Free-ranging eastern chipmunks (*Tamias striatus*) infected with bot fly (*Cuterebra emasculator*) larvae have higher resting but lower maximum metabolism

V. Careau, D. Garant, and M.M. Humphries

Abstract: Given the ubiquity and evolutionary importance of parasites, their effect on the energy budget of mammals remains surprisingly unclear. The eastern chipmunk (*Tamias striatus* (L., 1758)) is a burrowing rodent that is commonly infected by cuterebrid bot fly (*Cuterebra emasculator* Fitch, 1856) larvae. We measured resting metabolic rate (RMR) and cold-induced $V\mathrm{O}_2$-max (under heliox atmosphere) in 20 free-ranging individuals, of which 4 individuals were infected by one or two larva. We found that RMR was significantly higher in chipmunks infected by bot fly larvae (mean ± SE = 0.88 ± 0.05 W) than in uninfected individuals (0.74 ± 0.02 W). In contrast, $V\mathrm{O}_2$-max was significantly lower in chipmunks infected by bot fly larvae (4.96 ± 0.70 W) than in uninfected individuals (6.37 ± 0.16 W). Consequently, the aerobic scope (ratio of $V\mathrm{O}_2$-max to RMR) was negatively correlated with the number of bot fly larvae (infected individuals = 5.74 ± 1.03 W; noninfected individuals = 8.67 ± 0.26 W). Finally, after accounting for the effects of body mass and bot fly parasitism on RMR and $V\mathrm{O}_2$-max, there was no correlation between the two variables among individuals within our population. In addition to providing the first estimate of $V\mathrm{O}_2$-max in *T. striatus*, these results offer additional evidence that bot fly parasitism has significant impacts on the metabolic ecology of this host species.

Key words: aerobic scope, BMR, energetics, heliox, PMR, eastern chipmunk (*Tamias striatus*), summit metabolism, torpor.

Résumé : Malgré l'omniprésence et l'importance évolutive des parasites, notre compréhension demeure limitée quant à leurs impacts sur le budget énergétique des mammifères. Le tamia rayé (*Tamias striatus* (L., 1758)), un rongeur Sciuridé fouisseur, est communément infecté par les larves des mouches cutérébrides (*Cuterebra emasculator* Fitch, 1856). Nous avons mesuré le taux métabolique au repos (RMR) et maximal ($V\mathrm{O}_2$-max, induit par le froid dans une atmosphère héliox) de 20 tamias sauvages, dont quatre étaient infectés par une ou deux larves. Nous avons constaté que les tamias infectées par les larves parasites avaient un RMR significativement plus élevé (moyenne ± erreur type = 0,88 ± 0,05 W) que les individus non infectés (0,74 ± 0,02 W). Cependant, les tamias infectés par les larves avaient un $V\mathrm{O}_2$-max significativement plus bas (4,96 ± 0,70 W) que les individus non-infectés (6,37 ± 0,16 W). Par conséquent, le facteur aérobie (ratio de $V\mathrm{O}_2$-max sur RMR) était négativement corrélé au nombre de larves parasites (individus infectés : 5,74 ± 1,03 W; non infectés : 8,67 ± 0,26 W). Finalement, après la avoir pris en compte les effets de la masse corporelle et du parasitisme sur le RMR et le $V\mathrm{O}_2$-max, il n'y avait aucune corrélation entre les deux variables. En plus de documenter pour la première fois le $V\mathrm{O}_2$-max de *T. striatus*, nos résultats soulignent les effets importants qu'ont les larves des mouches cutérébrides sur l'éco-physiologie de cette espèce hôte.

Mots-clés : facteur aérobie, BMR, énergétique, héliox, PMR, tamia rayé (*Tamias striatus*), métabolisme maximal, torpeur.

Introduction

Given the ubiquity and evolutionary importance of parasites, it is surprising that their impacts on hosts' energy metabolism are still poorly understood (Degen 2006; Scantlebury et al. 2007). Parasites seem to impact the energy budget of their host in many different ways, from an effect to an absence of effect on resting metabolic rate (RMR), apparent dry matter digestibility, daily energy expenditure, and maximum metabolic rate ($V\mathrm{O}_2$-max) (Munger and Karasov 1994; Degen 2006; Scantlebury et al. 2007; Careau et al. 2010). This obvious complexity may arise from the fact that energy budgets are flexible: in response to an increase in energy devoted to immune system activation, animals can reduce another component of their budget (e.g., physical activity) to compensate (Degen 2006). This implies that the effects of parasites on host's energy metabolism are more likely to be detected in animals that are energetically constrained, such as during years of low food availability or for juveniles that must cope with the additional cost of growth

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(Careau et al. 2010). This flexibility also implies that measurements obtained from different components of the energy budget might differ, and therefore that measurement of multiple traits is required to better understand the impacts of parasites. For example, Meagher and O'Connor (2001) measured basal metabolic rate (BMR) and cold-induced V_{O_2}-max in two populations of deer mice (*Peromyscus maniculatus gracilis* (LeConte, 1855)) when infected by a nematode (*Capillaria hepatica* Bancroft, 1893). Although BMR was not affected by nematode infection, V_{O_2}-max of infected mice was significantly lower in one population, resulting in a lower metabolic scope (ratio of V_{O_2}-max to RMR or BMR).

