METHODS & REPORTING

Reporting guidelines for terrestrial respirometry: Building openness, transparency of metabolic rate and evaporative water loss data

Nicholas C. Wu[1,*], Lesley Alton[2], Rafael P. Bovo[3], Nicholas Carey[4], Shannon E. Currie[5], John R. B. Lighton[6], Andrew E. McKechnie[7,8], Patrice Pottier[9], Giulia Rossi[10], Craig R. White[2], Danielle Levesque[11]

[1]Hawkesbury Institute for the Environment, Western Sydney University, New South Wales 2753, Australia. [2]Centre for Geometric Biology, School of Biological Sciences, Monash University, Melbourne VIC 3800, Australia. [3]Department of Evolution, Ecology, and Organismal Biology, University of California Riverside, Riverside, CA, United States. [4]Marine Directorate for the Scottish Government, Aberdeen, United Kingdom. [5]Institute for Cell and Systems Biology, University of Hamburg, Martin-Luther-King Plz 3, 20146, Hamburg, Germany. [6]Sable Systems International, North Las Vegas, NV, United States. [7]South African Research Chair in Conservation Physiology, South African National Biodiversity Institute, South Africa. [8]DSI-NRF Centre of Excellence at the FitzPatrick Institute, Department of Zoology and Entomology, University of Pretoria, South Africa. [9]Evolution & Ecology Research Centre, School of Biological, Earth and Environmental Sciences, The University of New South Wales, Sydney, New South Wales, Australia. [10]Department of Biology, McMaster University, Hamilton, Ontario, Canada. [11]School of Biology and Ecology, University of Maine, Orono, ME, United States.
*Corresponding author. Email: nicholas.wu.nz@gmail.com

comparative physiology, energy metabolism, flow-through, indirect calorimetry, open science, research design

Respirometry is an important tool for understanding whole-animal energy and water balance in relation to their environment. Consequently, the growing number of studies using respirometry over the last decade warrants reliable reporting and data sharing for effective dissemination and research synthesis. We provide a checklist guideline on five key areas to facilitate the transparency and reproducibility of respirometry studies: 1) materials, set up, plumbing, 2) subject conditions/maintenance, 3) measurement conditions, 4) data processing, and 5) data reporting and statistics, each with explanations and example studies. Transparency in reporting and data availability has benefits on multiple fronts. Authors can use this checklist to design and report on their study, and reviewers and editors can use the checklist to assess the reporting quality of the manuscripts they review. Improved standards for reporting will enhance the value of primary studies and will greatly facilitate the ability to carry out higher quality research syntheses to address ecological and evolutionary theories.

MAIN TEXT

Measurements of energy and water exchanges date back to the 18th Century [2,3] and continue to be an important skill for evolutionary and ecological physiologists in the 21st Century. For example, the effects of temperature on energy and water budgets have been essential for predicting the impacts of rapid anthropogenic global heating on biodiversity, whether using species-specific empirical data [4-9] or for developing bioenergetic models [10-12]. Alongside this, the field of ecotoxicology and disease ecology relies on measurements of energy use (e.g. metabolic rate, MR) and water balance to evaluate species risk to novel toxins and pollutants [13,14] and emerging infectious diseases [15,16]. The same applies to other growing fields such as conservation physiology [17,18] and macrophysiology [19-22] in which, through the mechanistic lens of physiology, improve our understanding of ecological and evolutionary responses of different organisms to changes in their environment.

The most widespread approach to quantifying the amount of energy expended over a time in animals is indirect calorimetry [23-26]. This involves respirometric measurements of oxygen consumption ($\dot{V}_{O_2}$) and/or carbon dioxide production ($\dot{V}_{CO_2}$), from which MR can be estimated if the metabolic substrate can be inferred or reasonably assumed [26-28], but see Walsberg and Hoffman [29]. Combined with simultaneous measurements of evaporative water loss (EWL; see Glossary) and body temperature (T_b), respirometry remains an essential tool for several disciplines in biology in the contemporary era, allowing us to advance knowledge in key aspects of ecological and evolutionary theories, including size-scaling relationships, thermal adaptation, and phenotypic plasticity [6,30-37].

The need for standardised reporting is well-timed because of the exponential rise in empirical and synthesis studies using respirometry data [35,38-41]. A Web of Science search for terrestrial respirometry studies in the field of ecology and evolution (23 November 2023) showed a 158 % increase in whole-animal MR studies over the last 10 years (2012–2022; **Fig. 1a**) and 170 % increase in whole-animal water loss studies (2012–2022; **Fig. 1b**), while the overall publication rate in science is doubling almost every decade [42]. In the aquatic world, reporting of intermittent-flow respirometry experiments were generally poor and inconsistent [43]. It is highly likely this inconsistency applies to terrestrial respirometry, as we are aware with our own studies. For example, 22% of respirometry studies measuring avian MR do not provide the duration of the experiment [44]. Inconsistent and incomplete reporting can hamper the value of primary studies for comparative analyses [45]. Such studies may be excluded if key information is missing, such as acclimation time, body mass, sex, and

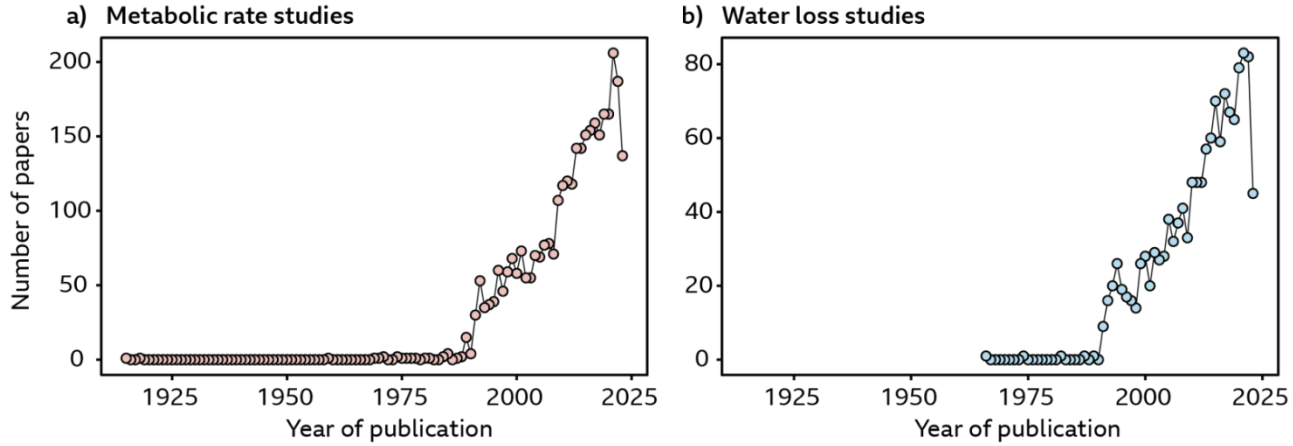

Fig. 1 | Number of studies published in the field of ecology and evolution relating to a) whole-animal metabolic rate, and b) water loss (via gravimetric, respirometry-based approaches). Data obtained from the Web of Science on 25 November 2023 using the search terms (respirometry OR gas exchange OR open-flow OR flow-through) AND (metabolic rate OR MR OR metabolism OR energy budget OR heat production) for metabolic rate studies and (respirometry OR gas exchange OR open-flow OR flow-through) AND (water loss OR evaporative OR skin resistance OR water budget OR desiccation rate OR hydroregulation) for water loss studies..

measurement duration example synthesis papers with exclusion criteria; [46,47].

To help facilitate the quality of reporting respirometry studies, we provide a checklist with five criteria to facilitate interpreting and replicating (see Glossary) empirical studies relating to measurements of whole-animal MR and EWL via flow-through respirometry for terrestrial air-breathing organisms (terrestrial respirometry). Note, many of the reporting descriptions in **Table 1** also apply to close-system respirometry, intermittent-closed respirometry, and gravimetric measurements of EWL. We broadly followed Killen et al. [43] with criteria specific to terrestrial respirometry.

The checklist comprises five main sections (**Fig. 2**) with specific reporting information and references that provide more detail, justification for the criteria, or example studies (**Table 1**). We do not intend to be overly prescriptive in how to design and undertake respirometry studies as there is already an extensive literature on respirometry designs, data processing, and calculations [23-26,48-52], yet we draw attention to some common reporting issues. The checklist does not include all possible elements, but it is formulated to aid in the design and reporting of most studies to increase transparency (see Glossary). The checklist is available on GitHub.

GLOSSARY

Basal metabolic rate (BMR): The metabolic rate (see MR) of a non-reproductive, inactive, unstressed, postprandial adult endotherm that is thermoregulating in a thermoneutral environment during the inactive phase of its circadian cycle (but not sleeping, or in torpor–equivalent to SMR for ectotherms).

Evaporative water loss (EWL): The rate of water loss via evaporation, typically expressed in absolute terms as mass of water lost per unit time (e.g. g H2O h-1), in relative terms as mass of water lost per mass of animal per unit time (e.g. g H2O g-1 h-1), or the mass of water lost per exposed surface area per unit time (e.g. g H2O cm-2 h-1). EWL is often represented as total evaporative water loss (TEWL*), which is the sum of the rate of evaporative water loss through the cutaneous surface (cutaneous evaporative water loss; CEWL) and the rate of evaporative water loss through respiration (respiratory evaporative water loss; REWL†). For moist-skinned organisms such as amphibians, CEWL is the main mode of water loss, whereas in many endotherms respiratory evaporation predominates (REWL)

* TEWL is sometimes used in the literature to refer to transepithelial (an equivalent of cutaneous) evaporative water loss.

