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

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ABSTRACT

Respirometry is an important tool for understanding whole-animal energy and water balance. Consequently, the growing number of studies using respirometry over the last decade warrants reliable reporting and data sharing for effective research synthesis and dissemination. 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 in the designing and reporting of their study, and reviewers and editors can use the checklist to assess reporting quality in 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, thus providing reliable evidence-based information for political actions in the face of global change.

INTRODUCTION

Quantifying energy and water fluxes between animals and their environments is vital for understanding their physiology, behaviour, ecology, and evolution (Bergmann, 1848; Kleiber, 1961; Ricklefs and Wikelski, 2002; Lovegrove, 2019; Pettersen and Metcalfe, 2024). Accurate

measurements of energy and water exchanges, the former dating back to the 18th Century (Lavoisier and Seguin, 1789; Townson, 1799), remain an essential skill for evolutionary and ecological physiologists as we enter the second quarter of the 21st Century. In this context, given the pervasive effects of temperature on energy and water budgets, it is not surprising that thermal physiology has become a field essential for predicting the impacts of rapid anthropogenic global heating on biodiversity, whether using species-specific empirical data (Mckechnie and Swanson, 2010; Seebacher et al., 2015; Conradie et al., 2020) or validating predictions and improving the development of increasingly sophisticated biophysical modelling approaches (Kearney and Porter, 2009; Riddell et al., 2021; Briscoe et al., 2023; Kearney and Enriquez-Urzelai, 2023). Alongside this, the growing field of ecotoxicology and disease ecology relies on measurements of energy (e.g. metabolic rate, MR) and water balance to accurately evaluate environmental risk to novel toxins and pollutants (Sokolova and Lannig, 2008; Baas and Kooijman, 2015) and species risk to emerging infectious diseases (Agugliaro et al., 2020; Wu, 2023). The same applies to other growing fields such as conservation physiology (Wikelski and Cooke, 2006; Cooke et al., 2013) and macrophysiology (Chown et al., 2004; Ruf and Geiser, 2015; Chown and Gaston, 2016; Wu et al., 2024) in which, through the mechanistic lens of physiology, improve our understanding on adaptations of different organisms in different environments. All of this emphasises that measurements of energy and water fluxes remain as essential tools for a number of disciplines in biology in the contemporary era.

Respirometry experiments (see next section for details) provide insights into the energy production, MR, water balance, and adaptation strategies of organisms to function in different environments. Briefly, the most widespread approach to quantifying the amount of energy expended over a specific period of time, such as MR, in animals is indirect calorimetry, which involves respirometric measurements of oxygen consumption ($\dot{V}_{O_2}$; where V indicates volume and the dot over the V indicates rate [volume/time]) 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 (reviewed by Lighton (2019), but see Walsberg and Hoffman (2005) and next section). Combined with simultaneous measurements of evaporative water loss (EWL i.e. water lost through the process of evaporation from the surface – skin and the respiratory tract – of an organism) and body temperature (T_b), respirometry remains the mainstay of experimental research into comparative physiology. For instance, respirometry provides the basis for quantifying energy and water expenditures under standardised conditions, including standard metabolic rate (SMR) in ectotherms and basal metabolic rate (BMR) in endotherms (Londono et al., 2015; Chabot et al., 2016), cutaneous and respiratory water loss (see Glossary) (Senzano and Andrade, 2018), upper limits to

cold tolerance and heat tolerance in resting endotherms (Bozinovic and Rosenmann, 1989; Swanson and Liknes, 2006; McKechnie et al., 2021), and, when combined with devices such as treadmills, exercise wheels or wind tunnels, maximum metabolic rates (MMR) and EWL during exercise (Fedak et al., 1974; Norberg, 1996; Wiersma et al., 2007; Clemente et al., 2009). In this way, respirometric data have provided the basis for most analyses of variation in animal energy and water balances, including scaling with body mass, thermal adaptation and phenotypic plasticity (Lovegrove, 2000; Chown and Nicolson, 2004; McKechnie and Wolf, 2004; Angilletta, 2009; Mckechnie and Swanson, 2010; Genoud et al., 2018; White et al., 2022), which highlights the crucial role of respirometry experiments and reliable measurements.

Advances in technology have made the tools for measurement of energy and water balances more affordable and robust, resulting in a growing number of MR and EWL studies and also measurements taken outside of controlled conditions (Langer et al., 2018; Nowack et al., 2020; Reher et al., 2022). With the burgeoning field of biologging, it has increasingly providing novel insights into the energy and water budgeting of free-ranging individuals, applying knowledge of physiological principles in appropriate ecological settings (Chmura et al., 2018; Cooper et al., 2019). Nevertheless, it requires careful validation at the individual level with measurements of, for example, energy budgets under controlled conditions (Halsey and Bryce, 2021). Following Killen et al. (2021) recent commentary on guidelines for reporting aquatic intermittent flow respirometry, we identified a number of considerations relevant for flow-through respirometry in terrestrial organisms in general and additional considerations specific to endotherms. In this commentary, we provide an overview of air-based respirometry, highlight common omissions in the literature, and provide a detailed-checklist to improve the standardisation and reporting of future studies.

WHAT IS RESPIROMETRY?

Respiration is a fundamental biological process that involves the exchange of gases, such as O_2 and CO_2, between an organism and its environment to generate energy. Thus, respirometry experiments are essential in a number of biological disciplines, providing insights into the energy production, MR, and adaptation strategies of organisms to cope with environmental changes. Such experiments measure the rate of respiration in living organisms, typically focusing on the consumption of O_2 or the production of CO_2. The experimental setup for respirometry typically involves a respirometer (**Fig. 1**), a device that measures changes in voltages that are converted by equations into meaningful gas concentrations. For instance, consumption of O_2 or production of CO_2 is inferred by monitoring the decrease in O_2 or increase in CO_2 levels over time. While respirometry itself is not designed to measure EWL directly, it can be complemented with other techniques (e.g., gravimetric

measurements; Wygoda, 1984), or the experimental setup can be modified to include gas measurements (water vapor density or pressure) to gain some insights into water loss along with respiratory parameters.

Although definitions vary, MR is usually taken to be the rate at which an animal expends energy (see Glossary). In living organisms, the common currency for energy is adenosine triphosphate (ATP), which is generated by the oxidation of nutrient substrates (carbohydrates, fats, and proteins). When an animal is in steady state and does not perform external work or change its body composition (by growth, digestion of food, or excretion), all energy transferred to ATP is eventually released as heat. MR under these conditions can be measured directly by quantifying the rate of heat production using direct calorimetry (e.g., Hofelich et al., 2001; Walsberg and Hoffman, 2005, 2006; Zhang, 2010; Kaiyala and Ramsay, 2011; Regan et al., 2013). But direct measurements of MR are rare. For those animals whose energy needs are fulfilled predominantly by aerobic respiration, it is more common to measure their metabolic heat production indirectly by quantifying their $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, or both (Depocas and Hart, 1957; Hill, 1972; Withers, 1977, 2001; Clark et al., 2013; Gerrits and Labussière, 2015; Lighton, 2019). Indirect calorimetry is based on Hess's Law of Constant Heat Sums, which states that the heat released in a chemical reaction depends only on the nature of the initial reactants and final products, and not on intermediate steps. Therefore, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ are related to heat production – or, equivalently, MR or energy expenditure (EE) – through the stoichiometry of the chemical reactions involved in the oxidation of nutrient substrates.

Knowledge of the nutrient substrates being oxidised, as well as the mode of nitrogen excretion, is required to convert measures of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ to rates of heat production using conversion factors (called oxycaloric or thermal coefficients, or energy equivalents) established in the literature and available in reference books (Gnaiger, 1983; Withers, 1992; Schmidt-Nielsen, 1997; Lighton, 2019; Butler et al., 2022) which provides a detailed accounts of which oxycaloric coefficients to use and when. When measuring $\dot{V}_{O_2}$ and/or $\dot{V}_{CO_2}$, it is typically assumed that an animal is either: (a) catabolising only lipids when fasting; (b) catabolising materials in proportion to their abundance in their diet; or (c) catabolising materials with a carbohydrate-to-lipid ratio as indicated by their respiratory exchange ratio, RER (the ratio of CO_2 released to O_2 consumed) (Walsberg and Hoffman, 2005) (see below).

The suites of techniques used to measure MR indirectly are collectively referred to as respirometry (or indirect calorimetry; some examples presented in **Fig. 1**). The technique is, in principle, quite straightforward. In flow-through respirometry (**Fig. 1c**), $\dot{V}_{O_2}$ can be determined by containing an animal within a chamber and passing air through the chamber. $\dot{V}_{O_2}$ is then calculated

from the difference between the rate at which oxygen enters the chamber (the product of incurrent flow rate, $\dot{V}_{\mathrm{I}}$, and the fractional oxygen concentration of incurrent air, $F_{\mathrm{I}}\mathrm{O}_2$) and the rate at which oxygen leaves the chamber (the product of excurrent flow rate, $\dot{V}_{\mathrm{E}}$, and the fractional oxygen concentration of excurrent air $F_{\mathrm{E}}\mathrm{O}_2$):

$$\dot{V}_{\mathrm{O}_2} = \dot{V}_{\mathrm{I}} \cdot F_{\mathrm{I}}\mathrm{O}_2 - \dot{V}_{\mathrm{E}} \cdot F_{\mathrm{E}}\mathrm{O}_2 \quad \text{(eq. 1)}$$

This equation (eq. 1), while useful for explaining the concepts underlying flow-through respirometry, is not practical to implement. Usually just one flow rate – incurrent or excurrent – is measured or controlled, and this requires a different equation. Flow rates, whether incurrent or excurrent, should always be converted to standard temperature and pressure or STP, where STP is usually defined at 273.15 K and 101.325 kPa in comparative physiology. Perhaps the simplest case is for a system in which the chamber is supplied with dry, CO_2-free air, and both CO_2 and water vapour are removed from the excurrent air (eq. 2):

$$\dot{V}_{\mathrm{O}_2} = \dot{V}_{\mathrm{I}}(F_{\mathrm{I}}\mathrm{O}_2 - F_{\mathrm{E}}\mathrm{O}_2)/(1 - F_{\mathrm{E}}\mathrm{O}_2) \quad \text{(eq. 2)}$$

where $\dot{V}_{\mathrm{I}}$ is the flow rate of air entering the chamber in a "push" system, and $F_{\mathrm{I}}\mathrm{O}_2$ and $F_{\mathrm{E}}\mathrm{O}_2$ are the incurrent and excurrent fractional concentrations of O_2, respectively. $\dot{V}_{\mathrm{O}_2}$ inherits the units of $\dot{V}_{\mathrm{I}}$, e.g., ml min^{-1}. The equation required for any given respirometry system depends on the configuration of the system; the relevant equations are provided by Lighton (2019); see also below. Examples of commonly used arrangements include mask respirometry systems (an example of a "pull mode" system, **Fig. 1c** 'pull') in which air is drawn at a known rate through a mask into which the animal breathes (e.g., Withers, 1977; Langman et al., 1995) and "push mode" systems (**Fig. 1c** 'push') in which air is pumped through an animal chamber at a known rate (e.g., Seymour et al., 1998; White et al., 2011; Seymour et al., 2013). Other respirometry systems are described Withers (1977) for terrestrial vertebrates, and Wightman (1977) and Worland and Block (1994) for terrestrial invertebrates, but here we mainly discuss the most common respirometry systems (closed and flow-through respirometry; **Fig. 1**).

