Rigid Allometries of Metabolic Costs and Aerobic Capacity in a Seasonal Environment

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Resumo

Energia é um recurso fundamental, necessário para o funcionamento de todos os processos nos organismos. A taxa de absorção, transformação e acumulação de energia no corpo afetam o desempenho e a aptidão dos animais. A composição corporal (contribuições relativas de, por exemplo, músculo e gordura), que pode mudar sazonalmente, afeta o nível da taxa metabólica e o seu escalonamento com a massa corporal, a relação entre a taxa metabólica e o tamanho do corpo. Espera-se que a taxa de metabolismo varie ao longo do tempo em relação às flutuações na disponibilidade de alimentos (energia) em ambientes sazonais. Utilizando uma população selvagem de ratos (*Apodemus sylvaticus*), investigamos a variação sazonal dos limites do processamento de energia ao nível do organismo: custos de manutenção (taxa metabólica basal, BMR) e capacidade aeróbica (taxa metabólica máxima, VO_2max). Com novos dados experimentais, testámos a hipótese de que, durante a época reprodutiva, quando os animais recolhem mais energia para afetar à reprodução, têm um nível metabólico mais elevado.

Palavras-chave: Alometria Intraespecífica, Respirometria, Reprodutibilidade, Sazonalidade, Metabolismo, Taxa Metabólica Máxima, Taxa Metabólica Basal.

Abstract

Energy is a fundamental resource, necessary to run all processes in organisms. The rate of energy absorption, transformation and build up to the body affect animal performance and fitness. Body composition (relative contributions of e.g. muscle and fat) that seasonally may change affects the level of metabolic rate and its scaling with body mass, the relationship between metabolic rate and body size. The rate of metabolism is expected to vary over time in relation to fluctuations in food (energy) availability in seasonal environments. Using a wild population of mice (*Apodemus sylvaticus*) here we investigated seasonal variation in limits to organismal level energy processing: maintenance costs (basal metabolic rate, BMR) and aerobic capacity (maximum metabolic rate, VO_2max). With novel experimental data we tested the hypothesis that during the reproductive season, when animals collect more energy to allocate to reproduction, they have higher metabolic levels.

Keywords: Intraspecific Allometry, Respirometry, Repeatability, Seasonality, Metabolism, Maximum Metabolic Rate, Basal Metabolic Rate.

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List of Abbreviations

FCUP	FACULTY OF SCIENCES OF THE UNIVERSITY OF PORTO
UP	UNIVERSITY OF PORTO
MR	METABOLIC RATE
VO_2MAX	MAXIMUM METABOLIC RATE
BMR	BASAL METABOLIC RATE
M_{SUM}	SUMMIT VO_2MAX
FMS	FIELD METABOLIC SYSTEM

1. Introduction

Metabolism is a set of fundamental biological processes that underlie all life functions. It can refer to the sum of chemical reactions that occur in living beings that convert food into usable forms of energy that can be used to build the basic necessary building blocks such as proteins. Metabolic rate (MR) is the rate of energy used by an organism per unit of time, or more specifically the rate at which an organism expends energy to carry out all life functions, such as breathing, digestion, and maintaining body temperature, but also running and reproducing (Speakman, 2005). It reflects how quickly an organism converts food into energy that can vary based on factors like size, age, activity level, and environmental conditions, among others (White & Seymour, 2003). Basal metabolic rate (henceforth referred to as BMR), or maintenance metabolism, is the minimum energy required to maintain vital physiological functions in endothermic animals at rest, excluding periods of hibernation or torpor (Hosken, 2003). It supports essential processes such as respiration, circulation, and cell maintenance. Maximum metabolic rate (MMR or VO_2max), or aerobic capacity, is an organism's ability to upregulate energy turnover to invest in other than basic functions. VO_2max is associated with the maximal rate at which O_2 can be transported from the environment to the tissue mitochondria, potentially allocating excess energy to fitness-related functions (Painter, 2005). Maximum metabolic rate may refer to one of two things: exercise-induced maximum metabolic rate (which will be referred here solely as VO_2max), the highest metabolic rate achieved while running, jumping, swimming or otherwise exercising, and thermogenic VO_2max or summit metabolic rate (summit MR or Msum), which is the highest metabolic rate achieved in response to cold temperatures, usually as a result of responses such as shivering. In endotherms VO_2max tends to be higher than Msum (Swanson & Garland, 2009).

Seasonality plays a crucial role in shaping animal physiology (Arnold, 2020). In temperate regions, many small mammal species exhibit pronounced seasonal variations in their life history traits, including reproduction, growth, and survival. This creates the opportunity of selection to operate on life history optimization, related to energy turnover, resulting in specific adaptations. These adaptations are often linked to changes in food availability, predation pressure, and environmental conditions across seasons (Shingleton, 2010). During colder seasons, food becomes scarcer, which creates a trade-off for small mammals between energy allocation to thermoregulation and maintaining body functions (BMR) or reproduction, in conditions with depleted resources (Zub et al., 2009). The seasonal environmental changes may influence basal metabolic

rate and aerobic capacity of mammals, with research showing that these animals exhibit physiological acclimation in response to the environmental changes associated with seasonality (Corp et al., 1997). Studies on mice, *Apodemus sylvaticus*, have shown that individuals that were acclimatized to winter and colder temperatures displayed reduced resting metabolic rate, compared to mice acclimatized to warmer, summer environments (Corp et al., 1997).

Allometric studies began in the early 20th century with Julian Huxley's work on relative growth, proposing that biological traits scale differently with size (Huxley, 1932). A relevant example is how metabolic rate scales with body size according to allometric relationships (scaling coefficient < 1). Allometry is the study of the relationship between the size of an organism and its biological traits, such as metabolism (and metabolic rate), lifespan and general fitness (White & Kearney, 2014), often following allometric scaling power laws, such as Kleiber's 3/4 power law (Kleiber, 1932), which states that metabolic rate scales with body mass to the 3/4 power. This pattern was believed to reflect constraints in energy distribution, O_2 transport, and resource use, influencing growth, reproduction, and ecological interactions (Ballesteros et al., 2018). The study of allometry helps explain such constraints, energy allocation, and ecological interactions across species.

However, recent studies have challenged this view, suggesting that metabolic scaling can be plastic and influenced by various factors including temperature, diet, and activity level. Studies on evolutionary life history theory explore how natural selection shapes the timing and allocation of energy to different life stages based on ecological and evolutionary pressures. It posits that organisms must balance trade-offs between investing energy in reproduction versus survival, as well as timing reproduction to maximize fitness (Glazier, 2005).