Cuterebrid bot flies (Diptera; Cuterebridae), whose larval forms infect mammals, comprise one of the most-studied groups of insect parasites (Slansky 2007). Gravid bot flies typically lay eggs near burrow entrances of small mammals (Catts 1982). Eggs stick to the passing host and subsequently hatch in response to the host's body heat. Larvae enter the host through the mouth, nose, eyes, or a wound, migrate to the thoracic or abdominal regions where they develop in a subcutaneous capsule (a warble), breathing and excreting through a pore through the skin. The larvae grow to maturity (~1 g) in 4–10 weeks after which they exit their host to pupate in the ground (Catts 1982). Because bot flies commonly infect livestock, domestic animals, and sometimes humans, they have been studied for over 150 years (Slansky 2007).

The effects of bot fly parasitism on rodent metabolism have been studied only two times. In the first study on this topic, the effect of a bot fly larva on adult, captive white-footed mice (*Peromyscus leucopus* (Rafinesque, 1818)) metabolism was surprisingly small—the metabolic rate of infected individuals was only 3% greater than that of experimentally deparasitized mice (Munger and Karasov 1994). In a recent study on free-ranging eastern chipmunks (*Tamias striatus* (L., 1758)), we found no effect of bot fly parasitism on RMR in adults during 2 years of high food productivity (Careau et al. 2010). In juveniles, however, we found that RMR strongly increased with the number of bot fly larvae hosted—each bot fly larvae resulted in a ~7.6% increase in the RMR of its host. These studies highlight the importance of considering the ecological context when studying the cost of parasitism, as negative effects on hosts are more likely to emerge during periods of high energetic demand (e.g., growing juveniles) and (or) in harsh environmental conditions (e.g., low food availability).

Surviving winter in northern latitudes is challenging for small mammals because low food availability is combined with low ambient temperatures (T_a), which presumably requires low maintenance costs and high thermogenic capacity (Scholander 1955; Bartholomew 1964). On one hand, survival during food shortages should be enhanced by a longer fasting endurance associated with a low RMR (Mueller and Diamond 2001). On the other hand, a high RMR may enhance thermoregulatory abilities necessary for maintaining a constant body temperature (T_b) at low T_a (Rezende et al. 2004). Indeed, a positive functional relationship between RMR and maximum aerobic capacity is a key assumption of the aerobic capacity model (Bennett and Ruben 1979; Hayes and Garland 1995). Although this model posited positive selection on exercise-induced rather than cold-induced V_{O_2}-max, the two measures of maximum aerobic capacity appear to be positively related (Hayes and Chappell 1990; Chappell and Bachman 1995). Furthermore, whereas RMR and cold-induced V_{O_2}-max are generally positively correlated at the interspecific level in rodents (Koteja 1987; Bozinovic 1992; Hinds and Rice-Warner 1992; Rezende et al. 2004), support for such a relationship at the individual level is scarce and contradictory (Hayes 1989; Chappell and Bachman 1995).

In this study, we quantified RMR and cold-induced V_{O_2}-max in *T. striatus* in the northern part of the species range where individuals rely on torpor to survive the cold temperatures and food scarcity that persist throughout long winters (Munro et al. 2005; Landry-Cuerrier et al. 2008). Our initial objective was to provide the first estimate of cold-induced V_{O_2}-max in this species. Our second objective was to test whether individual variation in RMR, V_{O_2}-max, and metabolic scope (ratio of V_{O_2}-max to RMR) was correlated with the number of bot fly larvae hosted, after accounting for factors such as body mass (M_b) and age. Finally, we wanted to assess if RMR and V_{O_2}-max were positively correlated among individuals within our population.

Materials and methods

Study population and animals

From 29 September to 10 October 2009, we measured metabolic rate in 20 eastern chipmunks sampled from a population located on the Ruiter Valley Land Trust (Sutton Mountains, Quebec; 45°05′N, 72°26′W; supplementary Table S1[1]). This population was closely monitored since 2005 on a site that contained 228 Longworth traps distributed in a circular grid pattern over 25 ha of mature American beech (*Fagus grandifolia* Ehrh.) forest (Landry-Cuerrier et al. 2008; Munro et al. 2008). On their first capture, chipmunks were permanently marked with numbered ear tags (National Band and Tag Company 1005-1) and a Trovan® PIT tag inserted in the interscapular region. We sampled individuals of different ages, ranging from juvenile (age = 0, born in spring 2009) to adults of up to 4 years old (individuals first captured as juveniles in 2005). 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).

When analysing cold-induced V_{O_2}-max data available in rodents, Rezende et al. (2004) tested whether interspecific variation in metabolic rate was related to environmental characteristics such as maximum and minimum annual mean T_a, latitude, altitude, and precipitation. To facilitate the integration of our new estimate on chipmunks to this Rezende et al.'s (2004) compilation, we provide the environmental variables that corresponds to our new measurement on chipmunks using data from a nearby weather station (Environment Canada's Sutton Station (data available from http://climate.weatheroffice.gc.ca); 45°04′N, 72°41′W; altitude = 243.8m; situated at ~20 km from our site). Over the 5 years that preceded this study (from 2005 to 2009), the annual mean daily minimum and maximum T_a were 1.7 °C and 11.6 °C, and

