Guidelines for the reporting of methods for estimating metabolic rates using aquatic intermittent closed respirometry

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SUMMARY STATEMENT

We show that reporting of intermittent-closed respirometry methods in peer-reviewed articles has been inconsistent and incomplete, and present the first guidelines for reporting intermittent-closed respirometry methods to enhance study replicability.

ABSTRACT

Interest in the measurement of metabolic rates is growing rapidly, due to the relevance of metabolism in understanding organismal physiology, behaviour, evolution, and responses to environmental change. The study of metabolism in aquatic organisms is experiencing an especially pronounced expansion, with more researchers utilizing intermittent-closed respirometry as a research tool than ever before. Despite this, there remain no published guidelines on the reporting of methodological details when using intermittent-closed respirometry. Using a survey of the existing literature, we show that this lack of recommendations has led to incomplete and inconsistent reporting of methods for intermittent-closed respirometry over the last several decades. We also provide the first guidelines for reporting such methods, in the form of a checklist of details that are the minimum required for the interpretation, evaluation, and replication of experiments using intermittent-closed respirometry. This should increase consistency of the reporting of methods for studies that use this research technique. With the steep increase in studies using intermittent-closed respirometry over the last several years, now is the ideal time to standardise the reporting of methods so that data can be properly assessed by other scientists and conservationists.

INTRODUCTION

Estimating metabolic rates of animals has been a core element of research in comparative physiology for decades (Kleiber, 1947; Rolfe and Brown, 1997). Metabolic rates have also been studied in the context of physiological and behavioural ecology (Killen et al., 2013; Mathot et al., 2019; Metcalfe et al., 2016), as well as in the examination of broad ecological phenomena across levels of biological organisation (Brown et al., 2004; Hatton et al., 2019). The study of metabolic rates has recently received even greater attention due to the need to understand plastic and evolutionary responses to environmental change, particularly in aquatic ecosystems (Jutfelt et al., 2018; Norin and Metcalfe, 2019; Pörtner et al., 2017). This increased attention has occurred alongside technological advances in methods of respirometry, which measure rates of gas exchange as a proxy for metabolic rate (Nelson, 2016). The rise of commercially available respirometry components has further facilitated the estimation of metabolic rates from a variety of organisms. These factors have been particularly consequential for respirometry on animals that breathe water because, historically, this has been more difficult to conduct as compared to respirometry on air-breathers. As such, there are more scientists using aquatic respirometry as a research tool than ever before, with more than 60% of the papers in this field being generated in the last 10 years alone (Figure 1).

The most widely-accepted method for estimating metabolic rates of water-breathing organisms is automated intermittent-closed respirometry (Steffensen, 1989; Svendsen et al., 2016). Although the technique has mainly been developed on fishes, it is suitable for almost any water-breathing organism and involves placing the animal in a respirometry chamber where it is exposed to periodic, alternating, “closed” and “flush” phases. During the closed phase, the respirometer is effectively sealed and there is a decline in oxygen concentration in the water due to oxygen uptake by the animal, which can be used as a proxy for whole-animal metabolic rate (Nelson, 2016). Such closed respirometry would, eventually, cause hypoxia and accumulation of waste products in the respirometer, but this is avoided by the flush phase when the respirometer is flushed with clean, aerated water. The alternation of these phases means that real-time rates of oxygen uptake can be recorded in successive closed phases over extended periods, with animals left undisturbed. This can provide an accurate picture of dynamic changes in metabolic rate over time, due to factors such as initial handling stress, circadian rhythms, metabolic costs of digestion, among others (Jourdan-Pineau et al., 2010; Steffensen, 1989). It can also reveal when the undisturbed animal is potentially functioning at basal rates of metabolism (standard metabolic rate, SMR, for ectotherms) (Chabot et al., 2016). This ability to track changes in metabolic rate in real time is a major improvement over the technique of flow-through respirometry (Ultsch et al., 1980), which measures rates of oxygen uptake from a continuous flow of water through a respirometer chamber, from the difference in oxygen concentration at inflow and outflow. Changes in oxygen concentration at the outflow, however, lag behind changes in metabolic activity of the fish due to wash-out effects, which can confound estimates of metabolic rate (Steffensen, 1989). Intermittent-closed respirometry is, therefore, the best available method to estimate metabolic rate in water-breathing animals, with a wide range of applications.