Intraspecific allometry is the relationship in scaling between body size and morphological, physiological, or other traits within a single species. Variation in size influences functional traits, often through the aforementioned power-law relationships that describe disproportionate changes in traits relative to body size (Glazier, 2005). Reflecting the underlying physiological constraints, these patterns allow us to interpret how organisms grow, allocate resources, and adapt to environmental pressures. The allometries we focused on in this study was the relationship between basal metabolic rate and body mass as well as maximum metabolic rate and body mass.

There are still, however, some gaps in our understanding of how specifically seasonality interacts with the metabolic rates of the mice and relates to their allometries, specifically

how seasonal factors influence the relationship (allometry) between metabolic rate and body mass. Larger individuals tend to have higher total metabolic rates but use less energy when counted per volume of body mass (Schmidt-Nielsen, 1984). It is generally believed that genetic and functional constraints determine that relationship, but possibly also environmental pressures may alter that pattern. There is the possibility of these seasonal pressures overriding the genetic ones, as environmental "cues" such as temperature and photoperiod changes may induce modifications to the expected scaling of metabolic rate with body mass (Konarzewski, 2013).

In this thesis I investigate how the level and scaling of metabolic costs and aerobic capacity vary seasonally, and how its relation to body size changes across different seasons in *Apodemus sylvaticus*. We expect significant relationships between overall weight and metabolic rates, with larger individuals having overall higher levels of both basal and maximum metabolic rates levels. Additionally, we anticipate seasonal differences in metabolic rates, expecting higher rates in more active seasons, i.e. like summer. I also hypothesize that environmental factors may override genetic and functional constraints on these relationships between metabolic rate and animal weight, resulting in varying allometric scaling. It is expected that scaling between BMR and body mass as well as between VO_2max and body mass will be steeper during summer due to exposure to stronger energetic trade-offs during reproduction. Alternatively, thermoregulatory costs during winter may impact these restraints. By investigating these aspects across multiple seasons, we aim to contribute to our understanding of how metabolic costs and aerobic capacity adapt to changing environmental conditions in small mammal species.

2. Materials and Methods

2.1. Study Organism and Area

Our study species is *Apodemus sylvaticus*, also commonly known as wood mouse, nocturnal rodents of the Muridae family found in Europe from the south-west all the way north to south-west Scandinavia and east to Greece throughout Ukraine, as well as in some parts of North Africa and many Mediterranean islands. This animal is usually readily available and easy to capture as it populates greenlands, forests, cultivated land as well as other spaces with vegetation. We studied a population from Quinta do Corgo forest (41.340; -8.8690), Vila do Conde, Portugal. In Figure 1 we can see an *Apodemus sylvaticus* individual as well as the entrance to the forest in Quinta do Corgo.



Figure 1. Entrance to our study area, Quinta do Corgo forest and our study species, *Apodemus sylvaticus*. [Google Maps photo of Quinta do Corgo forest]. Retrieved May 4, 2025, from https://maps.app.goo.gl/MY6VqHVSNNPokL2s6. *Apodemus sylvaticus* Photograph: © naturpel / CC BY-NC 4.0 https://creativecommons.org/licenses/by-nc/4.0/); source: BioDiversity4All via iNaturalist.org.

To start the project, we went into the forest and marked roughly square 40000 m^2 (200 m by 200 m square) area with one hundred points, distanced every 20 m, permanently marked with a stick and georeferenced in Gaia GPS (TrailBehind Inc., 2025) and Androzic (Novikov, 2016), two GPS softwares that allowed us to register and save grids for future reference during sample collections as shown in Figure 2.

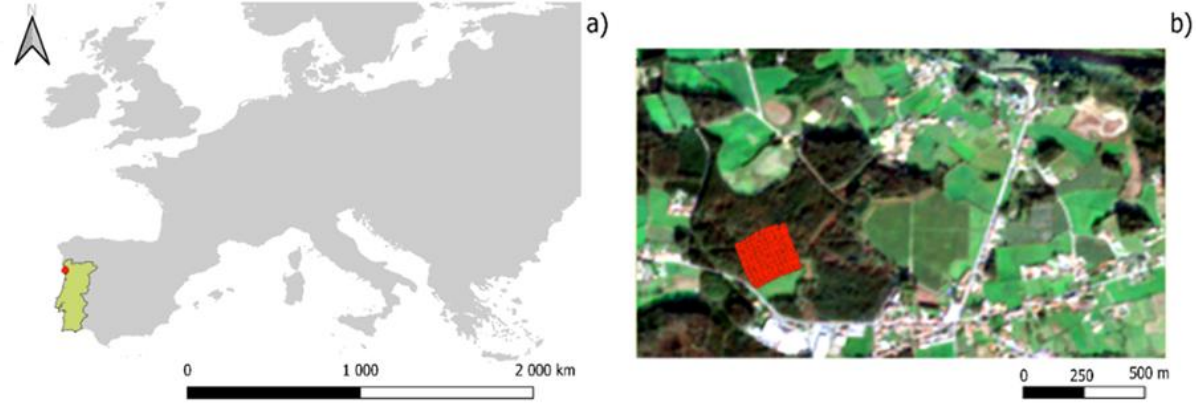


Figure 2. Map of Europe (a), and the study area in Quinta do Corgo forest (b). Last image acquired from Copernicus Sentinel Data (2025), Sentinel-2 Quarterly Mosaics, True Colour Cloudless, European Space Agency. Available at: htCloudless,er.dataspace.copernicus.eu/

We conducted experiments in four main sessions: from 16th to 26th of April (spring), 8th to 18th of July (summer), 1st to 13th of October (Autumn) and from 13th to 23rd of January (Winter). Later, during March 2025 we did one additional session. Temperature

in our study area during each experimental month was recorded with loggers placed in different positions (ground level, 1 m underground in burrows and 2 m above ground) and different habitats (oak, open field and eucalyptus).

Three days before each trapping session, trapping locations were pre-baited with sunflower seed. The following step was to start placing two mouse traps (Sherman foldable live traps, baited with sunflower seeds) beside each marked spot along four lines, for a total of 80 traps (4 full lines at the same time) each trapping day. This is done every evening for 10 to 12 days of each trapping season. In the morning following each trap placement session, we retrieve the traps and check for mice and their condition, such as if they are young or adult (by checking main age indicators, such as size and fur colour), male or female and, if they are female, check for pregnancy. No pregnant females and young animals were included in the experiment. After retrieving the traps and mice we brought them to the lab (Association BIOPOLIS; ~2 km distance from the forest) and began the maximum metabolic rate testing. Individuals recaptured within the same season were only taken for re-testing if they had been back in the wild for at least three days since first caught.

2.2. Metabolic Rates

Metabolic rate testing can be separated in 2 clear steps: VO_2max testing and BMR testing. This workflow can be seen represented in Figure 3.