[1]Supplementary Table S1 is available with the article through the journal Web site (http://nrcresearchpress.com/doi/suppl/10.1139/z2012-008).

annual precipitation as rain was 1092 mm. During the month prior to V_{O_2}-max sampling, the mean daily T_a recorded was 14 °C (range = 6.3–19.5 °C). The mean of the daily minimum and maximum were 8.3 °C (range = 0–15.5 °C) and 19.8 °C (range = 7.5–25.0 °C). During sampling, the mean daily T_a was 9.5 °C (range = 5.5–13.3 °C), whereas the mean of the daily minimum and maximum T_a were 6.0 °C (range = 3.5–8 °C) and 12.9 °C (range = 7.5–18.5 °C). During the 10 days following our sampling, T_a started to reach below freezing point regularly (daily minimum mean for 10 days after final sample = –2.3 °C, range = 5.5–1.5 °C). For three individuals with thermosensitive collars on our site, first torpor bouts occurred within the month following our study (date of first torpor bout, 23 October, 28 October, and 18 November; M.M. Humphries, unpublished data). These observations suggest that chipmunks were likely getting acclimatized to cold during sampling of V_{O_2}-max.

Four of the 20 sampled individuals (20%) had active bot fly infections, with three chipmunks hosting one bot fly larvae each, and one chipmunk hosting two larvae (Table S1).[1] The bot fly species specific for *T. striatus* is *Cuterebra emasculator* Fitch, 1856 (Bennett 1955). Once settled in a warble (an encapsulated pocket produced by the host in response to the presence of a bot fly in its subdermal tissue; Slansky 2007), bot fly larvae molt to a second instar that measure up to 11 mm in length, weigh from 0.01 to 0.1 g, and are greyish white (Bennett 1955). Mature third-instar larvae measure up to 25 mm, weigh, on average, 1.15 g (range = 1.00–1.30 g), and are dark brown (Bennett 1955). The larvae we detected on chipmunks were all third instars, which are obvious and easily detected during a visual inspection of small mammals (Lemaître et al. 2009).

The combination of a modest overall sample size for the study (n = 20) and a moderate prevalence of bot fly larvae (20%) resulted in only four individuals with bot fly larvae being included in the study. However, our ability to detect significant impacts of parasitism was enhanced by the fact that a limited number of potentially confounding variables were present in our data. Indeed, we compared this small sample of infected animals to a larger group of noninfected controls (n = 16), derived from the same, homogenous sample of wild-captured chipmunks, of known-age and gender, all captured and measured at the same location within a single 11-day period. The age, gender, and mass distributions of the infected chipmunks (50% one-year-olds and 50% three-year olds, 50% male, mean mass 88.5 g) were similar to the noninfected chipmunks (56% one-year-olds, 6% two-year-olds, 25% three-year olds, 25% male, mean mass 89.4 g).

Resting metabolic rate (RMR)

To minimize time spent in captivity, only those chipmunks captured after 1400 were transported in Longworth traps to a nearby (~1 km) laboratory facility at sunset. Because the capture success was low after 1400, on the last day of sampling we also kept individuals captured at 1200 to increase sample size. We measured O_2 consumption while chipmunks were resting using an open-circuit respirometry system modified from the system detailed in Careau et al. (2010). All RMR measurements were taken between 1730 and 0200 (Table S1),[1] a period over which metabolism and body temperature are relatively stable in eastern chipmunks (Wang and Hudson 1971; Levesque and Tattersall 2010). Previous studies on eastern chipmunks have shown that a clear metabolic burst (2–3.5× the RMR) usually occurs between 0400 and 0800 and is accompanied by an increase in body temperature of 1–3 °C (Wang and Hudson 1971; Levesque and Tattersall 2010).

For RMR, each individual was weighed and placed in a 650 mL Plexiglas cylindrical metabolic chamber. Chambers were placed in a constant-temperature cabinet regulated at 30 °C, which is thermoneutral for chipmunks (Wang and Hudson 1971). A manifold and two mass-flow meters (Sidetrack model 844; Sierra Instruments, Monterey, California, USA) provided a constant flow of 450 mL·min^{-1} of dry, CO_2-free air to each chamber, as well as to two baseline airflows. The outflows of each chamber and the two baselines were directed to a computer-controlled multiplexor, which allowed us to sequentially sample baselines and the chambers using two O_2 analyzers (model FC-1; Sable Systems International, Henderson, Nevada, USA). A 100 mL·min^{-1} subsample of baseline air or chamber outflow was dried and pulled through the O_2 analyzers without removing CO_2 (Koteja 1996). After 10 min of initial baseline recording, the system switched to chambers and recorded O_2 consumption for an hour, and a 10 min final baseline was recorded.

Resting state of each individual was confirmed by inspection of respirometry curves and observations of the animals themselves when opening the environmental cabinet at the end of the run. All chipmunks settled down in the chamber and were either sleeping, resting, or calm at the end of the run, except one that was grooming. Animals were provided with apple and peanut butter at all times other than when in metabolic chambers and they were released at their original trap location the following morning. This feeding, combined with the short period for which chipmunks were held in captivity, causes us to classify our metabolic measurements as thermoneutral RMR rather than basal metabolic rate (BMR).

