

Supplementary Materials and Methods

Field Monitoring in Terrestrial and Aquatic Environments

Species were monitored daily using very-high-frequency (VHF) telemetry for box turtles, or long-distance (15m) observations of mesocosms for painted turtles. Box turtles were located daily with BD-2 transmitters (Holohil; Ontario, Canada) for 12 days each (range: 11 – 14 days) and GPS coordinates recorded (62s Garmin GPS, \pm 3-10m resolution; Ontario, Canada). Based on our daily field observations, we assume that box turtles did not occupy bodies of water during this time and therefore only use the heating and cooling tau in air (see section *Mechanisms of the Cardiovascular Heat Exchange Framework* below). For painted turtles, all mesocosms (Frigid Units Inc, Toledo, OH, USA) had 1 m water depth, were 2 m diameter, consisted of nearby substrate (5-10cm, with rocks and organic material), had water from a nearby pond where turtles were present, a basking platform in the center with rocks, and food that consisted of local flora and fauna.

Field Body Temperatures and Corresponding Heart Rate

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, main text). Body temperatures of box turtles, which have been previously characterized as thermoconformers (Parlin et al., 2017), closely match ambient temperatures and thus have a cyclic pattern to their daily body temperature while painted turtles, which are partial thermoregulators (Rowe et al. 2014), have rapid changes in body temperature throughout the day. When we compared the body temperatures of box turtles and painted turtles between the beginning and middle of the active season we found significant differences between the species (Generalized linear mixed models:

$\chi^2_{(1)} = 5.66$, $P = 0.02$) and in the interaction between species and season ($\chi^2_{(1)} = 4.57$, $P = 0.03$).

Painted turtles in the middle of their active season ($T_b = 26.12 \pm 1.22$ °C) had significantly higher body temperatures than the beginning of the active season ($T_b = 23.12 \pm 1.99$ °C), and, than both the box turtle beginning ($T_b = 20.84 \pm 1.84$ °C) and middle ($T_b = 20.61 \pm 1.91$ °C) portions of their active season (lsmeansTukey-adj: painted turtle middle active season vs. all other comparisons, $P < 0.05$; all other pairwise comparisons $P > 0.05$).

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 and decreased rapidly (Fig. 2), concurrent with basking and water entry 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.

In tandem with body temperature, heart rate plays a significant role in the energy expenditure as higher heart rates tend to correlate with higher expenditure. When we compared the heart rates of box turtles and painted turtles, we found that there was a significant difference between species ($\chi^2_{(1)} = 6.92$, $P < 0.001$) and for the interaction of species and season ($\chi^2_{(1)} = 4.16$, $P =$

0.04). Box turtles in the beginning (HR = 18.29 ± 5.01 BPM) and middle (HR = 17.58 ± 4.98 BPM) portions of their active season had lower heart rates (lsmeans_{Tukey-adj}: box turtle beginning vs. box turtle middle, $P > 0.05$) than painted turtles. Painted turtle HR also did not differ due to season (beginning HR = 26.90 ± 7.18 BPM; middle HR = 31.38 ± 6.81 BPM) (lsmeans_{Tukey-adj}: painted turtle beginning vs. painted turtle middle, $P > 0.05$; all other pairwise comparisons $P < 0.05$). When we plotted heart rate as a function of body temperature for box turtles (Fig. S1A) and painted turtles (Fig. S1B) both species had a relatively similar curvilinear relationship. In both species heart rate increased with increasing body temperature, but when we include the 95% confidence intervals (CI; red lines) of the NLS curve (blue line) based on the heart rates of turtle hearts at fixed temperatures, HRs of box turtles were primarily within the CI, with relatively few above the upper limit, while painted turtle HRs were similarly distributed above, within, and below the CI.

Heart Rate Calculation

HR-loggers recorded ECG 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), and stored all of the raw data on the device, which could be wirelessly downloaded (e-Obs GmbH, Gruenwald, DE). We manually calculated heart rate for box turtles and used a mixture of quality-controlled algorithm and manual calculation for painted turtles. Manual calculations were done in Excel to visualize the ECG trace (Fig. S2) and we measured multiple R-R intervals in each recording and calculated an average. The quality-controlled algorithm was a non-commercial stand-alone variation of Star-Oddi's heart rate algorithm written in Python that processes the ECG recording and outputs heart rate and an associated quality index. This stand-

alone version has been used in Græsli et al. (2020) to re-calculate heart rate in Moose based on raw ECG signal recorded by loggers. The heart rate and the quality index [QI] is calculated from a set of rules (see Bjarnason et al., 2019 for details) 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). We included recordings graded QI = 0, 1 and 2 and excluded recordings graded QI = 3. We then tested the algorithm against manual calculations on several occasions and found similar values for heart rate based on the quality index score (see next section for details).

Data Selection of Heart Rate Measurements for Turtles

Biologging devices will log ECG recordings regardless of the data quality, and confounding variables include arrhythmia, low body temperatures, aliasing, and electromyography (EMG) interference due to activity.

We used the following thresholds for eliminating data for analyses in the study. First, we did not include data outside of our calibration curve for each species (box turtle: 15-30 °C; painted turtle: 20-35 °C). Second, if any of the remaining data produced a negative VO₂ measurement we removed those data as well. As a result, we reduced the dataset from 29376 down to 23538 measurements for painted turtles and from 30438 down to 18378 for box turtles. Based on the computer algorithm, we spot-checked 10-25 measurements per turtle per day to ensure that the heart rate was being accurately calculated. Many of the data were QI = 0 or 2, and for our randomly selected turtle day we found no difference (student t-test; $t_{561} = -0.98$, $p = 0.32$) between hand calculation (mean HR = 25.86 ± 9.61 BPM) or the computer algorithm (mean HR = 26.44 ± 10.26 BPM). Our final threshold was removing data that had a QI score of 3, which

was the algorithm definition of poor quality. Many of these measurements were over 100 BPM or under 5 BPM, and when we spot-checked these data points the recording had too much noise to reliably discern R-R intervals (See Figure S2 for raw ECG traces and corresponding QI score).

Indirect Calorimetry Measurements

Individuals were monitored in a temperature controlled environmental chamber (Percival model 141VL; Perry, IA, USA) and allowed 1 hour to equilibrate prior to the start of measurements at each temperature after allowing 20 minutes for the environmental chamber to reach the target test temperature. All testing temperatures were randomized and there was never greater than a 10 °C change in magnitude between trials. After we pumped dry air for 24-hours through the plexiglass chamber (7080 mL), we placed turtles into the chamber 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 re-entered the chamber. Flow rate was set to 250 mL·min⁻¹. 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):

$$M_s O_2 = \frac{FR(F''iO_2 - F''eO_2)}{1 - F''iO_2} \quad (1)$$

where $M_s O_2$ is the measured rate of O₂ consumption, FR is the STP-corrected mass flow rate, $F''iO_2$ is the initial fractional concentration of water-free, CO₂-free air that provides the baseline O₂ concentration for the system, and $F''eO_2$ 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).

