted a representative day of body temperature changes for box turtles and painted turtles to emphasize the differences in thermoregulatory strategy and emphasize the degree of temperature change in terrestrial and aquatic environments by each species. For changes in body and ambient temperature we reported the entire range and the central 50% of the data for each species. We also quantified the maximum temperature differences between changes in internal

and shell temperature and the maximum changes in the respective temperature between each 5minute sampling interval.

We report the mean field metabolic rate (FMR) and calculated the percent change from night-to-day in each species during each season. To compare seasonal and species level differences we use the R-package 'glmmTMB' v0.2.2.0 (Brooks et al. 2017) for linear mixed effects analysis of season, day-night, and species on FMR. Within each species, we compare season and day-night (i.e., sun-up or sun-down based on time of year) as fixed effects without an interaction into the model. To compare between species we included *species*, *day-night*, and season without interactions into the models. For random effects we used *turtle ID* for all models and incorporated *time-of-day* as an autoregressive term to correct for temporal autocorrelation in all models. P-values were obtained by likelihood ratio tests with season, day-night, and species against a null model for each respective comparison. Using the CHEF, we associated the active forms of heat exchange for each species where body temperature and heart rate were higher or lower than predicted but never within predicted in a table with the range of body temperatures and time of day that were associated with each active form of heat exchange. We compare the percentage of active and passive forms of heat exchange for each thermoregulatory strategy with a two-proportion z-test with the null assumption of a 50 percent probability for each form of heat exchange using the prop.test() function in the base 'stats' package of R. Data were analyzed with R (R core Team, 2018) and statistical comparisons were made at the $\alpha = 0.05$.

Results

Field Body Temperatures

Plots of body and ambient temperature of box turtles and painted turtles through the day show that each species possesses a different thermoregulatory strategy (Fig. 1). Body temperatures of box turtles, which have been previously characterized as thermoconformers, closely match ambient temperatures and thus have a cyclic pattern to their daily body temperature. Temperature changes (°C) in box turtles between each 5-minute sampling interval ranged from -2.12 to 2.32° C (central 50% from -0.06 to 0.00 °C) while ambient temperature changes ranged from -2.94 to 3.91° C (central 50% from -0.07 to 0.04 °C). Painted turtles had body temperatures that often increased rapidly (Fig. 1), concurrent with basking events. Changes in painted turtle body temperature ranged from -10.10 to 3.43° C (central 50% from -0.06 to 0.00 °C) while ambient temperature changes ranged from -15.44 to 6.91° C (central 50% from - 0.06 to 0.03 °C). We also found that body temperatures were consistently higher than ambient conditions in both species during nighttime hours. At night, box turtles had body temperatures greater than ambient for 99.5% of the recordings during the beginning (1.09 ± 0.68 °C) and 99.6% of the recordings during the middle (1.24 ± 0.38 °C) of the active season while painted turtles had body temperatures greater than ambient for 99.2% of the recordings in the beginning (0.72 ± 0.71 °C) and 96.1% of the recordings during the middle (0.57 ± 0.37 °C) of the active season.

Field Metabolic Rate

Field metabolic rate (FMR) in box turtles was approximately 24.5% of the FMR measured for painted turtles in the beginning of the active season, and 29.7% of the painted turtle FMR in the middle of the active season (Table 1). FMR in a representative day was similar for both species between the beginning (May – June) and middle (August) of their active seasons. Additionally, there was a non-significant 65% increase in mean FMR for box turtles from night to day during the beginning and middle of the active seasons while painted turtles had a non-significant 12% decrease in the beginning and 0.1% increase during the middle of the active season (Table 1). We found no significant effect of *season* or *day-night* on FMR in box turtles $(X_2^2 = 2.5057, p = 0.2857)$ or on FMR in painted turtles $(X_2^2 = 5.826, p = 0.05431)$, although nearly so. We did find a significant effect of *species* on FMR when comparing painted turtles and box turtles $(X_3^2 = 27.923, p = 3.77e^{-06})$ indicating higher FMR in painted turtles than box turtles. In general, energy consumption rate in painted turtles remained relatively consistent throughout the day while box turtles had a semi-cyclic pattern (Fig. 2). Hourly variance was also much lower in box turtles than painted turtles during all hours of the day.