Despite its increasingly wide usage, there are no guidelines for reporting the methods used in intermittent-closed respirometry. There are several guides to best practice for measuring and analysing various types of metabolic rates (Chabot et al., 2016; Jutfelt et al., 2018; Norin and Clark, 2016; Steffensen, 1989; Svendsen et al., 2016), but methodological details can vary widely among researchers. Equally important is that the *reporting* of methods also differs greatly across peer-reviewed studies, with important details often not mentioned. A lack of methodological detail, or inaccurate and vague descriptions, are problematic because they: (1) make it difficult for readers to evaluate data reliability and judge interpretation of results; (2) can give a misleading impression of what was actually done, and (3) hinder replication of the experiments. Notably, data on metabolic

rates are increasingly used in meta-analyses (Holtmann et al., 2017; Jerde et al., 2019; Killen et al., 2016) so proper methodological documentation would be useful in understanding sources of residual variation across studies. A standard set of guidelines for reporting methods in intermittent-closed respirometry studies would make it easier for journal editors and reviewers to decide whether a given study warrants publication in the first place. Finally, a list of important methodological details would be extremely useful for students and researchers who are new to this field of research and are using this technique for the first time. With the exponential increase in the number of published studies using respirometry (Figure 1), it is the ideal time to establish and institute standard guidelines for accurate methodological reporting.

Focussing on studies with fishes, we present guidelines for reporting methods used in aquatic intermittent-closed respirometry. We provide a checklist of details that should represent minimum requirements for accurate reporting and evaluation of methods, and for experimental replication. We also provide a quantitative analysis of previous reporting of methods among studies using aquatic intermittent-closed respirometry in fishes. Our aim is to highlight the specific areas in which reporting of methodological details can be improved and to provide an explicit list of details which can be referenced when writing or evaluating research papers.

METHODS

Checklist of Essential Criteria

We generated a list of 50 criteria that we deemed important for understanding, interpreting, and replicating experiments using aquatic intermittent-closed respirometry (Table 1; Figure 2). While the criteria are focussed on studies with fishes, most criteria could be applied to studies with any aquatic organism. Criteria were divided into the following categories: (1) equipment and setup; (2) measurement conditions; (3) details specific to the measurement of background oxygen uptake and adjustments; (4) details specific to the measurement of “minimum” metabolic rates, including SMR and routine metabolic rate (RMR); (5) details specific to the measurement of maximum metabolic rate (MMR), and (6) data handling and analysis. We restricted our criteria to cover methods involved in the most basic forms of aquatic intermittent-closed respirometry, namely, the measurement of minimum and maximum metabolic rates. Methods unique to other applications, such as protocols for measuring critical oxygen tensions (P_{crit}) are not covered here (Claireaux and Chabot, 2016; Ultsch and Regan, 2019).

Literature Survey and Criteria Scoring

We performed a survey of the literature to determine variation in the reporting of methods and the extent to which various criteria are (or are not) reported. Using Web of Science, we used the topic search term [fish AND ("standard metabolic rate" OR "resting metabolic rate" OR "routine metabolic rate") AND "maxim* metabolic rate"] in January 2021. This survey was not meant to be exhaustive but was meant to be representative of the methodological reporting across research using fish intermittent-closed respirometry as a whole. This search returned 120 research articles, which were then screened by reading titles and abstracts. Articles were excluded from further analysis if they were review articles, meta-analyses, or any other study that did not estimate metabolic rates using intermittent-closed respirometry. In addition, we excluded articles if they used flow-through respirometry or closed respirometry. Finally, to increase consistency in the criteria scored across studies, studies were only included in further analysis if they measured both SMR (or RMR) and MMR. This led to 72 studies being assessed (Table S1).