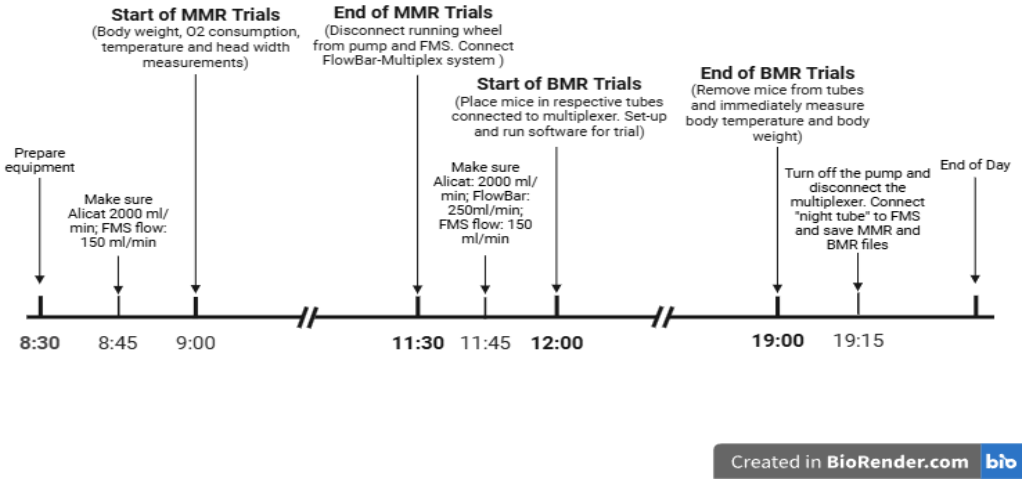


Figure 3. Visual representation of the timeline of a workday. Created in https://BioRender.com

As detailed in Figure 4 we first measured the mice's weight and place animals, one by one, inside a closed, plastic running wheel, 25 cm in diameter connected to a pump with its air flowing through dry silica gel and Drierite desiccant (gypsum, Calcium Sulfate by W.A. Hammond Corp.) which passes through an Alicat mass flow PC Series Standard Pressure Controller to keep flow rate at a constant value of 2000 $mLmin^{-1}$. With the mice inside the wheel three different stages of exercise where stimulated, in sequence: acclimation to wheel (200 to 260 seconds), slow walk (160 to 240 seconds) and fast run (around 240 seconds), by manually and gradually increasing rotational speed of the wheel after acclimation period. The maximum speeds are adjusted manually and depend on the animal's fitness, with some requiring slower speeds, due to inability to keep up. During this time we record all significant information about the running mice's behaviour, while a Field Metabolic System 3 (FMS3) records the O_2 consumption of the animal. O_2 consumption (mLh^{-1}) was measured in an open-flow, positive pressure, respirometric system. Air flow rate through the chamber was 2000 $mLmin^{-1}$ (exact air flow was controlled by Alicat mass flow controller). The experiment was terminated when animals did not increase O_2 consumption for 1 minute or could not keep up running with rotation of the wheel. After the mouse finishes running, we remove it from the wheel and immediately measure their internal body temperature with a thermocouple. Once this procedure is complete, we place them in a cage (Eurostandard type III, polycarbonate Tecniplast cages; 425 x 276 x 153 mm) with food (sterilized pellets 4RF21, Mucedola srl Complete feed for mice and rats) and water, where they rested until the last mouse has been tested and everything is ready for BMR testing, around midday. This method allows for tracking of VO_2max (maximum metabolic rate), which relates to the individual's ability to support elevated energy demands. Such maximum metabolic rates can vary significantly between individuals.

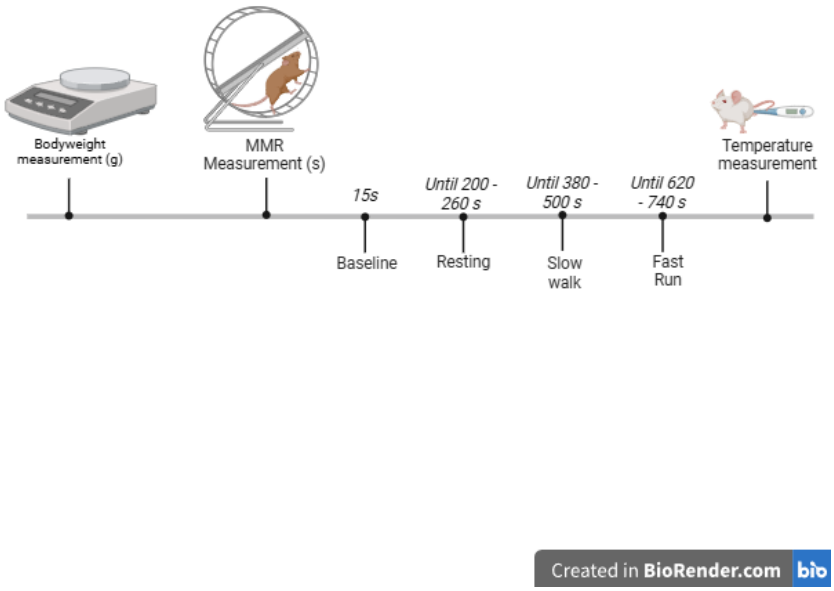


Figure 4. Visual representation of the VO_2max protocol. Created in https://BioRender.com

After finishing the last VO_2max tests, we connect the mass flow controller to a Sable Systems RM-8 Respirometry Flow Multiplexer and FlowBar-8 (8 channel respirometry system optimized to measure BMR of 7 mice simultaneously) and prepare the software for BMR trials. We then place each mouse inside their respective tube connected to the multiplexer and FMS3 for 7 hours, where air flow will remain constant. During this trial we measure their O_2 consumption, just like during the VO_2max trials step, and upon completion we remeasure animals body weight and body temperature once again (Figure 5). This BMR measurement represents the minimum metabolic rate under thermoneutral, post-absorptive and resting conditions (baseline maintenance energy expenditure).