Active Mechanisms of Heat Exchange

We had a total of 18380 corresponding heart rate, body temperature, and ambient temperature recordings for box turtles (n = 9) and another 22006 corresponding heart rate, body temperature, and ambient temperature recordings for painted turtles (n = 8). The CHEF associated 74.64% of the recordings with passive forms of heat exchange for box turtles, and the remaining 25.36% as having an active role of cardiovascular function in heat exchange (Table 2). There was a significant difference from the predicted 50% probability between passive and active forms of heat exchange ($X^2 = 8927.9$, df = 1, p = 2.2e⁻¹⁶). Box turtles had only 9.88% of

active cardiovascular adjustments related to gaining heat with 6.87% being *Bradycardic Heating* (i.e., T_b higher than expected and bradycardia) and the remaining 3.01% being for *Active Heat Gain Reduction* (i.e., T_b lower than expected and bradycardia). Box turtles had more frequent active cardiovascular adjustments during heat loss, where 3.52% of the classifications were for *Active Heat Loss Reduction* (i.e., T_b higher than expected and bradycardia) and 11.96% for *Bradycardic Cooling* (i.e., T_b lower than expected and bradycardia). *Tachycardic Heating, Heating Rapid Response, Cooling Rapid Response,* and *Tachycardic Cooling* were not observed by the CHEF for any of the box turtles monitored.

For painted turtles, only 24.36% of the recordings were classified as passive forms of heat exchange while the remaining 75.64% identified an active role for cardiovascular function in heat exchange (Table 2). There was a significant difference from the predicted 50% probability between active and passive forms of heat exchange ($X^2 = 11572.0$, df = 1, p = $2.2e^{-1}$ ¹⁶). All forms of active heat exchange were observed for painted turtles during heating and cooling. During heat gain, painted turtles had 0.03% of the observations identified as Tachycardic Heating (i.e., T_b higher than predicted and tachycardia), 1.93% as Bradycardic Heating(i.e., T_b higher than predicted and bradycardia), 0.14% as Heating Rapid Response (i.e., T_b lower than predicted and tachycardia), and 7.80% as Active Heat Gain Reduction (i.e. T_b lower than predicted and bradycardia). The CHEF classified the active cardiovascular adjustments in the morning hours (0900 to 1230 hours) for *Tachycardic Heating* and *Heating* Rapid Response while Bradycardic Heating and Active Heat Gain Reduction were observed during the afternoon hours (1200 to 1600 hours). During heat loss, painted turtles had 2.12% of the observations identified as Cooling Rapid Response (i.e., T_b higher than expected and tachycardia), 61.34% as Active Heat Loss Reduction (i.e., T_b higher than expected and bradycardia), 0.04% as Tachycardic Cooling (i.e., Tb lower than expected and tachycardia), and 2.24% as *Bradycardic Cooling* (i.e., T_b lower than expected and bradycardia).

Discussion

Thermoregulation has been assumed to favor optimizing physiological function while minimizing energetic demand in order to maximize fitness (Huey & Slatkin, 1976; Brewster, Sikes, & Gifford 2013), but the physiologically optimal may not always be ecologically relevant (Martin & Huey, 2008). We observed extremely large maximal changes in ambient and body

temperature (10 - 15 °C) for painted turtles, while box turtles experienced moderate changes in ambient and body temperature between consecutive measurements. The larger decrease in temperature for painted turtles is likely due to shifts from terrestrial to aquatic environments following basking events. Frequently, painted turtles tolerated body temperatures as high as 35° C for extended periods of time before diving back into the mesocosm. We speculate that the extremely high temperatures are likely for digestion and assimilation rather than optimal locomotive performance as the temperatures are well above this species' thermal preference (Thermal preference range of 25.2 – 30.5 °C; Rowe, Converse, & Clark 2014). The ability to bask during the day and retain heat at night in the water may allow for painted turtles to be more efficient at digestion, thus increasing the energy available for growth and reproduction. Conversely, box turtles had cyclic changes in body temperature that were closely related to the ambient temperature and never exceeded more than 7° C between external and internal temperatures. Tachycardia was never observed in box turtles in any of the CHEF scenarios, likely contributing to the low daily FMR. We speculate that the reliance on passive forms of heat exchange minimizes the need for tachycardia to regulate temperature, thus contributing to the low-energy lifestyle of box turtles.