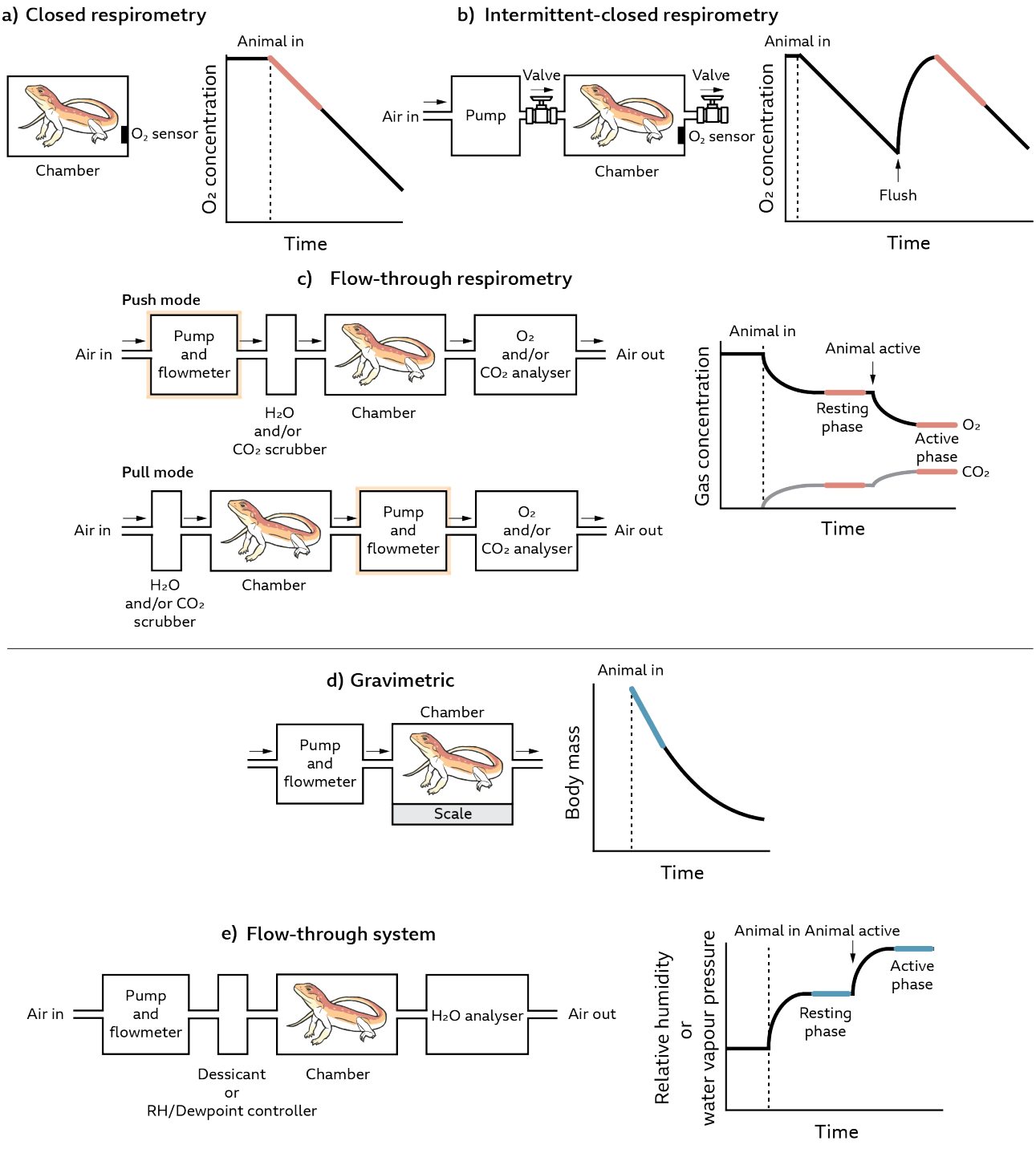

Figure 1. Example schematic diagrams of system configurations commonly used for measuring metabolic rate (as rate of oxygen, O_2, consumption and/or carbon dioxide, CO_2, production), and evaporative water loss (as change in mass or water vapour pressure). (**a**) A closed respirometry system with a sealed chamber containing an internal oxygen sensor for measuring O_2 concentration in the chamber. The rate of O_2 consumption is calculated as the change in O_2 concentration over time. Closed-system respirometry is suitable for small, inactive ectotherms. (**b**) An intermittent-closed respirometry system with an air pump to periodically flush the otherwise sealed chamber with new air to return to initial condition. After flushing, the chamber is sealed, and the decline in O_2 is measured with an internal oxygen sensor. Intermittent-closed respirometry is suitable for small animals that do not easily settle in the chamber because the system can be repeatedly flushed and sealed until the metabolic rate of the animal reaches a steady state. (**c**) Two system configurations for flow-through respirometry where air is either pushed (push mode) or pulled (pull mode) through the chamber at a known flow rate and the O_2 and/or CO_2 concentration of the excurrent air is measured by an external gas analyser placed after the chamber. Both modes have benefits and disadvantages depending on the question, animal, and set-up. Often the incurrent air is scrubbed of H_2O and CO_2 with the scrubbers placed before the chamber, but scrubbers can also be placed prior to the gas analyser to simplify metabolic rate calculations (see Lighton (2019) for details including options for physical and mathematical scrubbing). The rate of O_2 consumption or CO_2 production can

be calculated from the flow rate and the difference between the incurrent and excurrent gas concentrations. Flow-through respirometry is suitable for medium/large, active animals, but can also be applied to small animals, such as *Drosophila*, if using a high-sensitivity CO_2 analyser (Lighton, 2007; Videlier et al., 2019; Alton and Kellermann, 2023). Evaporative water loss can be estimated (**d**) gravimetrically by measuring change in body mass over a known flow rate over time, or via (**e**) an open-flow system with a known flow rate, a desiccant or relative humidity/dewpoint controller, and H_2O analyser. Note, only open-flow systems allow for distinction of metabolic rate and EWL when animal are inactive and active. Highlighted sections of the raw trace represents where values are typically extracted, either the first minute/hour of recording (in closed systems), or periods of stable rate of O_2 consumption, CO_2 production or water loss.

Within these broad configurations, many creative variations are possible: masks might be fashioned from large buckets to fit over the trunk of an elephant (e.g., Langman et al., 1995; Langman et al., 2012), be made to resemble a flower to measure the MR of hovering hummingbirds (e.g., Bartholomew and Lighton, 1986), or be constructed by the animal itself to measure the MR of calling mole crickets within their singing burrows (e.g., White et al., 2008). Large "masks" can also be used to measure the MR of diving animals by training animals to surface within them (e.g., Halsey et al., 2007; White et al., 2011). Tree cavities, with a single entrance as an inlet and a small outlet hole, have also been used as flow-through chambers to non-invasively measure MR in wild primates (e.g., Dausmann et al., 2009). Flow-through chambers have been constructed from components as diverse as Kjeldsens butter cookie containers to measure the MR of Namib desert golden moles while resting, running, and burrowing (e.g., Seymour et al., 1998) and bespoke 6-m^3 chambers constructed from plate steel to measure the MR of estuarine crocodiles weighing up to 389 kg (e.g., Seymour et al., 2013). Test tubes have been used to test the influence of tree hollow shelter on EWL for Brazilian tree-frogs, *Corythomantis greeningi* (Navas et al., 2002). Hardware and homeware stores are often a good source of usable chambers for more modestly sized (and less dangerous) animals (e.g., Wu et al., 2015).

The rate of oxygen consumption is considered a practical, if approximate, measure of MR because the amount of heat produced for each litre of oxygen consumed by metabolism (i.e., the energy equivalent of oxygen) is relatively constant irrespective of whether carbohydrate, fat, or protein is oxidised (maximum error ~10%). The energy equivalent of carbon dioxide is more variable than that of oxygen (maximum error ~32%) and so measuring only $\dot{V}_{CO_2}$ as a proxy for MR is less accurate. When both oxygen consumption and carbon dioxide production are measured, the ratio of $\dot{V}_{CO_2}/\dot{V}_{O_2}$ gives the RER, which indicates the proportional composition of nutrient substrates being catabolised. Likewise, if the mean food quotient of an animal's diet is known, and if the animal is neither gaining nor losing body mass, its RER can be predicted. An RER of 1.0 indicates that only carbohydrates are being catabolised, while an RER of 0.71 indicates only fats are being catabolised. Intermediate RER values arise from mixtures of metabolic substrates, including proteins (Lighton, 2019).

When measuring gas exchange rates, beware of the dilution effect of water vapor. In all cases, prior to gas analysis, water vapor must be either (a) thermally or chemically scrubbed from the airstream to remove its dilution effect, or (b) its concentration measured, and its dilution effect removed mathematically by application of Dalton's law of partial pressures in conjunction with barometric pressure. This latter option is preferred because it allows the rate of evaporative water loss (EWL or $\dot{V}_{H_2O}$) to be measured as well (Lighton, 2019). Measurement of barometric pressure also increases the accuracy of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ measurements by allowing correction of measurement fluctuations caused by variations in barometric pressure.

