

RESEARCH ARTICLE

Cardiovascular contributions and energetic costs of thermoregulation in ectothermic vertebrates

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ABSTRACT

Ectothermic vertebrates use a suite of physiological and behavioral mechanisms to thermoregulate, which result in various thermoregulatory strategies from thermoconformity to thermoregulation. Here, we present a novel synthesis of theoretical and empirical methods to determine cardiovascular contributions to heat transfer in free-living ectothermic vertebrates. We start by identifying the fundamental components of heat transfer and the cardiovascular mechanisms for physiological modulation of heat exchange, and then integrate these components into a single, integrative framework: the cardiovascular heat exchange framework (CHEF). We demonstrate that this framework can identify details of the thermoregulatory strategy in two turtle species, most notably the preponderance of instances where turtles use physiological mechanisms to avoid overheating, suggesting vulnerability to climate change. As modulated physiological contributions to heat flow incur a greater energy demand than relying on unmodulated passive heat transfer, we then asked whether we could characterize the energetic costs of thermoregulation. We measured field metabolic rate (FMR) in free-living turtles and used the CHEF to determine FMR while actively or passively thermoregulating. Comparing an individual's actual FMR to the rate calculated assuming absence of thermoregulation revealed that painted turtles, a partial thermoregulator, elevate their daily energy expenditure (DEE) by about 25%, while box turtles, a thermoconformer, have a DEE that is nearly unchanged as a result of thermoregulation. This integrative framework builds a new paradigm that provides a mechanism to explain correlations between energy demand and thermoregulatory strategy, quantifies the energetic costs of thermoregulation, and identifies the role of cardiovascular contributions to thermoregulation in free-living animals.

KEY WORDS: Thermoconformer, Thermoregulator, Biologging, Field metabolic rate, Heart rate, Modeling heat transfer, Daily energy expenditure, Big data

INTRODUCTION

Since the seminal work by Cowles and Bogert (1944) there has been a continuing interest in the thermal ecology of reptiles. Temperature influences the rates of biochemical reactions within an organism that in turn influence performance (Seebacher and Franklin, 2005; Angilletta, 2009), thus highlighting the importance of regulating internal temperature via heat exchange. External sources of heat

exchange between an animal and its habitat result from the temperature differences between the organism (i.e. the system) and its environment (i.e. its surroundings) (Clarke, 2017). This passive heat gain and loss, which can be achieved by behavioral adjustments in ectothermic vertebrates, leads to thermal flux proportional to temperature differences between organism and environment and has been well documented (Angilletta, 2009; Bels and Russell, 2019). Variation in the contributions of conductive, convective and radiative pathways of heat transfer can also alter the rates at which an individual heats and cools (Gates, 1980). However, internal mechanisms, via modulation of physiology, that influence heat transfer rates are an important aspect of thermoregulation that has been difficult to study under field conditions in free-living animals until the recent development of miniaturized biologging technology.

Thermoregulation encompasses strategies ranging from strict thermoconformity, solely relying on differences in ambient conditions to determine body temperature (T_b), to highly precise thermoregulation, in which T_b is maintained within a relatively narrow thermal preference (Angilletta, 2009); yet, the degree to which ectotherms thermoregulate varies and not all reptiles rely on a single strategy. Further, thermoregulation is dependent on the thermal landscape of the environment, which leads to variation in the difficulty in maintenance of preferred T_b (Sears and Angilletta, 2015; Sears et al., 2016). Maintaining high T_b has benefits for increased growth (Autumn and De Nardo, 1995) and reproductive output (Congdon and Tinkle, 1982; Krawchuk and Brooks, 1998). However, as the thermal quality of the habitat begins to deviate farther from the thermal preference to thermally limiting conditions, the energetic costs of thermoregulation begin to increase (Huey and Slatkin, 1976; Blouin-Demers and Nadeau, 2005; Brewster et al., 2013). Some species can modify their thermoregulatory behavior to switch between thermoconformity and thermoregulation (Randriamahazo and Mori, 2004) while others utilize facultative endothermy to increase activity at lower temperatures (Tattersall et al., 2016). Thus, changing environmental conditions can lead to varying degrees of thermoregulation (Angilletta, 2009).

Heating and cooling rates of ectotherms are also determined in part by the thermal mass of the individual and the composition of tissues (Grigg et al., 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 conductivity of tissues (Gates, 1980). Smaller organisms have a larger surface area to volume ratio, leading to generally faster rates of heat transfer (Stevenson, 1985a,b). Many ectotherms heat twice as fast as they cool (Smith, 1975a; Seebacher and Franklin, 2005), suggesting the importance of physiological contributions to rapidly increasing T_b to extend activity periods when conditions are thermally limiting (Grigg and Seebacher, 1999; Seebacher, 2000).

Similarly, in ectothermic vertebrates, heart rate (f_H) at a given temperature during a heating phase is usually higher than f_H during a

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cooling phase (Galli et al., 2004; Zaar et al., 2004; Seebacher and Franklin, 2005). For example, under both laboratory and field conditions, several species (e.g. *Terrapene ornata*, *Alligator mississippiensis*, *Crocodylus porosus*, *Pogona barbata* and *Varanus varius*) have higher f_H during heating than during cooling (Betha, 1972; Spray and May, 1972; Smith, 1976; Seebacher, 2000; Franklin and Seebacher, 2003). Further, ectotherms can also alter patterns of blood flow, and thus thermal conductivity (Smith, 1975b; Lillywhite et al., 1999). The combination of cardiac output and distribution of blood flow has a significant role in altering rates of heat exchange during heating and cooling phases (Lillywhite et al., 1999). Thus, in addition to unmodulated, passive heat transfer (e.g. basking; Gates, 1980), modulated, physiological heat transfer (e.g. altered cardiovascular function; Seebacher and Grigg, 2001) likely plays an important role in the maintenance of T_b within tolerable thermal limits. Observed cardiovascular responses span the spectrum of thermoregulatory strategies. For instance, 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 in cooler microclimates (Zaar et al., 2004). Alternatively, as a response to heat stress, moving to a cooler location and increasing f_H increases the rate of heat transfer from the body (reverse hysteresis; Grigg and Seebacher, 1999), thus increasing the rate at which an individual can lower T_b . Reptiles can also reduce their f_H to lower their heat transfer rates in a thermally hostile environment (Grigg and Seebacher, 1999). This reduction of f_H while in thermally hostile conditions can permit prolonged activity by reducing the rate at which T_b increases.

Any behavioral and physiological mechanism used for thermoregulation has energetic requirements, including costs of movement to select favorable microclimates (Sears et al., 2016) or cardiovascular adjustments (Dzialowski and O'Connor, 1999; Seebacher and Franklin, 2007). The 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 and Slatkin, 1976; Blouin-Demers and Nadeau, 2005). Presumably, thermoconformity is correlated with less energy consumption than physiological thermoregulation (Blouin-Demers and Nadeau, 2005). However, the energetic demands of thermoregulation and the cardiac contribution to heat exchange in free-living reptiles are usually studied independently. While logging f_H and T_b individually in free-living animals is not new (Mech, 1983), understanding the cardiovascular contributions to temperature regulation has been challenging. However, the continuous device miniaturization and reduction of battery size in biologging technologies has permitted the collection of high-resolution field measurements in undisturbed, free-living animals. Laboratory experiments attempting to explain the physiological responses contributing to heating and cooling rates (e.g. Smith, 1975a, 1976) have been limited by the inability to incorporate *in situ* field responses. Biologging devices provide the opportunity to monitor physiological mechanisms of free-living organisms (Wilmers et al., 2015) that may not be captured under laboratory conditions, with data collection at high resolution. Using T_b and f_H as proxies, these approaches have been successfully used to estimate field metabolic rate (FMR: often reported as daily energy expenditure, DEE; Green and Frappell, 2007; Green, 2011, Halsey et al., 2019). The robustness of estimating FMR from f_H and T_b has been shown in *Varanus rosenbergi* where activity and feeding state had little impact on the estimation (Clark et al., 2006). In addition to estimation of FMR, logged f_H , T_b and ambient temperature (T_a) measurements are essential to determine

modulated and unmodulated 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 suggest a physiological mechanism, measured as f_H , that alters the heating or cooling rate.