Individual and Group Respirometry Equations

The relationship between heart rate, body temperature, and respirometry measurements has been well documented for ectotherms. Green (2011) outlined recommendations for estimating metabolic rate using heart rate and body temperature as proxies. There are issues of multicollinearity and singularity when using heart rate and body temperature that should be considered, and most studies report the r^2 value, slope, intercept, and sum of squared errors for prediction (SEE) for their linear regression equations (e.g., Piercy et al., 2015) to explain as much variation in the data as possible. Other important considerations when using heart rate and body temperature as proxies include the potential for a rapid cardiac response (Seebacher and Franklin, 2003) that could overestimate metabolic rate in the field. Clark et al. (2006) approached this issue by only using body temperature to estimate oxygen consumption when the heating was greater than 20% change between measurements. In our study, we compare the regression coefficients between linear and polynomial equations, and then present the comparison of FMR calculated using individual and group equations for each turtle.

We created group equations and individual equations for Eastern box turtles (*Terrapene carolina*) and painted turtles (*Chrysemys picta*) from laboratory measurements. Each turtle had heart rate (HR) and internal body temperature (T_b) recorded simultaneously while recording oxygen consumption rate across a range of HR and T_b . Here, we present data for two group equations using pooled data for each species. We present metabolic rate calculated from both individual calibration equations derived from HR and T_b to ensure accuracy of the group

equations, necessitated as we did not make these measurements on the box turtles in 2014. Therefore, we analyzed data collected in 2015 (not presented in the manuscript) to ensure that the group equation produced similar results as each individual equation. To further assess accuracy, we also perform this analysis with our 2017 painted turtle data presented in the manuscript. We tested the group and individual equations on eight painted turtles monitored in 2017 and eight box turtles monitored during 2015. Raw data for the equations were removed if they were found to be extreme outliers based on an analysis of normality in which we used Q-Q plots to identify points outside 1.5 times the interquartile range. This included high or low values that likely resulted due to activity in the chamber. We first compared linear and polynomial regressions and found that a polynomial regression equation was the best-fit using heart rate, body temperature, and body mass as predictors. We included body mass as a predictor because of the effect that the whole animal mass has on internal changes in body temperature due to thermal inertia. The polynomial regressions had higher correlation coefficient (box turtle polynomial equation: $r^2 = 0.62$, painted turtle polynomial equation: $r^2 = 0.64$) than similar competing linear regression equations (box turtle linear equation: $r^2 = 0.43$, painted turtle linear equation: $r^2 = 0.31$) and were therefore used throughout.

Output for the group and individual oxygen consumption estimates in box turtles (Fig. S2) and painted turtles (Fig. S3) were highly overlapping in most cases. We then compare two data sets of field metabolic rate, one derived from group equation estimates and the other from pooled individual estimates for the beginning and middle of the active season. Then, we use a repeated-measures ANOVA to compare the seasons for each data set. For the 2015 box turtle field data, we found that the group equation showed no significant difference in the metabolic rate between the beginning and middle of the active season ($F_{1,5} = 2.094$, $p = 0.208$) and the pooled individual

estimates had similar results ($F_{1,5} = 1.232$, $p = 0.317$). For the 2017 painted turtle field data, we found no significant difference in the seasonal metabolic rate between the beginning and middle of the active season for the group equation estimates ($F_{1,6} = 0.001$, $p = 0.975$) or the pooled individual estimates ($F_{1,6} = 1.968$, $p = 0.21$). Future studies should address the variability in using body temperature and heart rate as proxies for oxygen consumption by presenting both the coefficient of determination and predicted output of individual and group equations whenever possible.

Estimation of Field Metabolic Rate

We calculated the field metabolic rate for each species from logged heart rate and body temperature using calibration curves generated from simultaneous respirometry measurements and logged heart rate and body temperature. As we did not calibrate heart rate and body temperature with respirometry measurements for box turtles in 2014, we used the box turtles from 2015 ($n = 11$) to obtain these measurements. Given the nearly identical results between individual and group equations, we applied the respective group calibration curves to the field collected data for each species. Our laboratory measurements of metabolic rate used to calibrate field metabolic rate from HR and T_b measurements are similar to previously reported values for box turtles (Gienger and Urdiales, 2017) and painted turtles (Stockhard and Gatten, 1983).

Mechanisms of the Cardiovascular Heat Exchange Framework: Organism-Environment

An important component of the *organism-environment* mechanism is the determination of faster or slower heat transfer in a living animal relative to a deceased animal. Therefore, calculating the heat transfer of a deceased organism (deceased tau, τ) and comparing the recorded

body temperature of a living animal identifies whether there was the potential of physiological contribution to heat transfer, such as cardiovascular modulations. Previous published work for quantifying heat transfer in living and deceased testudines used $^{\circ}\text{C}\cdot\text{min}^{-1}$ (e.g., Weathers and White, 1971; Spray and May, 1972), which is dependent on the step temperature, that is, the difference between starting and end temperatures (e.g., 20 – 40 $^{\circ}\text{C}$; Spray and May, 1972). For our study, we only used tau values from similar deceased species (i.e., *T. carolina* and *C. picta*). Smith (1976) outlined a conversion for $^{\circ}\text{C}\cdot\text{min}^{-1}$ to tau (τ) in equation (2) whereby:

$$\text{rate} \left(\frac{^{\circ}\text{C}}{\text{min}} \right) = \frac{\frac{1}{2} \text{Step Temperature}}{\tau} \quad (2)$$

rearranging equation (2) allows for the conversion of $^{\circ}\text{C}\cdot\text{min}^{-1}$ to τ . Therefore, the heating and cooling τ from previous work can be compared with the values we obtained for our study. We obtained the τ constant by heating a recently deceased turtle (*T. carolina* or *C. picta*) in an oven to 35 $^{\circ}\text{C}$ until the temperature was constant and then cooled the deceased animal in a 4 $^{\circ}\text{C}$ chamber until the temperature was constant for the cooling τ . This procedure was repeated for heating τ by removing the deceased animal from the cooling chamber to the oven and recording the temperature until it was constant. Temperatures were recorded using an implanted iButton (DS1922L) in the body cavity at two locations (anterior and posterior). The time constants were calculated using the procedure outlined by Dzialowski and O'Connor (2001) where the natural log of the difference between ambient temperature and body temperature (i.e., $\ln(T_b - T_a)$) is plotted against time. The slope of the regression in the semi-natural log plot indicates the thermal time constant (τ) when taking the negative reciprocal ($-1/\text{slope}$).