Each study was scored for whether they satisfied each criterion in the checklist. Studies were awarded a point for a given criterion if they gave a clear, unambiguous description of that methodological detail, without the need for reader assumptions or calculations. Importantly, scores were not based on the quality of a methodology itself – they were simply based on whether a given detail was provided. For example, if a paper stated that the respirometer was made of Swiss cheese, the criterion "provide material of respirometer" (criterion 5; Table 1) would be considered satisfied and a point would be awarded, without judgement of whether Swiss cheese is an appropriate material for respirometer construction. Methodological details for specific criteria were considered present if they were provided in the main article text, figures, tables, supplementary material, or in references to previously published work. When there were references to multiple prior studies for a given criterion, a point was not given if those prior sources provided inconsistent or contradictory descriptions. In some cases, the absence of a specific criterion made it impossible to assess other associated criteria, in which case a value of NA was assigned to criteria that were unable to be scored, and those instances were not included in calculating the mean average score for that paper, or for calculating the mean prevalence of that criteria across papers. While most studies were evaluated by one scorer, eight studies were evaluated by two scorers each, ensuring consistency across scorers and allowing refinement of criteria phrasing to minimise ambiguity. For each article, we also recorded the title, first author, year of publication, and journal.

Statistical Analysis

A generalised linear mixed model (GLMM) with a binomial distribution (logit link) was constructed to examine factors affecting methods reporting across published papers. The score for each criterion per paper (0 or 1) was used as the response variable, and criteria category, year, journal impact factor, and all interactions among these variables were initially included as explanatory variables. Paper ID (by title) and scorer were included as random effects. Non-significant interactions were dropped sequentially and the model re-run. All analyses were conducted using R v. 4.0.3 (R Development Core Team, 2020) using the function lmer in package lme4 (Bates et al., 2016).

RESULTS

Papers reported a mean of 54% of the listed criteria (Figure 3). While specific papers often scored highly within a particular category, all papers missed several of the listed criteria across categories. Even among criteria which were reported relatively frequently, there were key details that were often not provided. For example, animal mass at the time respirometry was conducted (as opposed to when the animals were brought into the lab) was not specifically mentioned in almost 40% of studies. Similarly, 17% of studies were not explicit about the temperature used during respirometry, and 51% did not mention how temperature was controlled. Almost 60% of studies did not mention the lowest water oxygen concentration that fish were exposed to during respirometer closed-phases.

Criteria associated with the measurement of background respiration were the least frequently reported (45%), while those concerning measurement of MMR were most frequently reported (76%, GLMM, effect of category, $p < 0.0001$). The reporting of criteria has improved over time, since publication of the first paper in our survey (published in 1993; Figure 4; GLMM, effect of year, $p = 0.002$). Reporting was not related to journal impact factor (2019 Clarivate Analytics).

There was wide variation in reporting frequency of criteria within categories, with some specific criteria being consistently under-reported (Table 1). Criteria relating to mixing circuit tubing (criteria 4, 6, and 12) were only mentioned in 8-13% of papers, and descriptions of probe calibration

(criterion 15) only appeared in 24% of papers. Few papers (20%) mentioned whether animals in adjacent respirometers were able to see each other during measurements with multiple chambers running simultaneously (criterion 23), and the total duration (i.e. in days) of all trials combined within a study (criterion 25) was infrequently reported as well (22% of papers).

DISCUSSION

We present a checklist of criteria that should be addressed when reporting the methods of experiments using aquatic intermittent-closed respirometry. Our quantitative analysis shows that reporting of methods has been relatively poor and inconsistent, including in our own published articles. The lack of consistency across studies is undoubtedly due to the lack of any publicly available guidelines for reporting the methodological details of intermittent-closed respirometry. Given the rapidly increasing number of studies being published in this field, we suggest that researchers use the checklist provided here when describing their methods, to prevent further under-reporting of important elements. The use of reporting guidelines for methods, in the form of checklists or flow-charts (Carp, 2012; Cowger et al., 2020; Michel et al., 2020), is widespread across the biological sciences, and is long overdue in comparative physiology and especially respirometry. Intermittent-closed respirometry is guaranteed to produce data, but the quality of that data is completely dependent on a myriad of methodological details and decisions made throughout data collection and analysis (Steffensen, 1989; Svendsen et al., 2016). Without describing these details when reporting methods, it is not possible for readers or reviewers to judge or replicate results. A clear list of important details will also be useful for planning experiments using intermittent-closed respirometry, especially for researchers that are new to the field.