† REWL is sometimes used in the literature to refer to resistance to evaporative water loss, but is now referred to as "r". Total resistance to EWL (rt) is the sum of the boundary layer resistance (rb) and the resistance provided by the animal integument (ri), expressed as s cm-1. rb is resistance of the moist air surrounding the animal empirically taken from an equivalent biophysical (e.g. agar, plaster, foam, etc.) model with the same size/shape of the animal. As there is no epithelial barrier (skin) in biophysical models to create resistance to water loss through the evaporating surface, thus ri = zero, and rb = rt. ri is the resistance provided by the animal integument, estimated after subtracting the amount of water lost by the animal (rt) from its biophysical ("no cutaneous") model (rb), thus ri = rt – rb.

Maximal metabolic rate (MMR): When induced by exercise, the energy expenditure (usually rate of oxygen consumption) during the maximum sustainable rate of exercise. When induced by cold for endotherms, the maximum rate of oxygen consumption during the maximum sustainable cold stress (often induced in a He-O2 atmosphere at temperatures above 0°C to avoid freezing injury to tissues).

Metabolic rate (MR): The rate of energy expenditure per unit time. Typically represented as the rate of O_2 consumption (ml h^{-1}), CO_2 production (ml h^{-1}), or converted to an energy equivalent such as Watts, or Joules or calories per unit time.

Replicability: Obtaining consistent results across studies aimed at answering the same scientific question, each of which has obtained its own data [1].

Reproducibility: Obtaining consistent results using the same input data, computational methods, and conditions of analysis [1].

Resting metabolic rate (RMR): The metabolic rate of an inactive animal when one or more of conditions required for measuring BMR or SMR cannot be met.

*Note that the abbreviation RMR is sometimes used in the literature to refer to routine metabolic rate, which is the MR averaged over a specified time interval, of an animal exhibiting spontaneous 'routine' behaviours, or a specified behaviour. Here, RMR refers to resting metabolic rate.

Standard metabolic rate (SMR): The metabolic rate of an inactive, unstressed, postprandial adult ectotherm measured under normothermic conditions during the inactive phase of its circadian cycle (but not sleeping, or in diapause, or brumating, or aestivating).

Transparency: The practice of openly and systematically sharing all aspects of the research process, including methodology, data, code, results, and interpretations. Importantly, transparency increases the replicability and credibility of the research.

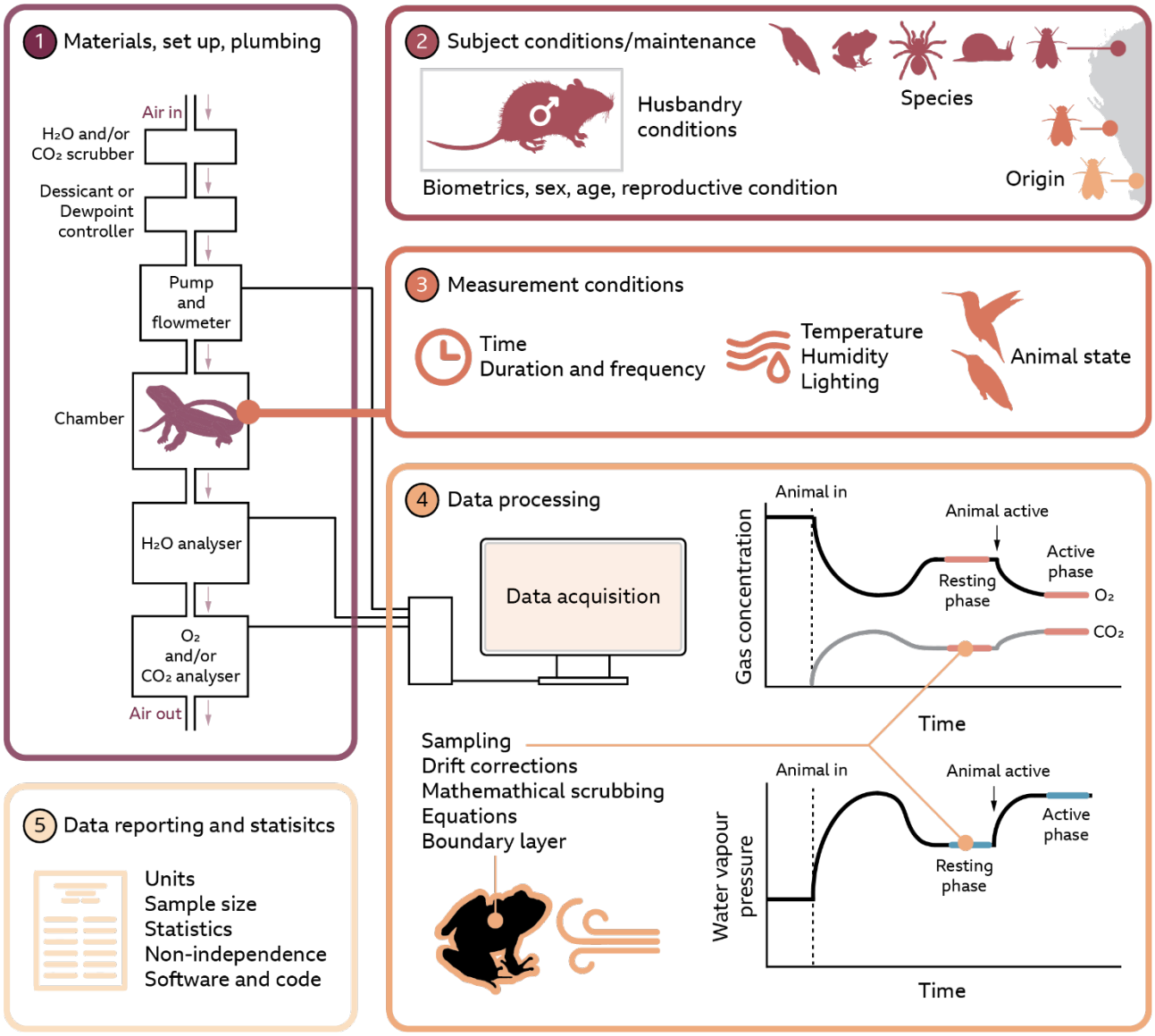

Fig. 2 | Visualisation of the five checklist guidelines with example reporting information. 1) Materials, set-up, plumbing shows an example schematic diagram of a "push" flow-through system. 2) Subject conditions/maintenance focus on the test subjects in question, their origin, and how they are housed/acclimated prior to experimentation. This may include the species, their origin, sex, age, and reproductive condition, as well as the husbandry conditions they are kept in. 3) Measurement conditions focus on reporting the raw data collected, how long and often the measurements were taken, the experiment conditions, and the state of the animals during the measurements. 4) Data processing focuses on reporting how the data were recorded, what mathematical corrections were used, how the data were converted, and the equations to calculate meaningful values from raw data. The highlighted sections of the raw gas and water vapour pressure trace represent where values are extracted, typically periods of stable rate of O_2 consumption, CO_2 production or water vapour pressure. 5) Data reporting and statistics focus on reporting how the data were analysed, the statistics and program used, and sharing data and code.

1. Materials, Set up, and Plumbing

The respirometry set up should be designed to answer your research question. Given the flexibility and modularity of respirometry set ups, many creative variations have been used. For example, masks have been fashioned from large buckets to fit over the trunk of an elephant [53,54], some made to resemble a flower to measure the MR of hovering hummingbirds [55,56], or burrows constructed by the animal itself [57,58]. Maximum metabolic rates (MMR; see Glossary) and EWL during exercise have been quantified in treadmills, exercise wheels, or wind tunnels [59-63]. Tree cavities, with a single entrance as an inlet and a small outlet hole, have also been used as flow-through chambers to measure MR in wild primates [64], and bespoke 6 m^3 chambers have been constructed to measure the MR of estuarine crocodiles weighing up to 389 kg [65]. Test tubes have been used to test the influence of tree hollow shelter on EWL of casque-headed tree frogs [66]. These creative set-ups can be summarised generally by the two ways air moves past an animal—pushing or pulling, each their associated advantage and disadvantage [48]. [26] provides comprehensive information on setting up push and pull systems which we highly recommend reviewing prior to designing respirometry experiments.

The more creative and elaborate the set-up, however, the more potential measurement errors can occur during the experiment. Every component such as the plumbing set up, materials used, the use of physical scrubbers, chamber size relative to flow rate can influence the reliability of the data collected (**Table 1**). For example, tubing material (e.g., Tygon, Silicone, PharMed, Teflon, Bev-a-Line) and size are

often not reported but can influence gas permeability, water vapour, and pressure resistance [26,67,68]. The same applies for the chamber/mask size, shape (cylinder, sub, rectangle), and material (plastic, acrylic, glass, metal). Chamber size is of particular importance because it influences the comfortability for the animal (size and behaviour) and flow rate which, in turn, influences washout characteristics [25,69-71]. This delicate balance between chamber design and the flow rate is highly subjective and context dependent, but reporting these features can increase the replicability of the experiments. Lighton and Halsey [48] provide calculations for optimising the chamber size and flow rates for the minimising lag-time for analysers. Lastly, drawing the set-up plumbing and equipment position is of great help for researchers designing their experiments and for reviewers when assessing the quality of the setup.