For previously recorded deceased *Terrapene carolina* with an airflow of 20 $\text{cm}\cdot\text{s}^{-1}$ (1/2 step temperature = 10 $^{\circ}\text{C}$; Spray and May, 1972), the heating time constant (heating τ = 73.69 min, n = 2; Spray and May, 1972) and cooling time constant (cooling τ = 71.69 min, n = 1; Spray and May,

1972); however, for our study we obtained the thermal time constants without airflow and found a relatively similar heating time constant (heating $\tau = 89.08$ min, $n = 1$ – this study) and cooling time constant (cooling $\tau = 73.53$ min, $n = 1$ – this study). While convection can play an important role in heat transfer (Bakken and Gates, 1975), we assume that unless sustained winds occur in the field and in a forested habitat, the thermal time constant determined under laboratory conditions without airflow would suffice for our analyses. For previously recorded deceased *Chrysemys picta* with no airflow the heating time constant (heating $\tau = 53.67$ min, $n = 1$; Spray and May, 1972) and cooling time constant (cooling $\tau = 51.15$ min, $n = 1$; Spray and May, 1972) closely matched the values we recorded for heating time constant (heating $\tau = 53.45$; this study) and cooling time constant ($\tau = 52.26$, $n = 1$; this study). It is important to note that these thermal time constants are determined for air. Given that *C. picta* is a semi-aquatic species, it is important to also determine the thermal time constants when under water. Smith et al. (1981) outlined the relation between thermal time constants in air and water as:

$$\tau_{air} = \tau_{water} + \left(\frac{M \cdot c}{h \cdot A} \right) \quad (3)$$

where M (kg) is the body mass, c ($\text{cal} \cdot \text{kg}^{-1} \cdot ^\circ\text{C}^{-1}$) is the mean specific heat capacity, A is the surface area, and h is the convection heat transfer coefficient with a lumped contribution from radiation and the compensation for the changing relative humidity during air warming or cooling. For our *C. picta* ($M = 0.231$ kg; $A = 254$ cm^2 ; $h = 0.026$ $\text{cal} \cdot \text{cm}^{-2} \cdot \text{min}^{-1} \cdot ^\circ\text{C}^{-1}$; $c = 850$ $\text{cal} \cdot \text{kg}^{-1} \cdot ^\circ\text{C}^{-1}$ – Parlin and Schaeffer, 2019), the thermal time constants in water were calculated by rearranging equation (3) and solving for τ_{water} (heating $\tau_{water} = 25.77$ min; cooling $\tau_{water} = 23.57$ min). Given that *C. picta* in our study were in semi-natural enclosures that mimic ponds, we assume that there was negligible water velocity that could influence heating and cooling. The heating and cooling τ in our study were based on data from two deceased adult turtles, one for each species, used to

determine the passive exchange of heat between the organism and the environment. The deceased adult turtles were of similar size and mass to those monitored in the field.

For painted turtles, to determine whether the heating and cooling was occurring in water or air, the mesocosms had operative models painted to similar absorptivity as turtles placed at varying water depths (i.e., 0cm, 45cm, 90cm, and basking platform), which provided an estimate of thermal stratification in the mesocosm. We used the external ECG-logger that recorded ambient temperature to identify mesocosm occupancy based on operative model temperatures. To sink the models, we either tied them to a brick or placed an external 50g weight. Temperature loggers within the operative models were programmed to record at 10-minute intervals, but to estimate occupancy at 5-minute intervals we approximated the model temperature by taking the average between two consecutive time points for each model. We then used the external logger (T_a) to approximate occupancy within the mesocosm as either at the bottom half (i.e., temperatures between the bottom and middle operative model), top half (i.e., temperatures between the middle and top operative model), or basking platform (i.e., temperatures above top and either above or below the basking operative model).

Supplementary Materials and Methods References

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CHEF Tutorial

R Markdown

Script by Adam F. Parlin

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Works in R version 3.6.2 (2019-12-12) -- "Dark and Stormy Night"

Platform: x86_64-w64-mingw32/x64 (64-bit)

This code can be downloaded from the Zenodo data repository as an R script:

<https://doi.org/10.5281/zenodo.5585986>

Libraries

```
library(ggplot2) #version 3.3.5
```

```
library(propagate) #version 1.0-6
```

```
library(dplyr) #version 1.0.6
```

There should be three CSV files that you will work with from the data repository:

HR Lab Calibration.csv

L1 - Example.csv

R3L1 - Example.csv

Heart Rate Prediction Curve

Heart Rate Calibration: Import heart rate lab data from Brown 1930, Barnes and Warren 1937, and Gatten 1974. Data includes heart rates across temperatures for excised turtle hearts, resting heart rate, and active heart rate. This includes both turtle species to increase the number of data points for the nls curve.

```
heart.dat<-read.csv('HR Lab Calibration.csv', header=T, sep=',')
head(heart.dat) #Check column names
```

```
##      Source  Tb      HR
## 1 Brown 1930  3.5  1.333333
## 2 Brown 1930  5.3  1.500000
## 3 Brown 1930  9.1  6.000000
## 4 Brown 1930  9.9  4.000000
## 5 Brown 1930 15.6 13.157895
## 6 Brown 1930 15.7 13.636364
```

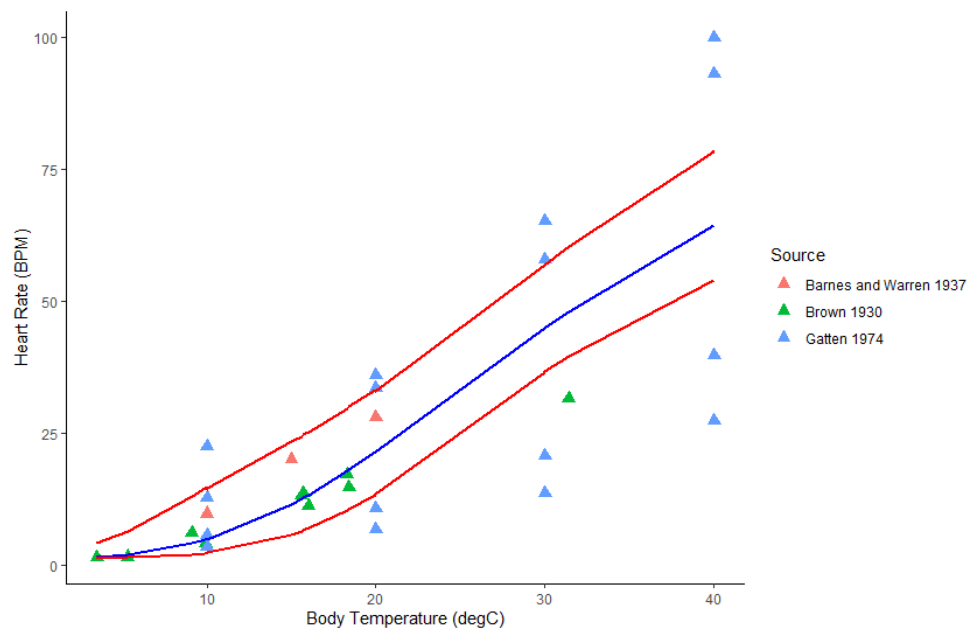
Non-linear regression: Based on the Reid (1996) equation, where the upper and lower limits are based on min and max values for heart rate from laboratory monitored turtles at ecologically relevant temperatures (i.e., non-frozen/non-overwintering).