In addressing specific methodological details, it is important for researchers to be as clear and explicit as possible, to eliminate any chance of misinterpretation. In our survey, for example, articles sometimes reported the body mass of the fish upon arrival at the lab or during holding conditions, but not at the time of respirometry. Body size affects both minimum and maximum metabolic rates (Jerde et al., 2019; Killen et al., 2016) and temporal variation in body mass may lead to inaccurate metabolic rate estimation. Similarly, the temperature or photoperiod during holding conditions were often given without explicit reference to conditions during respirometry, or how temperature conditions were maintained. Metabolic rates of ectothermic animals are profoundly influenced by temperature (Clarke and Johnston, 1999; Schulte, 2015) and photoperiod may also affect animal oxygen uptake (Biswas and Takeuchi, 2002). Another problem is that articles often refer to multiple prior studies for methodological details, but these references would contain inconsistent or contradictory information. The use of inaccurate or vague phrasing can also cause confusion, misunderstanding of what methods were actually performed and, potentially, the spread of incorrect information and terminology. Overall, while the use of a checklist for methodological details should improve the reporting of methods for intermittent-closed respirometry, it is ultimately dependent upon researchers to use clear and unambiguous language when describing their methods.

Overall, criteria associated with background bacterial oxygen uptake were the most inconsistently reported among studies. This is a critical oversight because the amount of bacterial respiration and the exact way it is measured, or incorrectly accounting for rates of background oxygen uptake, can greatly impact estimates of animal metabolic rates (Rodgers et al., 2016; Svendsen et al., 2016). A large proportion of papers failed to describe how bacterial respiration was controlled (e.g. by cleaning of respirometry chambers and setup), how it was measured and accounted for, or the proportion of animal metabolism that it represented. In fact, more than a quarter of papers

surveyed did not mention whether any form of background bacterial respiration measurement was performed. Without such details, it is extremely difficult to assess data validity. This is, therefore, a methodological element that researchers must perform properly and report clearly.

There were several other criteria that were consistently underreported. For example, details regarding the mixing circuit of respirometers were frequently neglected, despite this being a key component of intermittent-closed respirometry (Rodgers et al., 2016; Svendsen et al., 2016). Nearly 40% of papers did not provide any mention of whether mixing was performed or how it was accomplished (criterion 9). Proper mixing of the water in respirometers is critical to homogenise oxygen concentrations throughout chambers and accurately measuring oxygen uptake is simply not possible without effective mixing. Moreover, any tubing used in a mixing circuit needs to be as clean as possible, as short as possible, and made of relatively gas impermeable material. Any respiration from bacteria adhering to the surface, or gas exchange across the tubing, could have strong confounding effects that need to be corrected for or avoided. Moreover, the volume of the mixing circuit must be included in calculation of respirometer volume and therefore animal oxygen uptake rate.

Numerous criteria pertaining to conditions during measurement were reported infrequently. Although many studies measure multiple animals simultaneously during respirometry, with each animal within its own chamber, few studies mention whether the animals were visually shielded from each other. This could have various impacts on activity and metabolic rates that could differ among species, depending on their level of sociability or aggression (Killen et al., 2014; Nadler et al., 2016; Ros et al., 2006). Few studies reported the total duration taken to measure all animals in a study. This criterion may be especially important for studies with large sample sizes, leading to overlap with breeding seasons or significant changes in mass of small, rapidly growing animals. Another critical factor that was often not reported is the minimum level of oxygen concentration that occurred during closed phases of the intermittent respirometry cycle. If oxygen depletion by the animal actually causes hypoxia during closed phases, this may cause repeated reliance upon anaerobic pathways to meet energy requirements of metabolism, which would then interfere with estimates of metabolic rate using oxygen uptake as a proxy (Snyder et al., 2016). Additionally, repeated hypoxia may elicit an endocrine stress response, or stimulate swimming activity, also affecting metabolism and rates of oxygen uptake (Aboagye and Allen, 2014; Killen et al., 2012).