These data are critical for the development of new frameworks that join empirical and theoretical work to elucidate patterns and processes in thermoregulation in free-living animals, such as the work by Hertz et al. (1993) introducing the concept of thermal accuracy, thermal quality of the habitat and thermoregulatory effectiveness, or Rozen-Rechels et al. (2019) revisiting the thermo-hydroregulation framework that investigates functional adaptations to join environmental variation in water availability and temperature in an ecological and evolutionary context. The extensive theoretical and empirical work that has laid the foundation for analysis of organismal heat exchange describes the physical pathways (i.e. thermal conduction, convection, radiation, etc.) of the organism–environment interaction (Bakken and Gates, 1975; Gates, 1980; Blake et al., 2021). These quantitative models incorporate mathematical models or operative temperatures (T_{opt} or T_c), which are acquired using physical models of the study organism to measure temperature values (Hertz et al., 1993; Angilletta, 2009) that represent the temperature of an organism in the absence of metabolic heat production or evaporative cooling. These operative temperatures are then used to determine when T_b deviations from the operative temperature indicate physiological thermoregulation (Bakken, 1992; Bakken and Angilletta, 2014). Models of thermoregulation to date have excluded physiological responses because of technological limitations in obtaining these measurements. While these approaches have been informative (e.g. Fitzgerald and Nelson, 2011), the unification of physiological data with organism–environment temperature differences remains largely unexplored in free-living animals.

Here, we first present an integrative framework that allows for the differentiation of physiological mechanisms of heat exchange between the animal and the environment. While there is a wealth of information on heat transfer and thermoregulation in ectothermic vertebrates, we sought to integrate these approaches with novel biologging technology to clarify the role of physiological mechanisms for heat transfer in free-living individuals. To develop our framework, we started by identifying the fundamental components of heat transfer and the cardiovascular mechanisms for modulation of heat exchange, and integrated these components of heat transfer and cardiovascular response into a unified analytical approach. Our proposed cardiovascular heat exchange framework (CHEF) uses data from biologging devices that measure both temperature (organismal and ambient) and f_H to integrate empirical data with biophysical principles, gauging the role of cardiovascular modulation in thermoregulation of free-living ectotherms. We then assessed the utility of the CHEF using data from two species of turtles that occupy a similar climatic region, the Eastern box turtle (*Terrapene carolina carolina*, a terrestrial thermoconformer) and the painted turtle (*Chrysemys picta marginata*, a semi-aquatic partial thermoregulator). We asked whether the CHEF differentiates between the thermoregulatory strategies of these two species, predicting that painted turtles will display much more modulated heat exchange than box turtles, identified by the cardiovascular contribution to heat transfer rates. We then used the outcomes of the CHEF to ask several targeted questions, as examples of the utility of our approach as we further understanding of the physiological mechanisms underlying heating and cooling strategies (Fig. 1).

First, do we observe increased heating rates when T_b is below the lower thermal tolerance (LTT) during the day? Second, do we observe physiological mechanisms to limit heat gain when T_b is above the upper thermal tolerance (UTT)? Third, are physiological mechanisms engaged to reduce heat loss rates at night when thermoregulation is not feasible? Next, we use the logged f_H and T_b data to calculate FMR (Clark et al., 2006; Green and Frappell, 2007; Green, 2011) across the active season of each species, asking whether thermoregulatory strategy is associated with energy use. We further hypothesize that quantification of metabolic rate during identified periods of physiological thermoregulation will account

for the difference in FMR between these species. Finally, we elaborate on methods for applying the CHEF for future studies on other ectothermic vertebrates that are not testudines.

MATERIALS AND METHODS

Overview of the CHEF

The integrative framework we developed here is based on two system–surroundings relationships: organism–environment and cardiovascular–organism. We used measurements of T_b and T_a as well as f_H to differentiate between modulated and unmodulated heat transfer rates and cardiovascular function. Analysis of field-collected

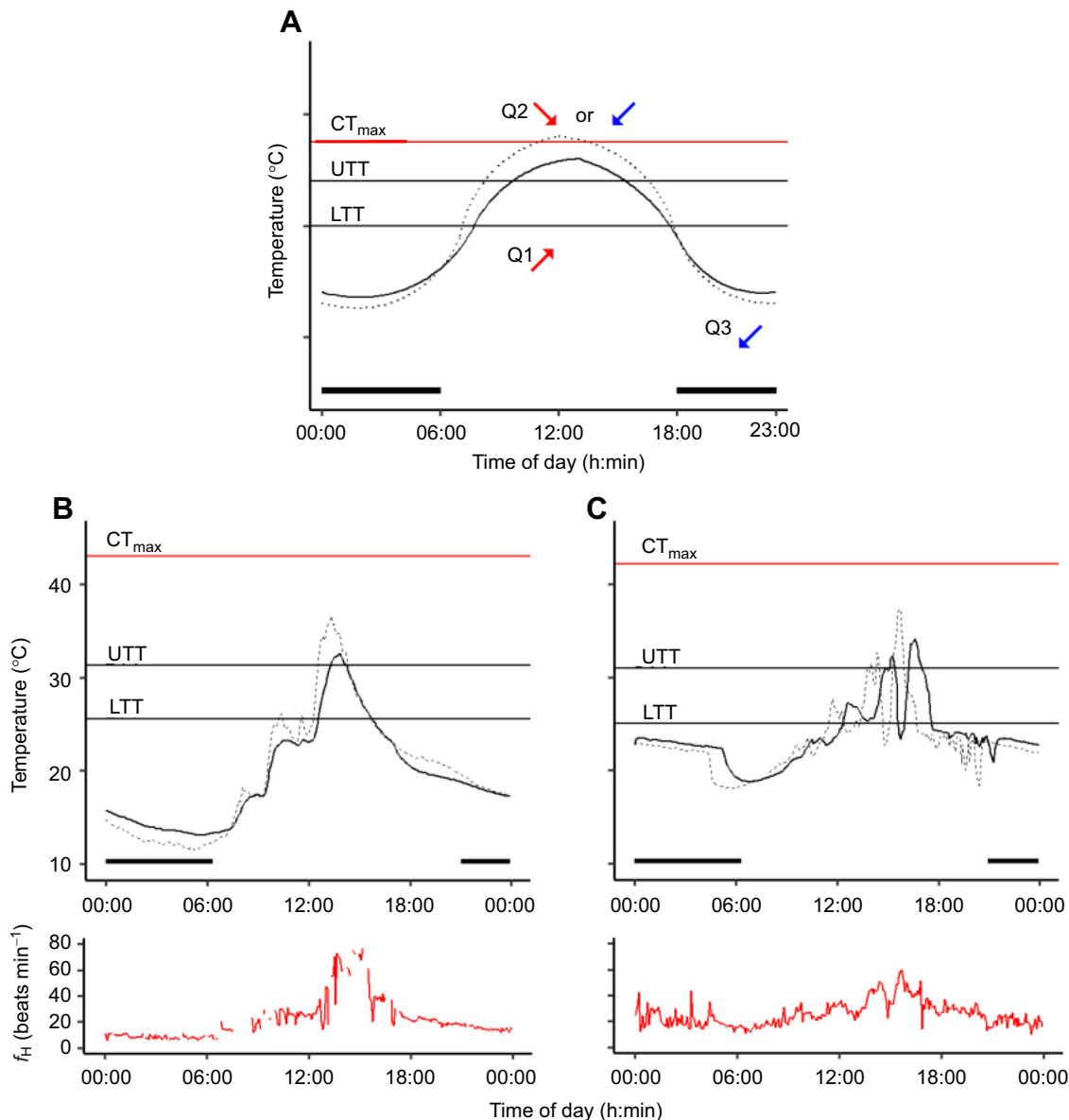


Fig. 1. A conceptual plot and field-collected data of a representative day comparing ambient (T_a) and body (T_b) temperatures. The presence of black bars represents night, the black solid line is T_b , the black dotted line is T_a , and horizontal lines are the upper (UTT) and lower (LTT) thermal tolerance (black) and critical thermal maximum (CT_{max} , red). (A) The hypothesis of the cardiovascular heat exchange framework (CHEF) is that physiological mechanisms are influencing heat transfer rates and thus temperature regulation. Q1–3 represent three questions that we used to assess the utility of the CHEF and which are based on differences between T_a , T_b and the predicted heat transfer rates (see Introduction, final paragraph). (B,C) Thermal profiles of box turtle (*Terrapene carolina carolina*) in June 2014 (B) and painted turtle (*Chrysemys picta marginata*) during June 2017 (C) at 5 min sampling intervals for T_b , T_a and heart rate (f_H , red). Gaps in f_H data indicate measurements that could not be calculated. Box turtles experienced gradual changes in T_b ranging from a maximum increase of 3.2°C to a maximum decrease of -2.1°C between consecutive sampling points whereas painted turtles had similar maximum increases in T_b of 3.4°C but higher maximum decreases in T_b as high as 10.1°C within a 5 min time interval. See Supplementary Materials and Methods for more details.