$$HR[p] = ((HR[\text{max}] - HR[\text{min}]) / (1 + Tb/C)^b) + HR[\text{min}]$$

```
heart.nls<-nls(HR~(100-1.3)/(1+(Tb/a)^-b)+1.3,
start=list(a=20,b=3),data=heart.dat) #nls() from {stats}
```

```
fitted.HR<-predictNLS(heart.nls, alpha = 0.05, interval="confidence") #uses
the predictNLS() from propagate R package
heart.dat$HR.lower<-fitted.HR[,c("2.5%")]
heart.dat$HR.upper<-fitted.HR[,c("97.5%")]
heart.dat$HR.fit<-fitted.HR[, "fit"]

ggplot(heart.dat, aes(x=Tb, y=HR, color=Source))+
  geom_point(size = 3, pch=17)+
  theme_classic()+labs(x="Body Temperature (degC)", y= "Heart Rate (BPM)")+
  geom_line(data= heart.dat, aes(y=HR.fit, x=Tb), col="Blue", lwd=1)+
  geom_line(data= heart.dat, aes(y=HR.upper, x=Tb), col="red", lwd=1)+
  geom_line(data= heart.dat, aes(y=HR.lower, x=Tb), col="red", lwd=1)
```



Body Temperature Prediction:

These predictions are species specific. The tau value should be determined for a deceased animal, as deviations of actual body temperatures from this predicted would indicate the influence of a physiological mechanism. The script will start with a single box turtle (R3L1) day, then analyze a single painted turtle (L1) day. The box turtle day was completely terrestrial, while the painted turtle day has variation in water and air, thus requiring different thermal time constants to calculate predicted body temperature.

```
#~~~~~#
# Box Turtle - R3L1 #
#~~~~~#
BoxT<-read.csv("R3L1 - Example.csv",
  header=T, sep=',',
  colClasses=c(date = "POSIXct"),
  na.strings=c("#N/A", "#DIV/0!"))
head(BoxT)
```


	date	turtle	Tex	Tb	HR	season
## 1	2015-05-24 00:00:00	R3L1	14.719	15.722	10.8618	beginning
## 2	2015-05-24 00:05:00	R3L1	14.508	15.659	10.5596	beginning
## 3	2015-05-24 00:10:00	R3L1	14.375	15.597	5.7404	beginning
## 4	2015-05-24 00:15:00	R3L1	14.203	15.534	10.5108	beginning
## 5	2015-05-24 00:20:00	R3L1	14.164	15.471	11.0947	beginning
## 6	2015-05-24 00:25:00	R3L1	14.117	15.346	10.5989	beginning

Newton's 2nd law, modified based on Sears and Angilletta (2015). NA occurs at the beginning of the dataframe because there is no reference temperature. The last row in the dataframe should also be removed since there is no future reference temperature. The lag function allows for determination of heat gain or loss between the animal and subsequent time interval.

$$Tb[t+1]t+1 = Ta[t+1] + (Tb[t] - Ta[t+1])e^{-(t/\tau)}$$

τ is based on a deceased animal. Use the lag() function from 'dplyr' R package.

```
BoxT$Tb.pred<-as.numeric(ifelse(BoxT$Tb < lag(BoxT$Tex, n = 1),
                                c(NA, tail(BoxT$Tex, -1) + ( head(BoxT$Tb, -
1) - tail(BoxT$Tex, -1) )*exp(-5/89.08)),
                                ifelse(BoxT$Tb > lag(BoxT$Tex, n = 1), c(NA,
tail(BoxT$Tex, -1) + ( head(BoxT$Tb, -1) - tail(BoxT$Tex, -1) )*exp(-
5/73.53)), "NA")))
```

Upper and lower bounds are based on the sampling accuracy of the data logger. Alternative options could include obtaining a mean +/- SE of τ across several deceased animals.

```
BoxT$Tb.lower<-(BoxT$Tb.pred - 0.0625)
BoxT$Tb.upper<-(BoxT$Tb.pred + 0.0625)

#Determines whether the recorded Tb is higher, lower, or within predicted
BoxT$Tb.predict<-ifelse(BoxT$Tb > BoxT$Tb.upper, "Above",
                        ifelse(BoxT$Tb < BoxT$Tb.lower, "Below", "Predicted"))
#Determines the predicted heart rate at each body temperature.
HR.fit_BoxT<-predictNLS(heart.nls,data.frame(Tb = BoxT$Tb))

#Upper and Lower bounds for predicted heart rate at the recorded body
temperature
BoxT$HR.upper<-HR.fit_BoxT[,c("97.5%")]
BoxT$HR.lower<-HR.fit_BoxT[,c("2.5%")]

#Determines whether recorded heart rate is above, below, or within predicted
BoxT$HR.predict<-ifelse(BoxT$HR > BoxT$HR.upper, "Above",
                        ifelse(BoxT$HR < BoxT$HR.lower, "Below",
                                ifelse(BoxT$HR < BoxT$HR.upper &
                                        BoxT$HR > BoxT$HR.lower, "Predicted",
"NA"))))
#NA is for missing values, such as low-quality measurement or inability to
calculate heart rate
```

```

#Determines whether turtle is gaining or losing heat relative to the environment
BoxT$Transfer<-ifelse(BoxT$Tb > lag(BoxT$Tex, k = 1), "Loss", "Gain")

#Classification of CHEF for Box Turtle R3L1:
BoxT$Exchange<-ifelse(BoxT$Transfer == "Gain" #for heat gain scenarios
    & BoxT$Tb.predict == "Above"
    & BoxT$HR.predict == "Above", "Tachycardic_Heating",
#option 1
    ifelse(BoxT$Transfer == "Gain"
        & BoxT$Tb.predict == "Above"
        & BoxT$HR.predict == "Predicted",
        "Unmodulated_Increased_Heat_Gain", #option 2
        ifelse(BoxT$Transfer == "Gain"
            & BoxT$Tb.predict == "Above"
            & BoxT$HR.predict ==
        "Below", "Bradycardic_Heating", #option 3
            ifelse(BoxT$Transfer == "Gain"
                & BoxT$Tb.predict == "Below"
                & BoxT$HR.predict == "Above",
        "Heating_Rapid_Response", #option 4
                ifelse(BoxT$Transfer == "Gain"
                    & BoxT$Tb.predict ==
        "Below"
                    & BoxT$HR.predict ==
        "Predicted", "Unmodulated_Reduced_Heat_Gain", #option 5
                    ifelse(BoxT$Transfer ==
        "Gain"
                        & BoxT$Tb.predict ==
        "Below"
                        & BoxT$HR.predict ==
        "Below", "Cardiac_Heating_Reduction", #option 6
                        ifelse(BoxT$Transfer
        == "Gain"
                            &
        BoxT$Tb.predict == "Predicted"
                            &
        BoxT$HR.predict == "Above", "Increased_Heart_Rate_Heating", #option 7
                            ifelse(BoxT$Transfer == "Gain"
                                &
        BoxT$Tb.predict == "Predicted"
                                &
        BoxT$HR.predict == "Below", "Decreased_Heart_Rate_Heating", #option 8
                            ifelse(BoxT$Transfer == "Gain"