Field metabolic rate (FMR) estimated from body temperature and heart rate measurements for the box turtles was roughly a quarter of the energy used when compared to the painted turtles for beginning and middle of the active seasons. Gatten (1974) reported similar trends for body temperature, heart rate, and oxygen consumption (\dot{V}_{O_2}) in a semi-aquatic (*Trachemys scripta elegans*) and a terrestrial (*Terrapene ornata*) turtle in the lab, and our laboratory measurements of metabolic rates do match previously established values for box turtles (Geinger & Urdiales, 2017) and painted turtles (Stockhard & Gatten, 1983). Although not significant, mean FMR increased 65% from night-to-day for box turtles while painted turtles only had a slight decrease of 12%. Increases in the basal metabolic rate have been hypothesized to result in an increased capacity to control the rate of body temperature change (Bartholomew & Tucker, 1963) thus prolonging the time that an organism can remain within the thermal preference for activity or digestion. It is plausible that by maintaining a higher FMR painted turtles are able to become active sooner in the day and rely less on ambient conditions being thermally suitable. Box turtle activity is largely independent of ambient conditions, allowing individuals to be active when body temperatures are as low as 14° C (Parlin, Nardone,

Dougherty, Rebein, Safi & Scaheffer 2018). However, rising ambient temperatures and extreme weather conditions due to the climate crisis (IPCC, 2018) could negatively impact daily activity for box turtles. While the box turtles used less energy overall, the consequences of frequently hotter days may increase midday inactivity or force individuals to risk overheating thus incurring energy deficits. Unlike the painted turtles, box turtles may not be able to become active earlier in the day and rely less on ambient conditions given their thermoregulatory strategy and cardiovascular role in heat exchange. The direct link between maintaining higher FMR and becoming active sooner remains to be explored in depth for both terrestrial and aquatic environments.

Cardiovascular contributions to heat exchange in both species were more likely to occur during heat loss than heat gain. The CHEF identified all possible scenarios of active heat exchange during heat gain and heat loss for painted turtles while box turtles never experienced tachycardia during heat gain or heat loss. A less active cardiovascular role during heat gain may be a strategy to prevent the risk of overheating (Grigg & Seebacher, 1999). Both these species occupy temperate regions that are characterized as humid continental where summers range from warm to hot with occasional humidity, and winters are cold and sometimes severe with precipitation distributed throughout the year (Karl & Koss, 1984). Although for some species 'hotter is better' (Angilletta, 2009), the consequences of reaching upper thermal limits and possibly overheating may outweigh the benefits of higher body temperatures in temperate regions. Thus, maintaining body temperatures in the middle of these thresholds (e.g., thermal preference) may invoke an active cardiovascular role in reducing heat gain during the day and minimizing heat loss at night to prevent body temperatures from reaching critical thresholds that impede function.

The disconnect between ecologically relevant and physiologically optimal temperatures ultimately leads to trade-offs in thermoregulatory strategy and heating-cooling rates (Angilletta, Cooper, Schuler & Boyles 2010). The life history of an organism plays a significant role in regulation of temperature and the subsequent impacts on heat exchange mechanisms. Laboratory experiments have shown that terrestrial turtles and tortoises cooler faster than they heat while aquatic turtles heat faster than they cool (Spray & May 1972; Craig, 1973), but Pages, Fuster & Palacios (1991) showed that *Mauremys caspica*, a semi-aquatic turtle, cooled faster than it heated, speculating that heat stress in the habitat from colonizing warmer waters could lead to a

delay in heat gain and an increase of heat loss. Other species may have a higher cooling rate than heating rate to increase the potential foraging time during thermally stressful conditions in open areas (Swingland & Fraizer, 1978). We found that at night there were instances of heat gain for both species. There is potential for microclimates to potentially influence heat transfer in both species. For instance, the different thermal properties of water, air, and soil (Geiger, Aron, & Todhunter 2009) could lead to selection of favorable microclimate conditions even at night when we expected the turtles to be inactive. Although we had no direct observations of activity at night, incorporation of those data would prove useful for further disentangling the cardiovascular role in thermoregulation using the CHEF during the day or night.

Overall, the CHEF was able to successfully integrate empirical data and biophysical principles to gauge the active role of cardiovascular adjustments in thermoregulation and maintenance of heat exchange in free-living turtles that use different thermoregulatory strategies. Our study provides a basis for the integration of theoretical principles and empirical data to better understand the physiological processes and energetics regulating temperature and heat exchange in the field. Advances in biologging technology permit observation of fine-scale physiological processes in free-living animals, offering new opportunities to ask novel questions of animal-habitat interactions. As technology continues to improve and becomes more cost-effective, we hope that the incorporation of multiple devices and parameters will be more feasible to test the CHEF and improve the accuracy as well as interpretation.

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Tables

Table 1. Mean (\pm SE) for field metabolic rate (FMR) during beginning and middle of the active season. Box turtle FMR was approximately 24.5% of the painted turtle FMR in the beginning of the active season, and 29.7% during the middle of the active season. Mean percent change in FMR from night to day was around a 65% increase for *T. carolina* during the beginning (late May and early June) and middle (August) of the active season while *C. picta* had a 12% decrease in FMR from night to day during the beginning of the active season and a negligible change of 0.101% during the middle of the active season.