There are several criteria unique to the estimation of either SMR/RMR or MMR that are often not reported. Regarding SMR/RMR, the total number of oxygen uptake measurements (i.e. number of closed phases) used in the derivation of the metabolic rate estimate (criterion 35) was reported in only 29% of studies. Methods for statistically estimating SMR, for example, including the use of quantiles or frequency distributions, require a large number of repeated measures and so the total number of slopes used in their derivation should be provided. Included within this are any slopes that were disregarded during acclimation to the respirometer (criterion 33) or periods of increased activity (criterion 37; including whether such periods were included in quantile- or frequency distribution-based methods of calculating SMR). Although reporting for MMR was relatively good when compared to the other criteria categories, there were still important details that were often neglected. For example, when measuring oxygen uptake immediately after exhaustion, many studies did not report whether the animal was exposed to air before placement in the chamber. The time taken to initiate measurements of oxygen uptake (e.g. in seconds, after the cessation of exercise) was also often not provided. This is important because data processing procedures can bias estimates of MMR (Zhang et al., 2020), including the duration of the slope used to estimate MMR

and the specific method of determining the maximum rate of oxygen uptake during recovery after exercise (criterion 49). Our survey revealed that both criteria were relatively underreported but, given emerging awareness of their importance, it is vital that authors provide these details going forward.

While the reporting of methods for intermittent-closed respirometry has generally been inconsistent, our analysis also indicates a steady improvement in reporting since the early 1990s. We hope the development of guidelines and the availability of a reporting checklist will hasten this trend towards systematically clear and accurate reporting of methods. As metabolic rates increasingly become a focus for understanding the ability of animals to cope with environmental change, it is more important than ever to ensure reliable and replicable data, particularly in cases where data may be used to inform conservation efforts. The availability of a checklist of important methodological details should also be useful to new researchers entering this rapidly developing field.

COMPETING INTERESTS

The authors declare no competing interests.

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DATA AVAILABILITY

Data are available along with this submission and will be uploaded to the Mendeley Data Repository.

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459 **TABLE 1.** Checklist of criteria that should be provided when reporting methods for aquatic
460 intermittent-closed respirometry. Also shown is the prevalence of each criteria in the existing
461 literature.

Number	Criterion and Category	Prevalence (% of papers)
	EQUIPMENT	
1	☐ Provide body mass of animals at time of respirometry	61.1
2	☐ Provide volume of respirometer	94.4
3	☐ Provide volume of tubing in mixing circuit	8.5
4	☐ Provide ratio of animal body mass to volume of respirometer (plus associated tubing in mixing circuit)	15.3
5	☐ Provide material of respirometer (e.g. glass, acrylic, etc.)	54.2
6	☐ Provide material of tubing	12.7
7	☐ Provide type of oxygen probe and data recording	90.3
8	☐ Provide sampling frequency of water oxygen concentration	44.4
9	☐ Describe how chamber mixing was achieved	62.0
10	☐ Describe placement of oxygen probe (in recirculation circuit or directly in chamber)	56.9
11	☐ Provide flow rate during flushing and recirculation, or confirm that chamber returned to normoxia during flushing	28.2
12	☐ Declare whether volume of tubing in mixing circuit was included in calculations of oxygen uptake	12.7
13	☐ Provide timing of flush/closed cycles	87.5
14	☐ Provide wait (delay) time excluded from closed measurement cycles	68.1
15	☐ Describe frequency and method of probe calibration (for both 0 and 100% calibrations)	23.6
16	☐ Mention whether software temperature compensation was used during recording of water oxygen concentration	15.3
	MEASUREMENT CONDITIONS	
17	☐ Provide temperature during respirometry	83.3
18	☐ Describe how temperature was controlled	48.6
19	☐ Provide photoperiod during respirometry	34.7
20	☐ Provide minimum water oxygen level or concentration reached during closed phases	40.3
21	☐ Describe whether chambers were visually shielded from external disturbance	47.9
22	☐ Describe how many fish were measured during a given respirometry trial	68.1
23	☐ If multiple animals were measured simultaneously, describe whether they were able to see each other during measurements	20.0
24	☐ Provide duration of animal fasting before placement in respirometer	84.7
25	☐ Provide duration of all trials combined (number of days to measure all animals in the study)	22.2
26	☐ Provide acclimation time to the laboratory before respirometry measurements	81.9
	BACKGROUND RESPIRATION	
27	☐ Mention whether background respiration was measured and accounted for, and if so, how this was done	73.6