2. Subject Conditions/Maintenance

Reporting where the test subjects were collected, their biological metrics (e.g., species, sex, reproductive status, age) and housing/maintenance condition (e.g., lighting, temperature, water access, food quality and quantity) are important because these factors can influence comparisons of MR and EWL measurements (**Table 1**; **Fig. 2**). For instance, MR shows substantial variation between individuals, populations, and species, and understanding this variation is an active research area [36,72-75]. Even when measurement errors are accounted for [76,77], variation in MR within species may still exist that can be explained by selection [78,79]. This is the case when test subjects from different populations and/or localities may exhibit different physiology due to local adaptation to their environment [80-82]. Therefore, comparative studies require such information from primary studies to formally deal with intraspecific variation, spatial autocorrelation, and environmental predictors.

Once the subjects are collected and housed, an important consideration is how long the test subjects are acclimated to their experimental condition before measurements of MR and/or EWL. Whether it is pollutant, pathogen, temperature exposure, food or water ration, it is important to report the magnitude and length of the different treatments (and whether a step process was used) for understanding the effects of acclimation within the study and allowing meaningful comparisons between studies [83,84]. For example, the BMR of sparrows can reach back to pre-acclimation levels after 8 weeks acclimated from 15 to 30 °C [85]. If measurements were taken at 4 weeks, MR would be reported as significantly higher than pre-acclimation MR [85].

3. Measurement Conditions

Reporting what data were collected, how it was collected, and the conditions the animals were subjected to during the experiment is important for the replicability of the experimental procedure (**Table 1**; **Fig. 2**). A common issue in studies measuring energy expenditure and water loss under standardised conditions, including standard metabolic rate (SMR; see Glossary) in ectotherms, basal metabolic rate (BMR; see Glossary) in endotherms [86,87], and EWL rate [88] is insufficient time [89-92]. Many animals experience handling stress and require a substantial amount of time (e.g. up to 30 minutes for a 30 g mouse) for MR and EWL to drop back down to resting levels [93]. This time can depend on both temperament of the animal, as well as their mass and the temperature exposure [94]. Similarly, resting MR can differ significantly depending on the time of day, particularly in species with pronounced circadian activity patterns [95]. It is therefore important to ensure that the length of time for the measurement, as well as the time that the data are collected, match what is attempted to be measured [96].

It is also important to consider in endotherms how the conditions mentioned above (mass, circadian phase, and resting state) influence body temperature and, thus, the interpretation of MR measurements. Body temperature can fluctuate by a few degrees, or up to 30°C or more over the course of a 24-hour period, depending on the species studied [95,97]. This includes both non-steady state changes such as torpor [98,99] or steady-state circadian rhythms [100-102]. Where possible we recommend the measurement of body temperature alongside MR, which is now made easier with relatively non-invasive measurement devices such as temperature-sensitive passive integrated transponders and temperature-sensitive transmitters [103,104]. This will enable distinctions to be made between metabolic states and ensure that the most accurate/relevant data are included in analyses, and can even enable detection of novel mechanisms of energy budgeting [105,106].

Even for studies in which EWL is not quantified, chamber humidity is an important consideration when measurements are conducted at air temperatures approaching or exceeding body temperature for endotherms. As noted by Lasiewski et al. [70], the humidity animals experience in chambers depends on incurrent humidity, chamber volume, flow rate as well as water loss from the subject. Even when incurrent air is dry, low flow rates (particularly when combined with large chamber volume) can result in high chamber humidity due to subject evaporation through respiration or skin, impeding evaporative heat loss or water loss and resulting in different thermoregulatory or evaporative responses compared to those observed in drier air with high flow rates [70,107-109]. Assuming adequate mixing of air in chambers and considering the effects of boundary layer resistance over the evaporating surfaces [which can be achieved by using sufficiently high flow rates for a given chamber volume [26]], subjects experience humidity equivalent to that of the excurrent air [70]. For this reason, in studies investigating responses to experimentally manipulated humidity, the excurrent – not incurrent – humidity is the variable that needs to be maintained at approximately constant levels. When subjects are exposed to multiple setpoint air temperatures during a set of measurements, for instance, regular adjustments of flow rate or incurrent humidity are necessary to maintain a constant chamber humidity [107]. When using this approach, however, it is essential to take the equilibrium time of the system into account [26,70] after each adjustment of flow rate or incurrent humidity.

Further considerations for studies where EWL is measured via flow-through methods include a) the requirement to exclude evaporation from excreta, usually

achieved by placing the subject on a mesh platform elevated above a layer of mineral oil or liquid paraffin deep enough to cover any excreta that falls into it [110], b) the need to use chambers constructed from material that does not adsorb water vapour [111], and c) the need to report the subject's posture [112] and position [113], as the subject surface exposed to evaporation may alter measured EWL. Humidity should also be reported in absolute terms (g H_2O m^{-3} air or partial pressures in kPA). If RH values are reported, it is essential that it is accompanied by the corresponding air temperature to permit the calculation of absolute humidity, water vapor deficits and related variables. Finally, in studies involving high air temperatures and experimental humidity levels, condensation in tubing and analysers must be avoided at all cost as, per most manufacturers, liquid water can permanently damage the sensors. Freeman et al. [107] for instance, placed their respirometry setup in a room within which air temperature was maintained at 35°C to accommodate excurrent dew points of up to ~ 30 °C. Particular care should be taken when tubing is in contact with the floor or in the proximity of heat exchangers.

4. Data Processing

Accurate descriptions of how the raw data were treated, what transformations were used, and details on the sampling procedure and data processing are important in both standardizing the findings as well as allowing for replicability (**Fig. 2**). These include data acquisition (what software and at what sampling intervals and if multiplexing how long on each chamber), measurement of and corrections for baseline drift, measurements of time lags between the parameters, sample selection, equations and calculations, as well as any data exclusion procedures (**Table 1**). How data are transformed, what time intervals are selected, and what criteria are used to exclude data e.g., activity during resting measurements; [93] can impact the findings and are therefore important to report. Hayes et al. [90] and Jacobs and McKechnie [96] provide concrete examples of the effects of sample length on estimates of metabolic rate. In both cases estimates for BMR were 12–30 % higher when calculated using 10–15 min intervals compared to 60 min intervals, while 30 min intervals were equivalent [96]. Similarly, correcting for analyser drift or lag-time between analysers can impact the results and should be calculated or accounted for [25,48], particularly when instantaneous measurements are of interest [114]. Lastly, if the equations for calculating $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, and EWL are formulised instead of providing references to existing equations [23-26,49,50], we recommend mathematical notations following Edwards and Auger-Méthé [115].

5. Data Reporting and Statistics

Describing how the data were analysed and presented is important for the interpretation and the reproducibility (see Glossary) of the study (**Table 1**). There are excellent resources available specially focusing on reporting statistics in experiment biology and more broadly in life sciences [116-119]. Here, we highlight advantages of sharing data and code to understand how the data were analysed and interpreted, as well as giving the data new light for synthesis studies. Open research is not just a theoretical ideal but a practical necessity to assess, replicate, and compile research findings [120-122]. Central to comparative physiology is the synthesis of original data, which should be made available along with the analytical methods. The encouragement of data and code sharing by journals, with some mandating data sharing, has been a welcome improvement in science [123-125]. The establishment of stable platforms for publishing data and code such as Dryad, Zenodo, Figshare, or the Open Science Framework has also been instrumental in promoting data availability. Sharing data in such repositories has been shown to offer numerous advantages, including enhanced citation counts [126-129].

Although data sharing has become more accepted, how the data is shared can vary in their quality. A particular challenge in compiling data for comparative studies is the inconsistency in units and terminology in the measurement and reporting of data. Differences in data presentation, such as reporting absolute rates versus mass-specific or rates normalized to temperature, can impede meaningful comparisons. Similarly, water loss is sometimes reported either as the rate of water loss or as integument resistance to water loss (r; see Glossary), but not both [113,130,131]. Converting between the two units is difficult if conversion parameters are not provided in the methods section or raw data (e.g., vapor density gradient). Addressing these challenges necessitates clear and standardized metadata guidelines [132]. In addition, following best practices for sharing data, which include providing the raw data, metadata detailing each column, code detailing all data processing steps, and a README file describing how to navigate materials in the repository is typically needed for reproducibility [133-135].

In recent years, open-source packages for processing respirometry and analysing data have become available, which allow the sharing of reproducible and succinct code detailing the processing methods. The adoption of such open-source packages, especially with command line code, is highly recommended, as they can streamline the analysis of respirometry data, provide standardised ways to perform common but complex tasks (e.g. applying adjustments from blank controls, unit conversions, standardising rates by mass or area, etc.), and provide a transparent and standardised workflow. Given that the analysis of respirometry data often involves onerous manual processing, such open-source code minimizes manual data transformations and ensures reproducibility [136,137]. Several packages are available for processing and analysis of respirometry data, from those aimed chiefly towards aquatic respirometry such as *respR* [136], to time series analysis tools such as *LoLinR* [138]. Unique aspects of terrestrial flow-through respirometry however, such as the irregular nature of discontinuous gas exchange data (see section 3 Measurement Conditions and 4 Data Processing), highlights the need to develop tools tailored specifically towards these data.

CONCLUSION

Standardised reporting can 1) provide important teaching opportunities for students/researchers new to respirometry

on best practices in reporting and interpreting metabolic and water loss data, 2) increase the transparency of data curation for comparative studies and meta-analysis to reduce sampling bias and incorrect interpretation [34,118,139], and 3) assist the efficiency of peer-review process by providing a clear checklist for reviewing studies with respirometry experiments [140]. Overall, the goal of our checklist is to provide a comprehensive guide that will help both new and experienced researchers design, execute, and report respirometry experiments with consistency.