Cold-induced V_{O_2}-max

Immediately after RMR measurements, we rapidly moved the metabolic chambers from 30 °C into a commercial freezer (model FFC0522GB1; Frigidaire) regulated at –20 °C. Owing to thermal inertia, air temperature within the freezer at the beginning of trials was –5 °C (ranged from –3 to –7 °C, measured with a thermocouple; model HH203A; Omega Engineering), but declined rapidly. At the end of trials, air temperature in the freezer was stable at –20.3 ± 3.3 °C.

As metabolic chambers were placed into the freezer, they were flushed with a mixture of helium and O_2 (heliox, 19.51% O_2). Cold-exposure into a heliox atmosphere is a widely used method to measure the thermogenic capacity of wild endotherms, as this protocol has the advantages that it does not require the repetitive training, which is impractical for sampling numerous wild individuals. Because the thermal conductivity of helium is ~6× greater than that of nitrogen, it is possible to elicit maximum thermogenesis at moderate temperatures to reduce the risk of peripheral cold injuries (Rosenmann and Morrison 1974; Thomas et al. 1998). Wang and Hudson (1971) reported that the thermal conductance in eastern chipmunks was 0.120 mL O_2·g^{-1}·h^{-1}·°C. Assuming that our chipmunks had a similar thermal conductance, and

that a 90 g chipmunk has a surface of 204 cm^2 (i.e., = $10 \cdot M_b^{0.67}$, in g; Schmidt-Nielsen 1984), then the thermal conductance under heliox atmosphere can be predicted to be ~6.82 $W \cdot m^2 \cdot °C$ (following Thomas et al. 1998), which is 2.3× higher than in air. Assuming a normal T_b of 39.3 °C (Careau et al. 2012), the "functional" temperature that chipmunks experienced when exposed to heliox at –20 °C can be estimated at –98 °C (using the following equation: $T_b - (T_b - T_a) \times 2.3$). However, T_a inside chambers was likely higher than –20 °C. Extrapolating the slope of thermal conductance reported by Wang and Hudson (1971), suggests the mean V_{O_2}-max observed in our study (see Results) would be elicited at –63.5 °C in normal air.

Flow rate was maintained constant with air-calibrated mass-flow controllers (Side-track model 844; Sierra Instruments, Monterey, California, USA) set to maximum capacity. Because the mass-flow meters were calibrated with air, their flow rate of 818 and 856 $mL \cdot min^{-1}$ was converted to 1056 and 1105 $mL \cdot min^{-1}$, respectively, using a heliox equivalent flow of 1.291 calculated from the charts accompanying the Sierra Instruments. Our heliox mixture was slightly hypoxic (19.51% O_2) and at the time V_{O_2}-max was reached (see below), the O_2 concentration averaged 18.13% ± 0.06% (range = 17.83%–18.79%). Although this may have reduced aerobic capacity of individuals, eastern chipmunks and fossorial mammals in general are less sensitive to hypoxic and hypercapnic conditions as O_2 concentration may be as low as 10% and CO_2 concentration can exceed 10% in burrow atmospheres (Williams and Rausch 1973; Kuhnen 1986; Levesque and Tattersall 2009).

We started V_{O_2}-max trials by recording baseline for 5 min while heliox replaced normal air in the chambers, and then switched to record O_2 concentration in the chambers. As temperature decreased, metabolic rate increased consistently during the first 30 min, reached a plateau, and started to decrease steadily (Fig. 1). We continued the tests until the animal's metabolic rate was obviously declining (see Fig. 1). Runs lasted 90 ± 20 min (range = 33–117 min), but V_{O_2}-max (defined as the highest continuous 10 min, see below) was usually reached after 50 ± 2.7 min (range = 17–64 min). Immediately after the conclusion of the test, we measured rectal temperatures with a thermocouple probe inserted 1 cm deep into the rectum (model HH203A; Omega Engineering). The mean T_b at the end of trials was 29.7 ± 0.6 °C (range = 24.0–35.1 °C), indicating that chipmunks were hypothermic. By running up to three cycles (RMR then V_{O_2}-max) between 1730 and 0400, we were able to measure up to six animals each night.

We used the Expedata software (Sable Systems) to read chamber O_2 concentration (at 1 s intervals), correct for drift between consecutive baseline measures, and to calculate individual O_2 consumption according to eq. 4*a* of Withers (1977). We did not scrub the chamber outflows of CO_2 and assumed a RQ of 0.8 to calculate O_2 consumption and convert data from mL $O_2 \cdot min^{-1}$ to Watts (W) (Koteja 1996; Speakman 2000). RMR was calculated from the lowest average of continuous O_2 consumption recorded for 5 min (i.e., 300 one-second samples, using "nadir" function in Expedata). V_{O_2}-max was calculated from the highest level O_2 consumption sustained over 10 min under heliox atmosphere (i.e., 600 one-second samples, using "zenith" function in Expedata). Chipmunks were active during cold exposure, which probably effectively mixed air within the chamber. However, this activity may have created the pattern of spikes and dips in the metabolic traces (see Fig. 1), as the animal may have had its nose adjacent to the outflow port. We thus chose to calculate V_{O_2}-max over 10 min instead of 1 min to avoid including artefacts resulting from movements in our measure. We did not apply the instantaneous correction (Bartholomew et al. 1981) to our metabolic curves because the calculated time constant of our system (~35 s) was short relative to the period over which V_{O_2}-max was averaged (10 min). Metabolic scope was calculated as the ratio of V_{O_2}-max to RMR. The time required to elicit V_{O_2}-max was calculated as the number of 1 s samples between the first samples (beginning of the test) and the samples at which V_{O_2}-max was measured.