biologged data uses Eqn 1 (see ‘First component of the CHEF: organism–environment mechanism’, below) to estimate the T_b of an organism independent of metabolic heat production or evaporative cooling (i.e. equivalent to a deceased animal) for the organism–environment relationship, and Eqn 2 (see ‘Second component of the CHEF: cardiovascular–organism mechanism’, below) to estimate f_H independent of extrinsic or intrinsic factors that could increase or decrease contractility (i.e. ectothermic vertebrate hearts measured across a range of temperatures) for the cardiovascular–organism relationship. Below, we outline the two major components of this framework and describe how they are conceptually linked. Then, we implement the CHEF using a case study of free-living ectothermic vertebrate species in which f_H , T_b and T_a were recorded.

First component of the CHEF: organism–environment mechanism

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–surroundings relationship involves the organism (i.e. system, measured as T_b) and the environment (i.e. surrounding, measured as T_a). In the simplest case, the recorded temperatures identify either a scenario of heat gain, where there is an expectation of heat flow leading to an increase in T_b because the T_a is greater than the organism, or heat loss, where the inverse is true.

For this first system–surroundings relationship, the rate of heat gain or loss in a free-living animal is compared with heat exchange between a corpse and T_a . Rates of heat exchange in a living organism that differ from those of a deceased organism are attributed to active physiological mechanisms. T_b is predicted at each subsequent measurement interval ($t+1$) using the previously recorded body temperature ($T_{b(t)}$) as the starting T_b and the external ambient temperature ($T_{a(t+1)}$) as the new step response. We used Newton’s second law of cooling to predict the T_b at time ($t+1$) using the heating and cooling constant (τ , τ) of a deceased animal, which represents passive heat transfer between the organism and the environment, and used the modified equation based on Sears and Angilletta (2015):

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

where $T_{b(t+1)}$ is the predicted T_b at the next sampling interval ($t+1$); $T_{b(t)}$ is the recorded starting T_b ; $T_{a(t+1)}$ is the T_a at the subsequent sampling time; τ is the thermal time constant described by Smith (1976; see Supplementary Materials and Methods) based on a deceased animal; and t is the time in minutes between temperature step change (equal to the sampling interval of the devices). In scenarios of heat gain, the increasing T_b can fit one of three results: (1) within predicted (heating no differently from a corpse), (2) higher than predicted (increased heating rate, $T_b \uparrow$), or (3) lower than predicted (reduced heating rate, $T_b \downarrow$). Similarly, for heat loss in the living organism, the decreasing T_b can be one of three results: (1) within predicted (cooling no differently from a corpse), (2) higher than predicted (reduced cooling rate, $T_b \uparrow$), or (3) lower than predicted (increased cooling rate, $T_b \downarrow$).

Second component of the CHEF: cardiovascular–organism mechanism

The rates of biochemical reactions depend on the organism’s T_b . Thus, passive and active effects on f_H can be separated by understanding the relationship between the heart (i.e. system, measured as f_H) and the organism’s temperature (i.e. surrounding, measured as T_b). Independent of intrinsic factors, cardiac

contraction rates increase as temperature increases. In ectothermic vertebrates, tachycardia, a higher-than-normal f_H , and bradycardia, a lower-than-normal f_H , can occur at any recorded T_b , can influence patterns of circulation in response to physiological challenges, and can thus influence heat transfer rates.

As temperature increases, the f_H of ectothermic vertebrates increases in a curvilinear response (Fig. S1) until an upper frequency limit is reached (Lillywhite et al., 1999). The upper limit for f_H would likely align with the maximal rate near the critical thermal maximum, or lethal thermal maximum, where the cardiac muscle would fail to contract (e.g. 40°C in turtles; Barnes and Warren, 1937). To predict f_H from T_b , we used a modified equation from Reid (1996) that used laboratory-informed measurements:

$$f_H(p) = \frac{f_{H,\max} - f_{H,\min}}{1 + \left(\frac{T_b}{C}\right)^B} + f_{H,\min}, \quad (2)$$

where $f_{H(p)}$ represents the predicted f_H as a function of T_b , $f_{H,\max}$ is maximum possible heart rate for the organism, $f_{H,\min}$ is the minimum recorded f_H for the organism in a laboratory setting, T_b is body temperature, C is body temperature at the mid-point of the f_H range, and B is a slope coefficient. A 95% confidence interval (CI) for non-linear regression would indicate the range of beats per minute that an ectothermic vertebrate heart would experience at a given temperature. Therefore, values that are above the 95% CI indicate tachycardia while values below the 95% CI indicate bradycardia. As for T_b , f_H can be higher (tachycardia), lower (bradycardia), or within a predicted range based on an established relationship between f_H and temperature. Tachycardia ($f_H \uparrow$) and bradycardia ($f_H \downarrow$) can be observed during heating or cooling scenarios identified using the first system–surroundings relationship, and represent physiological mechanisms for adjusting the rates of heat transfer between the organism and the environment.

Identifying scenarios of heat gain and loss

When the organism is heating at a faster rate than a null model (increased heating scenario), we define three categories: tachycardic heating ($T_b \uparrow; f_H \uparrow$), bradycardic heating ($T_b \uparrow; f_H \downarrow$) and increased heat gain ($T_b \uparrow$) based on the cardiovascular response. In tachycardic heating, the presence of tachycardia and a higher heating rate results in a higher than predicted T_b . In this scenario, we predict a direct effect of f_H on increased circulation and thus heat transfer to the organism, with environmental conditions having a secondary role in the increased heating. In bradycardic heating, the presence of bradycardia and higher heating rate results in a higher than predicted T_b . In this scenario, we predict a direct effect of f_H on reduced circulation to limit the extent of increased heat gain, and environmental conditions can have a secondary role in the increased heating. In increased heat gain, when f_H is within predicted values and there is a higher than predicted T_b , there is no direct effect of f_H on 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 ($T_b \downarrow; f_H \uparrow$), cardiac heating reduction ($T_b \downarrow; f_H \downarrow$) and decreased heat gain ($T_b \downarrow$) based on the cardiovascular response. In heating rapid response, the presence of tachycardia and a lower heating rate results in a lower than predicted T_b during heat gain (i.e. although T_b increases, it does so less than predicted). In this scenario, we predict a physiological or environmental cause for the reduced heat transfer. Elevated f_H may act as a physiological mechanism to reduce heat gain by dumping excess heat in a cooler

microclimate. Alternatively, a negative heat load could be an environmental factor reducing heat gain and potentially causing a cardiac reflex as a response. In cardiac heating reduction, the presence of bradycardia and a lower heating rate results in a lower than predicted T_b . In this scenario, we predict a direct effect of f_H on reduced circulation and thus a reduced heat transfer rate to the organism. Lastly, in decreased heat gain, f_H is within the predicted range and there is a lower heating rate, resulting in a lower than predicted T_b . In this scenario, we predict no direct effect of f_H on heat transfer, and that the primary cause is due to environmental conditions such as presence in a cooler microclimate.

When an organism is cooling at a faster rate than a null model (increased cooling scenario), we define tachycardic cooling ($T_b \downarrow; f_H \uparrow$), bradycardic cooling ($T_b \downarrow; f_H \downarrow$) and increased heat loss ($T_b \downarrow$). In tachycardic cooling, the presence of tachycardia and a higher cooling rate results in a lower than predicted T_b . In this scenario, we predict a direct effect of f_H on the increased cooling rate because of increased circulation, accompanied by environmental conditions that facilitate the increased cooling. In bradycardic cooling, the presence of bradycardia and a higher cooling rate results in a lower than predicted T_b . In this scenario, we predict a direct effect of environmental conditions, e.g. evaporative cooling or conduction, on the increased cooling despite a lower than predicted f_H . In increased heat loss, f_H is within predicted values and T_b is lower than predicted. In this scenario, we predict no direct effect of f_H on the heat transfer rate, and instead a direct effect of environmental conditions via presence in cooler microclimates that increase the rate of heat loss.