REFERENCES

1 National Academies of Sciences, E., and Medicine. *Reproducibility and replicability in science*. (National Academies Press, 2019).
2 Lavoisier, A.-L. & Seguin, A. Premier mémoire sur la respiration des animaux. *Mémoires de l'Académie des Sciences* **566** (1789).
3 Townson, R. *Tracts and observations in natural history and physiology*. (J. White, 1799).
4 Bozinovic, F. & Rosenmann, M. Maximum metabolic rate of rodents: physiological and ecological consequences on distributional limits. *Funct. Ecol.* **3**, 173-181 (1989).
5 Swanson, D. L. & Liknes, E. T. A comparative analysis of thermogenic capacity and cold tolerance in small birds. *J. Exp. Biol.* **209**, 466-474 (2006).
6 Mckechnie, A. E. & Swanson, D. L. Sources and significance of variation in basal, summit and maximal metabolic rates in birds. *Curr. Zool.* **56**, 741-758 (2010).
7 Seebacher, F., White, C. R. & Franklin, C. E. Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat. Clim. Change* **5**, 61-66 (2015).
8 McKechnie, A. E., Gerson, A. R. & Wolf, B. O. Thermoregulation in desert birds: scaling and phylogenetic variation in heat tolerance and evaporative cooling. *J. Exp. Biol.* **224**, jeb229211 (2021).
9 Alton, L. A. & Kellermann, V. Interspecific interactions alter the metabolic costs of climate warming. *Nat. Clim. Change* **13**, 382-388 (2023).
10 Kearney, M. & Porter, W. Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. *Ecol. Lett.* **12**, 334-350 (2009).
11 Riddell, E. *et al.* Exposure to climate change drives stability or collapse of desert mammal and bird communities. *Science* **371**, 633-636 (2021).
12 Briscoe, N. J. *et al.* Mechanistic forecasts of species responses to climate change: the promise of biophysical ecology. *Glob. Chang. Biol.* **29**, 1451-1470 (2023).
13 Sokolova, I. M. & Lannig, G. Interactive effects of metal pollution and temperature on metabolism in aquatic ectotherms: implications of global climate change. *Clim. Res.* **37**, 181-201 (2008).
14 Baas, J. & Kooijman, S. A. Sensitivity of animals to chemical compounds links to metabolic rate. *Ecotoxicology* **24**, 657-663 (2015).
15 Agugliaro, J., Lind, C. M., Lorch, J. M. & Farrell, T. M. An emerging fungal pathogen is associated with increased resting metabolic rate and total evaporative water loss rate in a winter-active snake. *Funct. Ecol.* **34**, 486-496 (2020).
16 Wu, N. C. Pathogen load predicts host functional disruption: A meta-analysis of an amphibian fungal panzootic. *Funct. Ecol.* **37**, 900-914 (2023).
17 Wikelski, M. & Cooke, S. J. Conservation physiology. *Trends Ecol. Evol.* **21**, 38-46 (2006).
18 Cooke, S. J. *et al.* What is conservation physiology? Perspectives on an increasingly integrated and essential science. *Conserv. Physiol.* **1**, cot001 (2013).
19 Chown, S., Gaston, K. & Robinson, D. Macrophysiology: large-scale patterns in physiological traits and their ecological implications. *Funct. Ecol.* **18**, 159-167 (2004).
20 Ruf, T. & Geiser, F. Daily torpor and hibernation in birds and mammals. *Biol. Rev.* **90**, 891-926 (2015).
21 Chown, S. L. & Gaston, K. J. Macrophysiology–progress and prospects. *Funct. Ecol.* **30**, 330-344 (2016).
22 Wu, N. C. *et al.* Global exposure risk of frogs to increasing environmental dryness. *EcoEvoRxiv*, doi:https://doi.org/10.32942/X2ZG7S (2024).
23 Depocas, F. & Hart, J. S. Use of the Pauling oxygen analyzer for measurement of oxygen consumption of animals in open-circuit systems and in a short-lag, closed-circuit apparatus. *J. Appl. Physiol.* **10**, 388-392 (1957).
24 Koteja, P. Measuring energy metabolism with open-flow respirometric systems: which design to choose? *Funct. Ecol.* **10**, 675-677 (1996).
25 Withers, P. C. Design, calibration and calculation for flow-through respirometry systems. *Aust. J. Zool.* **49**, 445-461 (2001).
26 Lighton, J. R. B. *Measuring metabolic rates: A manual for scientists*. (Oxford University Press, 2019).
27 Withers, P. C. Measurement of VO2, VCO2, and evaporative water loss with a flow-through mask. *J. Appl. Physiol.* **42**, 120-123 (1977).
28 Walsberg, G. E. & Hoffman, T. C. Using direct calorimetry to test the accuracy of indirect calorimetry in an ectotherm. *Physiol. Biochem. Zool.* **79**, 830-835 (2006).
29 Walsberg, G. E. & Hoffman, T. C. Direct calorimetry reveals large errors in respirometric estimates of energy expenditure. *J. Exp. Biol.* **208**, 1035-1043 (2005).
30 Lovegrove, B. G. The zoogeography of mammalian basal metabolic rate. *Am. Nat.* **156**, 201-219 (2000).
31 Chown, S. & Nicolson, S. *Insect physiological ecology: mechanisms and patterns*. (Oxford University Press, 2004).
32 Angilletta, M. J. *Thermal adaptation: a theoretical and empirical synthesis*. (Oxford University Press, 2009).
33 McKechnie, A. E. & Wolf, B. O. The allometry of avian basal metabolic rate: good predictions need good data. *Physiol. Biochem. Zool.* **77**, 502-521 (2004).
34 Genoud, M., Isler, K. & Martin, R. D. Comparative analyses of basal rate of metabolism in mammals: data selection does matter. *Biol. Rev.* **93**, 404-438 (2018).
35 White, C. R., Alton, L. A., Bywater, C. L., Lombardi, E. J. & Marshall, D. J. Metabolic scaling is the product of life-history optimization. *Science* **377**, 834-839 (2022).
36 McNab, B. K. *The physiological ecology of vertebrates: a view from energetics*. (Cornell University Press, 2002).
37 McNab, B. K. Complications inherent in scaling the basal rate of metabolism in mammals. *The Quarterly Review of Biology* **63**, 25-54 (1988).
38 Arnold, P. A., Delean, S., Cassey, P. & White, C. R. Meta-analysis reveals that resting metabolic rate is not consistently related to fitness and performance in animals. *J. Comp. Physiol. B* **191**, 1097-1110 (2021).
39 Le Galliard, J. F. *et al.* A worldwide and annotated database of evaporative water loss rates in squamate reptiles. *Glob. Ecol. Biogeogr.* **30**, 1938-1950 (2021).
40 Wu, N. C. & Seebacher, F. Physiology can predict animal activity, exploration, and dispersal. *Comm. Biol.* **5**, 1-11 (2022).
41 Mathot, K. J., Dingemanse, N. J. & Nakagawa, S. The covariance between metabolic rate and behaviour varies across behaviours and thermal types: meta-analytic insights. *Biol. Rev.* **94**, 1056-1074 (2019).
42 Bornmann, L. & Mutz, R. Growth rates of modern science: A bibliometric analysis based on the number of publications and cited references. *JASIST* **66**, 2215-2222 (2015).