28	☐	Specify method used to measure background respiration (e.g. parallel measures with empty respirometry chamber, measurements before and after for all chambers while empty, both)	66.2
29	☐	If background respiration was measured at beginning and end, describe how many slopes and for what duration	32.8
30	☐	Describe how were changes in background respiration modelled over time (e.g. linear, exponential, parallel measures)	42.0
31	☐	Provide level of background respiration (e.g. as a percentage of SMR)	23.6
32	☐	Describe method and frequency of system cleaning (e.g. system bleached between each trial, UV lamp)	30.6
		STANDARD OR ROUTINE METABOLIC RATE	
33	☐	Provide acclimation time after transfer to chamber, or alternatively, time to reach beginning of metabolic rate measurements after introduction to chamber	65.3
34	☐	Provide duration over which metabolic rate was estimated	87.5
35	☐	Provide total number of slopes measured and used to derive metabolic rate (e.g. how much data were used to calculate quantiles)	29.2
36	☐	Describe how metabolic rate was estimated (e.g. quantile method for SMR, average of lowest 10%, etc.)	92.8
37	☐	Specify whether any outlier data were removed (e.g. data during acclimation, or slopes with poor r^2 [and if so what % of the data], data during periods of high activity [e.g. daytime])	25.0
		MAXIMUM METABOLIC RATE	
38	☐	Specify method used (e.g. critical swimming speed respirometry, swim to exhaustion in swim tunnel, or chase to exhaustion)	98.6
39	☐	Specify what value was taken as MMR (e.g. the highest rate of oxygen uptake value after transfer, average of highest values)	86.1
40	☐	Specify when MMR was measured in relation to SMR (i.e. before or after)	87.1
41	☐	Specify how absolute aerobic scope and/or factorial aerobic scope is calculated (i.e. using raw SMR and MMR, mass-adjusted SMR and MMR, or mass-adjusting aerobic scope itself)	71.2
		IF MMR MEASURED POST-EXHAUSTION:	
42	☐	Describe length of activity challenge or chase (e.g. 2 min, until exhaustion, etc.)	92.1
43	☐	Specify whether further air-exposure was added after exercise	56.5
44	☐	Provide time (in seconds) until transfer to chamber after exhaustion, or alternatively, time to start of first oxygen uptake recording	39.1
		DATA HANDLING AND STATISTICS	
45	☐	Provide sample size	94.4
46	☐	Describe how oxygen uptake rates were calculated (software or script, equation, units, etc.)	75.0
47	☐	Provide r^2 threshold for slopes used for SMR/RMR, or mean r^2	34.7
48	☐	Duration of slopes used to calculate MMR (e.g. 1 min, 5 min, etc.)	49.3
49	☐	Specify slope estimation method for MMR (e.g. rolling regression, sequential discrete time frames)	20.9
50	☐	Describe any mass-corrections or adjustments	73.6