When an organism is cooling at a slower rate than a null model (decreased cooling scenario), we define cooling rapid response ($T_b \uparrow; f_H \uparrow$), cardiac heat retention ($T_b \uparrow; f_H \downarrow$) and decreased heat loss ($T_b \uparrow$). In cooling rapid response, the presence of tachycardia and a lower cooling rate results in a higher than predicted T_b during heat loss (i.e. although T_b decreases, it does so less than predicted). In this scenario, we predict two likely causes for the reduced heat loss. First, there may be an indirect environmental effect from a positive heat load which causes a rapid increase in f_H during heat loss. Second, f_H may be increased as the animal is near or above their upper thermal tolerance in an attempt to increase heat loss through increased circulation, which is however insufficient to offset the thermally hostile conditions. In cardiac heat retention, the presence of bradycardia and a lower cooling rate results in a higher than predicted T_b . In this scenario, we predict a direct effect of f_H on reduced circulation and thus a reduced heat transfer rate, potentially combined with environmental conditions that also facilitate the decreased heat loss. In decreased heat loss, f_H is within predicted values but cooling rate is low, resulting in a higher than predicted T_b . In this scenario, we predict no effect of f_H on heat transfer rates, and that environmental conditions, specifically cooler microclimates, are driving the reduced heat loss. When the T_b is within the predicted range during heating or cooling, all forms of heat exchange are unmodulated, and we predict that adjustments of f_H are related to movement or inactivity.

Application of the CHEF: a case study with two turtle species

We monitored Eastern box turtles, *Terrapene carolina carolina* (Linnaeus 1758) ($n=9$; 382–536 g), and painted turtles, *Chrysemys picta marginata* Agassiz 1857 ($n=8$; 196–314 g), in Southwest Ohio near Miami University (39.5°N, 84.7°W). A second set of box turtles ($n=11$) were used to calibrate respiratory and biologged data as described below. Box turtles were captured from the wild and monitored from April until August in 2014 for 2 week periods

during the beginning (late May–early June) and middle (late July–early August) of their active season. We followed free-living box turtles at the Miami University Natural Areas (MUNA), the Miami University Ecology Research Center (ERC) and Hueston Woods State Nature Preserve (HWSNP). Habitat in Southwest Ohio is highly fragmented from agriculture and is composed of mid- to late-successional forests whereas the ERC also included agricultural fields.

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 in semi-natural mesocosm enclosures at the Miami University ERC. Mesocosms were made using aquaculture tanks (Frigid Units Inc., Toledo, OH, USA) that were 1 m in depth and 2 m in diameter, and that were placed next to each other at the inner edge of a deciduous forested area. The mesocosms were exposed to direct solar radiation for more than 6 h a day, similar to other ponds in the area. Each mesocosm held only two turtles during each study period. 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 5 cm of organic material and placed several rocks and leaf litter in each. Each tank was filled with pond water and local fauna 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 (A.F.P., personal observation) and turtles foraged for food (i.e. aquatic insect larvae) within the mesocosms. Both tanks were created and filled with water during late April and remained operational for the duration of the experiment.

For both species, we monitored T_b , T_a and f_H simultaneously with implanted biologging devices. We implanted a DS1922L iButton (Maxim Integrated, San Jose, CA, USA) into the left posterior region of the coelomic cavity, anterior to the hindleg in both species through a 2 cm incision to record T_b . We epoxied an ECG logger of similar thermal properties to the shell (Parlin and Schaeffer, 2019) with temperature-recording capability (e-Obs, Grünwald, Germany) to the top of the carapace to record T_a 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 to record f_H . We filled openings in the carapace with sterile bone wax to prevent secondary infection and covered the electrode leads with epoxy to prevent damage and to hold them in place. All biologging devices were programmed to record T_b , T_a and f_H simultaneously at 5 min intervals for the duration of monitoring and raw ECG traces were stored on devices (see Fig. S2 for example ECG traces). For a more detailed description of the surgery procedure with device settings, see Parlin et al. (2017). The surgical procedure and all animal use followed Miami University Institutional Animal Care and Use Committee protocols #906 and 952. Additional details about biologging equipment settings, sampling frequency and example ECG traces can be found in the Supplementary Materials and Methods. Research was conducted under permits 15-162 and 16-144 issued by the Ohio Department of Natural Resources, and permits 2014-23 and 2015-11 issued by the Ohio Department of Natural Resources State Nature Preserve for Hueston Woods.

First and second components of the CHEF for turtles

For the first system–surroundings relationship (organism–environment), we determined whether the recorded T_b of the turtles was above, below or within the predicted temperature, $T_{b(+1)}$, using Eqn 1. These recorded values were compared with the output

from Eqn 1 using the sampling resolution of the data logger (0.0625°C; Falcón et al., 2018) as the upper and lower intervals for defining above, below or within the predicted temperature. Each species had their own τ value calculated in air, and painted turtles had another τ calculated when in water (Supplementary Materials and Methods) where the deviation of recorded T_b from predicted T_b using τ of a deceased animal indicates the role of an internal effect (e.g. physiological contribution). For the second system–surroundings relationship (cardiovascular–organism), we determined whether the recorded f_H was above, below or within the predicted range by comparing recorded data with values calculated using Eqn 2 based on the recorded f_H at different T_b from field-monitored turtles in our study (Fig. S1). We generated a 95% CI around the mean f_H measured in previous laboratory studies (Brown, 1930; Barnes and Warren, 1937; Gatten, 1974) and fitted a non-linear regression with error propagation using Monte Carlo simulations. These studies were selected for their discrete testing of f_H over a range of thermal conditions. Recorded f_H values above the 95% CI indicate tachycardia while values below the CI indicate bradycardia at that given T_b .

Respirometry measurements

We used indirect calorimetry to measure the oxygen consumption rate (\dot{V}_{O_2}) of individuals of each turtle species and simultaneously measured f_H and T_b for correlation with \dot{V}_{O_2} (Green, 2011). Turtles used for measurements of \dot{V}_{O_2} were either field acclimated (2015 box turtles, $n=11$; Fig. S3) or held in mesocosms for 2 weeks (painted turtles, $n=8$; Fig. S4) prior to measurements. Metabolic rate was measured at four different temperatures to derive calibration curves relating metabolic rate to T_b and f_H in resting and fasted (48 h) individuals. We measured \dot{V}_{O_2} 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 T_b recorded in the field) and because of the lack of a discernible rate of O_2 consumption below the minimum tested temperature in both species. See Supplementary Materials and Methods for species-specific calculation of f_H , indirect calorimetry measurements set-up, and calibration of f_H and T_b with respirometry measurements.

Estimation of FMR

We calculated the field metabolic rate for each species from logged f_H and T_b using calibration curves generated from simultaneous respirometry measurements and logged f_H and T_b . As we did not calibrate f_H and T_b with respirometry measurements for box turtles in 2014, we used the box turtles from 2015 to obtain these measurements (see Supplementary Materials and Methods). Given the nearly identical results between individual and group equations (Figs S3 and S4), we applied the respective group calibration curves to the field-collected data for each species. Our laboratory measurements of metabolic rate used to calibrate FMR from f_H and T_b measurements are similar to previously reported values for box turtles (Gienger and Urdiales, 2017) and painted turtles (Stockard and Gatten, 1983).

While there is concern about the role activity state may have on estimation of metabolic rate from f_H and T_b , Clark et al. (2006) found that as long as the change in f_H was within 20% between measurements, there was no impact of the prediction of metabolic rate during activity or rest in *V. rosenbergi*. Further, using accelerometer data collected with f_H and T_b (Parlin et al., 2018), we found that f_H had no relationship with activity state or intensity of activity (see Supplementary Materials and Methods, Fig. S5).