43 Killen, S. S. *et al.* Guidelines for reporting methods to estimate metabolic rates by aquatic intermittent-flow respirometry. *J. Exp. Biol.* **224**, jeb242522 (2021).
44 Downs, C. T. & Brown, M. Is respirometry a standardized technique? A review of measurement of avian resting metabolic rates. *J. Therm. Biol.* **37**, 531-536 (2012).
45 Parker, T. H. *et al.* Transparency in ecology and evolution: real problems, real solutions. *Trends Ecol. Evol.* **31**, 711-719 (2016).
46 Pottier, P., Burke, S., Drobniak, S. M., Lagisz, M. & Nakagawa, S. Sexual (in) equality? A meta-analysis of sex differences in thermal acclimation capacity across ectotherms. *Funct. Ecol.* **35**, 2663-2678 (2021).
47 Downie, A. T., Wu, N. C., Cramp, R. L. & Franklin, C. E. Sublethal consequences of ultraviolet radiation exposure on vertebrates: Synthesis through meta-analysis. *Glob. Chang. Biol.* **29**, 6620-6634 (2023).
48 Lighton, J. & Halsey, L. Flow-through respirometry applied to chamber systems: pros and cons, hints and tips. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **158**, 265-275 (2011).
49 Malte, C. L., Nørgaard, S. & Wang, T. Closed system respirometry may underestimate tissue gas exchange and bias the respiratory exchange ratio (RER). *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **192**, 17-27 (2016).
50 Schoffelen, P. & Plasqui, G. Classical experiments in whole-body metabolism: open-circuit respirometry—diluted flow chamber, hood, or facemask systems. *Eur. J. Appl. Physiol.* **118**, 33-49 (2018).
51 Bakken, G. S. Time-resolved respirometry: equations for the simultaneous measurement of all respiratory gases and the calibration of oxygen consumption using variable inert gas flow rates. *J. Therm. Biol.* **16**, 313-315 (1991).
52 Arch, J., Hislop, D., Wang, S. & Speakman, J. R. Some mathematical and technical issues in the measurement and interpretation of open-circuit indirect calorimetry in small animals. *Int. J. Obes.* **30**, 1322-1331 (2006).
53 Langman, V. *et al.* Moving cheaply: energetics of walking in the African elephant. *J. Exp. Biol.* **198**, 629-632 (1995).
54 Langman, V. A., Rowe, M. F., Roberts, T. J., Langman, N. V. & Taylor, C. R. Minimum cost of transport in Asian elephants: do we really need a bigger elephant? *J. Exp. Biol.* **215**, 1509-1514 (2012).
55 Bartholomew, G. A. & Lighton, J. R. Oxygen consumption during hover-feeding in free-ranging Anna hummingbirds. *J. Exp. Biol.* **123**, 191-199 (1986).
56 Welch Jr, K. C. The power of feeder-mask respirometry as a method for examining hummingbird energetics. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **158**, 276-286 (2011).
57 White, C. R., Matthews, P. G. & Seymour, R. S. In situ measurement of calling metabolic rate in an Australian mole cricket, *Gryllotalpa monanka*. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **150**, 217-221 (2008).
58 Wu, N. C., Alton, L. A., Clemente, C. J., Kearney, M. R. & White, C. R. Morphology and burrowing energetics of semi-fossorial skinks (*Liopholis* spp.). *J. Exp. Biol.* **218**, 2416-2426 (2015).
59 Fedak, M. A., Pinshow, B. & Schmidt-Nielsen, K. Energy cost of bipedal running. *Am. J. Physiol.* **227**, 1038-1044 (1974).
60 Norberg, U. M. in *Avian energetics and nutritional ecology* 199-249 (Springer, 1996).
61 Wiersma, P., Chappell, M. A. & Williams, J. B. Cold-and exercise-induced peak metabolic rates in tropical birds. *Proc. Natl. Acad. Sci.* **104**, 20866-20871 (2007).
62 Clemente, C. J., Withers, P. C. & Thompson, G. G. Metabolic rate and endurance capacity in Australian varanid lizards (Squamata: Varanidae: Varanus). *Biol. J. Linn. Soc.* **97**, 664-676 (2009).
63 Anderson, J. F. & Prestwich, K. N. The physiology of exercise at and above maximal aerobic capacity in a theraphosid (tarantula) spider, *Brachypelma smithi* (FO Pickard-Cambridge). *J. Comp. Physiol. B* **155**, 529-539 (1985).
64 Dausmann, K., Glos, J. & Heldmaier, G. Energetics of tropical hibernation. *J. Comp. Physiol. B* **179**, 345-357 (2009).
65 Seymour, R. S. *et al.* Scaling of standard metabolic rate in estuarine crocodiles *Crocodylus porosus*. *J. Comp. Physiol. B* **183**, 491-500 (2013).
66 Navas, C. A., Jared, C. & Antoniazzi, M. M. Water economy in the casque-headed tree-frog *Corythomantis greeningi* (Hylidae): role of behaviour, skin, and skull skin co-ossification. *J. Zool.* **257**, 525-532 (2002).
67 Bloom, A. J., Mooney, H. A., Björkman, O. & Berry, J. Materials and methods for carbon dioxide and water exchange analysis. *Plant, Cell & Environment* **3**, 371-376 (1980).
68 Dixon, M. & Grace, J. Water uptake by some chamber materials. *Plant, Cell & Environment* **5**, 323-327 (1982).
69 Kendeigh, S. C. Effect of air temperature on the rate of energy metabolism in the English sparrow. *J. Exp. Zool.* **96**, 1-16 (1944).
70 Lasiewski, R. C., Acosta, A. L. & Bernstein, M. H. Evaporative water loss in birds—I. Characteristics of the open flow method of determination, and their relation to estimates of thermoregulatory ability. *Comp. Biochem. Physiol.* **19**, 445-457 (1966).
71 McNab, B. K. The relationship among flow rate, chamber volume and calculated rate of metabolism in vertebrate respirometry. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **145**, 287-294 (2006).
72 White, C. R. & Kearney, M. R. Determinants of inter-specific variation in basal metabolic rate. *J. Comp. Physiol. B* **183**, 1-26 (2013).
73 Norin, T. & Metcalfe, N. B. Ecological and evolutionary consequences of metabolic rate plasticity in response to environmental change. *Philos. Trans. R. Soc. B* **374**, 20180180 (2019).
74 Koch, R. E. *et al.* Integrating mitochondrial aerobic metabolism into ecology and evolution. *Trends Ecol. Evol.* **36**, 321-332 (2021).
75 Glazier, D. S. Variable metabolic scaling breaks the law: from 'Newtonian'to 'Darwinian'approaches. *Proc. R. Soc. B.* **289**, 20221605 (2022).
76 Konarzewski, M. & Książek, A. Determinants of intra-specific variation in basal metabolic rate. *J. Comp. Physiol. B* **183**, 27-41 (2013).
77 Konarzewski, M., Książek, A. & Łapo, I. B. Artificial selection on metabolic rates and related traits in rodents. *Integr. Comp. Biol.* **45**, 416-425 (2005).
78 Auer, S. K., Dick, C. A., Metcalfe, N. B. & Reznick, D. N. Metabolic rate evolves rapidly and in parallel with the pace of life history. *Nat. Commun.* **9**, 1-6 (2018).
79 Pettersen, A. K., Marshall, D. J. & White, C. R. Understanding variation in metabolic rate. *J. Exp. Biol.* **221**, jeb166876 (2018).
80 Angilletta, J., Michael J. Variation in metabolic rate between populations of a geographically widespread lizard. *Physiol. Biochem. Zool.* **74**, 11-21 (2001).
81 Kurbalija Novičić, Z. *et al.* Within-population genetic effects of mtDNA on metabolic rate in *Drosophila subobscura*. *J. Evol. Biol.* **28**, 338-346 (2015).
82 Bovo, R. P. *et al.* Beyond Janzen's hypothesis: How amphibians that climb tropical mountains respond to climate variation. *Integr. Org. Biol.* **5**, obad009 (2023).
83 Harri, M. N. The rate of metabolic temperature acclimation in the frog, *Rana temporaria*. *Physiol. Zool.* **46**, 148-156 (1973).
84 Sandblom, E., Gräns, A., Axelsson, M. & Seth, H. Temperature acclimation rate of aerobic scope and feeding metabolism in fishes: implications in a thermally extreme future. *Proc. R. Soc. B. Biol. Sci.* **281**, 20141490 (2014).
85 Barceló, G., Salinas, J., Cavieres, G., Canals, M. & Sabat, P. Thermal history can affect the short-term thermal acclimation of