	Field Metabolic Rate (mL $O_2/g \cdot hr^{-1}$)		Percent Change in FMR from Night to Day			
Species	Beginning	Middle	Beginning	Middle		
T. carolina	0.0086 ± 0.002	0.0096 ± 0.003	65.500 ± 9.500	64.800 ± 22.200		
C. picta	0.0351 ± 0.008	0.0323 ± 0.007	-12.20 ± 12.100	0.101 ± 2.090		

Table 2. Active cardiovascular adjustments and corresponding heat exchange for box turtles and painted turtles with the quantile range for body temperatures (°C) and time of day at which the CHEF determined body temperature and heart rate higher, lower, or within predicted. Box turtles did not experience any *Tachycardic Heating* (increased heating), *Heating Rapid Response* (reduced heating), or *Tachycardic Cooling* (increased cooling). Painted turtles experienced all forms of the active heat exchange despite some of the data being less than 1% of the total. The remaining 74.64% of the data for box turtles and 24.36% for painted turtles were all forms of passive heat exchange with the environment where either heart rate or body temperature were within predicted.

Exchange Type - Species	Abbreviation*	Body Temperature (°C) Quantile		Time of Day Quantile		Percentage
	-	Central 50%	Range	Central 50%	Range	-
Heat Gain – (T. carolina) Increased Heating (Tachycardic Heating)	$\mathrm{AH}^{\uparrow\cdot\uparrow}$	-	-	-	-	0.0%
Increased Heating (Bradycardic Heating)	$\mathrm{AH}^{\uparrow \cdot \downarrow}$	21.18 - 28.35	15.16 - 35.94	11 – 14	08 – 18	6.87%
Reduced Heating (Heating Rapid Response)	$\mathrm{AH}^{\downarrow\uparrow\uparrow}$	-	-	-	-	0.0%
Reduced Heating (Active Heat Gain Reduction)	$\mathrm{AH}^{\downarrow \cdot \downarrow}$	23.94 - 32.00	16.62 - 36.07	14 – 17	00 – 22	3.01%
Heat Loss – (Terrapene carolina)						
Reduced Cooling (Cooling Rapid Response)	$\mathrm{AC}^{\uparrow \cdot \uparrow}$	-	-	-	-	0.0%

Reduced Cooling (Active Heat Loss Reduction)	$\mathrm{AC}^{\uparrow \cdot \downarrow}$	18.36-23.01	14.29 – 35.26	09 – 14	00 – 23	3.52%
Increased Cooling (Tachycardic Cooling)	$\mathrm{AC}^{\downarrow \cdot \uparrow}$	-	-	-	-	0.0%
Increased Cooling (Bradycardic Cooling)	$\mathrm{AC}^{\downarrow \cdot \downarrow}$	22.51 - 27.58	16.21 – 34.94	17 – 20	00 - 23	11.96%
Heat Gain – (C. picta)						
Increased Heating (Tachycardic Heating)	$\mathrm{AH}^{\uparrow\cdot\uparrow}$	16.86 - 19.30	16.11 – 20.31	09 – 12.5	09 – 15	0.03%
Increased Heating (Bradycardic Heating)	$\mathrm{AH}^{\uparrow\cdot\downarrow}$	25.77 - 30.80	17.62 – 37.06	13 – 16	00 – 23	1.93%
Reduced Heating (Heating Rapid Response)	$\mathrm{AH}^{\downarrow\uparrow}$	16.76 – 18.81	14.86 - 20.52	09 – 11	07 – 17	0.14%
Reduced Heating (Active Heat Gain Reduction)	$\mathrm{AH}^{\downarrow\cdot\downarrow}$	25.08 - 30.77	15.49 - 38.42	12 – 16	00 – 23	7.80%
Heat Loss – (Chrysemys picta)						
Reduced Cooling (Cooling Rapid Response)	$\mathrm{AC}^{\uparrow\cdot\uparrow}$	16.81 – 18.51	12.11 - 20.78	06 - 12	00 - 23	2.12%
Reduced Cooling (Active Heat Loss Reduction)	$AC^{\uparrow\cdot\downarrow}$	22.71 - 27.13	15.93 - 36.63	04 – 19	00 – 23	61.34%

Increased Cooling (Tachycardic Cooling)	$\mathrm{AC}^{\downarrow\uparrow\uparrow}$	16.92 - 18.81	14.75 - 20.68	09 – 11	07 – 14	0.04%
Increased Cooling (Bradycardic Cooling)	$AC^{\downarrow \cdot \downarrow}$	24.27 – 28.96	16.12 - 36.69	13 – 16	00 – 22	2.24%

*The abbreviation (i.e., AC or AH) represents the Active-Cooling or Active-Heating response, followed by higher (\uparrow) or lower (\downarrow) than predicted body temperature, then a corresponding higher (\uparrow) or lower (\downarrow) than predicted heart rate.