```

```
& BoxT$Tb.predict == "Predicted"

& BoxT$HR.predict == "Predicted", "Unmodulated_Heating", #option 9

ifelse(BoxT$Transfer == "Loss" #For heat loss scenarios

& BoxT$Tb.predict == "Above"

& BoxT$HR.predict == "Above", "Cooling_Rapid_Response", #Option 1

ifelse(BoxT$Transfer == "Loss"

& BoxT$Tb.predict == "Above"

& BoxT$HR.predict == "Predicted", "Unmodulated_Reduced_Heat_loss", #option 2

ifelse(BoxT$Transfer == "Loss"

& BoxT$Tb.predict == "Above"

& BoxT$HR.predict == "Below", "Cardiac_Heat_Retention", #Option 3

ifelse(BoxT$Transfer == "Loss"

& BoxT$Tb.predict == "Below"

& BoxT$HR.predict == "Above", "Tachycardic_Cooling", #Option 4

ifelse(BoxT$Transfer == "Loss"

& BoxT$Tb.predict == "Below"

& BoxT$HR.predict == "Predicted", "Unmodulated_Increased_Heat_Loss", #Option
5

ifelse(BoxT$Transfer == "Loss"

& BoxT$Tb.predict == "Below"

& BoxT$HR.predict == "Below", "Bradycardic_Cooling", #Option 6

ifelse(BoxT$Transfer == "Loss"

& BoxT$Tb.predict == "Predicted"

& BoxT$HR.predict == "Above", "Increased_Heart_Rate_Cooling", #Option 7

ifelse(BoxT$Transfer == "Loss"
```

```

& BoxT$Tb.predict == "Predicted"

& BoxT$HR.predict == "Predicted", "Unmodulated_Cooling", #Option 8

ifelse(BoxT$Transfer == "Loss"

& BoxT$Tb.predict == "Predicted"

& BoxT$HR.predict == "Below", "Decreased_Heart_rate_Cooling",
"NA"))))))))))) #Option 9

```

Table showing number of occurrences for each of the CHEF classifications for box turtle R3L1

```

table(BoxT[-c(1,288),]$Exchange) #removes first and last rows

##
##          Bradycardic_Heating          Cardiac_Heating_Reduction
##                      4                      6
##      Cooling_Rapid_Response      Decreased_Heart_rate_Cooling
##                      1                      1
##      Decreased_Heart_Rate_Heating      Heating_Rapid_Response
##                      1                      11
##      Increased_Heart_Rate_Heating      Tachycardic_Cooling
##                      4                      3
##          Unmodulated_Cooling          Unmodulated_Heating
##                      47                      25
## Unmodulated_Increased_Heat_Gain Unmodulated_Increased_Heat_Loss
##                      17                      3
##      Unmodulated_Reduced_Heat_Gain      Unmodulated_Reduced_Heat_loss
##                      91                      38

#~~~~~#
# Painted Turtle - L1 #
#~~~~~#

#Painted turtles had their position in the mesocosm determined for presence
#in air or water. This
#is an important step as the thermal time constant varies between air and
#water.
PaintT<-read.csv('L1 - Example.csv',
                 header=T, sep=',',
                 colClasses=c(date = "POSIXct"))

head(PaintT)

##          date turtle      Tex      Tb      HR      season      position
## 1 2017-05-21 00:00:00      L1 22.914 22.757 24.63896 beginning Bottom_Half
## 2 2017-05-21 00:05:00      L1 22.906 23.320 25.87091 beginning Bottom_Half
## 3 2017-05-21 00:10:00      L1 22.898 23.445 31.62000 beginning Bottom_Half
## 4 2017-05-21 00:15:00      L1 22.891 23.507 42.47463 beginning Bottom_Half

```

```
## 5 2017-05-21 00:20:00      L1 22.906 23.445 12.61996 beginning Bottom_Half
## 6 2017-05-21 00:25:00      L1 22.836 23.445 17.95457 beginning Bottom_Half
```

Newton's 2nd law, modified to calculate the rate of temperature change whether the painted turtle was in air or water. NA occurs at the beginning of the dataframe because there is no reference temperature. The last row in the dataframe should also be removed since there is no future reference temperature.

$$Tb[t+1]t+1 = Ta[t+1] + (Tb[t] - Ta[t+1])e^{-(t/\tau)}$$

tau is based on a deceased turtle.

```
PaintT$Tb.pred<-as.numeric(ifelse(PaintT$Tb <= lag(PaintT$Tex, n = 1) &
PaintT$position == "Basking",
                                c(NA, tail(PaintT$Tex, -1) + (
head(PaintT$Tb, -1) - tail(PaintT$Tex, -1) )*exp(-5/53.45)), #tau[air] based
on Laboratory data

                                ifelse(PaintT$Tb >= lag(PaintT$Tex,n = 1) &
PaintT$position == "Basking",
                                c(NA, tail(PaintT$Tex, -1) + (
head(PaintT$Tb, -1) - tail(PaintT$Tex,-1) )*exp(-5/52.26)),

                                ifelse(PaintT$Tb <= lag(PaintT$Tex,
n = 1) & PaintT$position == "Bottom_Half" | PaintT$position == "Top_Half",
                                c(NA, tail(PaintT$Tex, -1) +
( head(PaintT$Tb, -1) - tail(PaintT$Tex, -1) )*exp(-5/25.77)), #tau[water]
converted from tau[air] based on Smith et al. (1981)

                                ifelse(PaintT$Tb >=
lag(PaintT$Tex, n = 1) & PaintT$position == "Bottom_Half" | PaintT$position
== "Top_Half",
                                c(NA, tail(PaintT$Tex,
-1) + ( head(PaintT$Tb, -1) - tail(PaintT$Tex,-1) )*exp(-5/23.57)), "NA")))))
```

Upper and lower bounds are based on the sampling accuracy of the data logger. Alternative options could include obtaining a mean +/- SE of tau across several deceased animals.

```
PaintT$Tb.lower<-(PaintT$Tb.pred - 0.0625)
PaintT$Tb.upper<-(PaintT$Tb.pred + 0.0625)

#Determines whether the recorded Tb is higher, lower, or within predicted
PaintT$Tb.predict<-ifelse(PaintT$Tb > PaintT$Tb.upper, "Above",
                           ifelse(PaintT$Tb < PaintT$Tb.lower,
"Below","Predicted"))

#Determines the predicted heart rate at each body temperature.
HR.fit_PaintT<-predictNLS(heart.nls,data.frame(Tb = PaintT$Tb))

#Upper and Lower bounds for predicted heart rate at the recorded body
```

temperature

```
PaintT$HR.upper<-HR.fit_PaintT[,c("97.5%")]
PaintT$HR.lower<-HR.fit_PaintT[,c("2.5%")]
```