Data analysis and questions tested

We tested the ability of the CHEF to identify scenarios of increased or decreased heat transfer for two turtle species that are known to have different thermoregulatory strategies. To test the usage of physiological and unmodulated heat exchange mechanisms, we compared the physiological heating–cooling rates (above and below thermal tolerance) with unmodulated rates, at night and during the day. We based the UTT on the 75th quantile and the LTT on the lower 25th quantile of thermal preference for each species. For the painted turtle, the LTT was 25.0°C and the UTT was 31.0°C (Rowe et al., 2014), and for the box turtle the LTT was 25.5°C and the UTT was 31.3°C (Parlin et al., 2017).

We then assessed the ability of the CHEF to identify increased, decreased and unmodulated heat transfer rates and used these data to address the three questions posed (Fig. 1A). First, do we observe increased heat transfer rates when T_b is below the LTT during the day (Fig. 1A, Q1 – increased heating); next, when T_b is above the UTT, are physiological mechanisms invoked to limit heat gain (Fig. 1A, Q2 – decreased heating or increased cooling); last, are physiological mechanisms reducing heat loss at night when thermoregulation is not feasible (Fig. 1A, Q3 – decreased cooling)? We applied data from the CHEF to identify the different scenarios of physiological and unmodulated heat exchange, reporting the quantile range of T_b and time of day when the scenarios were identified. We then grouped together the different scenarios into (1) increased heating, (2) decreased heating, (3) increased cooling, (4) decreased cooling and (5) passive to compare the occurrence of physiological and unmodulated heat exchange in a representative day based on predictions related to T_b crossing the UTT and LTT of each species (Fig. 1B,C) as an empirical test of the CHEF. For these analyses, we used the Poisson test of rates, which compares the rates of occurrence for count data. We adjusted P -values using a Bonferroni adjustment ($\alpha=0.0167$) for multiple comparisons.

Next, we analyzed FMR within and between each species using generalized linear mixed models (GLMM) with the R package ‘glmmTMB’ v.0.2.2.0 (Brooks et al., 2017). For FMR within each species, we included season, day–night (sun-up or sun-down based on time of year), and their interaction as fixed effects. For FMR comparison between species, we included season (beginning or middle of active season), species (box turtle or painted turtle), and their interaction as fixed effects. 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.

Last, we compared the energy expenditure when recorded f_H was higher, lower or within predicted ranges (Eqn 2; Fig. S1; Brown, 1930; Barnes and Warren, 1937; Gatten, 1974) to test whether cardiovascular modulations act as an important modulator of energetic costs of thermoregulation. Given that this analysis violated the assumptions of normality for regular and \log_{10} -transformed data, we used a Kruskal–Wallis test and Dunn’s pairwise comparisons with Bonferroni correction. We also compared the T_b and f_H between box turtles and painted turtles. For the T_b and f_H comparison between species, we included season, species and their interaction as fixed effects, and the aforementioned random effect and autoregressive term. We also report the range of T_b and T_a changes across each recording interval, the maximum temperature differences between T_b and T_a , and the maximum T_b change between 5 min sampling intervals for each species.

All data were analyzed with R v.3.6 (<http://www.R-project.org/>) and statistical comparisons were made at the $\alpha=0.05$ level. All data in subsequent sections are reported as means \pm s.e.m. All *post hoc* comparisons for GLMM were least-squares means, also known as

estimated marginal means, using the ‘emmeans’ R package (<https://CRAN.R-project.org/package=emmeans>), and all pairwise comparisons were Tukey adjusted ($\text{lsmeans}_{\text{Tukey-adj}}$).

RESULTS

Comparison of physiological and unmodulated heat exchange within turtle species

The CHEF was able to identify instances of physiological and unmodulated heat exchange and increased or decreased heat transfer between the animal and its surrounding environment. We had a total of 18,378 matching f_H , T_b and T_a recordings for box turtles ($n=9$; 2042 ± 232 measurements per turtle) and another 23,538 corresponding f_H , T_b and T_a recordings for painted turtles ($n=8$; 2943 ± 90 measurements per turtle). Applying the CHEF, we found a difference in the occurrence of modulated and unmodulated heat transfer mechanisms, wherein box turtles relied heavily on unmodulated thermoregulation (unmodulated: 88.68% of recordings), although we detected all forms of physiological heat transfer (Table 1). Painted turtles relied considerably more on modulated forms of heat transfer (modulated: 39.05% of recordings) than box turtles, with the majority of the observations being cardiac heat retention (Table 1) during a cooling phase, which is likely explained by the rapid shifts from terrestrial to aquatic habitat.

Given that both species relied more on unmodulated mechanisms of heat exchange compared with physiological, we tested whether examples of specific thermoregulatory mechanisms of modulated heat exchange occurred in response to various challenges found throughout the day. For these analyses, we grouped (1) tachycardic heating and bradycardic heating as increased heating, (2) heating rapid response and cardiac heat reduction as decreased heating, (3) tachycardic cooling and bradycardic cooling as increased cooling, (4) cooling rapid response and cardiac heat retention as decreased cooling, and (5) the remaining classifications as

unmodulated exchange and compared subsets of the day (‘specific’) with the general occurrence throughout the day (‘general’). The first question was whether physiological mechanisms were used to increase heat transfer to the animal when the T_b was below the LTT during the day (Fig. 1; Q1 – increased heating). Both painted turtles (specific: 1.04%, general: 3.93%; $P < 0.001$) and box turtles (specific: 1.30%, general: 6.66%; $P < 0.001$) did not use physiological mechanisms more often to increase heat transfer rates when below the LTT compared with their general occurrence throughout the day. Mechanisms for physiological modulation of heat gain were actually used significantly less during these periods for both species. The second question we asked was whether physiological mechanisms were used to limit heat gain to the animal or increase heat transfer away from the animal when T_b was above the UTT (Fig. 1; Q2 – decreased heating or increased cooling). Both painted turtles (specific: 16.47%, general: 4.30%; $P < 0.001$) and box turtles (specific: 23.31%, general: 5.34%; $P < 0.001$) had more occurrences of cardiac mechanisms limiting heat gain to the body or increasing heat transfer from the body when T_b was above the UTT compared with the general occurrence throughout the day. The third question we asked was whether physiological mechanisms are engaged to reduce heat loss rates at night (Fig. 1; Q3 – reduced cooling). Painted turtles (specific: 36.83%, general: 28.30%; $P < 0.001$) and box turtles (specific: 4.17%, general: 2.48%; $P < 0.001$) both had more occurrences of physiological mechanisms specifically reducing heat loss at night compared with their general occurrence throughout the day.

Daytime and night-time FMR

FMR in box turtles was approximately 24.5% of the FMR measured for painted turtles at the beginning of the active season (Fig. 2A), and 29.7% of the painted turtle FMR in the middle of the active season (Fig. 2B). We found that for box turtles there was no

Table 1. Cardiovascular adjustments and corresponding heat exchange for box turtles (*Terrapene carolina carolina*) and painted turtles (*Chrysemys picta marginata*) with the quantile range for body temperatures (T_b) and time of day at which the CHEF determined T_b and f_H to be higher, lower or within the predicted range