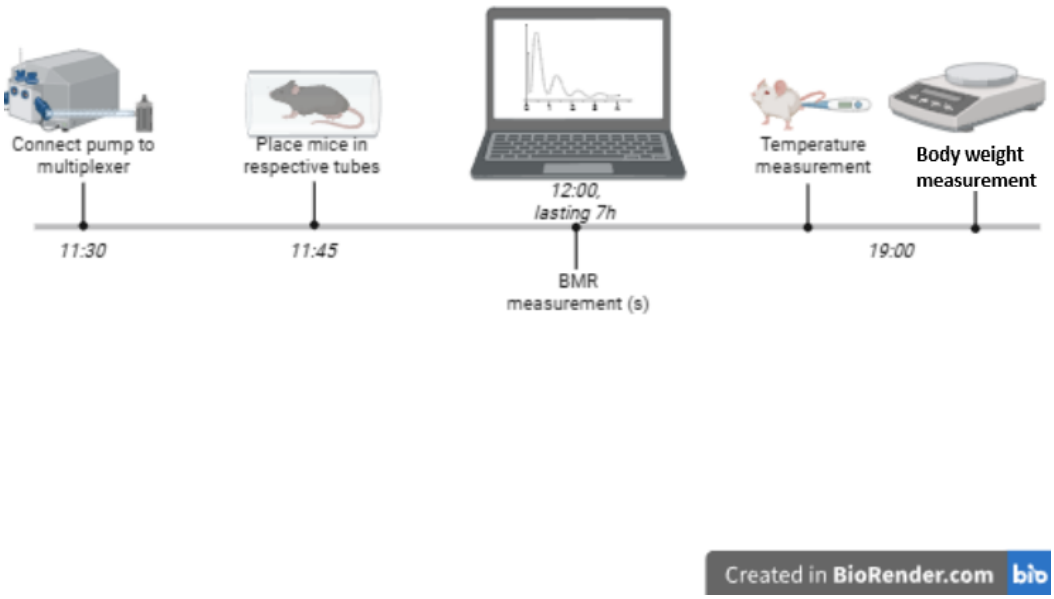


Figure 5. Visual representation of the BMR protocol with the expected starting time of each step. Created in https://BioRender.com

2.3. Statistical Analyses

2.3.1. Data Handling

Prior to metabolic rate calculations O_2 level data was transformed to fractional values (multiplied by 0.01), drift corrected, and for maximum metabolic rate calculation instantaneous correction (Z correction with factor of 0.08) was applied to account for delay in signal (air flush throughout the system) in ExpeData ver.1.9.27 (Sable Systems International). Metabolic rate was calculated from O_2 consumption estimates applying an equation, $VO_2 = \frac{Vi(FdO_2)}{1-FeO_2(1-RQ)}$ (eq. in: (Koteja 1996)) where: VO_2—oxygen consumption rate, Vi—flow rate measured before chamber, FdO_2—difference of O_2 fractional concentrations in dry air flow before and after passing through the chamber, FeO_2—fractional concentration of O_2 in dry air flow after the chamber, assuming $RQ = \frac{CO_2 eliminated}{O_2 consumed}$. The equation takes the following form when using general transform in ExpeData: “2000C1/(1-(0.2094-C1)(1-0.8))*60”. BMR was estimated from averages of the last 20 seconds of the recordings per individual, resulting in the 28 repeated estimates per individual from 7 hours long trials. BMR was defined as the mean of the 3rd, 4th and 5th such lowest values, defined by its high intra-individual consistency

(Labocha et al. 2004; Šíchová et al. 2014). VO_2max was defined as the average of the 20 seconds of the highest continuous O_2 consumption from the up to 15 minutes long wheel running trials.

2.3.2. Repeatability

With our data organized in our excel file, estimated repeatability for all measured traits utilizing the R package "*rptR_0.9.22*" (Stoffel et al., 2017). Since animals were captured and measured through four seasons (autumn, winter, summer and spring) estimated repeatability values were adjusted for seasonal variation (with season included as a factor). Significance of the sequence of measurements was also tested for VO_2max and BMR. The intraclass correlation is a descriptive statistic that describes how much different individuals of the same group share features and resemble each other over time (Koch, 1982). We run a mixed model analysis where our trait of interest is the dependent variable and the ID of the individual is a random factor, and we control for the session and sex of individual coded as fixed factors. For analysis of data within the same season, we sorted the date, so we have only two measurements of an individual within each season. If an animal was measured twice within a season in two or more different seasons (e.g.: twice in spring and twice in winter), it is only included once (1st available season). If animals were captured over twice within the season, we only take their first two measurements. For analysis of data between seasons, data is sorted so we have only two measurements of an individual between two consecutive seasons. If an animal was captured and measured in more seasons we include only the data of the first two available seasons. If an animal was measured within seasons more than once, we consider the first measurement of each season.

2.3.3. Level in Metabolic Rate Variation

We tested the variation in level of metabolic rate among seasons with a mixed model analyses with metabolic rate as dependent variable while month, sex and body mass were kept as independent variables. To account for repeated measurements, we included the ID of the individual as a random factor. Comparison of model performance was checked with AICc, Akaike information criterion with correction and R-Squared for mixed models. The AICc adjusts the traditional AIC for small sample sizes (Hurvich & Tsai, 1989), providing a balance between goodness-of-fit and model complexity (Burnham & Anderson, 2002). AICc calculations and model comparison were performed using the MuMIn R package (Barton, 2023). In mixed-effects models, R2 is partitioned into marginal R2, representing the variance explained by fixed effects, and conditional R^2, which includes both fixed and random effects. These are estimators of relative quality

for our dataset (Stoica, 2004) using the "*performance_0.10.0*" and "*MuMIn_1.47.1*" packages. We tested significance of effects of month, sex and body mass, and interactions between them, using linear mixed effects models in "*lme4_1.1-31*" and "*lmerTest 3.1-3*" R packages. We applied a Tukey post-hoc test for the significant factor effects, using the "*emmeans_1.8.2*" R package.

2.3.4. Allometric Scaling of Metabolic Rate

We compared scaling coefficients between seasons, dividing data into five sessions. We ran a mixed model analysis (same as above) within each of the seasons. Scaling coefficients describe how metabolic rate changes in proportion to body size and are typically expressed as exponents in power-law functions of the form $MR = aM^b$. A slope close to 0.75 is expected for interspecific comparisons of metabolic rate, according to Kleiber's historical prediction, but intraspecific values can differ depending on environmental, physiological and life-history factors, according to modern bioenergetic adaptive predictions. For scaling coefficients of BMR and VO_2max, we calculated 95% upper and lower 95% confidence interval limits. We interpret significance differences if the interval limits do not overlap.

3. Results

3.1. Environmental Temperature

The recorded temperatures during each month were organized in box and whisker plots, as seen in Figures 6, 7 and 8.