Measuring $\dot{V}_{H_2O}$ generally requires knowledge of the water vapor pressure (WVP) of the airstream. This can be obtained in several ways. Most commonly, the relative humidity (RH%) of the airstream is measured together with the RH sensor's temperature. Saturated WVP at the temperature of measurement is calculated using the Arden-Buck equation (eq. 3):

$$\mathrm{SWVP} = 0.61121e^{[18.678-(T/234.5)][T/(257.14+T)]} \quad \text{(eq. 3)}$$

where T is the RH sensor temperature in °C and SWVP is saturated water vapor pressure in kPa (Buck, 1981). WVP is then obtained by multiplying SWVP by RH% / 100. To measure actual $\dot{V}_{H_2O}$ there are two ways to proceed. First, divide WVP by BP (if it is measured) to yield fractional concentration of water vapor, and use the following equation (eq. 4):

$$\dot{V}_{H_2O} = \dot{V}_I(F_E H_2O - F_I H_2O)/(1 - F_E H_2O) \quad \text{(eq. 4)}$$

where the fractional concentrations are as defined for O_2 above, and $\dot{V}_I$ is the incurrent flow rate ($\dot{V}_{H_2O}$ will of course inherit the units of V_I). Knowing that each ml of water vapor at STP contains 0.803 mg of water, it is then trivial to calculate $\dot{V}_{H_2O}$ in units of water mass lost per unit time. In practice, the $(1 - F_E H_2O)$ term is usually close enough to unity that it can be ignored.

Alternatively, WVP can be converted to water vapor density (eq. 5):

$$\mathrm{WVD} = \mathrm{WVP} \,/\, (T \cdot R_w) \quad \text{(eq. 5)}$$

where WVD is water vapor density in μg ml^{-1}, R_w is the water vapor gas constant (461.5 J kg^{-1} * K) and other terms are as before. Then, simply multiply WVD by the flow rate; this yields $\dot{V}_{H_2O}$ in gravimetric units. For example, if the flow rate is in ml min^{-1}, then $\dot{V}_{H_2O}$ will be in μg min^{-1}. Excurrent $\dot{V}_{H_2O}$ should be subtracted from incurrent $\dot{V}_{H_2O}$ to yield the $\dot{V}_{H_2O}$ of the animal. See Lighton (2019) for more details and alternative methods.

Returning to metabolic measurement, it is generally necessary to measure both $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ to convert gas exchange data into accurate EE data. This is particularly so because neither gas

exchange parameter can usually be measured accurately in the absence of the other. For example, oxygen in the airstream passing over the animal will be diluted by carbon dioxide, which in turn will be concentrated by oxygen consumption. After accurate figures for $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ are obtained, actual EE can be calculated, for example with the Weir equation (eq. 6):

$$\mathrm{EE} = 0.06 \cdot (3.941 \cdot \dot{V}_{O_2} + 1.106 \cdot \dot{V}_{CO_2}) \quad \text{(eq. 6)}$$

where $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ are in ml min^{-1} and EE is in kcal h^{-1} (Weir, 1949). Again, the choice of equations can be quite complex, and depends (for flow-through respirometry) on whether measured air flow rates are pushed or pulled past the animal, which gas species are being measured, whether chemical scrubbers are used and other factors (see Lighton, 2019 for more details).

There is a curious exception to the requirement for the measurement of both $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ to yield accurate EE data. If only the fractional depletion of oxygen is measured in a flow-through system, the dilution effect of carbon dioxide almost exactly cancels the dependence on $\dot{V}_{CO_2}$ of the energy equivalent of O_2, greatly simplifying EE measurement (Kaiyala et al., 2019). In that case, where EE is in kcal min^{-1} and $\dot{V}_E$ is excurrent flow rate in L min^{-1} (eq. 7):

$$\mathrm{EE} = 5.0 \cdot \dot{V}_E \cdot (F_I O_2 - F_E O_2) \quad \text{(eq. 7)}$$

Given the potential sources of error in the measurement of either $\dot{V}_{O_2}$ or $\dot{V}_{CO_2}$ as a proxy for MR, it has been argued that both should be considered to be distinct from rate of energy expenditure (Nelson, 2016), and that $\dot{V}_{O_2}$ measured in O_2 units rather than energy units should be called respiration rate instead of MR (Chabot et al., 2016). We endorse that opinion. Despite this, the term "metabolic rate" continues to be used interchangeably with $\dot{V}_{O_2}$ in many studies, which consider data for rates of oxygen consumption or carbon dioxide production without converting to units of energy expenditure (e.g., White et al., 2019; White et al., 2022).

Flow-through respirometry is a versatile technique but becomes trickier to employ as the body mass of the experimental animal decreases. Eventually the gas exchange signals from the animal may be drowned in instrumental noise and drift. An alternative to flow-through respirometry for small animals is closed-box respirometry, in which the subject animal is held within a sealed container and rates of oxygen consumption are determined either by measuring the change in oxygen concentration within the chamber (e.g., Bartholomew and Casey, 1978) (closed respirometry: **Fig. 1a**), or by periodically ventilating the chamber (Kristín and Gvoždík, 2012) (intermittent-closed respirometry: **Fig. 1b**). Although this method has the advantage of being able to quantify low MRs with relatively insensitive (and therefore relatively inexpensive) equipment, it does tend to overestimate resting MRs because rates are integrated over the total measurement

period and may therefore be contaminated with undetected activity (e.g., Lighton and Fielden, 1995; Addo-Bediako et al., 2002; Chown et al., 2007; Kristín and Gvoždík, 2012). This observation of a possible method-dependent bias in the estimation of MR provides a clear example of the importance of accurate and thorough reporting, so that readers of respirometry studies have a good understanding of the design of the system used to measure MR.

CHECKLIST FOR REPORTING

The need for standardised reporting is timely because of the exponential rise in empirical and synthesis studies using respirometry data (Arnold et al., 2021; Le Galliard et al., 2021; White et al., 2022; Wu and Seebacher, 2022). 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. 2a**) and 170 % increase in whole-animal water loss studies (2012–2022; **Fig. 2b**). In the aquatic world, reporting of intermittent-flow respirometry experiments were generally poor and inconsistent (Killen et al., 2021). It is highly likely this inconsistency applies to terrestrial respirometry, as we are aware with our own studies.

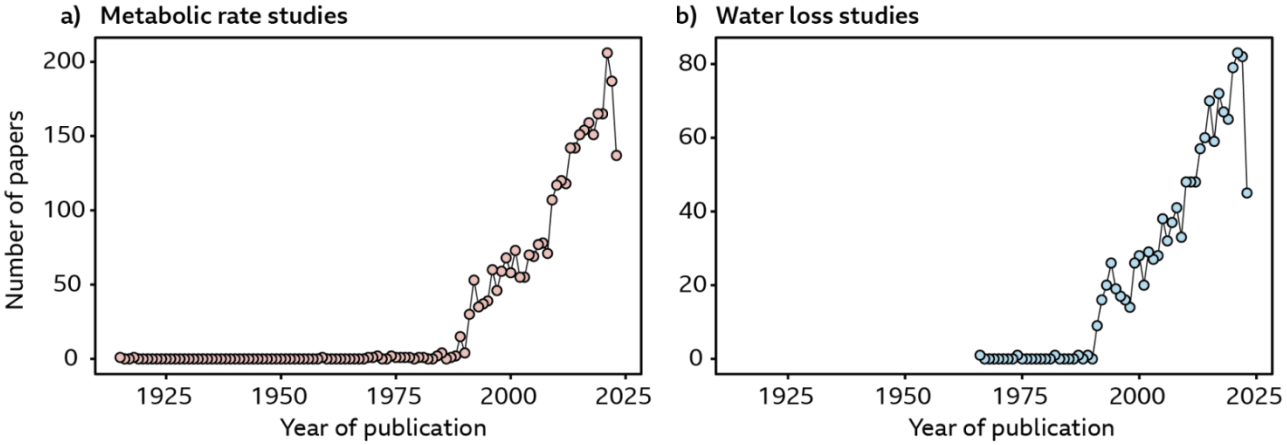

Figure 2. Number of studies published in the field of ecology and evolution relating to **a**) whole-animal metabolic rate, and **b**) water loss. Data obtained from the Web of Science on 25 November 2023 using the search terms (respirometry OR gas exchange OR open-flow OR flow-through) AND (metabolic 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.

Here, we provide a checklist of five criteria to facilitate interpreting and replicating empirical studies relating to measurements of whole-animal MR and EWL via aerial respirometry. We broadly followed Killen et al. (2021) with criteria specific to aerial respirometry. The checklist is comprised of five main sections with specific reporting information and references that provides more detail, justification for the criteria, or example studies (**Table 1**): 1) Materials, set up, plumbing: describes the equipment and materials used for the experiment. The choice of

experimental set up has a significant influence on the validity and interpretation of the results; 2) Subject conditions/maintenance: describes the test subjects housing or maintenance condition (e.g., lighting, temperature, food access) and relevant biological metrics (e.g., reproductive status, age) that can influence MR and EWL measurements; 3) Measurement conditions: describes what data is collected, how it was collected, and the conditions during the experiment; 4) Data processing: describes how the raw data was treated, what transformations were used, and details on the sampling procedure; 5) Data reporting and statistics: describes how the data is analysed and presented in a clear, transparent manner. Lighton (2019) provides comprehensive information on setting up various respirometry systems which we highly recommend reviewing prior to designing respirometry experiments.

IMPORTANT CONSIDERATIONS IN DESIGNING AND CONDUCTING RESPIROMETRY STUDIES

We do not intend to be overly prescriptive in how to design and undertake respirometry studies yet wish to draw attention to common issues that may arise. The checklist does not include all possible elements, but it is formulated to aid in the design and reporting of most studies. We expand here on a few items that can often slip through both the design and review processes. The most important is that all aspects of the system and the experiments should be designed to answer the questions being asked. A question involving instantaneous changes in MR (see Bartholomew et al. (1981) for an overview) requires significantly faster response times than one on a steady-state process and in all cases the individual characteristics of the study species (such as body mass, circadian activity phases, and propensity to rest in the chamber) need to be accounted for.