Exchange type (species)	Abbreviation	T_b (°C)		Time of day (h:min)		% Recordings
		Central 50%	Range	Central 50%	Range	
Heat gain (<i>T. carolina carolina</i>)						
Tachycardic heating	HG ($T_b \uparrow \cdot f_H \uparrow$)	22.38–31.44	15.24–35.26	12:00–15:00	10:00–16:00	0.35
Bradycardic heating	HG ($T_b \uparrow \cdot f_H \downarrow$)	22.00–28.08	16.98–35.14	12:00–14:00	09:00–18:00	1.71
Heating rapid response	HG ($T_b \downarrow \cdot f_H \uparrow$)	26.42–32.86	16.56–34.10	14:00–16:00	10:00–18:00	0.01
Cardiac heating reduction	HG ($T_b \downarrow \cdot f_H \downarrow$)	23.36–29.66	17.60–35.20	14:00–17:00	09:00–22:00	0.16
Heat loss (<i>T. carolina carolina</i>)						
Cooling rapid response	HL ($T_b \uparrow \cdot f_H \uparrow$)	18.26–19.62	13.80–32.75	09:00–11:00	04:00–23:00	2.34
Cardiac heat retention	HL ($T_b \uparrow \cdot f_H \downarrow$)	16.92–19.64	12.78–35.14	03:00–12:00	00:00–23:00	3.13
Tachycardic cooling	HL ($T_b \downarrow \cdot f_H \uparrow$)	27.72–31.87	22.55–33.50	15:00–16:30	14:00–18:00	0.13
Bradycardic cooling	HL ($T_b \downarrow \cdot f_H \downarrow$)	24.48–28.77	18.79–34.51	16:00–19:00	00:00–22:00	2.34
Heat gain (<i>C. picta marginata</i>)						
Tachycardic heating	HG ($T_b \uparrow \cdot f_H \uparrow$)	22.19–27.35	15.44–31.25	11:00–13:00	07:00–22:00	0.75
Bradycardic heating	HG ($T_b \uparrow \cdot f_H \downarrow$)	23.38–27.76	17.62–37.96	12:00–15:00	02:00–22:00	1.60
Heating rapid response	HG ($T_b \downarrow \cdot f_H \uparrow$)	19.82–26.15	14.87–30.63	10:00–16:00	00:00–23:00	1.02
Cardiac heating reduction	HG ($T_b \downarrow \cdot f_H \downarrow$)	25.65–30.44	16.81–38.18	13:00–17:00	00:00–23:00	1.87
Heat loss (<i>C. picta marginata</i>)						
Cooling rapid response	HL ($T_b \uparrow \cdot f_H \uparrow$)	19.80–24.71	12.11–31.91	05:00–13:00	00:00–23:00	12.01
Cardiac heat retention	HL ($T_b \uparrow \cdot f_H \downarrow$)	22.19–27.35	15.74–36.69	04:00–19:00	00:00–23:00	19.94
Tachycardic cooling	HL ($T_b \downarrow \cdot f_H \uparrow$)	21.03–25.64	15.62–31.13	10:00–18:00	01:00–23:00	0.54
Bradycardic cooling	HL ($T_b \downarrow \cdot f_H \downarrow$)	25.13–29.42	15.95–35.52	14:00–17:00	00:00–23:00	1.32

The abbreviations represent the heat gain (HG) or heat loss (HL), followed by higher (↑) or lower (↓) than predicted body temperature (T_b), then a corresponding higher (↑) or lower (↓) than predicted heart rate (f_H).

For box turtles, modulated thermoregulation applied to 11.32% of recordings, and for painted turtles, this was 39.05% of recordings. The remaining 88.68% of the data for box turtles and 60.95% for painted turtles were all forms of unmodulated heat exchange with the environment where either f_H or T_b was within the predicted range.

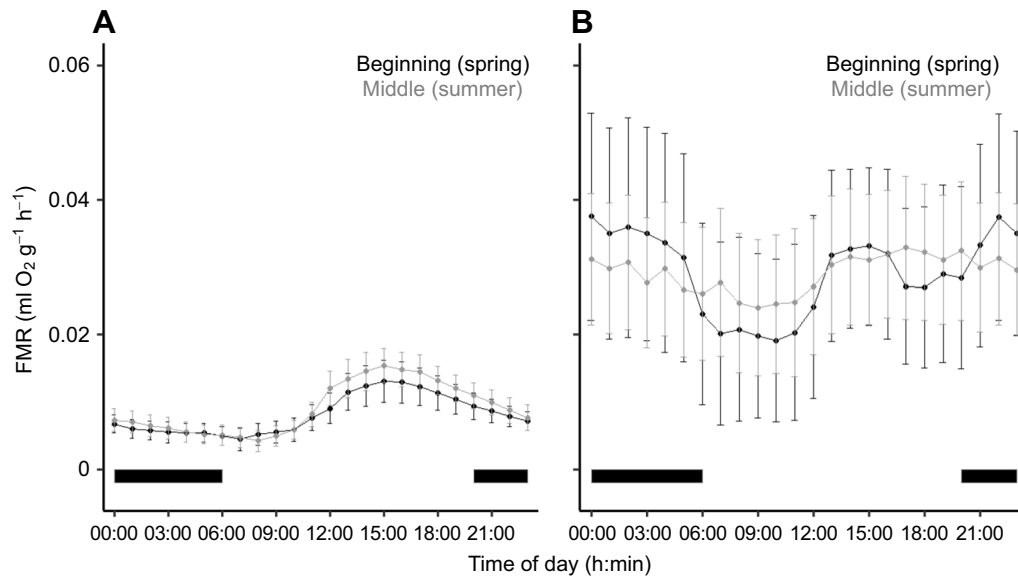


Fig. 2. Hourly mean (\pm s.e.m.) field metabolic rate (FMR) for turtles during the beginning and middle of the active season with an average photoperiod (black bars) for both seasons. (A) Box turtles (*T. carolina carolina*) and (B) painted turtles (*C. picta marginata*). In the beginning (late May–early June) of the active season, daily metabolic rate for box turtles (0.0086 ± 0.002 ml O_2 g^{-1} h^{-1}) was approximately 24.5% that of painted turtles (0.0351 ± 0.008 ml O_2 g^{-1} h^{-1}). During the middle (August) of the active season, daily metabolic rate for box turtles (0.0096 ± 0.003 ml O_2 g^{-1} h^{-1}) was 29.7% that of painted turtles (0.0323 ± 0.007 ml O_2 g^{-1} h^{-1}). Box turtles followed a similar daily rhythm with a low range of variance whereas painted turtles used a significantly higher amount of energy during any given hour with more variance but had lower metabolic rates during the daytime. In both species, FMR was not significantly different during the two measured seasons.

difference in energy expenditure between the seasons ($\chi^2_1=0.2142$, $P=0.64$), between day and night ($\chi^2_1=2.32$, $P=0.13$), and no significant interaction ($\chi^2_1=0.001$, $P=0.98$), which indicates a consistent and low energy expenditure throughout the active season for this thermoconformer. When we analyzed data for the painted turtle, a partial thermoregulator, we found that although there was no difference in energy expenditure between the seasons ($\chi^2_1=1.01$, $P=0.32$), there was a significant difference between day and night ($\chi^2_1=5.86$, $P<0.05$) and in their interaction ($\chi^2_1=13.25$, $P<0.001$). The difference in energy expenditure for the painted turtles was only observed at the beginning of the active season, where energy expenditure at night was higher than during the day (lsmeans_{Tukey-adj}: beginning-day versus beginning-night $P<0.05$; $P>0.05$ all other comparisons) while energy expenditure in the middle of the active season did not differ across the day. When we compared the energy expenditure of box turtles with that of painted turtles, we found that the only difference was between species ($\chi^2_1=70.58$, $P<0.001$) but not season ($\chi^2_1=0.68$, $P=0.40$) or their interaction ($\chi^2_1=1.38$, $P=0.24$). Hourly variance was also much lower in box turtles than in painted turtles during all hours of the day (Fig. 2).

Energetics of cardiovascular modulation

As f_H is tightly correlated with energy expenditure and it also influences heat exchange rates, we compared the energy expenditure in box turtles and painted turtles during physiological thermoregulation and while heat exchange is unmodulated. Using the CHEF, we identified the proportion of each day in which modulated or unmodulated heat exchange occurs and calculated the proportion of energy used as a result of physiological thermoregulation (Table 2). Box turtles, which rely almost exclusively on unmodulated heat exchange (Table 1), had higher energy expenditure associated with three of our four categories for physiological heat exchange (Table 2). However, the proportion of

time physiologically thermoregulating was quite low, and thus the increase in DEE as a result of these activities only accounted for a 1.39% increase in total DEE compared with that for a hypothetical turtle that relied on unmodulated exchange throughout the day. Box turtles also had higher energy expenditure associated with f_H when f_H was higher than (FMR: 0.0097 ± 0.0018 ml O_2 g^{-1} h^{-1}) or within (FMR: 0.0095 ± 0.0017 ml O_2 g^{-1} h^{-1}) predicted values compared with when it was lower (FMR: 0.0086 ± 0.0017 ml O_2 g^{-1} h^{-1}) than predicted (Kruskal–Wallis: $\chi^2_2=180.58$, $P<0.001$; Dunn’s test: above–predicted, $z=1.04$, $P=0.44$; above–below, $z=3.75$, $P<0.001$; predicted–below, $z=-13.26$, $P<0.001$). Painted turtles, which rely more on modulated heat exchange (Table 1), had elevated metabolic rates during every category of physiological heat exchange (Table 2). Painted turtles also had higher energy expenditure when f_H was higher (FMR: 0.035 ± 0.007 ml O_2 g^{-1} h^{-1}) and lower (FMR: 0.047 ± 0.009 ml O_2 g^{-1} h^{-1}) than predicted (FMR: 0.018 ± 0.004 ml O_2 g^{-1} h^{-1}) compared with when it was within predicted values (Kruskal–Wallis: $\chi^2_2=7895$, 33 , $P<0.001$; Dunn’s test: above–predicted, $z=41.42$, $P<0.001$; above–below, $z=-26.50$, $P<0.001$; predicted–below, $z=-88.09$, $P<0.001$). The proportion of time thermoregulating was higher than in box turtles, and thus the increase in DEE due to physiological thermoregulation accounted for nearly a 25% increase over a fully unmodulated turtle. The majority of this cost was due to modulating decreasing cooling rates, which accounted for nearly half of the DEE.