#Determines whether recorded heart rate is above, below, or within predicted

```
PaintT$HR.predict<-ifelse(PaintT$HR > PaintT$HR.upper, "Above",
                           ifelse(PaintT$HR < PaintT$HR.lower, "Below",
                                   ifelse(PaintT$HR < PaintT$HR.upper &
                                           PaintT$HR > PaintT$HR.lower,
                                           "Predicted", "NA")))
```

#Determines whether turtle is gaining or losing heat relative to the environment

```
PaintT$Transfer<-ifelse(PaintT$Tb > lag(PaintT$Tex, k = 1), "Loss", "Gain")
```

#Classification of CHEF for Painted Turtle L1:

```
PaintT$Exchange<-ifelse(PaintT$Transfer == "Gain" #for heat gain scenarios
                        & PaintT$Tb.predict == "Above"
                        & PaintT$HR.predict == "Above",
                        "Tachycardic_Heating", #option 1
                        ifelse(PaintT$Transfer == "Gain"
                                & PaintT$Tb.predict == "Above"
                                & PaintT$HR.predict == "Predicted",
                                "Unmodulated_Increased_Heat_Gain", #option 2
                                ifelse(PaintT$Transfer == "Gain"
                                        & PaintT$Tb.predict == "Above"
                                        & PaintT$HR.predict ==
                                        "Below", "Bradycardic_Heating", #option 3
                                        ifelse(PaintT$Transfer == "Gain"
                                                & PaintT$Tb.predict == "Below"
                                                & PaintT$HR.predict == "Above",
                                                "Heating_Rapid_Response", #option 4
                                                ifelse(PaintT$Transfer == "Gain"
                                                        & PaintT$Tb.predict ==
                                                        "Below"
                                                        & PaintT$HR.predict ==
                                                        "Predicted", "Unmodulated_Reduced_Heat_Gain", #option 5
                                                        ifelse(PaintT$Transfer ==
                                                                "Gain"
                                                                &
                                                                PaintT$Tb.predict == "Below"
                                                                &
                                                                PaintT$HR.predict == "Below", "Cardiac_Heating_Reduction", #option 6
                                                                ifelse(PaintT$Transfer == "Gain"
                                                                        &
                                                                        PaintT$Tb.predict == "Predicted"
                                                                        &
                                                                        PaintT$HR.predict == "Above", "Increased_Heart_Rate_Heating", #option 7
```

```

ifelse(PaintT$Transfer == "Gain"
      & PaintT$Tb.predict == "Predicted"
      & PaintT$HR.predict == "Below", "Decreased_Heart_Rate_Heating", #option 8
ifelse(PaintT$Transfer == "Gain"
      & PaintT$Tb.predict == "Predicted"
      & PaintT$HR.predict == "Predicted", "Unmodulated_Heating", #option 9

ifelse(PaintT$Transfer == "Loss" #For heat Loss scenarios
      & PaintT$Tb.predict == "Above"
      & PaintT$HR.predict == "Above", "Cooling_Rapid_Response", #Option 1
ifelse(PaintT$Transfer == "Loss"
      & PaintT$Tb.predict == "Above"
      & PaintT$HR.predict == "Predicted", "Unmodulated_Reduced_Heat_loss", #option
2
ifelse(PaintT$Transfer == "Loss"
      & PaintT$Tb.predict == "Above"
      & PaintT$HR.predict == "Below", "Cardiac_Heat_Retention", #Option 3
ifelse(PaintT$Transfer == "Loss"
      & PaintT$Tb.predict == "Below"
      & PaintT$HR.predict == "Above", "Tachycardic_Cooling", #Option 4
ifelse(PaintT$Transfer == "Loss"
      & PaintT$Tb.predict == "Below"
      & PaintT$HR.predict == "Predicted", "Unmodulated_Increased_Heat_Loss",
#Option 5
ifelse(PaintT$Transfer == "Loss"
      & PaintT$Tb.predict == "Below"
      & PaintT$HR.predict == "Below", "Bradycardic_Cooling", #Option 6

```

```

ifelse(PaintT$Transfer == "Loss"
& PaintT$Tb.predict == "Predicted"
& PaintT$HR.predict == "Above", "Increased_Heart_Rate_Cooling", #Option 7
ifelse(PaintT$Transfer == "Loss"
& PaintT$Tb.predict == "Predicted"
& PaintT$HR.predict == "Predicted", "Unmodulated_Cooling", #Option 8
ifelse(PaintT$Transfer == "Loss"
& PaintT$Tb.predict == "Predicted"
& PaintT$HR.predict == "Below", "Decreased_Heart_rate_Cooling",
"NA"))))))))))) #Option 9

```

Table showing number of occurrences for each of the CHEF classifications for painted turtle L1

```

table(PaintT[-c(1,288),]$Exchange) #removes first and last rows
##
##          Bradycardic_Cooling          Cardiac_Heat_Retention
##                      1                      62
##          Cardiac_Heating_Reduction          Cooling_Rapid_Response
##                      2                      2
##          Decreased_Heart_rate_Cooling          Heating_Rapid_Response
##                      10                      9
##          Tachycardic_Heating          Unmodulated_Cooling
##                      1                      25
##          Unmodulated_Heating          Unmodulated_Increased_Heat_Gain
##                      19                      16
##          Unmodulated_Increased_Heat_Loss          Unmodulated_Reduced_Heat_Gain
##                      12                      30
##          Unmodulated_Reduced_Heat_loss
##                      97