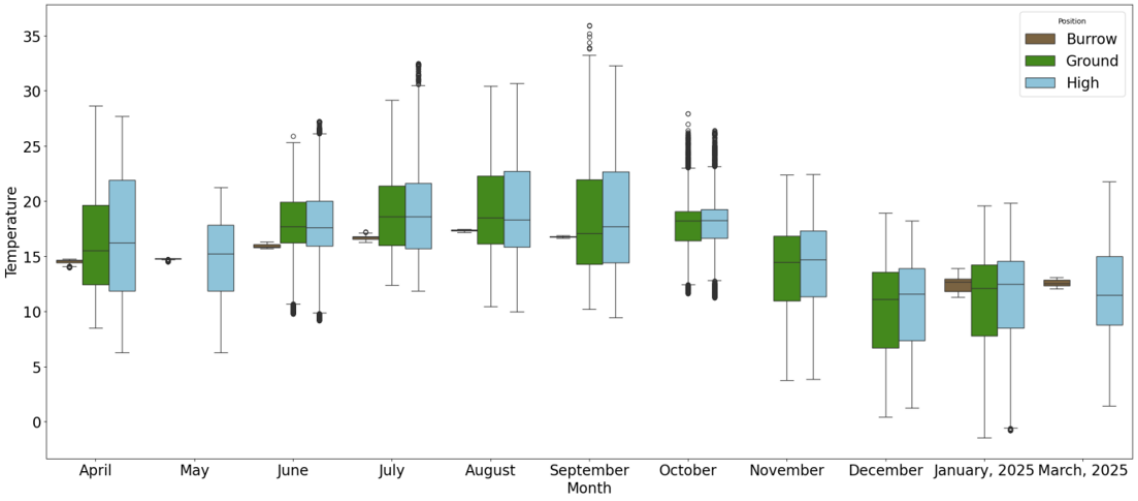


Figure 6. Box and whisker plot showcasing temperature values in the three different positions (burrows 1 meter below ground in brown, ground level in green, and high ground, 2 meters above ground in blue) for each month in the Oak

habitat. Missing burrow temperatures in October, November and December, missing ground temperatures in May and March 2025.

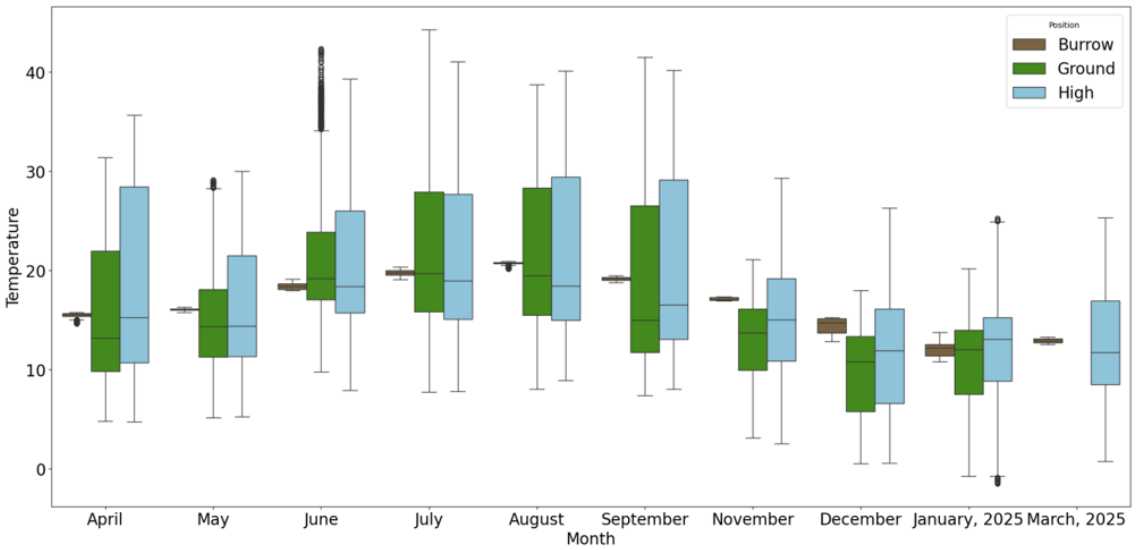


Figure 7. Box and whisker plot showcasing temperature values in the three different positions (burrows 1 meter below ground in brown, ground level in green, and high ground, 2 meters above ground in blue) for each month in the open field habitat. Missing ground temperatures in March 2025.

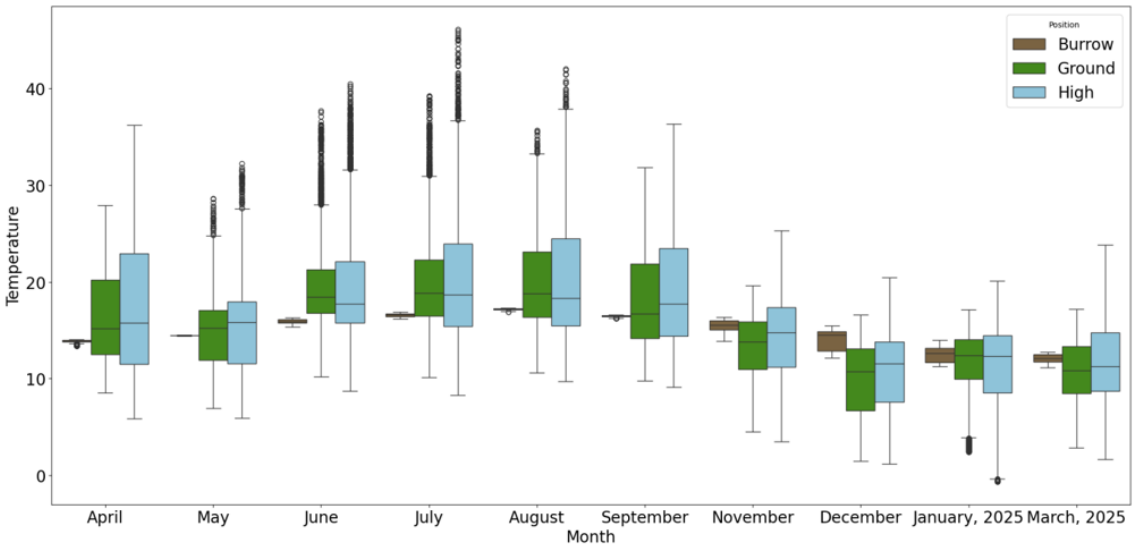


Figure 8. Box and whisker plot showcasing temperature values in the three different positions (burrows 1 meter below ground in brown, ground level in green, and high ground, 2 meters above ground in blue) for each month in the eucalyptus habitat.

3.2. Repeatability of Metabolic Rates

A total of 324 mice (155 female, 169 male) were captured over the course of the experiment (Figure 9). In all months but July the ratio of males and females was either 1:1 or very near to it, while in July there were 31 females, and 47 males investigated.