A common issue in studies measuring resting, standard, or basal MR is insufficient time. Many animals, endotherms in particular, experience handling stress and require a substantial amount of time for MR to drop back down to resting levels (Duarte et al., 2010). This time can depend on both temperament of the animal as well as their mass. Similarly, resting MR can differ significantly depending on the time of day, particularly in species with pronounced circadian activity patterns. 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 (e.g., Jacobs and McKechnie, 2014). 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 (Boyles et al., 2013). Where possible we recommend the measurement of body temperature alongside MR. 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 (e.g., Reher and Dausmann, 2021; Levesque et al., 2023). Temperature sensitive passive integrative transponders (or PIT tags) have made collecting body temperature (or a close proxy such as subcutaneous body temperature) significantly easier for most species (Currie et al., 2015; Whitfield et al., 2015; Oswald et al., 2018; Andreasson et al., 2023), and where the objective is to measure basal or standard MR, it is important to ensure that animals are fasted for sufficiently long that they become postabsorptive (reviewed in Secor, 2009), but not so long that they enter a state of metabolic depression associated with starvation (McCue, 2010).

Even for studies in which EWL is not measured, chamber humidity is an important consideration. As noted by Lasiewski et al. (1966), 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, impeding evaporative heat or water loss and resulting in different thermoregulatory or evaporative responses compared to those observed in drier air. A misconception still sometimes encountered in the literature is that subjects experience chamber humidity equivalent to that of the incurrent air; in reality they experience humidity equivalent to that of the excurrent air. For this reason, in studies investigating responses to experimentally manipulated humidity, the excurrent – not incurrent – humidity is the variable that needs to be regulated at 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 (Freeman et al., In press).

Further considerations for studies in EWL is measured 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, b) the need to use chambers constructed from material that does not adsorb water vapour, and c) the report on how incurrent air is diffused (uniformly or not) inside the chamber as well as the subject's posture (Pough et al., 1983) and position (Riddell et al., 2017) inside the chamber because the direction the air passes through the subject surface expose to evaporation may alter how much water evaporates from it. Humidity should also be reported in absolute terms (g H_2O m^{-3} air or partial pressures in kPA). If RH is reported, it is essential that each value is accompanied by the corresponding air temperature. This is because for the same RH values are presented under different temperatures, the water vapor gradient (the difference between subjects and the surrounding media) will differ substantially. Finally, in studies involving high air temperatures and experimental humidity levels, condensation must be avoided at all cost. Often, the only feasible way to avoid

condensation in tubing or analysers is to place the entire respirometry system in a heated room; for instance, Freeman et al. (In press) placed their analysers in a room within which air temperature was maintained at 35°C. Particular care should be taken when tubing is in contact with the floor or in the proximity of heat exchangers.

HOW TO IMPROVE DATA AND CODE AVAILABILITY

Open, reliable, reproducible, and transparent research is not just a theoretical ideal but a practical necessity to assess, replicate, and compile research findings (Fraser et al., 2018; O'Dea et al., 2021; Bertram et al., 2023). Central to comparative physiology is the synthesis of original data, which ideally should be made available along with the analytical methods. As the adage goes, "*a model is only as good as the data fed into it*", and the reliability and generalisability of comparative findings is directly linked to the scope of the data inputted into models. In fact, biases stemming from unavailable data can lead to spurious and misleading conclusions, particularly when samples are not representative (Culumber et al., 2019; Konno et al., 2020; Christie et al., 2021; Hughes et al., 2021; Nuñez et al., 2021; White et al., 2021). Therefore, the sharing of raw data and analytical methods is vital for future research aiming at establishing broad patterns in animal metabolism and water balance.

Despite the recognised importance of data sharing, raw data are typically not published alongside scientific articles, or are presented in non-reusable formats (Parr and Cummings, 2005; Moore et al., 2010; Roche et al., 2022). This lack of data sharing persists even though evidence indicates that journal policies mandating data and code sharing improve the adoption of open research practices (Vines et al., 2013; Culina et al., 2020; Roche et al., 2022). The establishment of stable platforms for publishing data and code such as Dryad (https://www.datadryad.org), Zenodo (https://www.zenodo.org), Figshare (https://figshare.com), or the Open Science Framework (https://osf.io/) has been instrumental in promoting data availability. In fact, sharing data in such repositories has been shown to offer numerous advantages, including enhanced citation counts (Piwowar et al., 2007; Piwowar and Vision, 2013; Vines et al., 2013; Gomes et al., 2022). However, to maximize the impact and reuse of data, adherence to certain standards is necessary (Poisot et al., 2019).

A particular challenge in comparative physiology is inconsistency in units and terminology, notably in the measurement and reporting of respiratory and water loss rates. Differences in data presentation, such as reporting absolute rates versus rates normalized to body size or 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, but not both (Tracy et al., 2007; Tracy et al.,

2008; Riddell et al., 2017). 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, as outlined in the present commentary. In addition, following best practices for sharing data, which include providing the rawest possible 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 (Reichman et al., 2011; Whitlock, 2011; Wilson et al., 2021).

In recent years, open-source packages for processing respirometry data have become available, which allow the sharing of reproducible and succinct code detailing the processing methods. The adoption of such open-source packages 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 ensure reproducibility (Harianto et al., 2019; Powers and Hampton, 2019). However, although researchers can adopt current packages such as *respR* (Harianto et al., 2019) or *LoLinR* (Olito et al., 2017) for respirometry data, these tools are primarily designed for aquatic settings, highlighting the need to develop functionalities tailored for terrestrial respirometry.

CONCLUSION

Standardised reporting has practical benefits beyond establishing a rigorous practice for researchers. 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 (Gerstner et al., 2017; Genoud et al., 2018; Schwanz et al., 2022; Leiva et al., 2023), and 3) assist the efficiency of peer-review process by providing a clear checklist for reviewing studies with respirometry experiments (Parker et al., 2018). As increasing novel ideas and hypotheses are developed in the field of ecology and evolution, respirometry experiments will be at the forefront of empirical physiology studies (Zera and Harshman, 2001; Brown et al., 2004; White et al., 2022). For example, flow-through stable isotope machines, which rely on basic respirometry principles for breath analyses, are becoming more accessible and enable novel measurements of fuel use and exercise metabolism (McCue and Welch, 2016; Welch Jr et al., 2016) in conditions once considered impossible (e.g., unencumbered forward flight; Hedh et al., 2020; Currie et al., 2023). Overall, the goal of our checklist is to provide a comprehensive guide

455 that will help both new and experienced researchers design, execute, and report respirometry
456 experiments with consistency.

457 CHECKLIST

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

Reporting criterion	Description	References
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 (**Fig. 1**)? 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.	(Kristín and Gvoždík, 2012; Lighton, 2019)
Air flow	Report the air flow as rate (volume over time) and/or velocity (distance 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.	(McNab, 2006)
Physical 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?	(Koteja, 1996; White et al., 2006; Lighton, 2019)
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.	(McNab, 2006; Wu et al., 2015; Nowack et al., 2020)
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).	(Cooper and Withers, 2014; Lighton, 2019)
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.	(Frappell et al., 1989)
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.	(Lighton, 2019)
CO_2 analyser	Report what type of analyser was used (e.g., infrared, nondispersive infrared) and provide the model and manufacturer.	(Lighton, 2019)
H_2O analyser	Report what type of analyser was used (e.g., chilled mirror, capacitive, infrared) and provide the model and manufacturer.	(Lighton, 2019)
Calibration	Describe how the flow meters, gas analysers, temperature probes, etc. were calibrated and how often. Include the concentrations of any span gases used.	(Withers, 2001)

Connectors	Provide details on the tubing material and connectors, as different materials can alter gas and humidity measurements due to potential leaking.	(Lighton, 2019)
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?	(Lighton, 2019)
Subsampling	If subsampling was used, provide the flow rate and explain how the flow rate was achieved and maintained.	(Lighton, 2019)
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.	(Lighton, 2019)
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.	(Smit and McKechnie, 2010; Burton et al., 2011; Bovo et al., 2023)
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.	(Terblanche et al., 2005; Weaver et al., 2023)
Age/life stage	Provide the life stage of the test subjects, and if known, provide the age.	
Sex	Report the number of test subjects of each sex and state whether sex ratios were equal or similar across experimental groups	
Reproductive condition	State the reproductive condition of the test subjects.	(Burton et al., 2011)
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.	(Kaiyala et al., 2010; Lighton, 2019)
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.	(Parker et al., 2018)
Baseline recording	Provide information on the background/baseline (empty chamber) recording including how often and how long the baseline was recorded for.	(Lighton and Halsey, 2011)
Time	State when the measurements were taken. MR fluctuates over the day and is affected by photoperiod.	(White et al., 2006; Page et al., 2011; Connolly and Cooper, 2014)
Lighting	Provide information on the lighting conditions during the experiment.	(Chew et al., 1965; Powers, 1991; Riccio and Goldman, 2000)

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.	(Cooper and Withers, 2010; Jacobs and McKechnie, 2014)
Test temperature	Provide the test temperature 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.	(Short et al., 2022)
Test humidity	Provide the test humidity 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 air temperatures are provided. In flow-through systems, the excurrent humidity (not the incurrent) is the humidity experienced by the subject.	(Gilson et al., 2021; Freeman et al., In press)
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.	
Fasted	State whether the test subjects were fasted prior to the experiment and for how long.	(McCue, 2006)
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.	(Preest et al., 1992; Senzano and Andrade, 2018)
Grouping	If more than one test subject was placed inside the chamber, provide the exact number of individuals.	(Rusli et al., 2016)
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.	(Jacobs and McKechnie, 2014)
Animal state	Describe the state of the animal when the measurement is 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? - Ideally, 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.	(Seymour et al., 1998; Duarte et al., 2010; Snelling et al., 2017; Wu et al., 2018; Videlier et al., 2019; Alton and Kellermann, 2023)
Multiple animals	When multiple animals are measured in sequence in one measurement period, provide the timing of switching between channels.	
Data processing		
Data acquisition	Provide information on the data acquisition systems/software.	(Lighton, 2019)
Baseline drift	Baseline measurements will fluctuate, especially for O_2 concentrations. State whether and how baseline drift was corrected.	(Lighton and Halsey, 2011)
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.	(Lighton and Halsey, 2011)
Mathematical scrubbing	If physical scrubbers were not used, provide details on how gas concentrations were mathematically scrubbed.	(Lighton, 2019)

Sampling	Describe and justify sample selection (mean, time period) as well as exclusion criteria (activity, posture, 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.	(Pough et al., 1983; Withers, 2001; Cooper and Withers, 2010)
Boundary layer	For EWL, state if the boundary layer was accounted for, either mathematically or empirically (e.g. from agar models) estimated.	(Riddell et al., 2017; Senzano et al., 2022)
Equations	Provide the equations or cite equations used for calculations of rates.	(Lighton, 2019)
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?	(Withers, 2001; Cooper and Withers, 2010)
Data exclusion	If data were excluded from the study due to experiment/measurement/animal issues, provide such information for transparency.	
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.	(Parker et al., 2018)
Units	Always report units in the paper.	
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.	(Packard and Boardman, 1988, 1999; White and Kearney, 2011; Lighton, 2019)
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.	(Parker et al., 2018)
Pseudoreplication	Report pesudoreplication if used. E.g. the number of tanks, rooms, chambers used, and the number of animals in each.	
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.	(Parker et al., 2018)

	· 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.	
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).	(Legendre, 1993; Noble et al., 2017)
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.	(Tedersoo et al., 2021; Gomes et al., 2022)

GLOSSARY

Metabolic rate measurements

Metabolic rate (MR): The rate of energy expenditure per unit time. Typically represented as the rate of O_2 consumption (ml h^{-1}), CO2 production (ml h^{-1}), or converted to an energy equivalent such as Watts, or Joules or calories per unit time.