basal metabolic rate in the passerine *Zonotrichia capensis*. *J. Therm. Biol.* **34**, 415-419 (2009).
86 Londono, G. A., Chappell, M. A., Castaneda, M. d. R., Jankowski, J. E. & Robinson, S. K. Basal metabolism in tropical birds: latitude, altitude, and the 'pace of life'. *Funct. Ecol.* **29**, 338-346 (2015).
87 Chabot, D., Steffensen, J. & Farrell, A. The determination of standard metabolic rate in fishes. *J. Fish Biol.* **88**, 81-121 (2016).
88 Senzano, L. M. & Andrade, D. V. Temperature and dehydration effects on metabolism, water uptake and the partitioning between respiratory and cutaneous evaporative water loss in a terrestrial toad. *J. Exp. Biol.* **221**, jeb188482 (2018).
89 Benedict, F. G. *Vital energetics. A study in comparative basal metabolism.* (Carnegie Institute, 1938).
90 Hayes, J. P., Speakman, J. R. & Racey, P. A. Sampling bias in respirometry. *Physiol. Zool.* **65**, 604-619 (1992).
91 Cooper, C. & Withers, P. Effects of measurement duration on the determination of basal metabolic rate and evaporative water loss of small marsupials: how long is long enough? *Physiol. Biochem. Zool.* **82**, 438-446 (2009).
92 Page, A. J., Cooper, C. E. & Withers, P. C. Effects of experiment start time and duration on measurement of standard physiological variables. *J. Comp. Physiol. B* **181**, 657-665 (2011).
93 Duarte, L. C., Vaanholt, L. M., Sinclair, R., Gamo, Y. & Speakman, J. R. Limits to sustained energy intake XII: is the poor relation between resting metabolic rate and reproductive performance because resting metabolism is not a repeatable trait? *J. Exp. Biol.* **213**, 278-287 (2010).
94 Winwood-Smith, H. S. & White, C. R. Short-duration respirometry underestimates metabolic rate for discontinuous breathers. *J. Exp. Biol.* **221**, jeb175752 (2018).
95 Aschoff, J. & Pohl, H. in *Fed. Proc.* 1541-1552.
96 Jacobs, P. J. & McKechnie, A. E. Experimental sources of variation in avian energetics: estimated basal metabolic rate decreases with successive measurements. *Physiol. Biochem. Zool.* **87**, 762-769 (2014).
97 Boyles, J. G. *et al.* A global heterothermic continuum in mammals. *Glob. Ecol. Biogeogr.* **22**, 1029-1039 (2013).
98 Willis, C. K. An energy-based body temperature threshold between torpor and normothermia for small mammals. *Physiol. Biochem. Zool.* **80**, 643-651 (2007).
99 Geiser, F. *Ecological physiology of daily torpor and hibernation*. (Springer, 2021).
100 Aschoff, J. Comparative physiology: diurnal rhythms. *Annu. Rev. Physiol.* **25**, 581-600 (1963).
101 Aschoff, J. Thermal conductance in mammals and birds: its dependence on body size and crcadian phase. *Comp. Biochem. Physiol. Part A Physiol.* **69**, 611-619 (1981).
102 Refinetti, R. & Menaker, M. The circadian rhythm of body temperature. *Physiol. Behav.* **51**, 613-637 (1992).
103 Toussaint, D. C. & McKechnie, A. E. Interspecific variation in thermoregulation among three sympatric bats inhabiting a hot, semi-arid environment. *J. Comp. Physiol. B* **182**, 1129-1140 (2012).
104 Nord, A. *et al.* Evaluation of two methods for minimally invasive peripheral body temperature measurement in birds. *J. Avian Biol.* **47**, 417-427 (2016).
105 Reher, S. & Dausmann, K. H. Tropical bats counter heat by combining torpor with adaptive hyperthermia. *Proc. R. Soc. B.* **288**, 20202059 (2021).
106 Levesque, D. L., Breit, A. M., Brown, E., Nowack, J. & Welman, S. Non-torpid heterothermy in mammals: another category along the homeothermy–hibernation continuum. *Integr. Comp. Biol.* **63**, 1039-1048 (2023).
107 Freeman, M. T. *et al.* Evolution of avian heat tolerance: the role of atmospheric humidity. *Ecology* **105**, e4279 (2024).
108 Van Dyk, M., Noakes, M. J. & McKechnie, A. E. Interactions between humidity and evaporative heat dissipation in a passerine bird. *J. Comp. Physiol. B* **189**, 299-308 (2019).
109 Powers, D. R. Effect of temperature and humidity on evaporative water loss in Anna's hummingbird (*Calypte anna*). *J. Comp. Physiol. B* **162**, 74-84 (1992).
110 McGuire, L. P., Mayberry, H. W. & Willis, C. K. White-nose syndrome increases torpid metabolic rate and evaporative water loss in hibernating bats. *Am. J. Physiol-Reg. I.* **313**, R680-R686 (2017).
111 Whitfield, M. C., Smit, B., McKechnie, A. E. & Wolf, B. O. Avian thermoregulation in the heat: scaling of heat tolerance and evaporative cooling capacity in three southern African arid-zone passerines. *J. Exp. Biol.* **218**, 1705-1714 (2015).
112 Pough, F. H., Taigen, T. L., Stewart, M. M. & Brussard, P. F. Behavioral modification of evaporative water loss by a Puerto Rican frog. *Ecology* **64**, 244-252 (1983).
113 Riddell, E. A., Apanovitch, E. K., Odom, J. P. & Sears, M. W. Physical calculations of resistance to water loss improve predictions of species range models. *Ecol. Monogr.* **87**, 21-33 (2017).
114 Bartholomew, G. A., Vleck, D. & Vleck, C. M. Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths. *J. Exp. Biol.* **90**, 17-32 (1981).
115 Edwards, A. M. & Auger-Méthé, M. Some guidance on using mathematical notation in ecology. *Method. Ecol. Evol.* **10**, 92-99 (2019).
116 Curran-Everett, D. & Benos, D. J. Vol. 287 R247-R249 (American Physiological Society, 2004).
117 Nakagawa, S. & Cuthill, I. C. Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biol. Rev.* **82**, 591-605 (2007).
118 Gerstner, K. *et al.* Will your paper be used in a meta-analysis? Make the reach of your research broader and longer lasting. *Method. Ecol. Evol.* **8**, 777-784 (2017).
119 Harrison, X. A. *et al.* A brief introduction to mixed effects modelling and multi-model inference in ecology. *PeerJ* **6**, e4794 (2018).
120 Fraser, H., Parker, T., Nakagawa, S., Barnett, A. & Fidler, F. Questionable research practices in ecology and evolution. *PloS one* **13**, e0200303 (2018).
121 O'Dea, R. E. *et al.* Preferred reporting items for systematic reviews and meta-analyses in ecology and evolutionary biology: a PRISMA extension. *Biol. Rev.* **96**, 1695-1722 (2021).
122 Bertram, M. G. *et al.* Open science. *Curr. Biol.* **33**, R792-R797 (2023).
123 Parr, C. S. & Cummings, M. P. Data sharing in ecology and evolution. *Trends Ecol. Evol.* **20**, 362-363 (2005).
124 Moore, A. J., McPeek, M. A., Rausher, M. D., Rieseberg, L. & Whitlock, M. C. The need for archiving data in evolutionary biology. *J. Evol. Biol.* **23**, 659-660 (2010).
125 Roche, D. G. *et al.* Slow improvement to the archiving quality of open datasets shared by researchers in ecology and evolution. *Proc. R. Soc. B.* **289**, 20212780 (2022).
126 Piwowar, H. A., Day, R. S. & Fridsma, D. B. Sharing detailed research data is associated with increased citation rate. *PloS ONE* **2**, e308 (2007).
127 Piwowar, H. A. & Vision, T. J. Data reuse and the open data citation advantage. *PeerJ* **1**, e175 (2013).
128 Vines, T. H. *et al.* Mandated data archiving greatly improves access to research data. *arXiv preprint arXiv:1301.3744* (2013).
129 Gomes, D. G. *et al.* Why don't we share data and code? Perceived barriers and benefits to public archiving practices. *Proc. R. Soc. B.* **289**, 20221113 (2022).

130 Tracy, C. R., Reynolds, S. J., McArthur, L., Tracy, C. R. & Christian, K. A. Ecology of aestivation in a cocoon-forming frog, *Cyclorana australis* (Hylidae). *Copeia* **2007**, 901-912 (2007).
131 Tracy, C. R., Christian, K. A., Betts, G. & Tracy, C. R. Body temperature and resistance to evaporative water loss in tropical Australian frogs. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **150**, 102-108 (2008).
132 Sansone, S.-A. *et al.* FAIRsharing as a community approach to standards, repositories and policies. *Nat. Biotechnol.* **37**, 358-367 (2019).
133 Reichman, O. J., Jones, M. B. & Schildhauer, M. P. Challenges and opportunities of open data in ecology. *Science* **331**, 703-705 (2011).
134 Whitlock, M. C. Data archiving in ecology and evolution: best practices. *Trends Ecol. Evol.* **26**, 61-65 (2011).
135 Wilson, S. L. *et al.* Sharing biological data: why, when, and how. *FEBS Lett.* **595**, 847–863 (2021).
136 Harianto, J., Carey, N. & Byrne, M. RespR—An R package for the manipulation and analysis of respirometry data. *Method. Ecol. Evol.* **10**, 912-920 (2019).
137 Powers, S. M. & Hampton, S. E. Open science, reproducibility, and transparency in ecology. *Ecol. Appl.* **29**, e01822 (2019).
138 Olito, C., White, C. R., Marshall, D. J. & Barneche, D. R. Estimating monotonic rates from biological data using local linear regression. *J. Exp. Biol.* **220**, 759-764 (2017).
139 Schwanz, L. E. *et al.* Best practices for building and curating databases for comparative analyses. *J. Exp. Biol.* **225**, jeb243295 (2022).
140 Parker, T. H. *et al.* Empowering peer reviewers with a checklist to improve transparency. *Nat. Eco. Evol.* **2**, 929-936 (2018).
141 Kristín, P. & Gvoždík, L. Influence of respirometry methods on intraspecific variation in standard metabolic rates in newts. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **163**, 147-151 (2012).
142 Bakken, G. S., Murphy, M. T. & Erskine, D. J. The effect of wind and air temperature on metabolism and evaporative water loss rates of Dark-eyed Juncos, *Junco hyemalis*: a standard operative temperature scale. *Physiol. Zool.* **64**, 1023-1049 (1991).
143 White, C. R., Portugal, S. J., Martin, G. R. & Butler, P. J. Respirometry: anhydrous drierite equilibrates with carbon dioxide and increases washout times. *Physiol. Biochem. Zool.* **79**, 977-980 (2006).
144 Elia, M., McDonald, T. & Crisp, A. Errors in measurements of CO2 with the use of drying agents. *Clin. Chim. Acta* **158**, 237-244 (1986).
145 Nowack, J., Dill, V. & Dausmann, K. H. Open-flow respirometry under field conditions: How does the airflow through the nest influence our results? *J. Therm. Biol.* **92**, 102667 (2020).
146 Rodgers, E. M. & Franklin, C. E. Aerobic scope and climate warming: testing the "plastic floors and concrete ceilings" hypothesis in the estuarine crocodile (*Crocodylus porosus*). *J. Exp. Zool. Part A* **335**, 108-117 (2021).
147 Cooper, C. E. & Withers, P. C. Physiological responses of a rodent to heliox reveal constancy of evaporative water loss under perturbing environmental conditions. *Am. J. Physiol-Reg. I.* **307**, R1042-R1048 (2014).
148 Frappell, P., Blevin, H. & Baudinette, R. Understanding respirometry chambers: what goes in must come out. *J. Theor. Biol.* **138**, 479-494 (1989).
149 Hill, R. W. Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. *J. Appl. Physiol.* **33**, 261-263 (1972).
150 Bakken, G., Erskine, D. & Murphy, M. A wind tunnel metabolism chamber with small volume and good air flow characteristics. *Journal of Physics E: Scientific Instruments* **22**, 1048 (1989).
151 Wilz, M. & Heldmaier, G. Comparison of hibernation, estivation and daily torpor in the edible dormouse, *Glis glis*. *J. Comp. Physiol. B* **170**, 511-521 (2000).
152 Smit, B. & McKechnie, A. E. Avian seasonal metabolic variation in a subtropical desert: basal metabolic rates are lower in winter than in summer. *Funct. Ecol.* **24**, 330-339 (2010).
153 Burton, T., Killen, S., Armstrong, J. D. & Metcalfe, N. What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proc. R. Soc. B. Biol. Sci.* **278**, 3465-3473 (2011).
154 Terblanche, J. S., Sinclair, B. J., Klok, C. J., McFarlane, M. L. & Chown, S. L. The effects of acclimation on thermal tolerance, desiccation resistance and metabolic rate in *Chirodica chalcoptera* (Coleoptera: Chrysomelidae). *J. Insect Physiol.* **51**, 1013-1023 (2005).
155 Weaver, S. J., McIntyre, T., van Rossum, T., Telemeco, R. S. & Taylor, E. N. Hydration and evaporative water loss of lizards change in response to temperature and humidity acclimation. *J. Exp. Biol.* **226** (2023).
156 Dezetter, M., Dupoué, A., Le Galliard, J. F. & Lourdais, O. Additive effects of developmental acclimation and physiological syndromes on lifetime metabolic and water loss rates of a dry-skinned ectotherm. *Funct. Ecol.* **36**, 432-445 (2022).
157 Alton, L. A. *et al.* Developmental nutrition modulates metabolic responses to projected climate change. *Funct. Ecol.* **34**, 2488-2502 (2020).
158 Medina-Báez, O. A., Lenard, A., Muzychuk, R. A., da Silva, C. R. & Diamond, S. E. Life cycle complexity and body mass drive erratic changes in climate vulnerability across ontogeny in a seasonally migrating butterfly. *Conserv. Physiol.* **11**, coad058 (2023).
159 Gray, E. M. Thermal acclimation in a complex life cycle: the effects of larval and adult thermal conditions on metabolic rate and heat resistance in *Culex pipiens* (Diptera: Culicidae). *J. Insect Physiol.* **59**, 1001-1007 (2013).
160 Pessato, A., Udino, E., McKechnie, A. E., Bennett, A. T. & Mariette, M. M. Thermal acclimatisation to heatwave conditions is rapid but sex-specific in wild zebra finches. *Sci. Rep.* **13**, 18297 (2023).
161 Videlier, M., Rundle, H. D. & Careau, V. Sex-specific among-individual covariation in locomotor activity and resting metabolic rate in *Drosophila melanogaster*. *Am. Nat.* **194**, E164-E176 (2019).
162 McLean, J. A. & Speakman, J. R. Effects of body mass and reproduction on the basal metabolic rate of brown long-eared bats (*Plecotus auritus*). *Physiol. Biochem. Zool.* **73**, 112-121 (2000).
163 Demarco, V. & Guillette Jr, L. J. Physiological cost of pregnancy in a viviparous lizard (*Sceloporus jarrovi*). *J. Exp. Zool.* **262**, 383-390 (1992).
164 Angilletta, M. & Sears, M. The metabolic cost of reproduction in an oviparous lizard. *Funct. Ecol.* **14**, 39-45 (2000).
165 Kaiyala, K. J. *et al.* Identification of body fat mass as a major determinant of metabolic rate in mice. *Diabetes* **59**, 1657-1666 (2010).
166 Connolly, M. & Cooper, C. How do measurement duration and timing interact to influence estimation of basal physiological variables of a nocturnal rodent? *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **178**, 24-29 (2014).
167 Chew, R. M., Lindberg, R. G. & Hayden, P. Circadian rhythm of metabolic rate in pocket mice. *J. Mammal.* **46**, 477-494 (1965).
168 Powers, D. R. Diurnal variation in mass, metabolic rate, and respiratory quotient in Anna's and Costa's hummingbirds. *Physiol. Zool.* **64**, 850-870 (1991).
169 Riccio, A. P. & Goldman, B. D. Circadian rhythms of body temperature and metabolic rate in naked mole-rats. *Physiol. Behav.* **71**, 15-22 (2000).
170 Cooper, C. E. & Withers, P. C. Effect of sampling regime on estimation of basal metabolic rate and standard evaporative water