Figure Legends

Figure 1. Scaled body temperature plot for a) box turtle (*T. carolina*) in June 2014 and b) painted turtle (*C. picta*) during June in 2017, both at 5-minute sampling intervals for body temperature (black line) and ambient temperature (grey line). *T. carolina* experience gradual changes in body temperature ranging from a maximum increase of $3.2 \,^{\circ}$ C to a maximum decrease of $-2.1 \,^{\circ}$ C between consecutive sampling points while *C. picta* had similar maximum increases in body temperature of $3.4 \,^{\circ}$ C but higher maximum decreases in body temperature as high as $10.1 \,^{\circ}$ C within a 5-minute time interval.

Figure 2. Hourly mean (\pm SE) field metabolic rate (FMR) for a) box turtles (*T. carolina*) and b) painted turtles (*C. picta*) during the beginning (black) and middle (grey) of the active season. Daily metabolic rate for *T. carolina* was approximately 25% of the daily energy and remained consistent throughout a 24-hr representative day with a low range of variance. *C. picta* used a significantly higher amount of energy during an hour and lowest during the daytime but had more hourly variance. In both species, field metabolic rate was similar and not significantly different during the beginning and middle of their active season.









Chapter 5

General Conclusions

Ectotherms are constantly exchanging heat with their environment and in turn are reliant on the surrounding ambient conditions to regulate their temperature (Angilletta, 2009; Clarke, 2017). The results of my studies provide an ecological context for cardiac adjustments during thermoregulation, providing new perspectives on heat gain and heat loss in ectothermic vertebrates.

These studies suggest that for these species whole-organismal function is largely independent of ambient conditions and demonstrate the relevance of cardiovascular function during heat exchange and thermoregulation. Incorporation of physiological parameters with heat exchange provides context for when we may observe active and passive mechanisms of heat transfer in free-living turtles. When comparing the two thermoregulatory strategies, the thermoconformer relied heavily on passive heat exchange and bradycardic cooling to mitigate heat loss at night, thus requiring less daily energetic expenditure than the partial thermoregulator. These results contrast previous assumptions that thermoregulation primarily favors optimizing physiological function while minimizing energetic demand in order to maximize fitness (Huey & Slatkin, 1979).

Energetic demands of thermoregulation have been inferred from temperature differences between the organism and the environment, where thermoregulatory costs increase as the thermal quality of the habitat begins to deviate from the thermally preferred temperatures (Blouin-Demers & Nadeau, 2005). However, these measurements of activity and performance in free-living ectotherms demonstrate a decoupling of ecologically relevant temperatures from physiologically optimal temperatures for ectothermic vertebrates. Despite the temperaturedependence of certain functions under laboratory conditions for ectotherms (e.g. Adams, Claussen, & Skillings 1989), field conditions impose different constraints that may not be reflected under controlled laboratory conditions. As such, free-living animals may be constrained more by upper thermal limits and risk of overheating than attempting to achieve and maintain a set-point range of body temperatures.

Cardiovascular adjustments for altering heat exchange rates were primarily found to mitigate heat loss at night and reduce heat gain during the day in both species. Although there is the capacity for tachycardic heating, it may not be necessary because passive heat exchange is

sufficient to increase body temperature. It seems that organisms may not consistently try to maintain optimal temperatures because the costs, such as missed opportunities or mortality, outweigh the physiological benefits (Angilletta 2009). By incorporating heart rate into our analysis of heat transfer and energetics, we found that the consequences of reaching upper thermal limits and possibly overheating appear to outweigh the benefits of higher body temperatures in temperate regions. Thus, individuals may invoke active cardiovascular contributions for reducing heat gain during the day. Likewise, we found that at night both species exhibited a tendency for bradycardia. It is plausible that by lowering heart rate at night each species was able to minimize heat loss and prevent body temperatures from decreasing to the nightly minimum.

To conclude, these studies show how some organismal function is independent of ambient conditions in the field, the extent of cardiovascular adjustments in heat exchange, and the energetic demands of varying thermoregulatory strategies. My studies add to the growing body of literature that is bridging the gap between theoretical and empirical work and provide a new perspective on the cardiovascular role in heat exchange and thermoregulation for free-living ectotherms. Future studies can build upon this integrative framework to better understand regulation of physiological processes and the impact of environmental stressors on organismal performance, especially under the threat of the climate crisis.

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