

Stress-induced rise in body temperature is repeatable in free-ranging Eastern chipmunks (*Tamias striatus*)

Vincent Careau · Denis Réale · Dany Garant ·
John R. Speakman · Murray M. Humphries

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Abstract In response to handling or other acute stressors, most mammals, including humans, experience a temporary rise in body temperature (T_b). Although this stress-induced rise in T_b has been extensively studied on model organisms under controlled environments, individual variation in this interesting phenomenon has not been examined in the field. We investigated the stress-induced rise in T_b in free-ranging eastern chipmunks (*Tamias striatus*) to determine first if it is repeatable. We predicted that the stress-induced rise in T_b should be positively correlated to factors affecting heat production and heat dissipation, including ambient temperature (T_a), body mass (M_b), and field metabolic rate (FMR). Over two summers, we recorded both T_b within the first minute of handling time (T_{b1}) and after 5 min of handling time (T_{b5}) 294 times on 140 individuals. The

mean ΔT_b ($T_{b5} - T_{b1}$) during this short interval was $0.30 \pm 0.02^\circ\text{C}$, confirming that the stress-induced rise in T_b occurs in chipmunks. Consistent differences among individuals accounted for 40% of the total variation in ΔT_b (i.e. the stress-induced rise in T_b is significantly repeatable). We also found that the stress-induced rise in T_b was positively correlated to T_a , M_b , and mass-adjusted FMR. These results confirm that individuals consistently differ in their expression of the stress-induced rise in T_b and that the extent of its expression is affected by factors related to heat production and dissipation. We highlight some research constraints and opportunities related to the integration of this laboratory paradigm into physiological and evolutionary ecology.

Keywords Autonomic nervous system · Daily energy expenditure · Emotional fever · Heat dissipation · Repeatability · Stress-induced hyperthermia · Thermoregulation

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V. Careau · D. Garant
Département de Biologie, Université de Sherbrooke,
Sherbrooke, QC J1K 2R1, Canada

V. Careau (✉)
Department of Biology, University of California
at Riverside, Riverside, CA 92521, USA
e-mail: vcareau@ucr.edu

D. Réale
Département des Sciences Biologiques, Université
du Québec à Montréal, Montréal, QC H3C 3P8, Canada

J. R. Speakman
Institute of Biological and Environmental Sciences,
University of Aberdeen, Aberdeen AB24 2TZ, UK

M. M. Humphries
Natural Resource Sciences, Macdonald Campus, McGill
University, Ste-Anne-de-Bellevue, QC H9X 3V9, Canada

Abbreviations

T_a	Ambient temperature
T_b	Body temperature
T_{b1}	Body temperature within 1 min of handling
T_{b5}	Body temperature after 5 min of handling
ΔT_b	Rise in body temperature during handling
FMR	Field metabolic rate

Introduction

Body temperature (T_b) is a key variable that is actively regulated in endotherms despite being influenced by multiple environmental factors that affect heat production and

dissipation (Schmidt-Nielsen 1990). If animals generate heat at a greater rate than it can be dissipated, the unavoidable result is hyperthermia (abnormally high T_b), that can be immediately fatal or more frequently impacts future survival and reproduction (Speakman and Król 2010). Therefore, physiological or behavioural strategies that elicit hyperthermia are intriguing phenomena. For example, fever induced by bacterial and viral infections is argued to be an adaptive elevation of the set-point at which T_b is regulated, that enhances immunocompetence at the expense of potential acute and long-term costs of hyperthermia (Kluger et al. 1996).

Endotherms routinely express a rise in T_b in response to handling stress. In fact, a standard procedure for recording T_b , handling and insertion of a rectal probe, leads to a rise in T_b within minutes (Borsini et al. 1989; Briese and De Quijada 1970). Such a rise in T_b in response to handling and other stressors is variously referred to as emotional hyperthermia (Briese 1995), emotional fever (Cabanac and Briese 1992), or stress-induced hyperthermia (Van der Heyden et al. 1997). This phenomenon has been recorded in humans (Briese 1995) and various other mammal species (Clark et al. 2003; Lee et al. 2000; Meyer et al. 2008; Oppermann and Bakken 1997; Parr and Hopkins 2000; Pedernera-Romano et al. 2010; Yokoi 1966). The stress-induced rise in T_b can be provoked by various factors such as handling, open-field tests, cage changes, social defeat (Hayashida et al. 2010), and even in anticipation of psychological stressors (Kluger et al. 1987). A standard laboratory procedure has been established to evaluate the stress-induced rise in T_b in singly housed laboratory mice that involves sequential recording of two rectal T_b spaced 5–10 min apart and calculating the difference between these (ΔT_b) (Van der Heyden et al. 1997). Given its simplicity, low-cost, wide application on model organisms (Bouwknicht et al. 2007), and its use as a “read-out” parameter of stress (Vinkers et al. 2010), it is surprising that the stress-induced rise in T_b has only been studied once among free-ranging animals in the field (Meyer et al. 2008).

There has been considerable debate whether the stress-induced rise in T_b is a hyperthermia or a fever (Oka et al. 2001; Vinkers et al. 2008). According to the Commission for thermal physiology of the international union of physiological sciences (2001), hyperthermia describes a T_b above the range specified for the normal active state of the species and can either be *forced* (implies a failure of thermoregulation, as total heat production exceeds the capacity for heat loss) or *regulated* (implies a fever, a rise in the set-point at which T_b is regulated accompanied by supportive changes in thermoeffector activities). Evidence interpreted as consistent with the stress-induced rise in T_b being a form of fever includes shivering and skin

vasoconstriction coinciding with a rise in T_b (Briese and Cabanac 1991) and the lack of a relationship between the stress-induced rise in T_b and ambient temperature (T_a) (Briese and Cabanac 1991; Long et al. 1990). Another set of evidence in support of the stress-induced rise in T_b being a form of fever are studies showing that it is inhibited by antipyretic drugs (Kluger et al. 1987). However, Vinkers et al. (2009) recently reported that the stress-induced rise in T_b and infection-induced fever are two distinct processes mediated largely by different neurobiological mechanisms. In the absence of empirical evidence in favour or against one of these scenarios, this phenomenon should be neutrally described as a stress-induced rise in T_b .

If the stress-induced rise in T_b is an adaptive mechanism involved during the “fight or flight” response, as previously suggested as an argument that it is a fever (Cabanac 2006; Oka et al. 2001), then individual differences in the stress-induced rise in T_b represent a biologically relevant level of variation (Wilson 1998). However, to our knowledge, the extent of consistent individual differences in (i.e. repeatability of) the stress-induced rise in T_b has never been explicitly studied, which is surprising given the longstanding interest of physiologists in individual variation (Berteaux et al. 1996; Chappell et al. 1996; Garland and Bennett 1990; Hayes et al. 1998; Labocha et al. 2004; Speakman et al. 2004; Szafranska et al. 2007). Repeatability is a key aspect of any trait that we assume to be a product (or target) of selection, as it places an upper limit on the possible heritability (Falconer and Mackay 1996). Individual variation can be seen as the raw material upon which natural selection acts, but also as the result of natural selection itself (Wilson 1998). In order to understand the evolutionary origins of the stress-induced rise in T_b , a first step is therefore to evaluate the degree of repeatability (Lessells and Boag 1987).