Energy expenditure (EE): Metabolic rate that is explicitly expressed in units of heat flux (e.g. kJ h^{-1}, Watts).

Basal metabolic rate (BMR): The metabolic rate 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).

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-O_2 atmosphere at temperatures above 0°C to avoid freezing injury to tissues).

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 (see below).

Routine metabolic rate: Metabolic rate, averaged over a specified time interval, of an animal exhibiting spontaneous 'routine' behaviours, or a specified behaviour.

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).

Specific dynamic action (SDA): The metabolic rate of a post-fed animal which includes the energy expenditure for digestion, absorption, and assimilation of a meal.

Evaporative Water Loss measurements

Total evaporative water loss (TEWL**)**: The sum of cutaneous (CEWL) and respiratory (REWL) water loss by evaporation, typically expressed in absolute terms as mass of water lost per unit time (e.g. g h^{-1}), or in relative terms as mass of water lost per mass of animal per unit time (e.g. g g^{-1} h^{-1}) or the mass of water lost per exposed surface area per unit time (e.g. g cm^{-2} h^{-1}).

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

CEWL: rate of evaporative water loss through the cutaneous surface. For moist-skinned organisms such as amphibians, CEWL is the main mode of water loss.

REWL: rate of evaporative water loss by respiration. For endotherms, water loss occurs primarily through respiration.

*Note that REWL is sometimes used in the literature to refer to resistance to evaporative water loss.

Total resistance to EWL (r_t): The sum of the boundary layer resistance (r_b) and the resistance provided by the animal integument (r_i), expressed as s cm^{-1}.

Boundary layer resistance (r_b): The 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 r_i = zero, and $r_b = r_t$.

Integument resistance (r_i): The resistance provided by the animal integument, estimated after subtracting the amount of water lost by the animal (r_t) from its biophysical ("no cutaneous") model (r_b), thus $r_i = r_t - r_b$ that is the time taken by water to evaporate through the animal surface.

*r_i is sometimes used in the literature to refer to skin resistance, r_s, or cutaneous resistance, r_c.

REFERENCES

Addo-Bediako, A., Chown, S. and Gaston, K. (2002). Metabolic cold adaptation in insects: a large-scale perspective. *Funct. Ecol.* **16**, 332-338.

Agugliaro, J., Lind, C. M., Lorch, J. M. and Farrell, T. M. (2020). 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.

Alton, L. A. and Kellermann, V. (2023). Interspecific interactions alter the metabolic costs of climate warming. *Nat. Clim. Change* **13**, 382-388.

Andreasson, F., Rostedt, E. and Nord, A. (2023). Measuring body temperature in birds–the effects of sensor type and placement on estimated temperature and metabolic rate. *J. Exp. Biol.* **226**, jeb246321.

Angilletta, M. J. (2009). Thermal adaptation: a theoretical and empirical synthesis. New York, USA: Oxford University Press.

Arnold, P. A., Delean, S., Cassey, P. and White, C. R. (2021). Meta-analysis reveals that resting metabolic rate is not consistently related to fitness and performance in animals. *J. Comp. Physiol. B* **191**, 1097-1110.

Baas, J. and Kooijman, S. A. (2015). Sensitivity of animals to chemical compounds links to metabolic rate. *Ecotoxicology* **24**, 657-663.

Bartholomew, G. A. and Casey, T. M. (1978). Oxygen consumption of moths during rest, pre-flight warm-up, and flight in relation to body size and wing morphology. *J. Exp. Biol.* **76**, 11-25.

Bartholomew, G. A. and Lighton, J. R. (1986). Oxygen consumption during hover-feeding in free-ranging Anna hummingbirds. *J. Exp. Biol.* **123**, 191-199.

Bartholomew, G. A., Vleck, D. and Vleck, C. M. (1981). 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.

Bergmann, C. (1848). Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse: Vandenhoeck und Ruprecht.

Bertram, M. G., Sundin, J., Roche, D. G., Sánchez-Tójar, A., Thoré, E. S. and Brodin, T. (2023). Open science. *Curr. Biol.* **33**, R792-R797.

Bovo, R. P., Simon, M. N., Provete, D. B., Lyra, M., Navas, C. A. and Andrade, D. V. (2023). Beyond Janzen's hypothesis: How amphibians that climb tropical mountains respond to climate variation. *Integr. Org. Biol.* **5**, obad009.

Boyles, J. G., Thompson, A. B., McKechnie, A. E., Malan, E., Humphries, M. M. and Careau, V. (2013). A global heterothermic continuum in mammals. *Glob. Ecol. Biogeogr.* **22**, 1029-1039.

Bozinovic, F. and Rosenmann, M. (1989). Maximum metabolic rate of rodents: physiological and ecological consequences on distributional limits. *Funct. Ecol.*, 173-181.

Briscoe, N. J., Morris, S. D., Mathewson, P. D., Buckley, L. B., Jusup, M., Levy, O., Maclean, I. M., Pincebourde, S., Riddell, E. A. and Roberts, J. A. (2023). Mechanistic forecasts of species responses to climate change: the promise of biophysical ecology. *Glob. Chang. Biol.* **29**, 1451-1470.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M. and West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology* **85**, 1771-1789.

Buck, A. L. (1981). New equations for computing vapor pressure and enhancement factor. *J. Appl. Meteorol. Climatol.* **20**, 1527-1532.

Burton, T., Killen, S., Armstrong, J. D. and Metcalfe, N. (2011). What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proc. R. Soc. B. Biol. Sci.* **278**, 3465-3473.

Butler, P., Brown, A., Stephenson, D. and Speakman, J. (2022). Animal physiology: an environmental perspective. Oxford, UK: Oxford University Press.

Chabot, D., Steffensen, J. and Farrell, A. (2016). The determination of standard metabolic rate in fishes. *J. Fish Biol.* **88**, 81-121.

Chew, R. M., Lindberg, R. G. and Hayden, P. (1965). Circadian rhythm of metabolic rate in pocket mice. *J. Mammal.* **46**, 477-494.

Chmura, H. E., Glass, T. W. and Williams, C. T. (2018). Biologging physiological and ecological responses to climatic variation: new tools for the climate change era. *Front. Ecol. Evol.* **6**, 92.

Chown, S. and Nicolson, S. (2004). Insect physiological ecology: mechanisms and patterns. Oxford, UK: Oxford University Press.

Chown, S., Gaston, K. and Robinson, D. (2004). Macrophysiology: large-scale patterns in physiological traits and their ecological implications. *Funct. Ecol.* **18**, 159-167.

Chown, S., Marais, E., Terblanche, J., Klok, C., Lighton, J. and Blackburn, T. (2007). Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. *Funct. Ecol.* **21**, 282-290.

Chown, S. L. and Gaston, K. J. (2016). Macrophysiology–progress and prospects. *Funct. Ecol.* **30**, 330-344.

Christie, A. P., Amano, T., Martin, P. A., Petrovan, S. O., Shackelford, G. E., Simmons, B. I., Smith, R. K., Williams, D. R., Wordley, C. F. and Sutherland, W. J. (2021). The challenge of biased evidence in conservation. *Conserv. Biol.* **35**, 249-262.

Clark, T. D., Sandblom, E. and Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *J. Exp. Biol.* **216**, 2771-2782.

Clemente, C. J., Withers, P. C. and Thompson, G. G. (2009). Metabolic rate and endurance capacity in Australian varanid lizards (Squamata: Varanidae: Varanus). *Biol. J. Linn. Soc.* **97**, 664-676.

Connolly, M. and Cooper, C. (2014). 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.

Conradie, S. R., Woodborne, S. M., Wolf, B. O., Pessato, A., Mariette, M. M. and McKechnie, A. E. (2020). Avian mortality risk during heat waves will increase greatly in arid Australia during the 21st century. *Conserv. Physiol.* **8**, coaa048.

Cooke, S. J., Sack, L., Franklin, C. E., Farrell, A. P., Beardall, J., Wikelski, M. and Chown, S. L. (2013). What is conservation physiology? Perspectives on an increasingly integrated and essential science. *Conserv. Physiol.* **1**, cot001.

Cooper, C. E. and Withers, P. C. (2010). 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.

Cooper, C. E. and Withers, P. C. (2014). 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.

Cooper, C. E., Withers, P. C., Hurley, L. L. and Griffith, S. C. (2019). The field metabolic rate, water turnover, and feeding and drinking behavior of a small avian desert granivore during a summer heatwave. *Front. Physiol.*, 1405.

Culina, A., van den Berg, I., Evans, S. and Sánchez-Tójar, A. (2020). Low availability of code in ecology: A call for urgent action. *PLoS Biol.* **18**, e3000763.

Culumber, Z. W., Anaya-Rojas, J. M., Booker, W. W., Hooks, A. P., Lange, E. C., Pluer, B., Ramírez-Bullón, N. and Travis, J. (2019). Widespread biases in ecological and evolutionary studies. *Bioscience* **69**, 631-640.