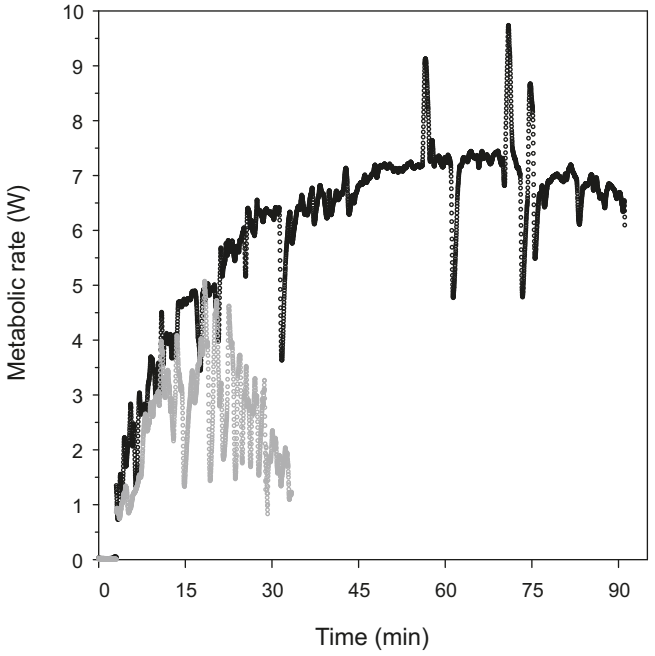


Fig. 1. Two extreme examples of temporal changes metabolic rate (W) of free-ranging eastern chipmunks (*Tamias striatus*) during cold-exposure in a heliox atmosphere. At time zero, chipmunks were transferred from their thermoneutral zone (30 °C) into cold (–20 °C) while air was flushed with heliox. Each circle represents a 1 s sample. After only ~15 min, the metabolic rate of the individual depicted by the grey circles (infected by one bot fly larvae) already reached its maximum at 3.2 W. In contrast, another individual (black circles; not infected by bot fly larvae) withstood the challenge for over 60 min and expressed twice as high thermogenic capacity (7.4 W).

Statistical analysis

We used multiple linear regressions (lm function in R version 2.13.1; R Foundation for Statistical Computing, Vienna, Austria) to test the effect of age, sex, time of day, Julian day, and number of bot fly larvae on M_b, RMR, V_{O_2}-max, and metabolic scope. We sequentially removed the least significant term from the model based on its P value until only significant effects (i.e., $P < 0.05$) remained in the model (i.e., backward procedure). We included M_b as a covariate in the models of RMR, V_{O_2}-max, and metabolic scope, thus our

Table 1. Descriptive statistics for body mass (M_b), resting metabolic rate (RMR), cold-induced maximum metabolic rate (V_{O_2}-max), and metabolic scope (ratio of V_{O_2}-max to RMR) in free-ranging eastern chipmunks (*Tamias striatus*) that were not infected ($n = 16$) or infected by one or two bot fly (*Cuterebra emasculator*) larva ($n = 4$).

		Descriptive statistics									
		Noninfected					Infected				
Traits	Units	Mean	SD	Min.	Max.	CV	Mean	SD	Min.	Max.	CV
M_b	g	89.35	5.25	74.89	97.03	0.06	88.52	7.14	84.28	99.18	0.08
RMR	W	0.739	0.085	0.608	0.901	0.12	0.883	0.101	0.742	0.984	0.11
V_{O_2}-max	W	6.37	0.63	5.53	7.44	0.10	4.96	1.39	3.21	6.15	0.28
Scope	ratio	8.69	1.05	6.90	10.84	0.12	5.74	2.05	3.57	8.08	0.36

Note: Min., minimum; Max., maximum; CV, coefficient of variation.

results should be interpreted on a mass-residual basis, as M_b was retained in the model if it was significant. Residuals from all models were normally distributed (Shapiro–Wilks' test: $W > 0.958$, $P > 0.52$). We used Pearson correlations to test the relationships among M_b and metabolic variables (RMR and V_{O_2}-max).

We tested the significance of the relationship between mass-residual V_{O_2}-max and RMR using a bivariate model in ASReml-R (Butler et al. 2007). This approach allows the estimation of the correlation between mass-residual traits in a one step process, which is more conservative than a two-step process where residuals are first calculated and then used for testing for the correlation. We ran a bivariate model in which M_b was fitted as a fixed effect to account for its effect on both variables. In this model, we also fitted a covariance term between the residuals (COV_{res}). Therefore, the correlation between mass-residual V_{O_2}-max and RMR can be calculated as the COV_{res} term divided by the square root of the product of the variance of residuals. We tested the significance of COV_{res} using a log-likelihood ratio test (LRT) that compares the log-likelihoods of a "full" model that includes the COV_{res} component versus a "reduced" model with the COV_{res} constrained to zero (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 1 df. We also ran another bivariate model with number of bot fly larvae fitted as a fixed effect (in addition to M_b) to test if the association between mass-residual V_{O_2}-max and RMR could be due to their mutual links with bot fly parasitism (see below). Means and COV estimates are presented with ±SE.