In this study, we investigated individual differences in the stress-induced rise in T_b , as well as individual and environmental correlates of the stress-induced rise in T_b , in a wild population of individually marked eastern chipmunks (*Tamias striatus*). We quantified the stress-induced rise in T_b as the ΔT_b recorded by a rectal T_b probe inserted in the first minute (T_{b1}) and after 5 min (T_{b5}) of a usual field handling procedure. We predicted that the stress-induced rise in T_b would be repeatable across multiple captures on the same individual, indicative of consistent individual differences in the stress-induced rise in T_b . We also evaluated potential correlates of the stress-induced rise in T_b , including season, time of day, age, sex, and reproductive status, as well as T_a and M_b reflecting heat dissipation potential and field metabolic rate (FMR) reflecting heat production.

Materials and methods

Study population and animals

In 2008 and 2009, we monitored an individually marked population of free-ranging eastern chipmunks in the Ruitter Valley Land Trust (Sutton Mountains, Québec, 45°05'N, 72°26'W). The study site contained 228 Longworth traps distributed in a grid pattern over 25 ha of mature American beech (*Fagus grandifolia*) forest (Munro et al. 2008). We systematically trapped the entire grid every week from early-May through early-October. Traps were baited with peanut butter around 0800 hours and visited at intervals of 2 h until sunset. At first capture, individuals were permanently marked with numbered ear tags (National Band and Tag Company 1005-1) and a Trovan® PIT tag inserted in the inter-scapular region. For all captures, we noted trap location, M_b (measured with a 300 g Pesola scale, ± 1 g) sex, and reproductive status (males scrotal or abdominal, females lactating or non-lactating). We assigned minimum known age to individuals according to whether they were first caught as a juvenile or as an adult (+1), as in Careau et al. (2010). We differentiated juvenile chipmunks from adults based either on an initial capture within a month following emergence when M_b was <80 g or, for individuals >80 g when first captured, on the absence of a darkened scrotum or developed mammae (Bennett 1972). We obtained measurements on juveniles as well as adults aged up to 4-year-old (since the monitoring of the population started in 2004). We divided the year into three seasons: spring (May and June), summer (July and August), and autumn (September and October). Animals could not be captured during winter hibernation. Hourly T_a data were obtained from two meteorological stations located at 32 and 37 km from our site (North of the site: Lac Memphremagog 45°16'00"N, 72°10'00"W; West of the site: Frelighsburg 45°03'01"N, 72°51'41"W; Environment Canada; <http://climate.weatheroffice.gc.ca>). Both stations gave T_a estimates that were highly correlated with each other and to a weather station located on the middle of our grid that was operational only in 2006 (Lac Memphremagog vs. Frelighsburg stations: $r = 0.96$, $n = 3719$; onsite station vs. Lac Memphremagog: $r = 0.97$, $n = 3719$; onsite station vs. Frelighsburg: $r = 0.98$, $n = 4366$; correlations estimated for each hourly estimate available for each station from May 1 to October 1 2006). We averaged T_a recordings at both stations and associated each T_b recording to the closest corresponding hourly T_a recording. Although our T_a variable ignores factors like solar radiation and wind speed, whose impacts are likely important on small mammals, the canopy of the dense forest at our site might have reduced the relative impact of these factors. The “noise” included by these factors makes

our test of the effect of T_a on the stress-induced rise in T_b conservative.

Body temperature (T_b) and stress-induced rise in T_b

We recorded rectal T_b over two summers (2008 and 2009) directly in the field, following a slightly modified version of the standard procedure for evaluating the stress-induced rise in T_b in singly housed mice (Van der Heyden et al. 1997). All T_b were taken by one person (VC) to minimize variation. Upon arriving at a trap with a closed door, a timer was started, the trap was lifted, and the chipmunk was transferred into a handling bag and taken by hand. It was possible to rapidly expose the anus of the chipmunk by pinching firmly the fur in the nape of the neck and holding one hind leg. A thermocouple probe was inserted 10 mm deep into the rectum (Omega engineering, model HH203A) and maintained until the display reached a stable value (~ 20 s). All initial T_b records (T_{b1}) used in the analysis were taken within 60 s of lifting the trap, otherwise the record was excluded. After the initial T_b was taken, the animal was put back in the handling bag for pit tag and ear tag identification, measurement of M_b , and assessment of reproductive status (see above). A final reading of temperature (T_{b5}) was taken exactly 5 min after the trap had been first lifted. After each test, the probe was disinfected with 95% ethanol. We calculated the elevation in T_b induced by handling as $T_{b5} - T_{b1}$ and hereafter refer to this variable as ΔT_b .

Ninety percent of T_b recordings occurred between 1000 and 1800 hours, a period during which the diurnal pattern of T_b is relatively stable at 39°C in captive chipmunks (Levesque and Tattersall 2010). We recognise that the best protocol would have included repeated T_b recordings at 10-min interval, as in singly housed laboratory mice (Van der Heyden et al. 1997). However, handling can be highly stressful for wild animals and some chipmunks become lethargic (or faint death) when handled over prolonged period (VC, *pers. obs.*). It has been shown in singly housed laboratory mice that $\sim 80\%$ of the rise in T_b is reached within 5 min of handling (Van der Heyden et al. 1997). Therefore, our protocol represents a necessary compromise between the length of capture under field conditions that required quick release of the animals and the full expression of the stress-induced rise in T_b . We currently do not have the data to suggest that an interval of 5 min is appropriate for assessing the magnitude of the stress-induced rise in T_b in chipmunks. If the peak value of the stress-induced rise in T_b is obtained later than 5 min after the onset of handling, then the significance of our ΔT_b measure may not be its magnitude but instead the speed at which the response occurs during stress, which itself may also be relevant from an evolutionary point of view. Our

results must therefore be tentatively interpreted until a comprehensive study is performed using temperature-sensitive transmitters or data loggers implanted into the abdominal cavity of animals to obtain a complete and continuous tracing of T_b before, during, and after capture.

Field metabolic rate (FMR)

We measured FMR using the doubly labelled water (DLW) technique (Butler et al. 2004; Lifson et al. 1975; Speakman 1997). This technique estimates the carbon dioxide (CO_2) produced by a free-ranging animal based on the differential washout of injected hydrogen (^2H) and oxygen (^{18}O) isotopes. It has been previously validated by comparison with direct calorimetry in a range of small mammals and provides an accurate measure of FMR over periods of several days (Speakman et al. 1994). The DLW technique has also been previously used successfully on eastern chipmunks (Humphries et al. 2002).