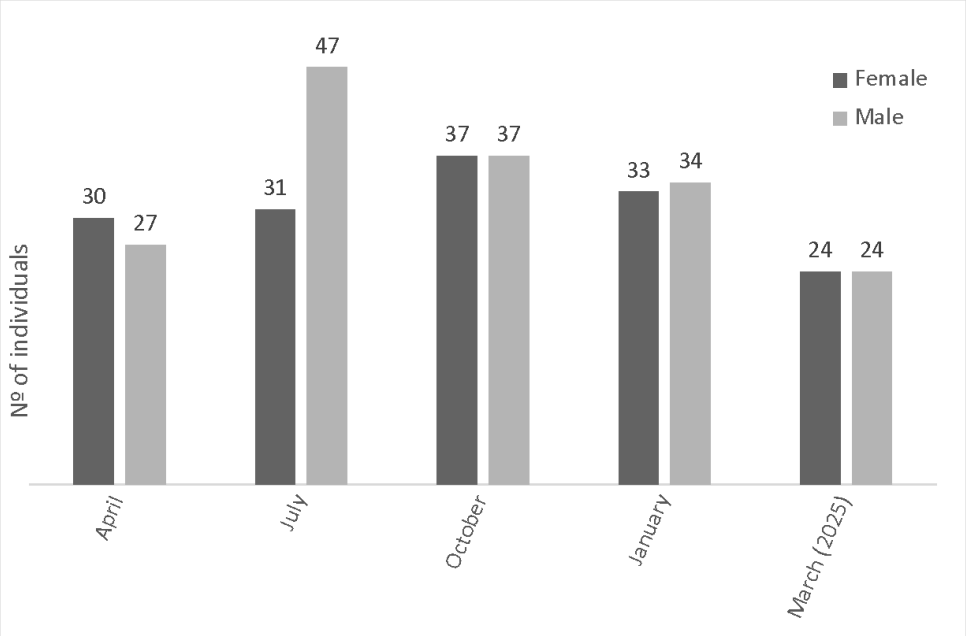


Figure 9. Number of female (dark columns) and male (light columns) mice captured in each experiment month. Total of 155 females and 169 males.

Repeatability value for replicated measurements between 1 - 8 days were relatively high for absolute body mass (BM, $\tau = 0.83$) and maximum metabolic rate (VO_2max, $\tau = 0.80$), while it was lower for basal metabolic rate (BMR, $\tau = 0.66$; Table 1). Repeatabilities for body mass accounted for metabolic rates were lower but statistically significant for both VO_2max ($\tau = 0.59$) and BMR ($\tau = 0.40$). Repeatability value for replicated measurements between 66-106 days were lower than short term repeatability, but still statistically significant for absolute body mass ($\tau = 0.60$), maximum metabolic rate ($\tau = 0.40$) and basal metabolic rate ($\tau = 0.30$). Repeatabilities for body mass accounted for metabolic rates were statistically significant for both VO_2max ($\tau = 0.29$) and BMR ($\tau = 0.44$).

Table 1. Repeatability estimates, intra-class correlation coefficients (τ), from this study for wild animals and from previous study for laboratory-based experiment. Estimates were retrieved from mixed models analyses that included trait of interest as dependent variable and sex, field session (or laboratory trial) and body mass (Cov.; before or after BMR measurements) as cofactors, and individual ID as random factor, for two repeated measurements per individual. N, refer to number of individuals with repeated measurements; VO_2max to body mass taken before metabolic rate measurements

(g); BMR and VO_2max, to basal and maximum metabolic rates (O_2 $mLmin^{-1}$); days, to number of days before repeated measurements; pPer, is derived from 1000 permutations; AICc, is second-order Akaike Information Criterion.

				without body mass			with body mass		
trait	*N*	*cofactors*	*days*	*τ (95%CI)*	p_{Per}	*AICc*	*τ (95%CI)*	p_{Per}	*AICc*
Field estimates (repeatability from this study)									
VO_2max	44	sex, session	1-8	0.83 (0.70-0.91)	0.001	-	-	-	-
BMR	44	sex, session	1-8	0.66 (0.44-0.80)	0.001	-192.1	0.40 (0.09-0.65)	0.011	-276.8
VO_{2max}	45	sex, session	1-8	0.80 (0.67-0.89)	0.001	-243.6	0.59 (0.31-0.78)	0.001	-290.3
VO_2max	45	sex, session	66-106	0.60 (0.37-0.76)	0.001	-	-	-	-
BMR	45	sex, session	66-106	0.30 (0.02-0.55)	0.029	-192.2	0.44 (0.15-0.65)	0.003	-267.9
VO_{2max}	47	sex, session	66-106	0.42 (0.16-0.63)	0.002	-223.2	0.29 (0.01-0.53)	0.030	-282.6
Laboratory estimates (repeatability from previous study)									
VO_2max	14	sex	42	0.80 (0.50-0.94)	0.002				
BMR	14	trial	42	0.96 (0.88-0.99)	0.001	-65.3	0.79 (0.45-0.93)	0.001	-84.4
VO_{2max}	13	trial	14	0.78 (0.38-0.93)	0.001	-57.5	0.64 (0.12-0.88)	0.020	-74.4

3.3. Seasonal Variation in the Level of Metabolic Rates

VO_2max variance was analyzed according to the month/session, sex and body mass. We first did this with only one measurement per animal, represented in Figures 10 and 11. We also analysed BMR variance according to these same parameters, so we can compare it with how VO_2max varies.

Maximum metabolic rate (log_{10} VO_2max) remained relatively stable across most months but declined sharply in January and March; sex differences were negligible, and VO_2max increased proportionally with body mass (Figure 10).

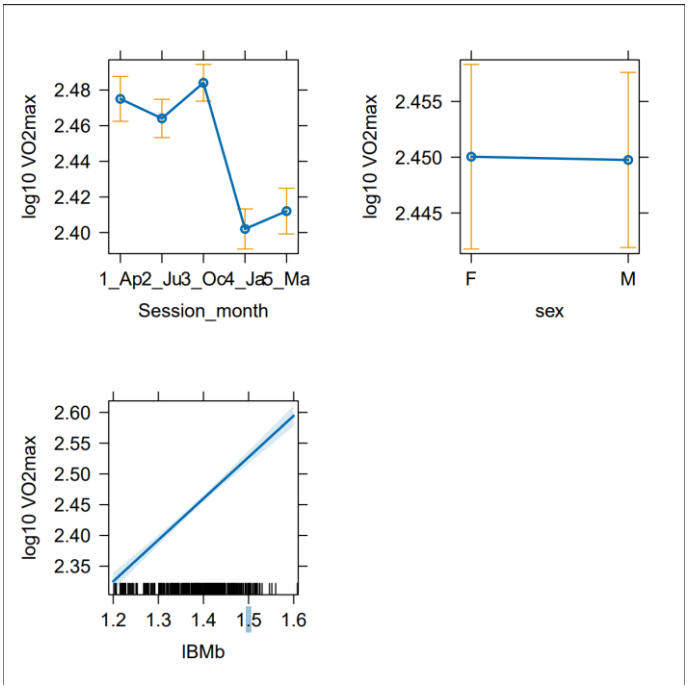


Figure 10. VO_2max variance over time with one measurement per animal, with log_{10} transformed measurements (O_2 $mLmin^{-1}$) according to session month (April, July, October and January 2024 and March 2025), sex (Female or Male), and log10 transformed body mass before BMR (lBMb) (g).