Results

M_b and RMR

The mean M_b in our sample was 89.6 ± 1.3 g (see Table 1 for complete descriptive statistics calculated separately for noninfected and infected individuals). M_b did not differ between sexes ($t_{[15]} = –0.16$, $P = 0.88$) and was not correlated with Julian day ($t_{[16]} = –0.42$, $P = 0.68$), number of bot fly larvae ($t_{[17]} = 0.74$, $P = 0.47$), or age ($t_{[18]} = 0.85$, $P = 0.41$).

The mean RMR in our sample was 0.768 ± 0.023 W (see Table 1 for complete descriptive statistics calculated separately for noninfected and infected individuals). The final model explained 67% of variance in RMR and included a significant effect of M_b (Table 2, Fig. 2*a*). Mass-independent RMR was not influenced by age, sex, or time of day (Table 2). Mass-independent RMR decreased with Julian day and increased with the number of bot fly larvae (Table 2, Fig. 3A). The correlation coefficient (r) between RMR and number of bot fly larvae was –0.62 ($P = 0.004$, estimated on raw data). The effect of bot fly parasitism on RMR was marginally nonsignificant after excluding the individual infected with two larvae ($t_{[15]} = 1.85$, $P = 0.085$).

Cold-induced V_{O_2}-max

The mean V_{O_2}-max in our sample was 6.08 ± 0.22 W (see Table 1 for complete descriptive statistics calculated separately for noninfected and infected individuals). The time required to elicit V_{O_2}-max was positively correlated with M_b ($r = 0.48$, $P = 0.031$) and V_{O_2}-max itself ($r = 0.77$, $P < 0.001$), suggesting that larger individuals with greater thermogenic capacity were able to withstand the cold challenge over a longer period.

The final model explained 52% of variance in V_{O_2}-max and included a significant effect of M_b (Table 2, Fig. 2*a*). Similarly to our RMR results, mass-independent V_{O_2}-max was not correlated with age, sex, or time of day (Table 2). In contrast to RMR results, however, mass-independent V_{O_2}-max did not vary with Julian day and was negatively correlated to the number of bot fly larvae (Table 2, Fig. 3*b*). The correlation coefficient (r) between V_{O_2}-max and number of bot fly larvae was –0.60 ($P = 0.005$, estimated on raw data). The effect of bot fly parasitism on V_{O_2}-max was still significant after excluding the individual infected with two larvae ($t_{[16]} = –2.86$, $P = 0.011$).

Metabolic scope

The mean metabolic scope in our sample was 8.1 ± 0.4 (see Table 1 for complete descriptive statistics calculated separately for noninfected and infected individuals). The final model explained 65% of variance in metabolic scope and included a significant effect of Julian day (Table 2). Metabolic scope was not correlated with sex, age, M_b, or time of day (Table 2). Metabolic scope was negatively related to the number of bot fly larvae (Table 2; Fig. 3C). The correlation coefficient (r) between metabolic scope and number of bot fly larvae was –0.72 ($P < 0.001$, estimated on raw data). The effect of bot fly parasitism on metabolic scope was still significant after excluding the individual infected with two larvae ($t_{[16]} = –2.82$, $P = 0.012$).

Correlation between V_{O_2}-max and RMR

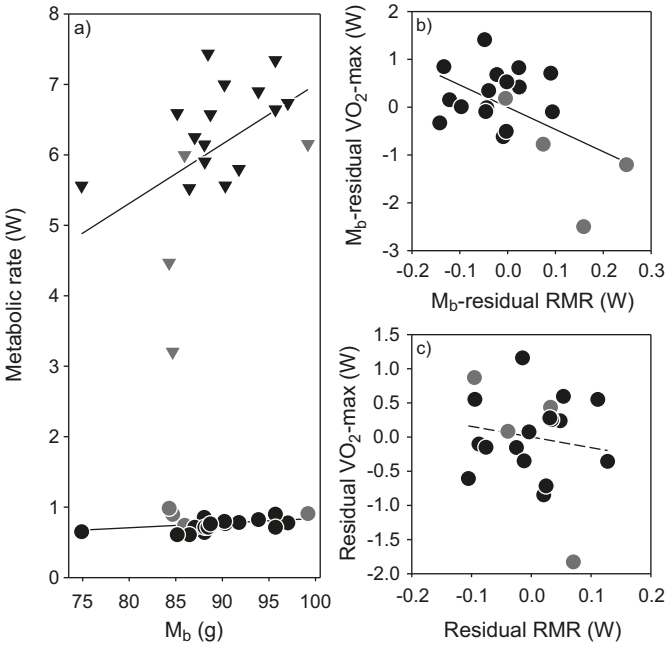
On a whole-animal basis, V_{O_2}-max and RMR were not significantly correlated ($r = –0.27$, $P = 0.25$). In the bivariate

Table 2. Statistical values obtained from multiple regression models of resting metabolic rate (RMR), cold-induced maximum metabolic rate (V_{O_2}-max), and metabolic scope (ratio of V_{O_2}-max to RMR) as a function of body mass (M_b), Julian day, age, sex, time of day, and number of bot fly (*Cuterebra emasculator*) larvae hosted in free-ranging eastern chipmunks (*Tamias striatus*).