The method we used implied two captures. On the initial capture, chipmunks were injected intra-peritoneally with 240 μl of DLW (37.78 and 4.57% enriched ^{18}O and ^2H). Following injection, subjects were held in the trap for a 1-h equilibration period (Speakman and Król 2005). Then, a first blood sample was collected via a clipped toenail for initial isotope analysis. Chipmunks were then released at the site of capture and recaptured, weighed and bled 1 to 3 days later, at as close as feasible to periods of 24-h interval, and a final blood sample was taken to estimate isotope elimination rates. Taking samples over multiples of 24-h period minimise the substantial day-to-day variability in FMR (Berteaux and Thomas 1999; Speakman et al. 1994). The range of absolute deviation from 24 h was 5–180 min (25th percentile = 24 min; median = 40 min, 75th percentile = 62 min). In 2008 and 2009, a total of 5 and 3 animals were blood sampled without prior injection to estimate background isotope enrichments of ^2H and ^{18}O (method C in Speakman and Racey 1987). Capillaries were flame sealed immediately after sample collection. Capillaries that contained the blood samples were vacuum distilled and water from the resulting distillate was used to produce CO_2 (methods in Speakman et al. 1990) and H_2 (methods in Speakman and Król 2010). The isotope ratios $^{18}\text{O}:^{16}\text{O}$ and $^2\text{H}:^1\text{H}$ were analysed using gas source isotope ratio mass spectrometry (Optima, Micromass IRMS and Isochrom μG , Manchester, UK). Samples were run alongside three lab standards for each isotope (calibrated to International standards) to correct delta values to ppm. Isotope enrichments were converted to values of FMR using a single pool model as recommended for this size of animal (Speakman 1993). We assumed evaporation of 25% of the water flux (equation 7.17 in Speakman 1997)

which minimizes error in a range of conditions (Visser and Schekkerman 1999).

The FMR data analysed here consist in a subset of 21 individuals (18 lactating females, 2 non-reproductive females, and 1 reproductive male) for which we applied the ΔT_b protocol on the final captures, just before the blood sampling started. The mean ΔT_b in this subsample was not significantly different from the complete sample (mean \pm SD = $0.32 \pm 0.45^\circ\text{C}$; $F = 0.09$; $P = 0.76$).

Data analysis

We tested the effects of year (2008 or 2009), season (spring, summer, and fall), time of day, time of day², T_a , age, sex, reproductive status, and M_b on T_{b1} , T_{b5} , and ΔT_b using multiple regression linear mixed models in R 2.7.1 (<http://www.r-project.org>) with the package ‘nlme’. We included individual identity (ID) as a random effect to estimate the significance of fixed effects while accounting for pseudoreplication resulting from repeated measurements. Model selection was performed using backward procedures, sequentially removing the least significant term from the model based on its P value until all remaining terms were significant at $\alpha = 0.05$. Although the mean T_a differed among seasons (mean \pm SD, spring = $19.6 \pm 4.8^\circ\text{C}$, range: $11.0\text{--}27.8^\circ\text{C}$; summer = $21.3 \pm 3.5^\circ\text{C}$, range: $14.8\text{--}27.5^\circ\text{C}$, fall = $18.3 \pm 4.9^\circ\text{C}$, range $5.5\text{--}27.3^\circ\text{C}$), the high variance present within seasons (attributed to day-to-day and hour-to-hour variations) allowed us to test the effects of T_a and season simultaneously without colinearity problems.

Stress-related variables are routinely affected by acclimation (habituation effects), and the stress-induced rise in T_b is no exception (Cabanac and Briese 1992; Thompson et al. 2003). To test whether chipmunks acclimated to the trapping and T_b recording procedures, we included the order of tests as an additional fixed effect. We also included the number of time each chipmunk was captured before each T_b recording (mean \pm SD = 36 ± 35 captures, range: 0–140). Finally, we calculated the time elapsed between any two T_b recordings on an individual. The first T_b record for each individual was given an arbitrary value of 740, which corresponds to twice the longest period elapsed between two recordings in our dataset. We never applied the temperature-recording procedure on any chipmunks prior to this study.

Once the fixed effects structure was determined, we calculated individual repeatability by calculating the proportion of variance attributed to ID as a random effect. We assessed the significance of random effects using log-likelihood ratio tests (LRT). A LRT test compares the log-likelihoods of a “full” model that includes ID as a random component versus a “reduced” model without ID (Pinheiro and Bates 2000). The test statistic is equal to twice the

difference in log-likelihoods between the two nested models and assumes that it follows a χ^2 -distribution with one degree of freedom (*df*). We also estimated repeatability of ΔT_b using intraclass correlation coefficient (τ) calculated on individuals with repeated measures only (Lessells and Boag 1987). In addition, we tested whether the rise in T_b was significant using a paired *t* test with the number of degrees of freedom equal to number of individuals measured.

Because we could not control for the time spent in the trap before we handled chipmunks, we tested whether the variability in ΔT_b was primarily due to variations in T_{b1} or mostly due to variation in T_{b5} . We expected T_{b1} or T_{b5} to be more strongly correlated to ΔT_b if the stress of trapping or handling is more important. Statistical analysis of the effect of FMR on ΔT_b was assessed by including julian day and M_b as covariates in a multiple regression model.

Results

We recorded T_{b1} and T_{b5} 294 times on 140 individuals over two complete summers (Fig. 1). T_{b1} was normally distributed and ranged from 37.7 to 41.1°C (mean \pm SD = 39.3 ± 0.6 , Fig. 2). T_{b1} was not influenced by season, time of day, time of day², age, order of test, and time since last capture (Table 1), but varied annually and was lower in males than in female and in reproductive than non-reproductive animals (Table 1). T_{b1} was negatively related to the number of time the individual was captured before T_b recording (Table 1). T_{b1} was also positively related to T_a (Fig. 3a) and M_b (Fig. 3b). After accounting for these significant sources of variation, T_{b1} was significantly repeatable with 31% of its variance attributed to individual differences (Table 1).

T_{b5} was normally distributed and ranged from 37.6 to 41.3°C (mean \pm SD = 39.6 ± 0.7 , Fig. 2). Similarly to T_{b1} , T_{b5} was not correlated to time of day, time of day², age, and time since last capture (Table 1). In contrast to T_{b1} , T_{b5} did not vary annually and but differed among seasons (lower in mid-summer than spring and fall), and was not affected by the number of previous capture but decreased with order of test (Table 1). Also in contrast to T_{b1} , the effects of sex and reproductive status were marginally non-significant (Table 1). T_{b5} was positively related to T_a (Fig. 3c) and M_b (Fig. 3d). After accounting for these significant sources of variation, T_{b5} was significantly repeatable, with 25% of its variance attributed to individual differences (Table 1).

Rectal temperature rose between T_{b1} and T_{b5} in 74.5% of the captures ($N = 219$ out of 294; data-points located above the line in Fig. 1). ΔT_b was negligible (i.e. less than our precision, 0.1°C) in 8.8% of the captures

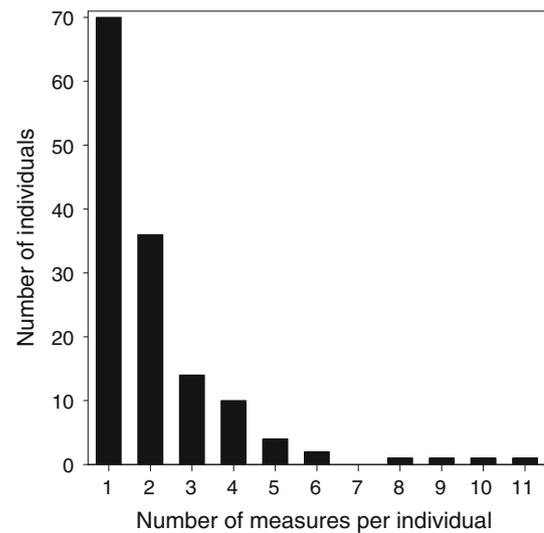


Fig. 1 Frequency distribution of the number of records per individual included in this study on free-ranging eastern chipmunks