```

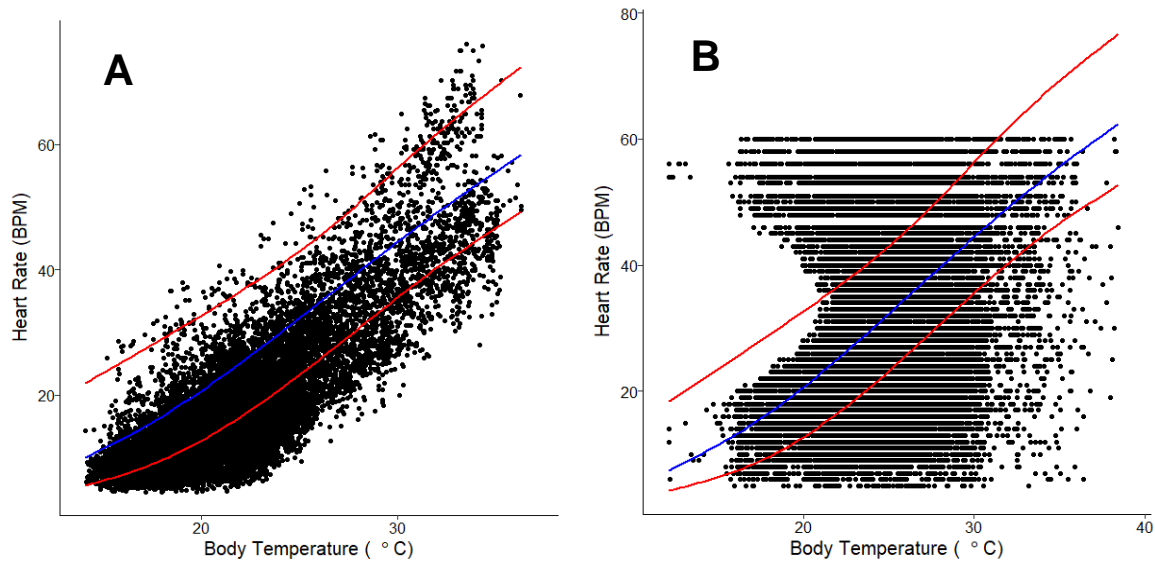



Fig. S1. Heart rate as a function of body temperature for (A) box turtles and (B) painted turtles with the fitted NLS regression (blue line) and confidence interval (95% CI) for turtle heart rates over a range of temperatures from 0 to 40°C. The regression line is generated from previously collected laboratory data, while the raw data were collected from turtles in our study. Data in (A) were all hand calculated and data in (B) were calculated using a quality control algorithm that was spot-checked with hand-calculations.

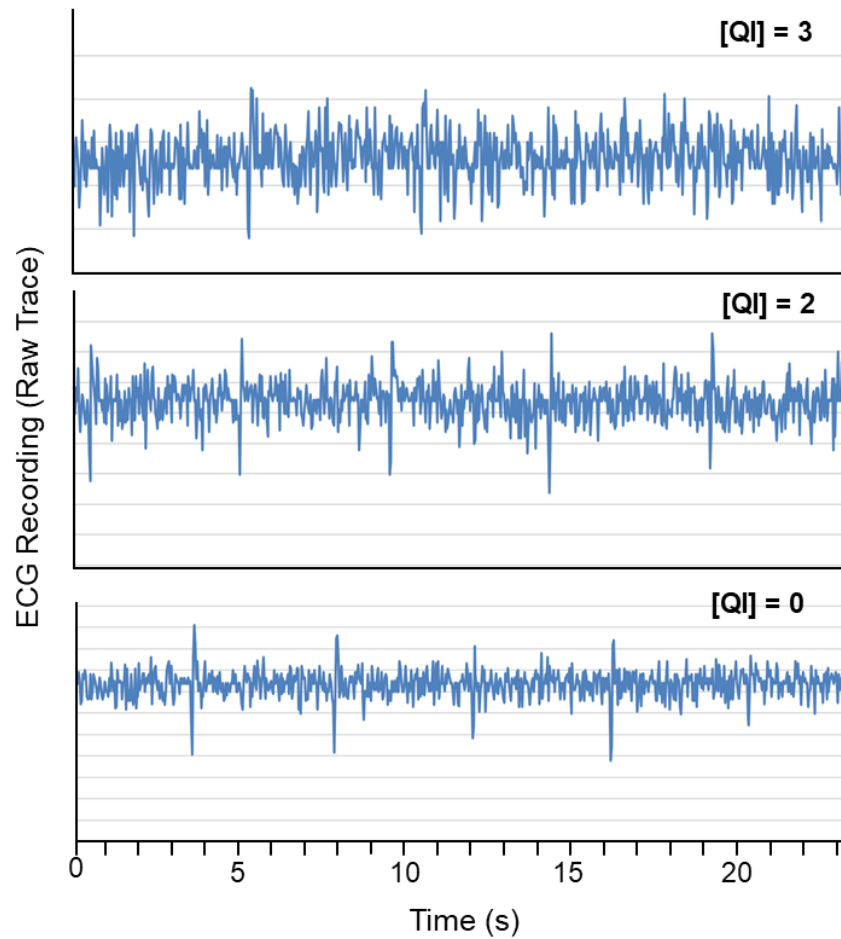


Fig. S2. Date and time of ECG recording from biologging device with corresponding QI score of 3 (top), 2 (middle), and 0 (bottom). The computer algorithm rarely categorized QI = 1 because of the arrhythmia that would occur between R-R intervals during the 30 second recordings. Data presented are from painted turtle ID L1 within a 30-minute time frame.