loss using flow-through respirometry. *Physiol. Biochem. Zool.* **83**, 385-393 (2010).
171 Short, J. C., Freeman, M. T. & McKechnie, A. E. Respirometry protocols for avian thermoregulation at high air temperatures: stepped and steady-state profiles yield similar results. *J. Exp. Biol.* **225**, jeb244166 (2022).
172 Gilson, L. N., Cooper, C. E., Withers, P. C. & Gagnon, M. M. Two independent approaches to assessing the constancy of evaporative water loss for birds under varying evaporative conditions. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **261**, 111041 (2021).
173 McCue, M. Specific dynamic action: a century of investigation. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **144**, 381-394 (2006).
174 Preest, M. & Pough, F. H. Interaction of temperature and hydration on locomotion of toads. *Funct. Ecol.* **3**, 693-699 (1989).
175 Rusli, M. U., Booth, D. T. & Joseph, J. Synchronous activity lowers the energetic cost of nest escape for sea turtle hatchlings. *J. Exp. Biol.* **219**, 1505-1513 (2016).
176 Seymour, R. S., Withers, P. C. & Weathers, W. W. Energetics of burrowing, running, and free-living in the Namib Desert golden mole (*Eremitalpa namibensis*). *J. Zool.* **244**, 107-117 (1998).
177 Snelling, E. P., Duncker, R., Jones, K. K., Fagan-Jeffries, E. P. & Seymour, R. S. Flight metabolic rate of *Locusta migratoria* in relation to oxygen partial pressure in atmospheres of varying diffusivity and density. *J. Exp. Biol.* **220**, 4432-4439 (2017).
178 Wu, N. C., Cramp, R. L. & Franklin, C. E. Body size influences energetic and osmoregulatory costs in frogs infected with *Batrachochytrium dendrobatidis*. *Sci. Rep.* **8**, 3739 (2018).
179 Lighton, J. in *Indirect calorimetry: Techniques, computations and applications* (eds Walter Gerrits & Etienne Labussière) 57-71 (Wageningen Academic Publishers, 2023).
180 Senzano, L. M., Bovo, R. P. & Andrade, D. V. Empirical estimation of skin resistance to water loss in amphibians: agar evaluation as a non-resistance model to evaporation. *J. Exp. Biol.* **225**, jeb243941 (2022).
181 Nickerson, D. M., Facey, D. E. & Grossman, G. D. Estimating physiological thresholds with continuous two-phase regression. *Physiol. Zool.* **62**, 866-887 (1989).
182 Packard, G. C. & Boardman, T. J. The misuse of ratios, indices, and percentages in ecophysiological research. *Physiol. Zool.* **61**, 1-9 (1988).
183 Packard, G. C. & Boardman, T. J. The use of percentages and size-specific indices to normalize physiological data for variation in body size: wasted time, wasted effort? *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **122**, 37-44 (1999).
184 White, C. R. & Kearney, M. R. Metabolic scaling in animals: methods, empirical results, and theoretical explanations. *Compr. Physiol.* **4**, 231-256 (2011).
185 Legendre, P. Spatial autocorrelation: trouble or new paradigm? *Ecology* **74**, 1659-1673 (1993).
186 Noble, D. W., Lagisz, M., O'dea, R. E. & Nakagawa, S. Nonindependence and sensitivity analyses in ecological and evolutionary meta-analyses. *Mol. Ecol.* **26**, 2410-2425 (2017).
187 Cinar, O., Nakagawa, S. & Viechtbauer, W. Phylogenetic multilevel meta-analysis: A simulation study on the importance of modeling the phylogeny. *Method. Ecol. Evol.* **13**, 383-395 (2022).
188 Tedersoo, L. *et al*. Data sharing practices and data availability upon request differ across scientific disciplines. *Scientific data* **8**, 192 (2021).

Author contributions: NCW and DLL conceived the idea, and all authors contributed to the development and write up of the manuscript. Co-authors were listed in alphabetical order by last name.

Competing interests: Authors declare no conflict of interest.

Funding information: LAA was supported by an ARC Discovery Project (DP220103553). DLL was supported by the United States Department of Agriculture-National Institute of Food and Agriculture (ME022406 through the Maine Agricultural & Forest Experiment Station). RPB was support by the São Paulo Research Foundation—FAPESP (#10/20061-6, #17/10338-0).

SUPPLEMENTARY MATERIALS

Respirometry Checklist

TABLES

Table 1 | A checklist of criteria for reporting the methods and results from respirometry experiments for terrestrial animals. Example references are provided for each criterion. Endotherm-specific details are provided in **bold**. A printable checklist is also provided in the electronic supplementary attachment.

Reporting criterion	Description	Refs
Materials, Set up, Plumbing		
Set-up type	For MR measurements, report whether the system was closed, intermittent-closed, or flow-through. If flow-through, was it a push or pull system? For EWL measurements, report whether the system was designed to measure change in body mass, change in desiccant mass, flux chamber, or change in water vapour pressure.	26,141
Air flow	Report the air flow as volume over time corrected to standard temperature and pressure (STP), and how the flow rate was achieved and maintained. Definitions for STP vary and so should be defined (in comparative physiology, it is usually defined as 273.15 K and 101.325 kPa). Air flow should refer to the flow experienced by the animal and not the flow through the analysers. See Subsampling below.	71,142
Physical and chemical scrubbing	Report whether air was scrubbed of H_2O and/or CO_2 physically or mathematically. If physically scrubbed, what type of scrubbers were used (e.g., Drierite) and where in the plumbing set-up were they placed? If mathematically scrubbed, what equation was used?	24,26,143, 144
Chamber design	Provide details on the chamber design including empty chamber size and volume, and what material(s) the chamber is made from. If objects were placed inside the chamber (e.g. mesh, platform, nest material), describe them. If a layer of mineral oil or similar is placed in the bottom of the chamber to prevent evaporation from excreta affecting EWL measurements, indicate the approximate depth of the layer.	58,71,110, 145,146
Incurrent air	State the source of the incurrent air (e.g. outdoor air, gas cylinders). If gas mixes were used, provide the gas composition (e.g., nitrogen-oxygen mix, helium-oxygen mix (helox), CO_2-free air).	26,147
Chamber mixing	Describe how chamber mixing was achieved. Important for the correct gas mixture, and also mediating washout times if animals are exercising/active/shivering in the chamber.	148