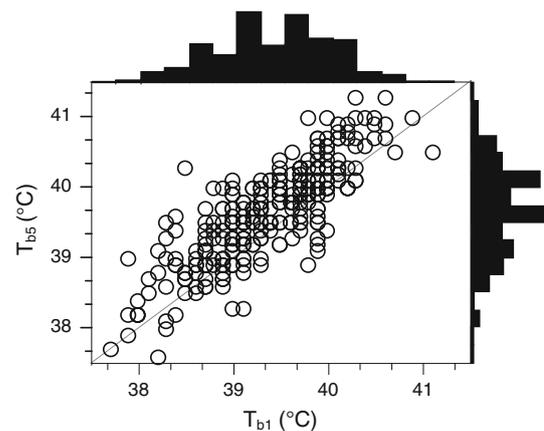


Fig. 2 Final body temperature (T_{b5} ; after 5 min of handling) as function of initial body temperature (T_{b1} ; within 1 min of handling) in free-ranging eastern chipmunks. The line represents the 1:1 ratio ($T_{b5} = T_{b1}$)

($N = 26$) and negative in 16.7% of the captures ($N = 49$; data-points located below the line in Fig. 2). Overall, ΔT_b was normally distributed and ranged from -0.9 to 1.8°C (mean \pm SD = $0.3 \pm 0.4^\circ\text{C}$). According to a paired *t* test, the rise in T_b was highly significant ($t = 11.09$, $df = 140$, $P < 0.001$). ΔT_b was more strongly correlated to T_{b5} ($r = 0.43$, $P < 0.0001$) than T_{b1} ($r = -0.16$, $P = 0.007$).

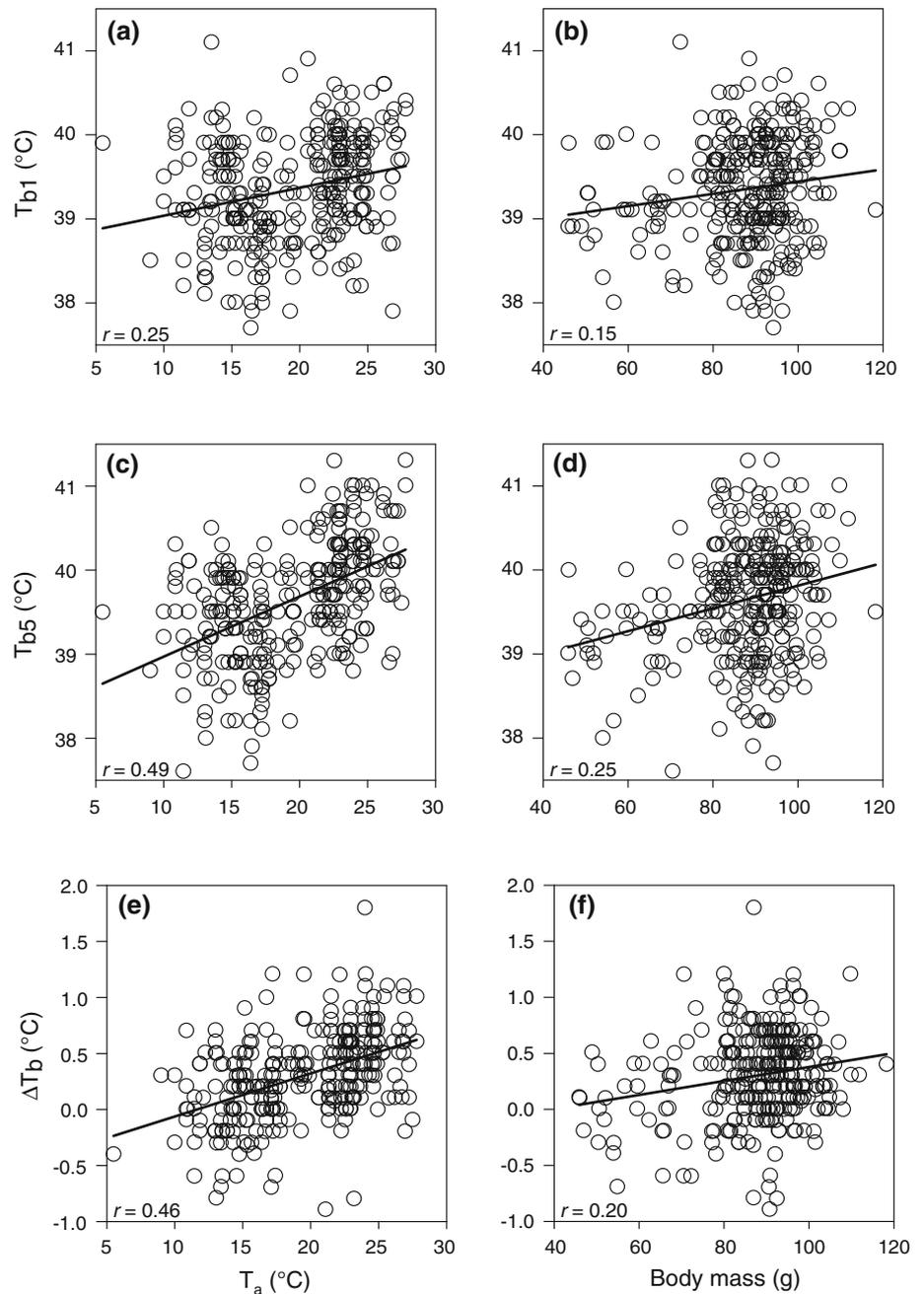
ΔT_b was lower in 2008 than in 2009 and varied seasonally (lower in summer relative to spring and fall; Table 1). The effects of the variables “order of test” and “number of captures prior to T_b recording” were non-significant on ΔT_b , whereas the variable “time since last T_b

Table 1 Linear mixed effects models for initial body temperature (T_{b1} ; within 1 min of handling), final body temperature (T_{b5} ; after 5 min of handling), and stress-induced rise in T_b ($\Delta T_b = T_{b1} - T_{b5}$) in relation to environmental and individual variables in free-ranging eastern chipmunks

Fixed effects	T_{b1}					T_{b5}					ΔT_b				
	Estimate	SE	df	t	P	Estimate	SE	df	t	P	Estimate	SE	df	t	P
Year (2009)	-0.428	0.070	149	-6.07	<0.0001	-0.162	0.115	145	-1.40	0.1622	0.113	0.055	149	2.04	0.0433
Season (spring)	-0.108	0.113	142	-0.95	0.3434	0.237	0.094	149	2.53	0.013	0.316	0.057	149	5.55	<0.0001
Season (fall)	0.033	0.099	142	0.33	0.7391	0.377	0.084	149	4.50	<0.0001	0.222	0.054	149	4.14	0.0001
Time of day	-0.166	0.139	144	-1.19	0.2360	0.022	0.014	147	1.55	0.1239	-0.002	0.008	146	-0.20	0.8415
(Time of day) ²	0.001	0.000	148	1.30	0.1957	0.005	0.005	142	0.92	0.3572	-0.002	0.003	145	-0.58	0.5654
T_a	0.039	0.008	149	5.04	<0.0001	0.073	0.007	149	9.87	<0.0001	0.039	0.004	149	8.96	<0.0001
Age	-0.067	0.059	145	-1.13	0.2618	-0.066	0.059	144	-1.12	0.2652	-0.008	0.038	143	-0.21	0.8342
Sex (M)	-0.190	0.095	138	-2.00	0.0474	-0.155	0.093	138	-1.66	0.0998	0.034	0.056	138	0.60	0.5464
Repro (yes)	-0.178	0.081	149	-2.19	0.0303	-0.158	0.085	148	-1.86	0.0646	0.013	0.051	144	0.25	0.8029
M_b	0.013	0.003	149	3.94	0.0001	0.013	0.003	149	4.30	<0.0001	0.005	0.002	149	2.65	0.0089
Order of test	-0.021	0.024	147	-0.86	0.3930	-0.083	0.018	149	-4.49	<0.0001	0.001	0.017	142	0.08	0.9398
Time since last record	-1.3E-04	1.1E-04	146	-1.18	0.2380	-4.0E-05	1.1E-04	143	-0.37	0.7126	9.5E-05	5.4E-05	148	1.75	0.0815
Number of captures before	-0.004	0.001	149	-3.24	0.0015	-0.002	0.001	146	-1.40	0.1641	0.001	0.001	147	0.75	0.4553
Random effects	σ	SE	df	χ^2	P	σ	SE	df	χ^2	P	σ	SE	df	χ^2	P
Individual ID	0.100	0.032	1	18.91	<0.0001	0.078	0.030	1	10.85	0.0010	0.046	0.014	1	16.84	<0.0001
Error	0.224	0.026				0.239	0.027				0.070	0.009			