	RMR				V_{O_2}-max				Scope			
	Estimate ± SE	*t*	df	*P*	Estimate ± SE	*t*	df	*P*	Estimate ± SE	*t*	df	*P*
M_b	**0.008±0.003**	**2.74**	**16**	**0.014**	**0.071±0.030**	**2.36**	**17**	**0.031**	0.016±0.053	0.30	15	0.766
Julian day	**–0.007±0.003**	**–2.16**	**16**	**0.046**	0.042±0.038	1.11	16	0.282	**0.144±0.058**	**2.50**	**17**	**0.023**
Age	0.006±0.016	0.36	14	0.722	0.031±0.177	0.18	14	0.863	–0.025±0.279	–0.09	14	0.931
Sex_{Female}	–0.001±0.023	–0.03	13	0.976	0.018±0.251	0.07	13	0.945	0.005±0.396	0.01	13	0.990
Time of day	0.026±0.055	0.48	15	0.638	–0.269±0.597	–0.45	16	0.658	–0.387±0.846	–0.46	16	0.654
Bot fly	**0.109±0.029**	**3.79**	**16**	**0.002**	**–0.973±0.301**	**–3.23**	**17**	**0.005**	**–1.907±0.475**	**–4.01**	**17**	**0.001**

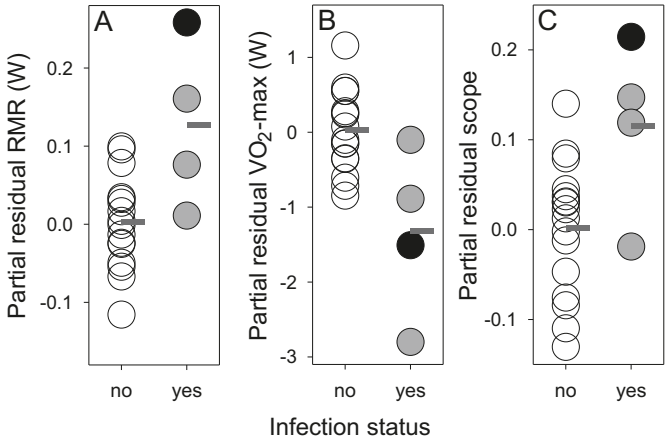
Note: Values in boldfaced type are statistically significant ($P < 0.05$). Statistics for nonsignificant terms are based on values obtained prior to being removed from the model.

Fig. 2. (*a*) Resting (circles) and maximum (triangles) metabolic rate in relation to body mass (M_b) in 20 free-ranging eastern chipmunks (*Tamias striatus*) during autumn 2009. In *b*, the relationship between maximum metabolic rate (V_{O_2}-max) and resting metabolic rate (RMR) is shown after both variables are corrected for M_b (residuals from a linear model including M_b), whereas in *c*, both variables are corrected for M_b in addition to the number of parasites (residuals from a linear model including M_b and number bot fly larvae). Individuals infected with one or two bot fly (*Cuterebra emasculator*) larva are shown in grey.



model controlling for M_b, however, the COV_{res} term was significant (COV_{res} = –0.0468 ± 0.0006, LRT χ^2 = 5.88, P = 0.015). This translated in a negative correlation between mass-residual V_{O_2}-max and RMR (r = –0.53; Fig. 2*b*). Since bot fly larvae simultaneously increased mass-residual RMR and decreased V_{O_2}-max (see above), we further assessed if this factor was responsible for the negative correlation obtained. Indeed, when adding number of bot fly larvae as a fixed effect in the bivariate model, the COV_{res} term was no longer significant (COV_{res} = –0.0080 ± 0.0002, LRT χ^2 = 0.42, P = 0.52) and the correlation was still negative but much weaker (r = –0.16; Fig. 2*c*).

Fig. 3. Partial residuals of (A) resting metabolic rate (RMR), (B) cold-induced maximum metabolic rate (V_{O_2}-max), and (C) metabolic scope (ratio of V_{O_2}-max to RMR) as function of infection status in 20 free-ranging eastern chipmunks (*Tamias striatus*). Individuals infected with one or two bot fly (*Cuterebra emasculator*) larva are shown in grey and black, respectively. Grey lines indicate the mean value in each category.



Discussion

This study provides the first quantification of V_{O_2}-max in eastern chipmunks and reports that individuals infected with bot fly larvae were doubly penalized, as they had simultaneously higher RMR but lower V_{O_2}-max, which resulted in a lower metabolic scope. Given only four infected individuals were included in our analysis, these results need to be interpreted with caution and should be replicated on a larger sample size in the future. Nevertheless, these four individuals were sufficiently divergent from noninfected individuals in metabolic traits (yet similar in age, mass, etc.) to suggest robust metabolic impacts of bot fly parasitism in chipmunks that are consistent with and extend beyond those reported by Careau et al. (2010).