DISCUSSION

Cardiovascular contributions and energetic costs of thermoregulation for two turtle species

In applying the CHEF to our study system, we first asked whether we could identify distinct scenarios of heat gain and loss, and whether these would differ between two somewhat divergent turtle species. Indeed, the more active of the two species (painted turtles) consistently demonstrated greater engagement of the cardiovascular

Table 2. Energy expenditure for box turtles (*Terrapene carolina carolina*) and painted turtles (*Chrysemys picta marginata*) based on occurrence counts of modulated and unmodulated heat exchange during heating and cooling

Species	Category	Count	Daily proportion (%)	FMR (ml O ₂ g ⁻¹ h ⁻¹)	DEE – passive day (ml O ₂ g ⁻¹ day ⁻¹)	DEE (ml O ₂ g ⁻¹ day ⁻¹)	Cost of physiological thermoregulation (% increase)
<i>T. carolina carolina</i>	Decreased cooling	593	3.227	0.006±0.002	–	0.005	–
	Decreased heating	460	2.503	0.012±0.002	–	0.007	–
	Increased cooling	648	3.526	0.012±0.001	–	0.010	–
	Increased heating	380	2.068	0.011±0.002	–	0.006	–
	Unmodulated	16,297	88.677	0.009±0.002	0.216	0.192	–
	Total	18,378			0.216	0.219	1.39
<i>C. picta marginata</i>	Decreased cooling	7520	31.948	0.044±0.009	–	0.340	–
	Decreased heating	679	2.885	0.041±0.008	–	0.028	–
	Increased cooling	438	1.861	0.043±0.009	–	0.019	–
	Increased heating	552	2.345	0.042±0.009	–	0.023	–
	Unmodulated	14,349	60.961	0.024±0.007	0.585	0.357	–
	Total	23,538			0.585	0.767	23.75

Both species relied on unmodulated exchange, which had the lowest field metabolic rate (FMR) relative to modulated forms (i.e. increased and decreased heat transfer). In a representative day, comparing the additional cost of physiological thermoregulation (daily energy expenditure, DEE) over unmodulated only (DEE–passive day) showed painted turtles had a 23.75% increase in DEE while box turtles had a 1.36% increase.

system in thermoregulation. When then we asked three targeted questions (Fig. 1A) and showed that neither species increased f_H to increase heat gain when below their LTT (Fig. 1A, Q1), the turtles did use physiological mechanisms to reduce heat gain above their UTT (Fig. 1A, Q2), and both species reduced heat loss at night by lowering f_H (Fig. 1A, Q3). When their T_b was low, we were able to show that both species rarely used cardiovascular function to increase heat gain during the day. These turtles even reduced engagement of cardiovascular mechanisms that would aid heat gain, thus avoiding modulated heating when relatively cold. This is in contrast to the concept that ‘hotter is better’ (Angilletta, 2009) proposed for many active thermoregulating reptiles. In the more actively thermoregulating painted turtles, we did not find any instances of tachycardic heating whereby the presence of tachycardia facilitates an increase in T_b over that predicted, which we would have expected based on the life history of the painted turtle (Rowe et al., 2014). Both species showed a large increase in modulated cardiovascular engagement to reduce heat gain when T_b was above their preferred range. These data strongly suggest that the consequences of reaching upper thermal limits and possibly overheating outweigh the benefits of higher T_b . Thus, maintaining T_b within preferred ranges (i.e. within T_{pref}) appears to be secondary to ensuring safety with bradycardia during heat gain to reduce the risk of overheating (Grigg and Seebacher, 1999). This may partly reflect the thermal properties of the shell, which represents around 30% of the total body mass. The shell differs from other biological tissue by having a higher specific heat capacity (Parlin and Schaeffer, 2019), increasing thermal inertia. The potential problem this presents is seen in Aldabra tortoises (*Aldabrachelys gigantea*), which continue to increase T_b when locating a cool microclimate after foraging in open areas, even leading to overheating-related death in some instances (Swingland and Fraizer, 1980). However, many ectotherms heat twice as fast as they cool (Smith, 1975a; Seebacher and Franklin, 2005), suggesting this may be a common concern. The slow movement of turtles likely increases risk in locating favorable refugia, favoring caution in the regulation of heating rates. We note that painted turtles frequently tolerated T_b as high as 35°C for extended periods of time before diving back into the water. We speculate that the extremely high temperatures are for digestion and assimilation of food (Angilletta, 2009) as these temperatures are well above this species’ thermal

preference when fasted (25.0–31.0°C; Rowe et al., 2014). Unlike the painted turtles that will select relatively consistent temperatures in a laboratory gradient (i.e. T_{pref}), box turtles will select warmer temperatures at night and cooler temperatures during the day (Parlin et al., 2017), which is in keeping with the observed patterns found in the modulated and unmodulated heat transfer in the field. Application of the CHEF to other ectothermic vertebrates will provide further insight into the relevance of overheating risk for animals with other natural histories and thermoregulatory strategies. In contrast, at night, both species engaged the cardiovascular system to reduce heat loss, presumably either to retain heat in preparation for the subsequent day or to facilitate night-time digestion. The heat capacity of the shell likely plays a role in helping to maintain higher nighttime T_b (Parlin and Schaeffer, 2019), which surprisingly stayed above T_a throughout the night. The CHEF successfully identified all possible scenarios of modulated heat exchange, differentiated between species with divergent thermoregulatory strategies and was able to elucidate mechanisms for regulation of T_b that would be impossible without the wealth of biologging data and the model application.

Using logged f_H and T_b , we estimated the FMR using established approaches (Clark et al., 2006; Green, 2011) and found support for our hypothesis that painted turtles have a much higher FMR than box turtles. This agrees with our prediction that thermoregulatory strategy would impact energy demand. Higher metabolic rate has been hypothesized to result in an increased capacity to control the rate of T_b change (Bartholomew and 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. We previously found that the activity of free-living box turtles is largely independent of ambient conditions whereby individuals maintain full activity across a wide range of T_b , including as low as 14°C (Parlin et al., 2018). Using the periods identified by the CHEF as modulated or unmodulated thermoregulation, we were able to directly determine the energy costs of each, and thus of physiological thermoregulation. As expected, painted turtles elevated their metabolic rate as a result of thermoregulation whereas box turtles showed essentially no difference between a fully passive animal and the monitored individuals. Further, physiological