Initial models included fixed effects of year (2008 or 2009), season (spring, summer, and fall), time of day (from 0900 to 1900 hours), time of day², ambient temperature (T_a), age (in years), sex, reproductive status (yes or no), body mass (M_b), order of tests (acclimation), time since last capture (days), and number of capture before T_b recording, but only significant terms ($P < 0.05$ in *bold*) were retained following model simplification (effect of non-significant variables are shown at the moment they were dropped from the model). Also shown is the variance (σ) associated with individual identity (ID) included as a random effect and its significance level (P) determined by a likelihood ratio test assuming a Chi-square distribution χ^2 with one degree of freedom

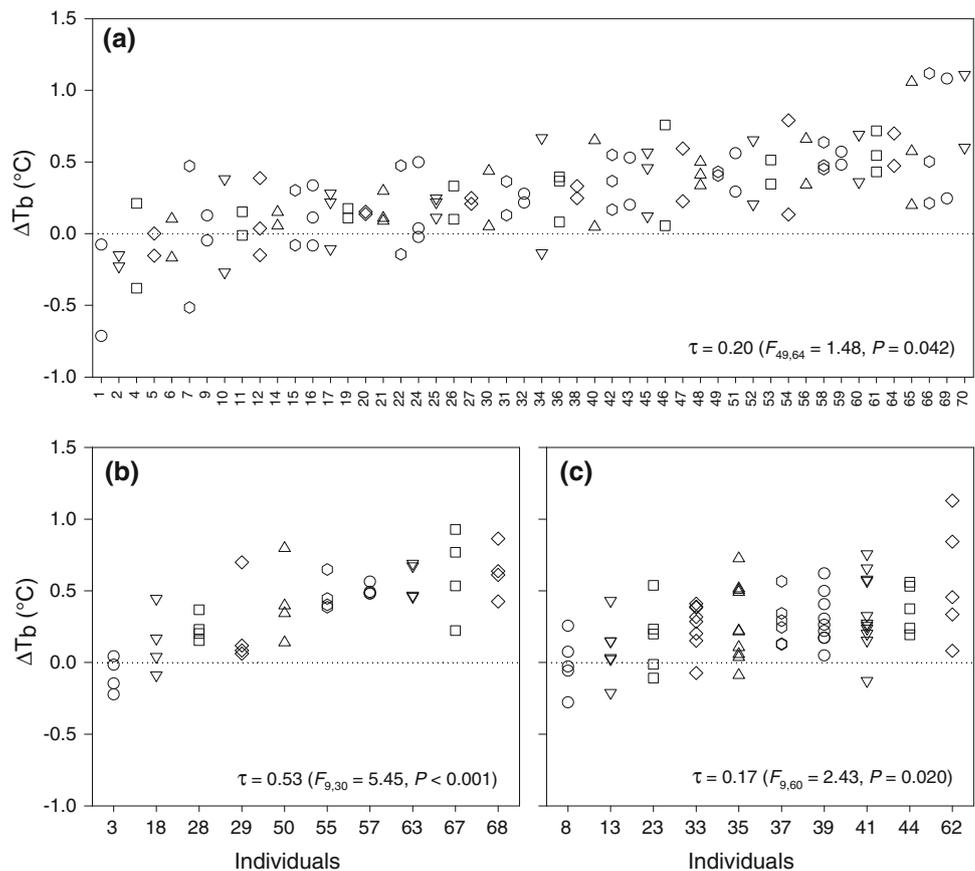
Fig. 3 Influence of ambient temperature (T_a ; left panels) and body mass (right panels) on (a and b) initial (T_{b1}), (c and d) final (T_{b5}) body temperature, and (e and f) stress-induced rise in T_b ($\Delta T_b = T_{b5} - T_{b1}$) in free-ranging eastern chipmunks. See Table 1 for statistical significance. A slight jitter was introduced in all panels to show overlapping dots



recording” was marginally non-significant (Table 1). Because of the stronger effects of T_a and M_b on T_{b5} than T_{b1} , ΔT_b was positively and significantly correlated with T_a (Fig. 3e) and M_b (Fig. 3f). After accounting for these significant sources of variation, ΔT_b was significantly repeatable, with 40% of its variance attributed to individual differences (Table 1). Figure 4a shows the extent of individual variation in ΔT_b for 50 chipmunks with 2 and 3 repeated records. Individuals with 4 ($n = 10$) and 5 or more ($n = 10$) repeated measures are shown in Fig. 4b, c, respectively.

The mean FMR (\pm SD) was 216.2 ± 64.6 kJ/day (range = 124.5–398.3 kJ/day) among the subsample of 21 individuals for which we quantified ΔT_b on the final capture. FMR was not a significant predictor of T_{b1} (estimate \pm SE = 0.53 ± 1.51 , $t = 0.35$, $P = 0.73$) but tended to be positively correlated with T_{b5} with julian day and M_b included in the model as a covariates (estimate \pm SE = 2.83 ± 1.45 , $t = 1.95$, $P = 0.07$). FMR was a significant positive predictor of ΔT_b (estimate \pm SE = 2.30 ± 0.74 , $t = 3.11$, $P = 0.006$) with julian day and M_b included in the model as covariates (Fig. 5).

Fig. 4 Individual variation in the stress-induced rise in body temperature (ΔT_b , final minus initial body temperature, adjusted for year, season, ambient temperature, and body mass) in free-ranging eastern chipmunks with (a) two or three, (b) four, and (c) five to 11 repeated measurements [total $n = 224$ on 70 individuals $\tau = 0.28$ ($F_{69,154} = 2.24$, $P < 0.001$)]. A slight jitter was introduced in all panels to show overlapping dots



Discussion

This study, representing the first exploration of individual variation in the stress-induced rise in T_b in a wild population, confirms that individuals consistently differ in the magnitude of their stress-induced rise in T_b (i.e. ΔT_b is repeatable) and that the stress-induced rise in T_b is affected by correlates of heat production and dissipation such as M_b , T_a , and energy expenditure.