Currie, S. E., Körtner, G. and Geiser, F. (2015). Measuring subcutaneous temperature and differential rates of rewarming from hibernation and daily torpor in two species of bats. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **190**, 26-31.

Currie, S. E., Johansson, L. C., Aumont, C., Voigt, C. C. and Hedenström, A. (2023). Conversion efficiency of flight power is low, but increases with flight speed in the migratory bat *Pipistrellus nathusii*. *Proc. R. Soc. B.* **290**, 20230045.

Dausmann, K., Glos, J. and Heldmaier, G. (2009). Energetics of tropical hibernation. *J. Comp. Physiol. B* **179**, 345-357.

Depocas, F. and Hart, J. S. (1957). 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.

Duarte, L. C., Vaanholt, L. M., Sinclair, R., Gamo, Y. and Speakman, J. R. (2010). 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.

Fedak, M. A., Pinshow, B. and Schmidt-Nielsen, K. (1974). Energy cost of bipedal running. *Am. J. Physiol.* **227**, 1038-1044.

Frappell, P., Blevin, H. and Baudinette, R. (1989). Understanding respirometry chambers: what goes in must come out. *J. Theor. Biol.* **138**, 479-494.

Fraser, H., Parker, T., Nakagawa, S., Barnett, A. and Fidler, F. (2018). Questionable research practices in ecology and evolution. *PloS one* **13**, e0200303.

Freeman, M. T., Coulson, B., Short, J. C., Ngcamphalala, C. A., Makola, M. O. and McKechnie, A. E. (In press). Evolution of avian heat tolerance: the role of atmospheric humidity. *Ecology*.

Genoud, M., Isler, K. and Martin, R. D. (2018). Comparative analyses of basal rate of metabolism in mammals: data selection does matter. *Biol. Rev.* **93**, 404-438.

Gerrits, W. and Labussière, E. (2015). Indirect calorimetry: Techniques, computations and applications: Wageningen Academic Publishers.

Gerstner, K., Moreno-Mateos, D., Gurevitch, J., Beckmann, M., Kambach, S., Jones, H. P. and Seppelt, R. (2017). 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.

Gilson, L. N., Cooper, C. E., Withers, P. C. and Gagnon, M. M. (2021). 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.

Gnaiger, E. (1983). Calculation of energetic and biochemical equivalents of respiratory oxygen consumption. In *Polarographic oxygen sensors: aquatic and physiological applications*, pp. 337-345: Springer.

Gomes, D. G., Pottier, P., Crystal-Ornelas, R., Hudgins, E. J., Foroughirad, V., Sánchez-Reyes, L. L., Turba, R., Martinez, P. A., Moreau, D. and Bertram, M. G. (2022). Why don't we share data and code? Perceived barriers and benefits to public archiving practices. *Proc. R. Soc. B.* **289**, 20221113.

Halsey, L. G. and Bryce, C. M. (2021). Proxy problems: why a calibration is essential for interpreting quantified changes in energy expenditure from biologging data. *Funct. Ecol.* **35**, 627-634.

Halsey, L. G., White, C. R., Enstipp, M. R., Jones, D. R., Martin, G. R. and Butler, P. J. (2007). When cormorants go fishing: the differing costs of hunting for sedentary and motile prey. *Biol. Lett.* **3**, 574-576.

Harianto, J., Carey, N. and Byrne, M. (2019). RespR—An R package for the manipulation and analysis of respirometry data. *Method. Ecol. Evol.* **10**, 912-920.

Hedh, L., Guglielmo, C. G., Johansson, L. C., Deakin, J. E., Voigt, C. C. and Hedenström, A. (2020). Measuring power input, power output and energy conversion efficiency in un-instrumented flying birds. *J. Exp. Biol.* **223**, jeb223545.

Hill, R. W. (1972). Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. *J. Appl. Physiol.* **33**, 261-263.

Hofelich, T., Wadsö, L., Smith, A. L., Shirazi, H. and Mulligan, S. R. (2001). The isothermal heat conduction calorimeter: a versatile instrument for studying processes in physics, chemistry, and biology. *J. Chem. Educ.* **78**, 1080.

Hughes, A. C., Orr, M. C., Ma, K., Costello, M. J., Waller, J., Provoost, P., Yang, Q., Zhu, C. and Qiao, H. (2021). Sampling biases shape our view of the natural world. *Ecography* **44**, 1259-1269.

Jacobs, P. J. and McKechnie, A. E. (2014). Experimental sources of variation in avian energetics: estimated basal metabolic rate decreases with successive measurements. *Physiol. Biochem. Zool.* **87**, 762-769.

Kaiyala, K. J. and Ramsay, D. S. (2011). Direct animal calorimetry, the underused gold standard for quantifying the fire of life. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **158**, 252-264.

Kaiyala, K. J., Wisse, B. E. and Lighton, J. R. (2019). Validation of an equation for energy expenditure that does not require the respiratory quotient. *PLoS One* **14**, e0211585.

Kaiyala, K. J., Morton, G. J., Leroux, B. G., Ogimoto, K., Wisse, B. and Schwartz, M. W. (2010). Identification of body fat mass as a major determinant of metabolic rate in mice. *Diabetes* **59**, 1657-1666.

Kearney, M. and Porter, W. (2009). Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. *Ecol. Lett.* **12**, 334-350.

Kearney, M. R. and Enriquez-Urzelai, U. (2023). A general framework for jointly modelling thermal and hydric constraints on developing eggs. *Method. Ecol. Evol.* **14**, 583-595.

Killen, S. S., Christensen, E. A., Cortese, D., Závorka, L., Norin, T., Cotgrove, L., Crespel, A. l., Munson, A., Nati, J. J. and Papatheodoulou, M. (2021). Guidelines for reporting methods to estimate metabolic rates by aquatic intermittent-flow respirometry. *J. Exp. Biol.* **224**, jeb242522.

Kleiber, M. (1961). The fire of life. An introduction to animal energetics. New York: London: John Wiley & Sons, Inc.

Konno, K., Akasaka, M., Koshida, C., Katayama, N., Osada, N., Spake, R. and Amano, T. (2020). Ignoring non-English-language studies may bias ecological meta-analyses. *Ecol. Evol.* **10**, 6373-6384.

Koteja, P. (1996). Measuring energy metabolism with open-flow respirometric systems: which design to choose? *Funct. Ecol.* **10**, 675-677.

Kristín, P. and Gvoždík, L. (2012). Influence of respirometry methods on intraspecific variation in standard metabolic rates in newts. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **163**, 147-151.

Langer, F., Havenstein, N. and Fietz, J. (2018). Flexibility is the key: metabolic and thermoregulatory behaviour in a small endotherm. *J. Comp. Physiol. B* **188**, 553-563.

Langman, V., Roberts, T., Black, J., Maloiy, G., Heglund, N., Weber, J.-M., Kram, R. and Taylor, C. (1995). Moving cheaply: energetics of walking in the African elephant. *J. Exp. Biol.* **198**, 629-632.

Langman, V. A., Rowe, M. F., Roberts, T. J., Langman, N. V. and Taylor, C. R. (2012). Minimum cost of transport in Asian elephants: do we really need a bigger elephant? *J. Exp. Biol.* **215**, 1509-1514.

Lasiewski, R. C., Acosta, A. L. and Bernstein, M. H. (1966). 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.

Lavoisier, A.-L. and Seguin, A. (1789). Premier mémoire sur la respiration des animaux. *Mémoires de l'Académie des Sciences* **566**.

Le Galliard, J. F., Chabaud, C., de Andrade, D. O. V., Brischoux, F., Carretero, M. A., Dupoué, A., Gavira, R. S., Lourdais, O., Sannolo, M. and Van Dooren, T. J. (2021). A worldwide and annotated database of evaporative water loss rates in squamate reptiles. *Glob. Ecol. Biogeogr.* **30**, 1938-1950.

Legendre, P. (1993). Spatial autocorrelation: trouble or new paradigm? *Ecology* **74**, 1659-1673.

Leiva, F. P., Barneche, D. R., Blackburn, T. M., Castañeda, L., Chown, S. L., Gaitán-Espitia, J. D., Gebauer, P., Gomez-Isaza, D. F., Hardy, I. C. W., Hermaniuk, A. et al. (2023). ShareTrait: a data portal for making trait data interoperable and reusable (v1.0.0).

Levesque, D. L., Breit, A. M., Brown, E., Nowack, J. and Welman, S. (2023). Non-torpid heterothermy in mammals: another category along the homeothermy–hibernation continuum. *Integr. Comp. Biol.* **63**, 1039-1048.

Lighton, J. and Halsey, L. (2011). Flow-through respirometry applied to chamber systems: pros and cons, hints and tips. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **158**, 265-275.

Lighton, J. R. (2007). Hot hypoxic flies: whole-organism interactions between hypoxic and thermal stressors in *Drosophila melanogaster*. *J. Therm. Biol.* **32**, 134-143.

Lighton, J. R. and Fielden, L. J. (1995). Mass scaling of standard metabolism in ticks: a valid case of low metabolic rates in sit-and-wait strategists. *Physiol. Zool.* **68**, 43-62.

Lighton, J. R. B. (2019). Measuring metabolic rates: A manual for scientists. New York: Oxford University Press.

Londono, G. A., Chappell, M. A., Castaneda, M. d. R., Jankowski, J. E. and Robinson, S. K. (2015). Basal metabolism in tropical birds: latitude, altitude, and the 'pace of life'. *Funct. Ecol.* **29**, 338-346.

Lovegrove, B. G. (2000). The zoogeography of mammalian basal metabolic rate. *Am. Nat.* **156**, 201-219.

Lovegrove, B. G. (2019). Fires of life: endothermy in birds and mammals. New Haven and London: Yale University Press.

McCue, M. (2006). Specific dynamic action: a century of investigation. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **144**, 381-394.

McCue, M. D. (2010). Starvation physiology: reviewing the different strategies animals use to survive a common challenge. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **156**, 1-18.

McCue, M. D. and Welch, K. C. (2016). 13 C-Breath testing in animals: theory, applications, and future directions. *J. Comp. Physiol. B* **186**, 265-285.

McKechnie, A. E. and Wolf, B. O. (2004). The allometry of avian basal metabolic rate: good predictions need good data. *Physiol. Biochem. Zool.* **77**, 502-521.