O_2 analyser	Report what type of analyser was used (e.g., paramagnetic, fuel-cell, zirconia-cell, infrared, oxygen-quenched fluorescence) and provide the model and manufacturer.	26,149
CO_2 analyser	Report what type of analyser was used (e.g., infrared, nondispersive infrared) and provide the model and manufacturer.	26
H_2O analyser	Report what type of analyser was used (e.g., chilled mirror, capacitive, infrared) and provide the model and manufacturer.	26
Calibration	Describe how the flow meters, gas analysers, temperature probes, etc. were calibrated and how often. Include the concentrations of any span gases used.	25
Connectors	Provide details on the tubing material and connectors, as different materials can alter gas and humidity measurements due to potential leaking.	26
Temperature recorder	Report how and where respirometer temperature was measured. **Endotherm**: Temperature should generally be measured inside the respirometer chamber due to heat production by the animal.	
Multiplexers	If multiplexers were used, describe how and where they were set-up. Was a digital-to-analogue converter used for automation?	26
Subsampling	If subsampling was used, provide the flow rate and explain how the flow rate was achieved and maintained.	26
Visualisation	Ideally, a schematic diagram of the plumbing and position of the equipment relative to the respirometry chamber will help facilitate the description of the set-up.	26,150,151
Subject conditions/maintenance		
Study species	State the study species (and strain if relevant).	
Origin	State the origin of collection such as where (coordinates) and when (dates) the animals were collected. Provide the habitat characteristics if relevant to discussing the environmental context of the study. For laboratory raised subjects, provide the number of generations since caught from the wild and the source of the original population.	82,152,153
Husbandry conditions	Describe the husbandry conditions relevant to the study including, but not limited to: enclosure, feeding schedule, maintenance duration, acclimation duration, treatment groups, etc.	154-157
Age/life stage	Provide the life stage of the test subjects, and if known, provide the age.	158,159
Sex	Report the number of test subjects of each sex and state whether sex ratios were equal or similar across experimental groups	160,161
Reproductive condition	State the reproductive condition of the test subjects.	153,162-164
Biometrics	Measure biometrics for the test subjects (e.g., fresh mass, length, body condition) immediately before or after the respirometry trial. Biometrics collected upon arrival to the laboratory or at the time of capture may not reflect the animals physiological state at the time of experimentation Moreover, dry body mass, lipid-free dried mass, non-skeletal body mass is not recommended because live animals tightly control their hydration and lipid levels. Therefore, the total mass (water, fat and everything else) measured at the start or end, or both, should be reported.	26,165
Measurement conditions		
Blinding	If possible, data recorders should be blind to the experimental treatment imposed on the subjects when gathering data. Also, report whether or not blinding was implemented.	140
Baseline recording	Provide information on the background/baseline (empty chamber) recording including how often and how long the baseline was recorded for. For multiplexed systems, check whether each system is baselined with air before and after the experiment, or was a separate system relied on.	48
Time	State when the measurements were taken. MR fluctuates over the day and is affected by photoperiod.	92,143,166
Lighting	Provide information on the lighting conditions during the experiment.	167-169
Duration and frequency	State the experiment and measurement duration and frequency. This is especially important for obtaining minimum MR and EWL. Reducing the frequency of sampling can underestimate BMR and EWL. Duration should include the total time the animal is in the respirometer and not just while the recording is happening.	96,170
Test temperature	Provide the test air temperature (T_a) and how it was maintained. If a stepped temperature change was used, provide details on the duration and rate of change between each temperature setpoint.	171
Test humidity	Provide the test humidity (incurrent and excurrent) and how it was maintained. Humidity should be reported as absolute values or partial pressures (g H_2O m^{-3} or kPa) rather than relative humidity (RH, %). If only RH values are available, it is critical that the corresponding T_a are provided. If converting to water vapour pressure and water vapour deficit from RH and T_a, provide the reference to the equations used.	107,172
Standard temperature and pressure	Given that definitions of standard temperature and pressure (STP) vary, it is important that a definition of STP should also be provided for transparency.	26
Fasted	State whether the test subjects were fasted prior to the experiment and for how long.	173
Hydration	Hydration state affects MR and EWL measurements. How was the hydration level controlled prior to experimentation? If wet-skinned animal (e.g. amphibians), make sure to gently dry excessive water droplets over the surface (skin) exposed to evaporation. This effect is exaggerated in smaller test subjects.	88,174
Grouping	If more than one test subject was placed inside the chamber, provide the exact number of individuals.	175

Measurements	State what measurements were obtained (see Glossary), when, and for long they were measured. If individuals were repeated, state the number of repeats per exposure. If multiplexors where used, providing the sampling period	96
Animal state	Describe the state of the animal when the measurement was taken [e.g., inactive, active, rest-phase, active-phase, post-exhaustion, digesting, torpid, aestivating, normothermic (for endotherms)]. - For resting states, state the recovery time from handling stress after being placed into the chamber. - If post-exhausted for MMR, how was this achieved? - Activity should be monitored visually or measured to confirm an animal is inactive or to account for variation in MR and EWL associated with variation in activity levels.	9,93,161,176-178
Multiple animals	When multiple animals are measured in sequence in one measurement period, provide the timing of switching between channels. Describe how the washout times for the respirometry system and multiplexed sampling period was matched.	48
Data processing		
Data acquisition	Provide information on the data acquisition systems/software.	26
Baseline drift	Baseline measurements will fluctuate, especially for O_2 concentrations. State whether and how baseline drift was corrected.	48
Time lag	The position of the equipment and length of plumbing (and if physical scrubbers were used post-respirometer chamber) will influence the time of the recording. State whether and how time lag was corrected.	48
Mathematical scrubbing	If physical scrubbers were not used, provide details on how gas concentrations were mathematically scrubbed. Report whether it is appropriate for the type of O_2 analyser used and how this was determined e.g. paramagnetic?	26,179
Sampling	Describe and justify sample selection (mean, time period) as well as exclusion criteria (activity, posture, excretion etc). **Endotherm**: Some mammals will lick their fur or the chamber during respirometry trials which will produce relatively high EWL. The use of video surveillance is recommended to monitor such activities.	25,91,112,148,170
Boundary layer	For calculating skin resistance from EWL, state how the boundary layer was accounted for, either mathematically or empirically (e.g. from agar models) estimated.	113,180
Equations	Show the equations for all calculations in addition to citing their sources.	23-26,49,50
Calculations	State how MR and EWL values were calculated (e.g., lowest value, lowest 10% of average, first hour slope, residuals around a linear regression). Differences in metabolic sampling can cause small but significant effects on minimum MR measurements. - For maximal or forced locomotion, define method of extraction e.g., MR at fastest speed, highest value, immediately post-exhaustion?	25,170,181
Data exclusion	If data were excluded from the study due to experiment/measurement/animal issues, provide such information for transparency. Indicate the criterion e.g. extreme values, outlier statistics.	
Data reporting and statistics		
Aims and hypotheses	In the Introduction, clearly state the aims and/or hypothesis for which the study was conducted and data were gathered.	140
Units	Always report units in the paper. Use only International System of Units (SI) or SI-derived units.	
Raw data	Supply raw data on the rate of O_2 consumption, CO_2 production or EWL in addition to converted values used in the paper. E.g. translating to energy equivalents, mass-corrected or mass-specific values, surface-specific values. And when presenting mass- or surface-specific values, remember that such data remove the effect of mass only in very specific (and usually not realistic) situations.	26,52,182-184
Sample size	Report sample sizes for all data, including subsets of data (e.g., each treatment group, other subsets), and sample size used for all statistical analyses.	140
Pseudoreplication	Report pesudoreplication if used. E.g. the number of tanks, rooms, chambers used, and the number of animals in each. Also report how pesudoreplication was statistically accounted for (e.g. random effect).	
Statistics	List each statistical test and analysis conducted in sufficient detail such that they can be replicated and fully understood by those experienced in those methods. Fully report outcomes from each statistical analysis. For most analyses, this includes, but is not limited to, basic parameter estimates of central tendency (e.g., means) or other basic estimates (regression coefficients, correlation) and variability (e.g., standard deviation) or associated estimates of uncertainty (e.g., confidence/credible intervals). Thorough and transparent reporting will involve additional information that differs depending on the type of analyses conducted. · For null hypothesis tests, this also should at minimum include test statistic, degrees of freedom, and p-value. · For Bayesian analyses, this also should at a minimum include information on choice of priors and MCMC (Markov chain Monte Carlo) settings (e.g. burn-in, the number of iterations, and thinning intervals). · For hierarchical and other more complex experimental designs, full information on the design and analysis, including identification of the appropriate level for tests (e.g. identifying the denominator used for split-plot experiments) and full reporting of outcomes (e.g. including blocking in the analysis if it was used in the design). Relevant information will differ among other types of analyses but in all cases should include enough information to fully evaluate the design and analysis.	140
Covariates	Provide a description of all covariates tested.	
Non-independence	State if the data presents sources of non-independence (e.g., group effect, repeated measures, spatial and temporal effects such as autocorrelations) and how they were accounted for in the analyses (e.g., random effects).	185-187

Softwares and packages	Cite all softwares and packages used in the data processing and analysis.	
Data	Include the data upon which analyses are based (as well as raw data) as supplementary materials with submission and archived in a permanently supported, publicly accessible database. Include a METADATA to describe what the naming conventions and abbreviations means. If additional data was obtained from other sources for comparison (e.g., database, publication), list and cite the sources.	129,188