Studying the stress-induced rise in T_b in the field rather than the laboratory introduces opportunities as well as limitations. Studying an abundant, marked field population facilitated a relatively large sample size (Fig. 1; 294 ΔT_b records on 140 individuals), which enabled the detection of consistent individual differences in the stress-induced rise in T_b . These individual differences have not been a focus of most prior research on this topic, but represent a promising avenue to explore the evolutionary causes and consequences of the stress-induced rise in T_b (Berteaux et al. 1996; Chappell et al. 1996; Garland and Bennett 1990; Hayes et al. 1998; Labocha et al. 2004; Speakman et al. 2004; Szafranska et al. 2007). However, studying the stress-induced rise in T_b in the field introduces additional sources of variation that are more easily avoided in captivity. For example, beyond the captures included in the

current analyses chipmunks have been trapped and handled multiple times for multiple purposes such as initial marking, pit tag insertion, open-field test (Montiglio et al. 2010), doubly labelled water technique (this study, Bergeron et al. 2011), and respirometry (Careau et al. 2010). Interestingly, the more an individual has been captured the lower its T_{b1} was, suggesting that chipmunks may habituate to being trapped (and thus had lower T_{b1}). In contrast, T_{b5} was not related to number of captures prior to T_b recording but instead decreased with the number of time their T_b was recorded. Although the relationship was marginally non-significant, ΔT_b was positively related to time elapsed since last T_b recording, suggesting that frequent, regular captures lead to habituation and attenuated the stress-induced rise in T_b , while infrequent captures do not attenuate the stress-induced rise in T_b . These results attest for the complexity of habituation trends to previous and concurrent handling events involving a mixture of overlapping and unique procedures realised over different temporal frames. Nevertheless, this potential contributor to unexplained variation in our results did not preclude detection of significant individual differences in the stress-induced rise in T_b and correlations with T_a , M_b , and FMR.

An additional source of variation is that we could not control the amount of time (up to 2 h) the individuals spent

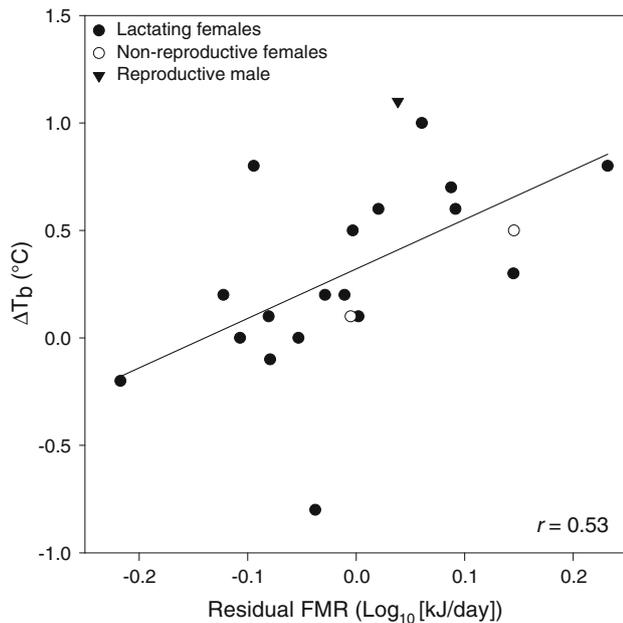


Fig. 5 Stress-induced rise in body temperature (ΔT_b) against field metabolic rate (FMR) in 21 free-ranging eastern chipmunks. Statistical analysis of the effect of FMR on ΔT_b was assessed by including Julian day and body mass (M_b) as a covariates in the model, but for illustrative purposes, here FMR values are plotted as residuals from the significant relationship between with M_b [$r^2 = 0.24$, $P = 0.022$, $\text{Log}_{10}(\text{FMR, in kJ per day}) = -0.93 + 1.65 \text{Log}_{10}(M_b)$]

in the traps before handling. Therefore, our results should be interpreted with care as long as the time course of trap-stress-induced rise in T_b remains unknown. One potential explanation for why some values of ΔT_b were negative could be the differential effects of the stress of being in a trap on T_{b1} versus the stress of being handled on T_{b5} (see above). However, it is notable that negative ΔT_b was not preferentially associated with an elevated initial T_{b1} (see Fig. 2). In fact, the range of T_{b1} (37.7–41.1°C) is only slightly higher than the range of diurnal T_b recorded in captive chipmunks at the same latitude (36.3–40.9°C; Danielle Levesque, personal communication). If time spent in the trap would be the explaining factor for negative values, then the T_{b1} of these observations would be higher than normal (already stressed in the trap), which seems not to be the case in our study. Interestingly, T_{b1} is less affected by T_a than T_{b5} , suggesting that handling causes the chipmunks to regulate T_b less tightly. However, even after controlling for T_a and stress-related variables, ΔT_b was repeatable, which indicates that some individuals are more likely to express negative ΔT_b than others (see Fig. 2). It may be possible, then, that negative values are included in the natural range of ΔT_b observed in response to the temperature-recording procedure. In some rat strains, negative ΔT_b can be apparently induced by restraint depending on time of day and the type of restraint (mesh vs. plexiglas restrainers) and is thought to be a physiological response to

severe overheating microenvironment (McGivern et al. 2009).

The most parsimonious explanation to our findings that the stress-induced rise in T_b increases as a function of T_a , M_b , and FMR is that it represents a forced hyperthermia (i.e. an incidental consequence of heat production that exceeds heat dissipation) rather than an active hyperthermia (i.e. fever, an increase in the set-point at which T_b is regulated). Under this interpretation, the occurrence of repeatable individual variation in the stress-induced rise in T_b would arise from differential propensity to over-heat in stressful circumstances associated with consistent individual differences in physiology, morphology (beyond M_b , which was accounted for as a covariate) or behaviour affecting heat production and dissipation during handling. Our findings that the stress-induced rise in T_b differed between years and seasons could be related to physiological and behavioural variations occurring at the population level. For example, eastern chipmunks are known to become much less active in summer, a phenomenon referred to as the summer “lull” (Elliot 1978; Yahner 1978). In our population, resting metabolic rate increases in fall compared to spring and summer (Careau, unpublished data). If these seasonal changes at the population level are somehow linked to propensity to overheat during captures, they could explain the annual and seasonal differences we observed in ΔT_b .

Given the debate that occurred as to whether the stress-induced rise in T_b represents an uncontrolled rise in T_b versus an increase in the set-point at which T_b is regulated, we also tentatively interpret our findings under the fever scenario. If the stress-induced rise in T_b represents an increase in the regulated set-point (Cabanac 2006), why, then, ΔT_b is positively correlated with T_a and M_b ? We suggest the possibility that the value of the set-point varies according to heat dissipation needs/capacity (Speakman and Król 2010). Although, most obviously, hyperthermia can be an outcome of insufficient heat dissipation, counter-intuitively, an up-regulated T_b can also be a means to facilitate heat loss. By increasing the thermal gradient between T_b and T_a , an up-regulated set-point increases heat flux. If the capacity to dissipate heat is an important limit to animal performance during brief period of intense activity (Speakman and Król 2010), then individuals with higher heat load should regulate T_b at a higher set-point than individuals with lower heat load. This should be the case for animals exposed to higher T_a and/or have higher FMR compared to those exposed to lower T_a and/or have lower FMR. Animals with lower surface-to-volume ratio (e.g. higher M_b) should also have higher heat load than those with higher surface-to-volume ratio (e.g. lower M_b), as long as T_a is lower than T_b , which probably is the case for most endotherms in most parts of the world during most

months of the year (Humphries and Careau 2011). Interestingly, it was recently found that infection-induced fever was modest or even absent in mice at low T_a in response to the same stimuli that induce fever at thermoneutral conditions (Rudaya et al. 2005). Two previous studies (Briese and Cabanac 1991; Long et al. 1990) reporting that SIH was independent of T_a interpreted their result as in favour of the stress-induced rise in T_b being a rise in the set-point (Oka et al. 2001). Briese (1992) later reported that the stress-induced rise in T_b was higher in a cold (8°C) than a warm (31°C) environment, whereas we obtained the opposite result (ΔT_b greater in warm T_a). Although there is no clear empirical consensus emerging on the effect of T_a on the stress-induced rise in T_b , the logic we proposed above implies that the independency of this phenomenon versus T_a does not constitute per se an argument in support of the view that it is an adaptive rise in set-point.