Mckechnie, A. E. and Swanson, D. L. (2010). Sources and significance of variation in basal, summit and maximal metabolic rates in birds. *Curr. Zool.* **56**, 741-758.

McKechnie, A. E., Gerson, A. R. and Wolf, B. O. (2021). Thermoregulation in desert birds: scaling and phylogenetic variation in heat tolerance and evaporative cooling. *J. Exp. Biol.* **224**, jeb229211.

McNab, B. K. (2006). 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.

Moore, A. J., McPeek, M. A., Rausher, M. D., Rieseberg, L. and Whitlock, M. C. (2010). The need for archiving data in evolutionary biology. *J. Evol. Biol.* **23**, 659-660.

Navas, C. A., Jared, C. and Antoniazzi, M. M. (2002). 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.

Nelson, J. (2016). Oxygen consumption rate v. rate of energy utilization of fishes: a comparison and brief history of the two measurements. *J. Fish Biol.* **88**, 10-25.

Noble, D. W., Lagisz, M., O'dea, R. E. and Nakagawa, S. (2017). Nonindependence and sensitivity analyses in ecological and evolutionary meta-analyses. *Mol. Ecol.* **26**, 2410-2425.

Norberg, U. M. (1996). Energetics of flight. In *Avian energetics and nutritional ecology*, pp. 199-249: Springer.

Nowack, J., Dill, V. and Dausmann, K. H. (2020). Open-flow respirometry under field conditions: How does the airflow through the nest influence our results? *J. Therm. Biol.* **92**, 102667.

Nuñez, M. A., Chiuffo, M. C., Pauchard, A. and Zenni, R. D. (2021). Making ecology really global. *Trends Ecol. Evol.* **36**, 766-769.

O'Dea, R. E., Lagisz, M., Jennions, M. D., Koricheva, J., Noble, D. W., Parker, T. H., Gurevitch, J., Page, M. J., Stewart, G. and Moher, D. (2021). Preferred reporting items for systematic reviews and meta-analyses in ecology and evolutionary biology: a PRISMA extension. *Biol. Rev.* **96**, 1695-1722.

Olito, C., White, C. R., Marshall, D. J. and Barneche, D. R. (2017). Estimating monotonic rates from biological data using local linear regression. *J. Exp. Biol.* **220**, 759-764.

Oswald, K. N., Evlambiou, A. A., Ribeiro, Â. M. and Smit, B. (2018). Tag location and risk assessment for passive integrated transponder-tagging passerines. *Ibis* **160**, 453-457.

Packard, G. C. and Boardman, T. J. (1988). The misuse of ratios, indices, and percentages in ecophysiological research. *Physiol. Zool.* **61**, 1-9.

Packard, G. C. and Boardman, T. J. (1999). 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.

Page, A. J., Cooper, C. E. and Withers, P. C. (2011). Effects of experiment start time and duration on measurement of standard physiological variables. *J. Comp. Physiol. B* **181**, 657-665.

Parker, T. H., Griffith, S. C., Bronstein, J. L., Fidler, F., Foster, S., Fraser, H., Forstmeier, W., Gurevitch, J., Koricheva, J. and Seppelt, R. (2018). Empowering peer reviewers with a checklist to improve transparency. *Nat. Eco. Evol.* **2**, 929-936.

Parr, C. S. and Cummings, M. P. (2005). Data sharing in ecology and evolution. *Trends Ecol. Evol.* **20**, 362-363.

Pettersen, A. K. and Metcalfe, N. B. (2024). Consequences of the cost of living: is variation in metabolic rate evolutionarily significant? *Philos. Trans. R. Soc. B* **379**, 20220498.

Piwowar, H. A. and Vision, T. J. (2013). Data reuse and the open data citation advantage. *PeerJ* **1**, e175.

Piwowar, H. A., Day, R. S. and Fridsma, D. B. (2007). Sharing detailed research data is associated with increased citation rate. *PloS ONE* **2**, e308.

Poisot, T., Bruneau, A., Gonzalez, A., Gravel, D. and Peres-Neto, P. (2019). Ecological data should not be so hard to find and reuse. *Trends Ecol. Evol.* **34**, 494-496.

Pough, F. H., Taigen, T. L., Stewart, M. M. and Brussard, P. F. (1983). Behavioral modification of evaporative water loss by a Puerto Rican frog. *Ecology* **64**, 244-252.

Powers, D. R. (1991). Diurnal variation in mass, metabolic rate, and respiratory quotient in Anna's and Costa's hummingbirds. *Physiol. Zool.* **64**, 850-870.

Powers, S. M. and Hampton, S. E. (2019). Open science, reproducibility, and transparency in ecology. *Ecol. Appl.* **29**, e01822.

Preest, M. R., Brust, D. G. and Wygoda, M. L. (1992). Cutaneous water loss and the effects of temperature and hydration state on aerobic metabolism of canyon treefrogs, *Hyla arenicolor*. *Herpetologica* **48**, 210-219.

Regan, M., Gosline, J. and Richards, J. (2013). A simple and affordable calorespirometer for assessing the metabolic rates of fishes. *J. Exp. Biol.* **216**, 4507-4513.

Reher, S. and Dausmann, K. H. (2021). Tropical bats counter heat by combining torpor with adaptive hyperthermia. *Proc. R. Soc. B.* **288**, 20202059.

Reher, S., Rabarison, H., Nowack, J. and Dausmann, K. H. (2022). Limited physiological compensation in response to an acute microclimate change in a Malagasy bat. *Front. Ecol. Evol.* **10**, 779381.

Reichman, O. J., Jones, M. B. and Schildhauer, M. P. (2011). Challenges and opportunities of open data in ecology. *Science* **331**, 703-705.

Riccio, A. P. and Goldman, B. D. (2000). Circadian rhythms of body temperature and metabolic rate in naked mole-rats. *Physiol. Behav.* **71**, 15-22.

Ricklefs, R. E. and Wikelski, M. (2002). The physiology/life-history nexus. *Trends Ecol. Evol.* **17**, 462-468.

Riddell, E., Iknayan, K., Hargrove, L., Tremor, S., Patton, J., Ramirez, R., Wolf, B. and Beissinger, S. (2021). Exposure to climate change drives stability or collapse of desert mammal and bird communities. *Science* **371**, 633-636.

Riddell, E. A., Apanovitch, E. K., Odom, J. P. and Sears, M. W. (2017). Physical calculations of resistance to water loss improve predictions of species range models. *Ecol. Monogr.* **87**, 21-33.

Roche, D. G., Berberi, I., Dhane, F., Lauzon, F., Soeharjono, S., Dakin, R. and Binning, S. A. (2022). Slow improvement to the archiving quality of open datasets shared by researchers in ecology and evolution. *Proc. R. Soc. B.* **289**, 20212780.

Ruf, T. and Geiser, F. (2015). Daily torpor and hibernation in birds and mammals. *Biol. Rev.* **90**, 891-926.

Rusli, M. U., Booth, D. T. and Joseph, J. (2016). Synchronous activity lowers the energetic cost of nest escape for sea turtle hatchlings. *J. Exp. Biol.* **219**, 1505-1513.

Schmidt-Nielsen, K. (1997). Animal physiology: adaptation and environment: Cambridge university press.

Schwanz, L. E., Gunderson, A., Iglesias-Carrasco, M., Johnson, M. A., Kong, J. D., Riley, J. and Wu, N. C. (2022). Best practices for building and curating databases for comparative analyses. *J. Exp. Biol.* **225**, jeb243295.

Secor, S. M. (2009). Specific dynamic action: A review of the postprandial metabolic response. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **179**, 1-56.

Seebacher, F., White, C. R. and Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat. Clim. Change* **5**, 61-66.

Senzano, L. M. and Andrade, D. V. (2018). 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.

Senzano, L. M., Bovo, R. P. and Andrade, D. V. (2022). Empirical estimation of skin resistance to water loss in amphibians: agar evaluation as a non-resistance model to evaporation. *J. Exp. Biol.* **225**, jeb243941.

Seymour, R. S., Withers, P. C. and Weathers, W. W. (1998). Energetics of burrowing, running, and free-living in the Namib Desert golden mole (*Eremitalpa namibensis*). *J. Zool.* **244**, 107-117.

Seymour, R. S., Gienger, C., Brien, M. L., Tracy, C. R., Charlie Manolis, S., Webb, G. J. and Christian, K. A. (2013). Scaling of standard metabolic rate in estuarine crocodiles *Crocodylus porosus*. *J. Comp. Physiol. B* **183**, 491-500.

Short, J. C., Freeman, M. T. and McKechnie, A. E. (2022). Respirometry protocols for avian thermoregulation at high air temperatures: stepped and steady-state profiles yield similar results. *J. Exp. Biol.* **225**, jeb244166.

Smit, B. and McKechnie, A. E. (2010). Avian seasonal metabolic variation in a subtropical desert: basal metabolic rates are lower in winter than in summer. *Funct. Ecol.* **24**, 330-339.

Snelling, E. P., Duncker, R., Jones, K. K., Fagan-Jeffries, E. P. and Seymour, R. S. (2017). 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.

Sokolova, I. M. and Lannig, G. (2008). Interactive effects of metal pollution and temperature on metabolism in aquatic ectotherms: implications of global climate change. *Clim. Res.* **37**, 181-201.

Swanson, D. L. and Liknes, E. T. (2006). A comparative analysis of thermogenic capacity and cold tolerance in small birds. *J. Exp. Biol.* **209**, 466-474.

Tedersoo, L., Küngas, R., Oras, E., Köster, K., Eenmaa, H., Leijen, Ä., Pedaste, M., Raju, M., Astapova, A. and Lukner, H. (2021). Data sharing practices and data availability upon request differ across scientific disciplines. *Scientific data* **8**, 192.

Terblanche, J. S., Sinclair, B. J., Klok, C. J., McFarlane, M. L. and Chown, S. L. (2005). The effects of acclimation on thermal tolerance, desiccation resistance and metabolic rate in *Chirodica chalcoptera* (Coleoptera: Chrysomelidae). *J. Insect Physiol.* **51**, 1013-1023.

Townson, R. (1799). Tracts and observations in natural history and physiology. London, UK: J. White.

