REPORTING CHECKLIST
Updated 14 May 2024

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 volume over time corrected to standard temperature and pressure (STP), and how the flow rate was achieved and maintained. Definitions for STP vary and so should be defined (in comparative physiology, it is usually defined as 273.15 K and 101.325 kPa). Air flow should refer to the flow experienced by the animal and not the flow through the analysers. See Subsampling below.	
Physical and chemical scrubbing	Report whether air was scrubbed of H_2O and/or CO_2 physically or mathematically. If physically scrubbed, what type of scrubbers were used (e.g., Drierite) and where in the plumbing set-up were they placed? If mathematically scrubbed, what equation was used?	
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 also 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, etc.	
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. For multiplexed systems, check whether each system is baselined with air before and after the experiment, or was a separate system relied on.	
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 air temperature (T_a) and how it was maintained. If a stepped temperature change was used, provide details on the duration and rate of change between each temperature setpoint.	
Test humidity	Provide the test humidity (incurrent and excurrent) and how it was maintained. Humidity should be reported as absolute values or partial pressures (g H_2O m^{-3} or kPa) rather than relative humidity (RH, %). If only RH values are available, it is critical that the corresponding T_a are provided. If converting to water vapour pressure and water vapour deficit from RH and T_a, provide the reference to the equations used.	
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.	
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 (see Glossary), when, and for long they were measured. If individuals were repeated, state the number of repeats per exposure. If multiplexors where used, providing the sampling period	
Animal state	Describe the state of the animal when the measurement was taken [e.g., inactive, active, rest-phase, active-phase, post-exhaustion, digesting, torpid, aestivating, normothermic (for endotherms)]. - For resting states, state the recovery time from handling stress after being placed into the chamber. - If post-exhausted for MMR, how was this achieved? - Activity should be monitored visually or measured to confirm an animal is inactive or to account for variation in MR and EWL associated with variation in activity levels.	

Multiple animals	When multiple animals are measured in sequence in one measurement period, provide the timing of switching between channels. Describe how the washout times for the respirometry system and multiplexed sampling period was matched.	
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. Report whether it is appropriate for the type of O_2 analyser used and how this was determined e.g. paramagnetic?	
Sampling	Describe and justify sample selection (mean, time period) as well as exclusion criteria (activity, posture, excretion etc). **Endotherm**: Some mammals will lick their fur or the chamber during respirometry trials which will produce relatively high EWL. The use of video surveillance is recommended to monitor such activities.	
Boundary layer	For calculating skin resistance from EWL, state how the boundary layer was accounted for, either mathematically or empirically (e.g. from agar models) estimated.	
Equations	Show the equations for all calculations in addition to citing their sources.	
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. Indicate the criterion e.g. extreme values, outlier statistics.	
Data reporting and statistics		
Aims and hypotheses	In the Introduction, clearly state the aims and/or hypothesis for which the study was conducted and data were gathered.	
Units	Always report units in the paper. Use only International System of Units (SI) or SI-derived units.	
Raw data	Supply raw data on the rate of O_2 consumption, CO_2 production or EWL in addition to converted values used in the paper. E.g. translating to energy equivalents, mass-corrected or mass-specific values, surface-specific values. And when presenting mass- or surface-specific values, remember that such data remove the effect of mass only in very specific (and usually not realistic) situations.	
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. Also report how pesudoreplication was statistically accounted for (e.g. random effect).	
Statistics	List each statistical test and analysis conducted in sufficient detail such that they can be replicated and fully understood by those experienced in those methods. Fully report outcomes from each statistical analysis. For most analyses, this includes, but is not limited to, basic parameter estimates of central tendency (e.g., means) or other basic estimates (regression coefficients, correlation) and variability (e.g., standard deviation) or associated estimates of uncertainty (e.g., confidence/credible intervals). Thorough and transparent reporting will involve additional information that differs depending on the type of analyses conducted. · For null hypothesis tests, this also should at minimum include test statistic, degrees of freedom, and p-value. · For Bayesian analyses, this also should at a minimum include information on choice of priors and MCMC (Markov chain Monte Carlo) settings (e.g. burn-in, the number of iterations, and thinning intervals). · For hierarchical and other more complex experimental designs, full information on the design and analysis, including identification of the appropriate level for tests (e.g. identifying the denominator used for split-plot experiments) and full reporting of outcomes (e.g. including blocking in the analysis if it was used in the design).	

	Relevant information will differ among other types of analyses but in all cases should include enough information to fully evaluate the design and analysis.	
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.	

Abbreviations

BMR: Basal metabolic rate
EWL: Evaporative water loss
MR: Metabolic rate
MMR: Maximal metabolic rate
RH: Relative humidity
STP: Standard temperature and pressure