The study of individual differences in the stress-induced rise in T_b could serve as a bridge between this laboratory paradigm and other sub-disciplines where inter-individual variation is already considered as an important ecological and evolutionary characteristic of wild populations [population biology (Bolnick et al. 2003), epidemiology (Lloyd-Smith et al. 2005), endocrinology (Williams 2008), behavioural ecology (Réale et al. 2007), and energy metabolism (Careau et al. 2008)]. Although identifying the mechanisms involved in the stress-induced rise in T_b are likely best explored in captivity, the study of this phenomenon into a natural context will yield novel insights about environmental effects and fitness consequences that affect the expression and evolution of the stress-induced rise in T_b in wild populations (Costa and Sinervo 2004). Our demonstration of consistent individual differences in the stress-induced rise in T_b is an important first step in defining the upper limit of its potential heritability (Falconer and Mackay 1996). Given the stress-induced rise in T_b is speculated to be an adaptive mechanism involved in the “fight or flight” response (Cabanac 2006; Oka et al. 2001), then the obvious next step is to evaluate selection on it and its heritability in the wild. Additional promising avenues for future research, in captivity or the field, include relating the stress-induced rise in T_b to personality traits (e.g. boldness, aggressiveness, and exploration) or coping styles (Agren et al. 2009; Carere and van Oers 2004), as well as conducting a more thorough evaluation of energetic correlates than was accomplished here.

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References

- Agren G, Lund I, Thiblin I, Lundeberg T (2009) Tail skin temperatures reflect coping styles in rats. *Physiol Behav* 96:374–382
- Bennett GF (1972) Further studies on the chipmunk warble, *Cuterebra emasculator* (Diptera: Cuterebridae). *Can J Zool* 50:861–864
- Bergeron P, Careau V, Humphries MM, Réale D, Speakman JR, Garant D (2011) The energetic and oxidative costs of reproduction in a free-ranging rodent. *Funct Ecol* 25:1063–1071
- Berteaux D, Thomas D (1999) Seasonal and interindividual variation in field water metabolism of female meadow voles *Microtus pennsylvanicus*. *Physiol Biochem Zool* 72:545–554
- Berteaux D, Thomas DW, Bergeron JM, Lapierre H (1996) Repeatability of daily field metabolic rate in female Meadow Voles (*Microtus pennsylvanicus*). *Funct Ecol* 10:751–759
- Bolnick DI, Svanback R, Fordyce JA, Yang LH, Davis JM, Hulsey CD, Forister ML (2003) The ecology of individuals: incidence and implications of individual specialization. *Am Nat* 161:1–28
- Borsini F, Lecci A, Volterra G, Meli A (1989) A model to measure anticipatory anxiety in mice? *Psychopharmacology* 98:207–211
- Bouwknicht JA, Olivier B, Paylor RE (2007) The stress-induced hyperthermia paradigm as a physiological animal model for anxiety: a review of pharmacological and genetic studies in the mouse. *Neurosci Biobehav Rev* 31:41–59
- Briese E (1992) Cold increases and warmth diminishes stress-induced rise of colonic temperature in rats. *Physiol Behav* 51:881–883
- Briese E (1995) Emotional hyperthermia and performance in humans. *Physiol Behav* 58:615–618
- Briese E, Cabanac M (1991) Stress hyperthermia: physiological arguments that it is a fever. *Physiol Behav* 49:1153–1157
- Briese E, De Quijada MG (1970) Colonic temperature of rats during handling. *Acta Physiologica Latinoamericana* 20:97–102
- Butler PJ, Green JA, Boyd IL, Speakman JR (2004) Measuring metabolic rate in the field: the pros and cons of the doubly labelled water and heart rate methods. *Funct Ecol* 18:168–183
- Cabanac M (2006) Adjustable set point: to honor Harold T. Hammel. *J Appl Physiol* 100:1338–1346
- Cabanac A, Briese E (1992) Handling elevates the colonic temperature of mice. *Physiol Behav* 51:95–98
- Careau V, Thomas D, Humphries MM, Réale D (2008) Energy metabolism and animal personality. *Oikos* 117:641–653
- Careau V, Humphries MM, Thomas D (2010) Energetic cost of bot fly parasitism in free-ranging eastern chipmunks. *Oecologia* 162:303–312
- Carere C, van Oers K (2004) Shy and bold great tits (*Parus major*): body temperature and breath rate in response to handling stress. *Physiol Behav* 82:905–912
- Chappell MA, Zuk M, Johnsen TS (1996) Repeatability of aerobic performance in Red Junglefowl: effects of ontogeny and nematode infection. *Funct Ecol* 10:578–585
- Clark DL, DeBow SB, Iseke MD, Colbourne F (2003) Stress-induced fever after postischemic rectal temperature measurements in the gerbil. *Can J Physiol Pharmacol* 81:880–883
- Commission for thermal physiology of the international union of physiological sciences (2001) Glossary of terms for thermal physiology, 3rd edn. *Japanese J Physiol* 51:245–280