Tracy, C. R., Christian, K. A., Betts, G. and Tracy, C. R. (2008). Body temperature and resistance to evaporative water loss in tropical Australian frogs. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **150**, 102-108.

Tracy, C. R., Reynolds, S. J., McArthur, L., Tracy, C. R. and Christian, K. A. (2007). Ecology of aestivation in a cocoon-forming frog, *Cyclorana australis* (Hylidae). *Copeia* **2007**, 901-912.

Videlier, M., Rundle, H. D. and Careau, V. (2019). Sex-specific among-individual covariation in locomotor activity and resting metabolic rate in *Drosophila melanogaster*. *Am. Nat.* **194**, E164-E176.

Vines, T. H., Andrew, R. L., Bock, D. G., Franklin, M. T., Gilbert, K. J., Kane, N. C., Moore, J.-S., Moyers, B. T., Renaut, S. and Rennison, D. J. (2013). Mandated data archiving greatly improves access to research data. *arXiv preprint arXiv:1301.3744*.

Walsberg, G. E. and Hoffman, T. C. (2005). Direct calorimetry reveals large errors in respirometric estimates of energy expenditure. *J. Exp. Biol.* **208**, 1035-1043.

Walsberg, G. E. and Hoffman, T. C. (2006). Using direct calorimetry to test the accuracy of indirect calorimetry in an ectotherm. *Physiol. Biochem. Zool.* **79**, 830-835.

Weaver, S. J., McIntyre, T., van Rossum, T., Telemeco, R. S. and Taylor, E. N. (2023). Hydration and evaporative water loss of lizards change in response to temperature and humidity acclimation. *J. Exp. Biol.* **226**.

Weir, J. d. V. (1949). New methods for calculating metabolic rate with special reference to protein metabolism. *J. Physiol.* **109**, 1.

Welch Jr, K. C., Péronnet, F., Hatch, K. A., Voigt, C. C. and McCue, M. D. (2016). Carbon stable-isotope tracking in breath for comparative studies of fuel use. *Ann. N. Y. Acad. Sci.* **1365**, 15-32.

White, C. R. and Kearney, M. R. (2011). Metabolic scaling in animals: methods, empirical results, and theoretical explanations. *Compr. Physiol.* **4**, 231-256.

White, C. R., Matthews, P. G. and Seymour, R. S. (2008). 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.

White, C. R., Portugal, S. J., Martin, G. R. and Butler, P. J. (2006). Respirometry: anhydrous drierite equilibrates with carbon dioxide and increases washout times. *Physiol. Biochem. Zool.* **79**, 977-980.

White, C. R., Grémillet, D., Green, J. A., Martin, G. R. and Butler, P. J. (2011). Metabolic rate throughout the annual cycle reveals the demands of an Arctic existence in Great Cormorants. *Ecology* **92**, 475-486.

White, C. R., Alton, L. A., Bywater, C. L., Lombardi, E. J. and Marshall, D. J. (2022). Metabolic scaling is the product of life-history optimization. *Science* **377**, 834-839.

White, C. R., Marshall, D. J., Chown, S. L., Clusella-Trullas, S., Portugal, S. J., Franklin, C. E. and Seebacher, F. (2021). Geographical bias in physiological data limits predictions of global change impacts. *Funct. Ecol.* **35**, 1572-1578.

White, C. R., Marshall, D. J., Alton, L. A., Arnold, P. A., Beaman, J. E., Bywater, C. L., Condon, C., Crispin, T. S., Janetzki, A. and Pirtle, E. (2019). The origin and maintenance of metabolic allometry in animals. *Nat. Eco. Evol.* **3**, 598-603.

Whitfield, M. C., Smit, B., McKechnie, A. E. and Wolf, B. O. (2015). Avian thermoregulation in the heat: scaling of heat tolerance and evaporative cooling capacity in three southern African arid-zone passerines. *The Journal of experimental biology* **218**, 1705-1714.

Whitlock, M. C. (2011). Data archiving in ecology and evolution: best practices. *Trends Ecol. Evol.* **26**, 61-65.

Wiersma, P., Chappell, M. A. and Williams, J. B. (2007). Cold-and exercise-induced peak metabolic rates in tropical birds. *Proc. Natl. Acad. Sci.* **104**, 20866-20871.

Wightman, J. A. (1977). Respirometry techniques for terrestrial invertebrates and their application to energetics studies. *N. Z. J. Zool.* **4**, 453-469.

Wikelski, M. and Cooke, S. J. (2006). Conservation physiology. *Trends Ecol. Evol.* **21**, 38-46.

Wilson, S. L., Way, G. P., Bittremieux, W., Armache, J.-P., Haendel, M. A. and Hoffman, M. M. (2021). Sharing biological data: why, when, and how. *FEBS Lett.* **595**, 847–863.

Withers, P. C. (1977). Measurement of VO2, VCO2, and evaporative water loss with a flow-through mask. *J. Appl. Physiol.* **42**, 120-123.

Withers, P. C. (1992). Comparative animal physiology. New York: Saunders College Publishing.

Withers, P. C. (2001). Design, calibration and calculation for flow-through respirometry systems. *Aust. J. Zool.* **49**, 445-461.

Worland, M. R. and Block, W. (1994). An automatic respirometer for use with small invertebrates. *Funct. Ecol.*, 419-426.

Wu, N. C. (2023). Pathogen load predicts host functional disruption: A meta-analysis of an amphibian fungal panzootic. *Funct. Ecol.* **37**, 900-914.

Wu, N. C. and Seebacher, F. (2022). Physiology can predict animal activity, exploration, and dispersal. *Comm. Biol.* **5**, 1-11.

Wu, N. C., Cramp, R. L. and Franklin, C. E. (2018). Body size influences energetic and osmoregulatory costs in frogs infected with *Batrachochytrium dendrobatidis*. *Sci. Rep.* **8**, 3739.

Wu, N. C., Alton, L. A., Clemente, C. J., Kearney, M. R. and White, C. R. (2015). Morphology and burrowing energetics of semi-fossorial skinks (*Liopholis* spp.). *J. Exp. Biol.* **218**, 2416-2426.

Wu, N. C., Bovo, R. P., Enriquez-Urzelai, U., Clusella-Trullas, S., Kearney, M., Navas, C. A. and Kong, J. D. (2024). Global exposure risk of frogs to increasing environmental dryness. *EcoEvoRxiv*.

Wygoda, M. L. (1984). Low cutaneous evaporative water loss in arboreal frogs. *Physiol. Zool.* **57**, 329-337.

Zera, A. J. and Harshman, L. G. (2001). The physiology of life history trade-offs in animals. *Annu. Rev. Ecol. Syst.* **32**, 95-126.

Zhang, W.-S. (2010). Construction, calibration and testing of a decimeter-size heat-flow calorimeter. *Thermochimica acta* **499**, 128-132.

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Reporting criterion	Description	Checklist
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.	
Air flow	Report the air flow as rate (volume over time) and/or velocity (distance 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.	
Physical 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?	
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.	
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).	
Chamber mixing	Describe how chamber mixing was achieved. Important for the correct gas mixture, and mediating washout times if animals are exercising/active/shivering in the chamber.	
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.	
CO_2 analyser	Report what type of analyser was used (e.g., infrared, nondispersive infrared) and provide the model and manufacturer.	
H_2O analyser	Report what type of analyser was used (e.g., chilled mirror, capacitive, infrared) and provide the model and manufacturer.	
Calibration	Describe how the flow meters, gas analysers, temperature probes, etc. were calibrated and how often. Include the concentrations of any span gases used.	
Connectors	Provide details on the tubing material and connectors, as different materials can alter gas and humidity measurements due to potential leaking.	
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?	
Subsampling	If subsampling was used, provide the flow rate and explain how the flow rate was achieved and maintained.	
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.	
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.	
Husbandry conditions	Describe the husbandry conditions relevant to the study including, but not limited to: enclosure, feeding schedule, maintenance duration, acclimation duration, treatment groups.	
Age/life stage	Provide the life stage of the test subjects, and if known, provide the age.	
Sex	Report the number of test subjects of each sex and state whether sex ratios were equal or similar across experimental groups	
Reproductive condition	State the reproductive condition of the test subjects.	
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.	
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.	
Baseline recording	Provide information on the background/baseline (empty chamber) recording including how often and how long the baseline was recorded for.	
Time	State when the measurements were taken. MR fluctuates over the day and is affected by photoperiod.	
Lighting	Provide information on the lighting conditions during the experiment.	
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.	
Test temperature	Provide the test temperature 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.	
Test humidity	Provide the test humidity 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 air temperatures are provided. In flow-through systems, the excurrent humidity (not the incurrent) is the humidity experienced by the subject.	
Standard temperature and pressure	Given that definitions of STP vary, it is important that a definition of STP should also be provided for transparency.	
Fasted	State whether the test subjects were fasted prior to the experiment and for how long.	
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.	
Grouping	If more than one test subject was placed inside the chamber, provide the exact number of individuals.	

Measurements	State what measurements were obtained, when, and for long they were measured. If individuals were repeated, state the number of repeats per exposure.	
Animal state	Describe the state of the animal when the measurement is 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? - Ideally, activity should be monitored visually or measured to confirm an animal is inactive or to account for variation in metabolic rate associated with variation in activity levels.	
Multiple animals	When multiple animals are measured in sequence in one measurement period, provide the timing of switching between channels.	
Data processing		
Data acquisition	Provide information on the data acquisition systems/software.	
Baseline drift	Baseline measurements will fluctuate, especially for O_2 concentrations. State whether and how baseline drift was corrected.	
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.	
Mathematical scrubbing	If physical scrubbers were not used, provide details on how gas concentrations were mathematically scrubbed.	
Sampling	Describe and justify sample selection (mean, time period) as well as exclusion criteria (activity, posture, 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.	
Boundary layer	For EWL, state if the boundary layer was accounted for, either mathematically or empirically (e.g. from agar models) estimated.	
Equations	Provide the equations or cite equations used for calculations of rates.	
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?	
Data exclusion	If data were excluded from the study due to experiment/measurement/animal issues, provide such information for transparency.	
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.	
Units	Always report units in the paper.	
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.	
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.	

Pseudoreplication	Report pesudoreplication if used. E.g. the number of tanks, rooms, chambers used, and the number of animals in each.	
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.	
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).	
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.	

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