The cold-induced V_{O_2}-max averaged 12.75 mL $O_2 \cdot g^{-1} \cdot h^{-1}$ in noninfected individuals, which is 103% higher than predicted, on average, for rodents (6.28 mL $O_2 \cdot g^{-1} \cdot h^{-1}$; Rezende et al. 2004). The RMR measured in the same individuals averaged 1.57 mL $O_2 \cdot g^{-1} \cdot h^{-1}$, which is also above the predicted line (44% higher, predicted = 1.09 mL $O_2 \cdot g^{-1} \cdot h^{-1}$). Our data are consistent with previous comparative studies, indicating that rodent species with higher-than-average V_{O_2}-max

have higher-than-average RMR (Koteja 1987; Bozinovic 1992; Hinds and Rice-Warner 1992; Rezende et al. 2004). Support for such a relationship at the individual level, however, is contradictory. Hayes (1989) first reported a significant positive correlation between $V\dot{O}_2$-max and RMR in deer mice (*Peromyscus maniculatus* (Wagner, 1845)), whereas Chappell and Bachman (1995) did not find such an association in Belding's ground squirrels (*Spermophilus beldingi* Merriam, 1888). In our study, we found no association between $V\dot{O}_2$-max and RMR among individuals within our population after controlling for M_b and parasites, perhaps because winter survival simultaneously requires a low RMR and high thermogenic capacities.

The metabolic scope of noninfected individuals (8.7) falls near the highest ever found in experiments measuring cold-induced $V\dot{O}_2$-max in rodents (mean ±SE = 5.9 ± 0.2, range = 3.0–10.1; Rezende et al. 2004). These numbers suggest the presence of (past) selective forces that led to the evolution of high metabolic rates and scope in *T. striatus*. An alternative explanation is that most data included in Rezende et al. (2004) were collected on summer-acclimatized animals, whereas we collected our data in autumn, about a month prior to torpor. It is also interesting to note that metabolic scope increased with Julian day in our population. During preliminary trials conducted during summer (on 15 and 16 June), the metabolic scope was 6.85 ± 0.25 (n = 4), which is 21% lower than in autumn, suggesting that some level of metabolic plasticity exists in preparation for winter.

The effect of bot fly larvae on $V\dot{O}_2$-max and metabolic scope suggests that parasitism is an important aspect of the metabolic ecology of chipmunks (Careau et al. 2010) and possibly other rodents infected by cuterebrid bot fly larvae (but see Munger and Karasov 1994). The correlations observed, however, are not evidence of cause and effect because it was impossible to determine if bot fly infection resulted in low $V\dot{O}_2$-max or vice versa. In a previous study, we showed that individuals with a high RMR in mid-summer were not predisposed to higher bot fly loads during autumn (Careau et al. 2010). In this case, each individual served as its own control because we compared RMR before and during bot fly infection, suggesting that the correlation between RMR and bot fly parasitism was not a sampling artefact, but rather reflects direct and (or) indirect metabolic costs of bot fly parasitism. In the absence of a before versus during comparison, or more rigorous experimental tests, the causality of the relationship between parasites and $V\dot{O}_2$-max or metabolic scope cannot be confirmed, as the possibility remains that individuals with low $V\dot{O}_2$-max or metabolic scope are more susceptible to develop infections by bot fly larvae.

Although we previously reported that RMR was higher in juveniles infected with bot fly larvae, we found no effect of bot fly parasitism on RMR in adults in this previous study (Careau et al. 2010). In the present study, we detected an effect of bot fly parasitism on RMR in a sample mostly constituted of adults (95%). One possible reason for this discrepancy is that our initial study was restricted to mast years (2006 and 2008) with high food availability and did not include measurements on adults during a nonmast year (2007; because adults ceased activity before the parasite season). In the present study conducted in 2009, the productivity of the main masting tree on our site (American beech) was very low (Bergeron et al. 2011). Yet adults were still active above ground during the parasite season and we detected an effect of bot fly parasitism on RMR. Our results, in combination with previous studies on bot fly parasitism (Munger and Karasov 1994; Careau et al. 2010), reinforce the idea that negative effects on hosts are more likely to emerge in periods of high energetic demand (e.g., growing juveniles) and (or) in harsh environmental conditions (e.g., low food availability).

Although the duration of the detrimental effect of bot fly parasitism on $V\dot{O}_2$-max is unknown, the effect of bot fly larvae on RMR in juvenile chipmunks can persist to adulthood (Careau et al. 2010). If the bot fly associated increase in RMR and reduction in $V\dot{O}_2$-max last throughout the winter (even though larvae have left their host), then it may potentially alter torpor expression. Indeed, one of the critical phases during torpor is the arousal when warming up to normothermic levels requires extreme elevation of metabolism. Two previous studies on eastern chipmunks reported that metabolic rate during torpor arousals can reach up to 10.2 mL $O_2 \cdot g^{-1} \cdot h^{-1}$ or 5.1 W for a 90 g mammal (Wang and Hudson 1971) and 14.0 mL $O_2 \cdot g^{-1} \cdot h^{-1}$ or 7.0 W for a 90 g mammal (D. Levesque, personal communication). These numbers, which are close to the mean $V\dot{O}_2$-max reported here, suggest that chipmunks need a large thermogenic capacity to warm up from torpid to normothermic body temperature. Given that torpor is a physiological adaptation aimed at conserving energy but requires high thermogenic capacity during arousals, having a high metabolic scope may be doubly advantageous, as it combines high thermogenic capacity ($V\dot{O}_2$-max) and low maintenance costs (RMR). This could explain why metabolic scope increased with Julian day in the month prior to torpor use. Interestingly, bot fly parasitism exerts a negative effect on a host's winter survival in our population (V. Careau, unpublished data), which may be linked to a reduction in $V\dot{O}_2$-max and (or) metabolic scope.

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