- Costa DP, Sinervo B (2004) Field physiology: physiological insights from animals in nature. *Annu Rev Physiol* 66:209–238
- Elliot L (1978) Social behavior and foraging ecology of the eastern chipmunk (*Tamias striatus*) in the Adirondack mountains. *Smithsonian Contrib Zool* 265:1–107
- Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics, 4th edn. Longman, Harlow, Essex
- Garland T Jr, Bennett AF (1990) Quantitative genetics of maximal oxygen consumption in a garter snake. *Am J Physiol* 259:R986–R992
- Hayashida S, Oka T, Mera T, Tsuji S (2010) Repeated social defeat stress induces chronic hyperthermia in rats. *Physiol Behav* 101:124–131
- Hayes JP, Bible CA, Boone JD (1998) Repeatability of mammalian physiology: evaporative water loss and oxygen consumption of *Dipodomys merriami*. *J Mammal* 79:475–485
- Humphries M, Careau V (2011) Heat for nothing or activity for free? Evidence and implications of activity-thermoregulatory heat substitution. *Integr Comp Biol* 51:419–431
- Humphries MM, Thomas DW, Hall CL, Speakman JR, Kramer DL (2002) The energetics of autumn mast hoarding in eastern chipmunks. *Oecologia* 133:30–37
- Kluger MJ, O'Reilly B, Shope TR, Vander AJ (1987) Further evidence that stress hyperthermia is a fever. *Physiol Behav* 39:763–766
- Kluger MJ, Kozak W, Conn CA, Leon LR, Soszynski D (1996) The adaptive value of fever. *Infect Dis Clin N. Am* 10:1–20
- Labocha MK, Sadowska ET, Baliga K, Semer AK, Koteja P (2004) Individual variation and repeatability of basal metabolism in the bank vole, *Clethrionomys glareolus*. *Proc Royal Soc Lond Ser B-Biol Sci* 271:367–372
- Lee BY, Padick DA, Muchlinski AE (2000) Stress fever magnitude in laboratory-maintained California ground squirrels varies with season. *Comp Biochem Physiol A* 125:325–330
- Lessells CM, Boag PT (1987) Unrepeatable repeatabilities: a common mistake. *Auk* 104:116–121
- Levesque DL, Tattersall GJ (2010) Seasonal torpor and normothermic energy metabolism in the Eastern chipmunk (*Tamias striatus*). *J Comp Physiol B-Biochem Syst Environ Physiol* 180:279–292
- Lifson N, Little WS, Levitt DG, Henderson RM (1975) D₂¹⁸O method for CO₂ output in small mammals and economic feasibility in man. *J Appl Physiol* 39:657–663
- Lloyd-Smith JO, Schreiber SJ, Kopp PE, Getz WM (2005) Super-spreading and the effect of individual variation on disease emergence. *Nature* 438:355–359
- Long NC, Vander AJ, Kluger MJ (1990) Stress-induced rise of body temperature in rats is the same in warm and cool environments. *Physiol Behav* 47:773–775
- McGivern RF, Zuloaga DG, Handa RJ (2009) Sex differences in stress-induced hyperthermia in rats: restraint versus confinement. *Physiol Behav* 98:416–420
- Meyer LC, Fick L, Matthee A, Mitchell D, Fuller A (2008) Hyperthermia in captured impala (*Aepyceros melampus*): a fright not flight response. *J Wildl Dis* 44:404–416
- Montiglio PO, Garant D, Thomas DW, Réale D (2010) Individual variation in temporal activity patterns in open-field tests. *Anim Behav* 80:905–912
- Munro D, Thomas DW, Humphries MM (2008) Extreme suppression of above-ground activity by a food-storing hibernator the eastern chipmunk (*Tamias striatus*). *Can J Zool* 86:364–370
- Oka T, Oka K, Hori T (2001) Mechanisms and mediators of psychological stress-induced rise in core temperature. *Psychosom Med* 63:476–486
- Oppermann R, Bakken M (1997) Effect of indomethacin on LPS-induced fever and on hyperthermia induced by physical restraint in the silver fox (*Vulpes vulpes*). *J Therm Biol* 22:79–85
- Parr L, Hopkins W (2000) Brain temperature asymmetries and emotional perception in chimpanzees, *Pan troglodytes*. *Physiol Behav* 71:363–371
- Pedernera-Romano C, Ruiz de la Torre JL, Badiella L, Manteca X (2010) Effect of perphenazine enanthate on open-field test behaviour and stress-induced hyperthermia in domestic sheep. *Pharmacol Biochem Behav* 94:329–332
- Pinheiro JC, Bates DM (2000) Mixed-effects models in S and S-PLUS. Springer Verlag, New York
- Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ (2007) Integrating animal temperament within ecology and evolution. *Biol Rev* 82:291–318
- Rudaya AY, Steiner AA, Robbins JR, Dragic AS, Romanovsky AA (2005) Thermoregulatory responses to lipopolysaccharide in the mouse: dependence on the dose and ambient temperature. *Am J Physiol-Regul Integr Comp Physiol* 289:R1244–R1252
- Schmidt-Nielsen K (1990) Animal Physiology. Cambridge University Press, Cambridge
- Speakman JR (1993) How should we calculate CO₂ production in doubly labeled water studies of animals? *Funct Ecol* 7:746–750
- Speakman JR (1997) Doubly labelled water: theory and practice. Chapman and Hall, London
- Speakman JR, Król E (2005) Validation of the doubly-labelled water method in a small mammal. *Physiol Biochem Zool* 78:650–667
- Speakman JR, Król E (2010) Maximal heat dissipation capacity and hyperthermia risk: neglected key factors in the ecology of endotherms. *J Anim Ecol* 79:726–746
- Speakman JR, Racey PA (1987) The equilibrium concentration of oxygen-18 in body water: Implications for the accuracy of the doubly-labelled water technique and a potential new method of measuring RQ in free-living animals. *J Theor Biol* 127:79–95
- Speakman JR, Nagy KA, Masman D, Mook WG, Poppitt SD, Strathearn GE, Racey PA (1990) Interlaboratory comparison of different analytical techniques for the determination of oxygen-18 abundance. *Anal Chem* 62:703–708
- Speakman JR, Racey PA, Haim A, Webb PI, Ellison GTH, Skinner JD (1994) Interindividual and intraindividual variation in daily energy expenditure of the pouched mouse (*Saccostomus campestris*). *Funct Ecol* 8:336–342
- Speakman JR, Król E, Johnson MS (2004) The functional significance of individual variation in basal metabolic rate. *Physiol Biochem Zool* 77:900–915
- Szafranska PA, Zub K, Konarzewski M (2007) Long-term repeatability of body mass and resting metabolic rate in free-living weasels, *Mustela nivalis*. *Funct Ecol* 21:731–737
- Thompson CI, Brannon AJ, Heck AL (2003) Emotional fever after habituation to the temperature-recording procedure. *Physiol Behav* 80:103–108
- Van der Heyden JAM, Zethof TJJ, Olivier B (1997) Stress-induced hyperthermia in singly housed mice. *Physiol Behav* 62:463–470
- Vinkers CH, van Bogaert MJ, Klanker M, Korte SM, Oosting R, Hanania T, Hopkins SC, Olivier B, Groenink L (2008) Translational aspects of pharmacological research into anxiety disorders: the stress-induced hyperthermia (SIH) paradigm. *Eur J Pharmacol* 585:407–425
- Vinkers CH, Groenink L, van Bogaert MJV, Westphal KGC, Kalkman CJ, van Oorschoot R, Oosting RS, Olivier B, Korte SM (2009) Stress-induced hyperthermia and infection-induced fever: two of a kind? *Physiol Behav* 98:37–43
- Vinkers CH, Penning R, Ebbens MM, Hellhammer J, Verster JC, Kalkman CJ, Olivier B (2010) Stress-induced hyperthermia in translational stress research. *Open Pharmacol J* 4:30–35
- Visser GH, Schekkerman H (1999) Validation of the doubly labeled water method in growing precocial birds: the importance of assumptions concerning evaporative water loss. *Physiol Biochem Zool* 72:740–749

- Williams TD (2008) Individual variation in endocrine systems: moving beyond the “tyranny of the Golden Mean”. *Philos Trans Royal Soc Lond B* 363:1687–1698
- Wilson DS (1998) Adaptive individual differences within single populations. *Philos Trans Royal Soc Lond B* 353:199–205
- Yahner RH (1978) The adaptive nature of the social system and behavior in the Eastern chipmunk, *Tamias striatus*. *Behav Ecol Sociobiol* 3:397–427
- Yokoi Y (1966) Effect of ambient temperature upon emotional hyperthermia and hypothermia in rabbits. *J Appl Physiol* 21:1795–1798