Basal metabolic rate (log10 BMR) peaked in January before decreasing in March. BMR was higher in females than males and showed a positive relationship with body mass (Figure 11).

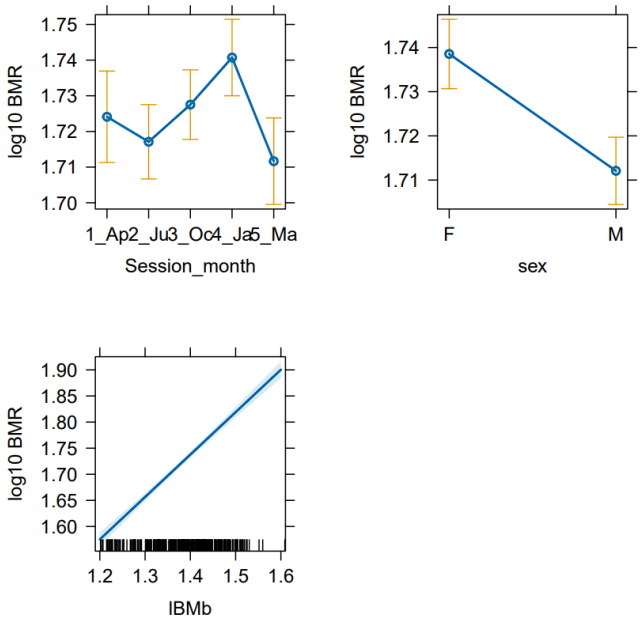


Figure 11. BMR variance over time, with log10 transformed measurements (O2 $mLmin^{-1}$) according to session month (April, July, October and January 2024 and March 2025), sex (Female or Male), and log10 transformed body mass before BMR (lBMb) (g).

We also made sure to verify if the results remained consistent for animals with more than one measurement, both for VO_2max and BMR, represented in Figures 12 and 13. This time males tended to have slightly higher VO_2max, and body mass again positively influenced VO_2max (Figure 12). BMR values remained consistent with the values for animals with only one measurement (Figure 13).

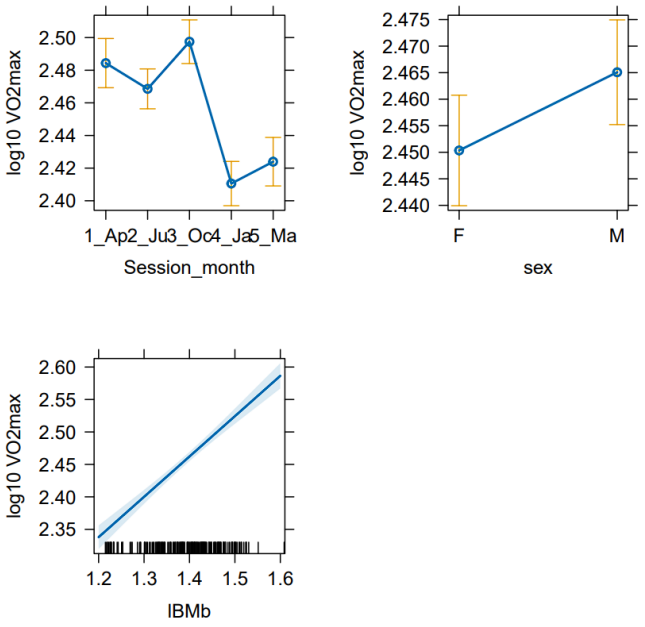


Figure 12. VO_2max variance over time for animals with more than one measurement, with log_{10} transformed measurements (O_2 $mLmin^{-1}$) according to session month (April, July, October and January 2024 and March 2025), sex (Female or Male), and log10 transformed body mass before BMR (g).

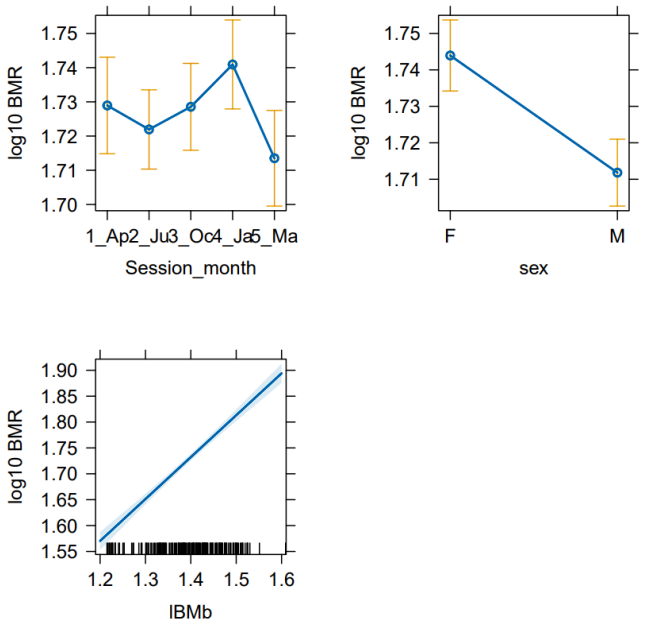


Figure 13. BMR variance over time for animals with more than one measurement, with log10 transformed measurements (O2 $mLmin^{-1}$) according to session month (April, July, October and January 2024 and March 2025), sex (Female or Male), and log10 transformed body mass before BMR (g).

3.4. Seasonal Variation in the Scaling of Metabolic Rates

After doing mixed model analysis to acquire statistical results for BMR and VO_2max (Table 2) we can infer that BMR values were generally higher in winter, with the scaling exponent (slope) varying between 1.08 and 0.69, being highest in April (session 1) and lowest in July (session 2) (Figure 14). The slopes for VO_2max also did vary somewhat, although it was much less than BMR, with the highest value being January (session 4) and the lowest being July (session 2) (Figure 15). In both cases the slope is lowest in the hottest months.

Table 2. Statistical Results (Mixed Models) for BMR and VO_2max within each session, including mean and range of body mass (Bmb_mean and Bmb_range) and metabolic rate (MR_mean and MR_range), as well as the slope associated with each session's results.