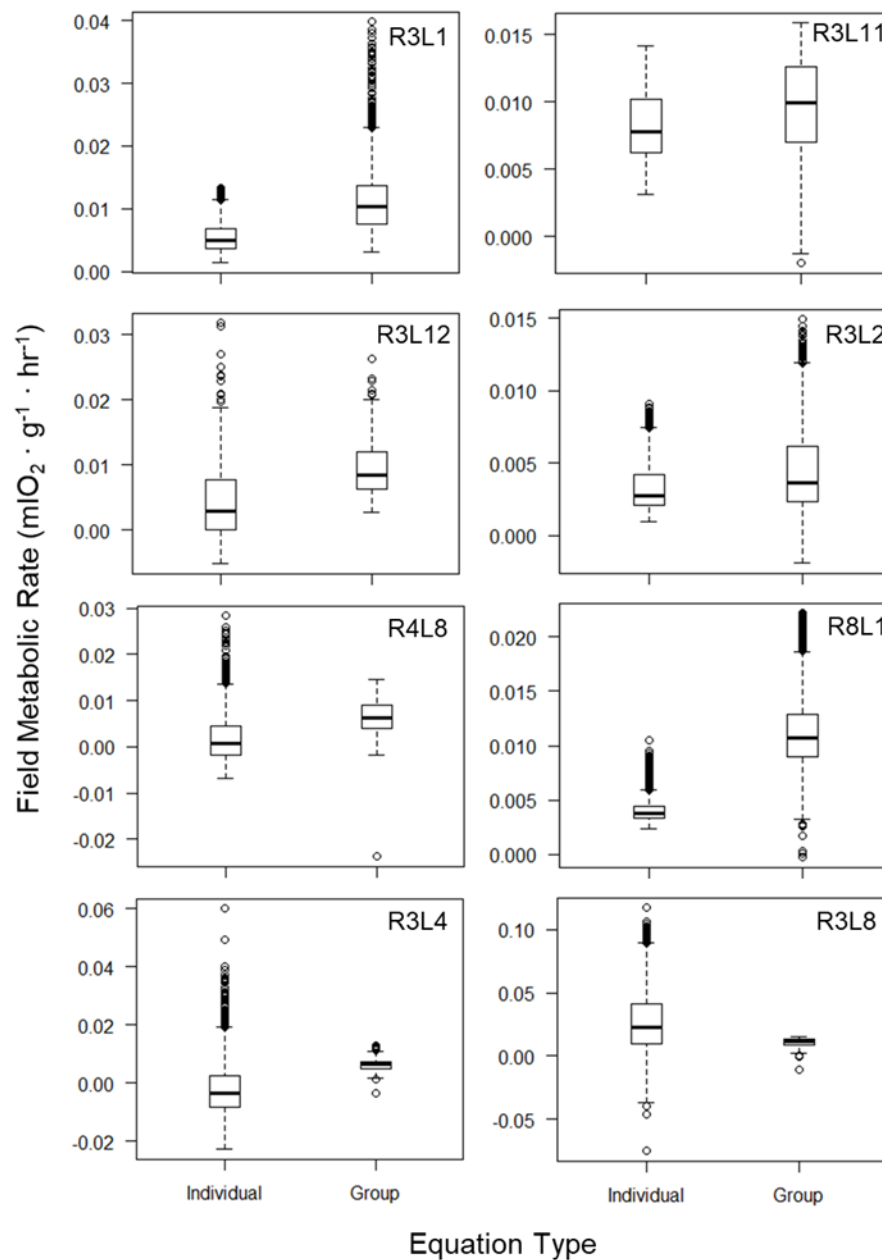


Fig. S3. Metabolic rate of box turtles monitored in 2015 with comparison of individual and group equations. The best fit were second degree polynomial regression equations and there was a high degree of overlap between equations. Box plots represent raw metabolic rate data using temperatures and heart rates monitored in the field. Turtles R3L8 and R3L4 had measurements that were removed from the group equation but were included in the individual equations. Turtle ID is in the upper-right corner of each individual plot.

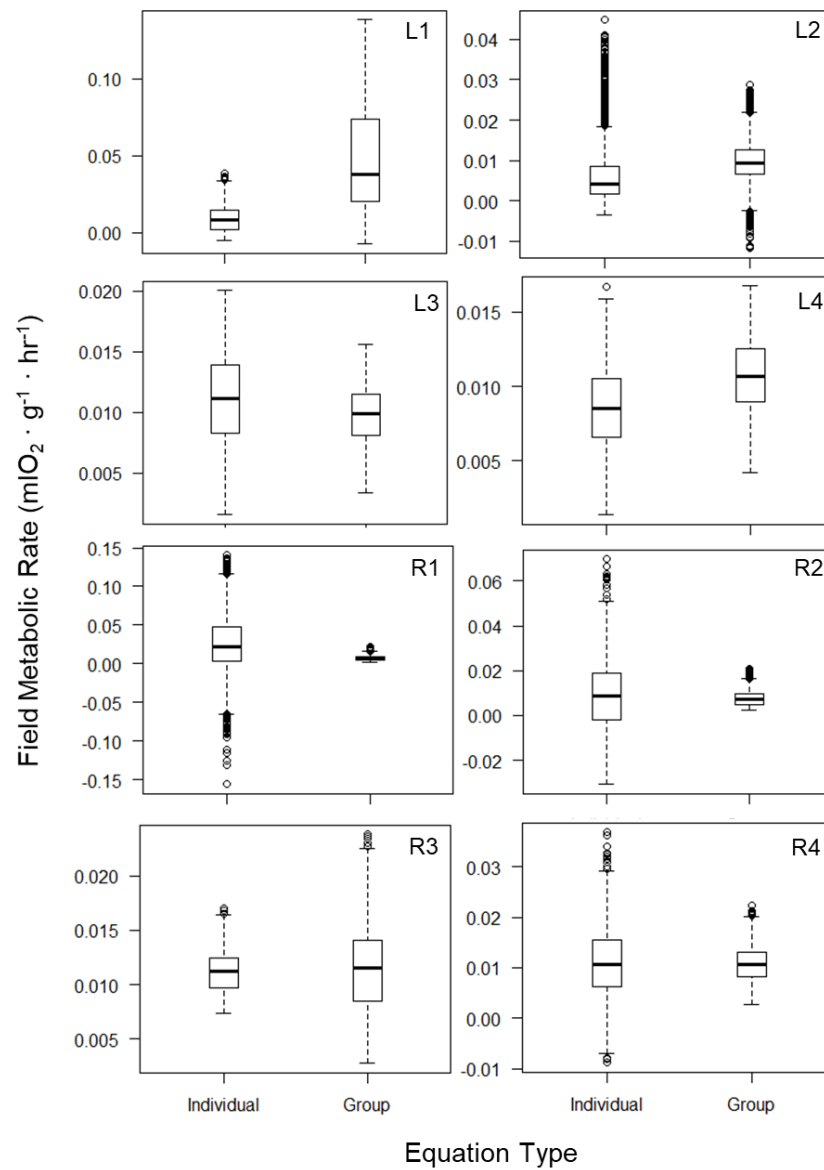


Fig. S4. Metabolic rate of painted turtles monitored in 2017 with comparison of individual and group equations. The best fit were second degree polynomial regression equations and there was a high degree of overlap between equations. Box plots represent raw metabolic rate data using temperatures and heart rates monitored in the field. Turtles R1 and R2 had measurements that were removed from the group equation but were included in the individual equations. Turtle ID is in the upper-right corner of each individual plot.

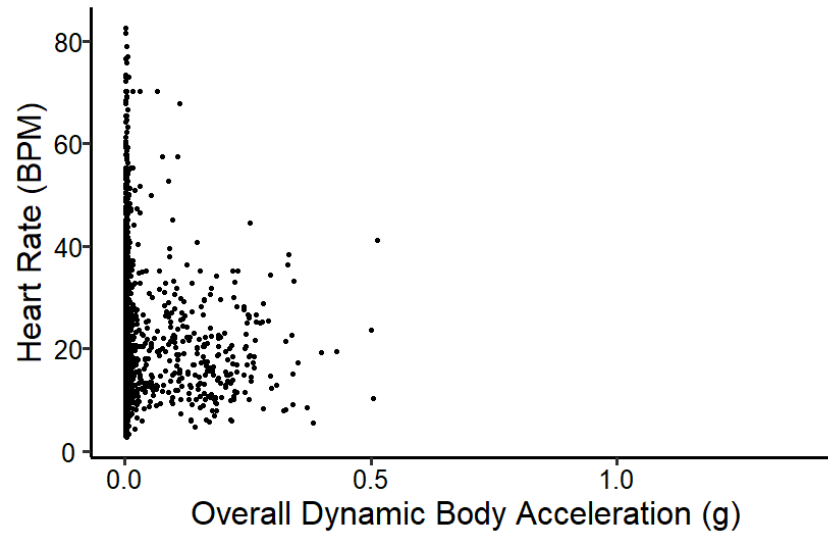


Fig. S5. Relationship between heart rate (BPM) and the corresponding raw overall dynamic body acceleration (ODBA), a proxy for activity, recorded from free-living box turtles in 2015. In no model was raw ODBA or classification of activity state (0 = inactive, 1 = active) different than a null model.