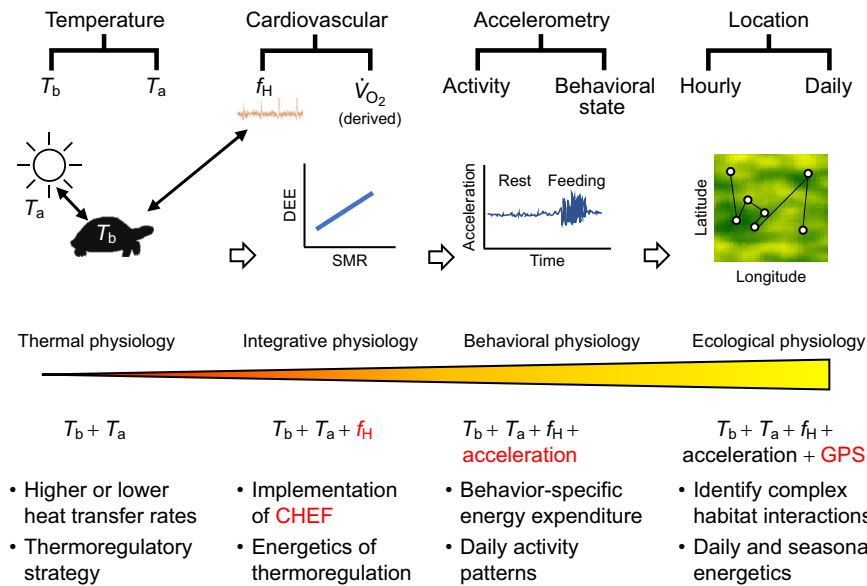


Fig. 3. Conceptual framework for linking together different measurements including temperature, physiology, accelerometry and location (top) that span physiological subdisciplines related to thermal, integrative, behavioral and ecological physiology (bottom). The CHEF can be implemented with T_b , T_a and f_H measurements. These measurements can also be calibrated under laboratory conditions prior to release to determine FMR to address questions related to energy management strategies (i.e. daily energy expenditure, DEE, as a function of standard metabolic rate, SMR; Halsey et al., 2019) and energetics of thermoregulation. The addition of accelerometry can link the aforementioned variables with activity (intensity) or behavioral state, and recording the location at hourly or daily intervals can permit comparison with animal–habitat interactions. Aspects of the CHEF, such as using only temperature measurements and accelerometry, can still provide valuable insight into the transfer of heat rates, thermoregulatory strategy and overall activity in the field. The continual miniaturization of biologging devices will allow for multiple, simultaneous measurements at high temporal resolution to provide unique perspectives specific to ectothermic vertebrates from thermal physiology to ecological physiology.

thermoregulation in painted turtles only elevated their metabolic rate by about 25% over that of a fully passive animal. To our knowledge, these are the only data that directly calculate the energy costs of physiological thermoregulation in free-living animals. Note, however, that our approach does not include the costs of moving to select a favorable site, nor the costs of being unable to perform other behaviors. These are still to be quantified. We remain interested in whether thermoconformers in general are freed of the energy costs of maintaining elevated T_b while still maintaining activity across such a wide range of T_b , as are the box turtles.

Guidelines and considerations for future studies

Merging theoretical concepts of biophysical modeling with empirical field measurements via the CHEF allows for differentiation of modulated and unmodulated mechanisms of heat transfer between the animal and the environment, and thus provides significant insight into the physiology of thermoregulation (Fig. 3). However, there are limitations that should be addressed. Although biologging devices have made great advances in recent years, among cardiovascular functions they generally remain limited to measurement of f_H . Other physiological mechanisms could contribute to the adjustment of rates of heat exchange, including peripheral resistance, blood flow distribution and blood viscosity (Dzialowski and O'Connor, 1999; Seebacher and Franklin, 2007). Although physiological responses such as vasodilation, blood flow, shunting and blood viscosity likely contribute to every proposed scenario, we argue that the value of the approach is demonstrated by our success in identifying relevant patterns of response. We hope that these additional physiological parameters that we are unable to account for in free-living individuals will add detail and refinement to our framework in the future. In addition to physiological variables, it is likely that infrequent behavioral adjustments, such as responses to predator presence,

also alter f_H , leading to variation that is not relevant to thermoregulation. The wealth of data that can be logged helps to ensure that unusual events will not overly skew the data and identification of active periods (e.g. day versus night or season) can help to ensure that the data are more representative. An additional issue is the challenge in attributing cause and effect for each of the proposed scenario combinations from the two system–surroundings relationships used by the CHEF. For example, in the case of bradycardic cooling, it is not immediately clear how a reduction in f_H is mechanically linked to a lower-than-expected T_b (increased cooling) compared with a corpse that represents the lack of physiological control. In other words, how does lower than expected f_H lead to faster cooling than no cardiovascular activity? In this case, it is likely that additional factors, such as increased evaporation that can hasten cooling, radiative heat loss at night and exploitation of microclimates may be important; however, these external variables remain an unidentified contribution. That bradycardic cooling was identified suggests that these unaccounted parameters are contributing to heat transfer and warrant further investigation; however, this scenario only accounted for about 1–2% of the identified occurrences. Finally, logging of f_H remains challenging for small ectotherms (e.g. <50 g). In these cases, another approach is to limit investigation to the first component of the framework, the organism–environment interaction, measuring only T_a and T_b . Unlike the calculation of the operative temperature at a single time point (Bakken, 1992; Bakken and Angilletta, 2014), the first system–surroundings component of the CHEF emphasizes temporal variation that determines whether the rate of temperature change is or is not as expected. This approach, independent of monitoring physiological mechanisms, provides important information for understanding the relevance of physiological thermoregulation of free-living ectotherms and also

to determine the ecological relevance under current and changing climatic conditions. For application of the CHEF to other study organisms, researchers should first consider device attachment, specifically internal or external to elucidate thermal, cardiovascular or other parameters (Fig. 3, top). A second consideration should be whether the study organism is primarily terrestrial, semi-aquatic or fully aquatic. A third consideration should be whether the organism has high or low activity, as measurements such as accelerometry (Fig. 3) may be necessary. A final consideration is whether the study organism is a resident, with a large or small home range, or a disperser, as this could impact energy expenditure and thermoregulation in heterogeneous landscapes (Fig. 3, Location). The combination of numerous parameters for a single individual allows researchers to go beyond basic thermal physiology questions and ask direct questions in the realm of integrative physiology, behavioral physiology and ecological physiology at the scale of the organism and interaction with the environment (Fig. 3, bottom).

Daily thermoregulation requires energy that is also necessary for survival, growth and reproduction. Therefore, differentiation between modulated and unmodulated heat transfer via f_H is one mechanism for evaluating the energy demand necessary to thermoregulate and the impact of thermoregulation on organismal energy balance. The CHEF that we present here shows the utility of integrating the extensive data that can be collected using biologging devices with models that use biophysical principles to assess the role of cardiovascular adjustments in heat exchange and thus thermoregulation in free-living ectotherms. The CHEF both identifies the thermoregulatory strategies chosen in our study species and reveals the ways in which cardiovascular physiology plays an important role in several important thermoregulatory responses. The CHEF also identifies a mechanism for detecting differences in energy demand in animals with two different thermoregulatory strategies. To our knowledge, these are the only direct measurements of the energy costs of thermoregulation currently available for undisturbed, free-living ectothermic vertebrates.

Conclusions

Rising T_a and extreme weather conditions due to the climate crisis (IPCC, 2018) could negatively impact daily activity by creating midday heat stress. Hotter days may either force midday inactivity or lead individuals to risk overheating, thus also incurring further energy costs for thermoregulation. Thermoregulation is expected to favor optimizing physiological function while minimizing energetic demand in order to maximize fitness (Huey and Slatkin, 1976; Brewster et al., 2013), but the physiologically optimal temperature may not always be ecologically relevant (Martin and Huey, 2008). The disconnect between ecologically relevant and physiologically optimal temperatures ultimately leads to trade-offs in thermoregulatory strategy and heating-cooling rates (Angilletta et al., 2010) that may preclude activity at optimal temperatures because of excessive risk. The life history of an organism plays a significant role in regulation of temperature and the subsequent impacts on heat exchange mechanisms; thus, studies that extend our findings to other reptiles are critically needed.

Application of the CHEF revealed not only a correlation between energy costs and thermoregulatory strategy but also how modulation of f_H is more important as a mechanism for keeping T_b below the upper critical limits than for increasing heating. The CHEF identifies a mechanism for both physiological increases and decreases in heat transfer rates as well as a method for identifying responses that are modulated (i.e. physiologically) or unmodulated (i.e. $T_b - T_a$ differences), providing a direct link to the energy costs of

thermoregulation. Our framework and implementation of a case study provides a basis for the integration of theoretical principles and empirical data to better understand the physiological regulation and energetics of temperature and heat exchange in the field. Advances in biologging technology will continue to permit observation of fine-scale physiological processes in free-living animals, offering new opportunities to ask novel questions of animal-habitat interactions (Fig. 3), thus improving the utility of the CHEF.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.F.P., P.J.S.; Formal analysis: A.F.P.; Data curation: A.F.P.; Writing - original draft: A.F.P., P.J.S.; Writing - review & editing: A.F.P., P.J.S.; Funding acquisition: A.F.P., P.J.S.

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Data availability

Data are available from the Zenodo Data Repository: <https://doi.org/10.5281/zenodo.5585986>

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