Session	Trait	BMb_mean	BMb_range	MR_mean	MR_range	Slope (95%CI)
1	BMR	24.28444	17.1, 31.9	53.73733	35.45, 76.72	1.075806 (0.91-1.18)
2	BMR	26.62794	16.9, 33.0	55.68791	36.26, 68.17	0.6857582 (0.52-0.88)
3	BMR	24.27397	15.0, 36.3	54.03194	30.67, 77.37	0.8541683 (0.73-0.98)
4	BMR	21.90323	15.9, 31.2	50.24081	33.28, 72.86	0.7038413 (0.57-0.82)
5	BMR	25.68222	16.8, 35.6	53.886	37.99, 71.68	0.8022719 (0.59-0.99)
1	VO_2max	24.28444	17.1, 31.9	298.6296	197.05, 374.57	0.6303844 (0.41-0.68)
2	VO_2max	26.62794	16.9, 33.0	313.3856	217.96, 415.89	0.6158193 (0.42-0.78)
3	VO_2max	24.27397	15.0, 36.3	304.2482	199.63, 442.31	0.7271927 (0.60-0.85)
4	VO_2max	21.90323	15.9, 31.2	241.7213	173.49, 353.76	0.7605887 (0.61-0.86)
5	VO_2max	25.68222	16.8, 35.6	270.1847	177.99, 365.77	0.6449448 (0.42-0.83)

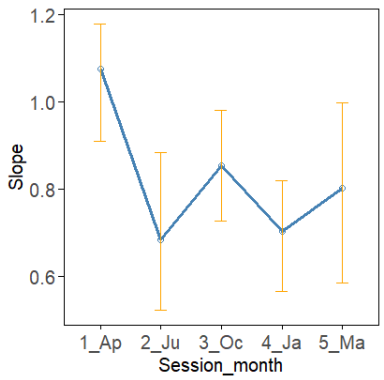


Figure 14. Variance over time of the slope associated with each session's results (April, July, October and January 2024 and March 2025) for BMR and body mass values.

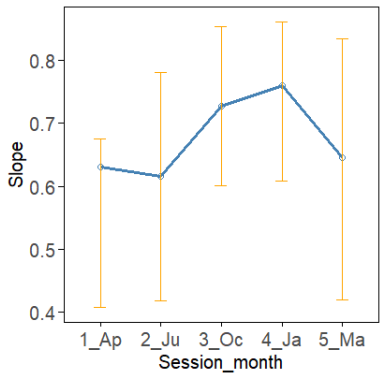


Figure 15. Variance over time of the slope associated with each session's results (April, July, October and January 2024 and March 2025) for VO_2max and body mass values.

4. Discussion

In the field, metabolic traits showed high short-term repeatability (1 to 8 days) with values as high as 0.83, especially for BMR and VO_2max, but these values are lower over longer intervals (66 to 106 days) with values not passing 0.60. Including body mass generally reduced repeatability, especially for VO_2max. Laboratory estimates were consistently higher, most likely due to reduced variation of external conditions.

Metabolic rates measured in natural field environments tend to be less consistent, reflecting more realistic plasticity in traits, then laboratory experiments. When comparing with laboratory settings, where environmental conditions are more controlled, we notice metabolic rates show higher repeatability. This implies variability in natural conditions plays a significant role in the changing of metabolic patterns and makes it harder to study potentially flexible, plastic, metabolic traits in populations found in the wild (Auer, 2016).

When looking at our results for variance in metabolic rate throughout the months, having one measurement per individual, we can see that VO_2max remains relatively high during April, July and October (spring, summer and autumn) and then falls at a steep decline towards January and early March (Winter). This suggests a potential seasonal modulation of VO_2max, possibly linked to environmental factors such as temperature or resource availability, as previously observed on small mammals (Arnold, 2020; Zub et al., 2009). Sexual dimorphism in VO_2max, when considering its relation with body mass and seasonal changes, were minimal. As expected, VO_2max showed a strong positive relation with body mass, consistent with general metabolic scaling principles, as supported in studies on allometry and energy allocation (White & Kearney, 2014; Ballesteros et al., 2018).

BMR, on the other hand, was highest during the month of January, after which it fell back down to low level in March. BMR, like VO_2max, was strongly positively correlated with body mass, but unlike VO_2max, BMR was influenced by the sex of the individual, with females having generally higher BMR. This may point to sex-specific energy requirements that may affect resting metabolism, in line with ideas of functional trait variation by sex and ecological pressures (Glazier, 2005).

The general trend for VO_2max over time for animals with more than one measurement in relation to body mass remained relatively similar. We did, however, also verify that unlike when we only considered a single measurement per animal, there was a difference females and males, although they also overlap somewhat, so this may be inconclusive, although it may also suggest some resemblance of an effect on metabolic rate that although less prevalent than in BMR, may also somewhat affect VO_2max.

While with VO_2max we did notice a somewhat minor change in the way it related with an individual's sex (for animals with more than one measurement, when compared with animals with one measurement only), BMR remained with very similar values throughout all 3 parameters. This may mean that BMR can represent a more intrinsic physiological baseline, while VO_2max is more plastic and sensitive to environmental or contextual factors. As would be expected (Corp et al., 1997; Fuglesteg et al., 2006), we also notice that repeatability in laboratory-maintained individuals is relatively similar to wild caught specimens that have only short time (< 24 hours) experience in the laboratory. The effect was observed in both BMR and VO_2max parameters, but higher for BMR. Repeatability of BMR is consistently two times higher in laboratory-maintained animals than in wild caught, when we include body mass as a covariable. For VO_2max this difference was evident when repeatability was measured between seasons, but not so within season tests.

We observed clear seasonal variation in the level but not in scaling of metabolic rate, with BMR and VO_2max changing in response to seasonal conditions, with BMR peaking in April but VO_2max peaking in October. The scaling coefficients of both BMR and VO_2max varied only slightly across sessions, possibly indicating that the relationship between body size and metabolic rate has a limited level of flexibility. However, taking how our 95% confidence interval overlaps between seasons into consideration, we can conclude that more data would be needed to detect truly significant changes.

5. Conclusion

This study investigated variation in basal and maximum energy rates in a wild mouse of *Apodemus sylvaticus* during various times of the year. Our results show that both BMR and VO_2max change not just with animals' weight but also with seasonal conditions, showing how the environment affects energy spending. This ranges from the changes in temperature, especially in winter which force the animals to spend more energy to regulate their own temperature with less availability of food.

VO_2max showed some steadiness through the reproductive season but dropped substantially in winter, possibly because of cooler weather and fewer resources and less mobility needed for home ranging. On the other hand, BMR was highest in winter, probably showing greater needs for body heat during chillier times. These findings back up the idea that how metabolism is not fixed but instead can change based on nature's challenges. Our results showed also some variation in scaling coefficients between metabolic rate and body mass. BMR has a consistently higher slope than VO_2max between seasons, but the slopes do not change consistently, and generally their confidence intervals overlap.

We found that repeatability of metabolic traits was generally lower in wild-caught animals than in laboratory conditions, showing how unpredictable natural surroundings can affect physiological traits. Importantly, our data suggests that while BMR may reflect a more intrinsic physiological baseline, VO_2max is more susceptible to environmental and possibly behavioural factors and changes predictably more over seasons.

Taken together, these findings contribute to a better understanding of how metabolic scaling works within a species facing natural changing conditions, showing us how metabolic rate is context-dependent. They also remind us of the importance of framing the context by the season and the weather and food conditions.

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