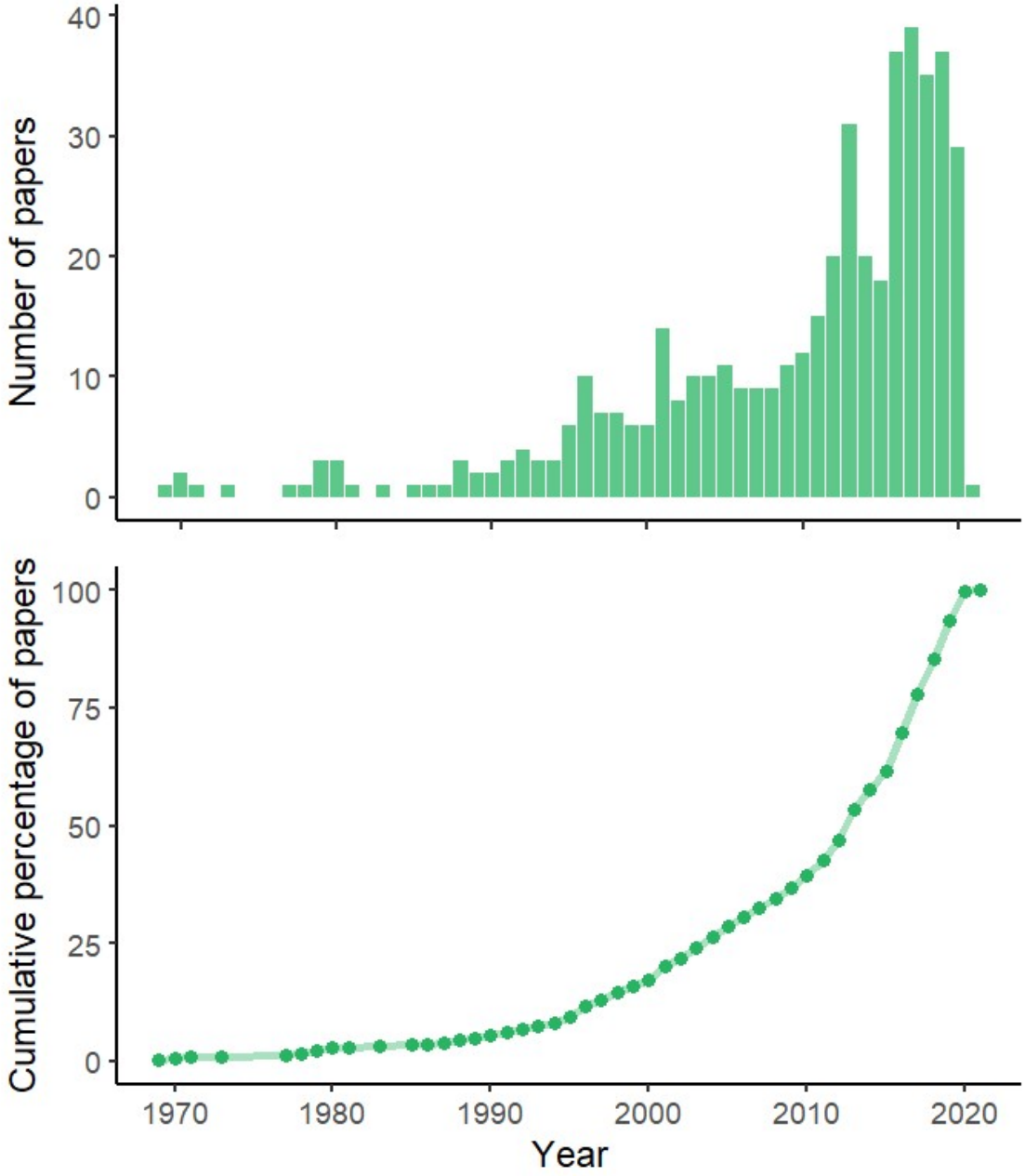

FIGURE 1. Research in aquatic respirometry has increased steeply over the last several decades. Top panel: the number of papers per year, returned by the topic search [aquatic AND respirometry] (Web of Science, February 2021); bottom panel: the cumulative percentage of all papers by year.

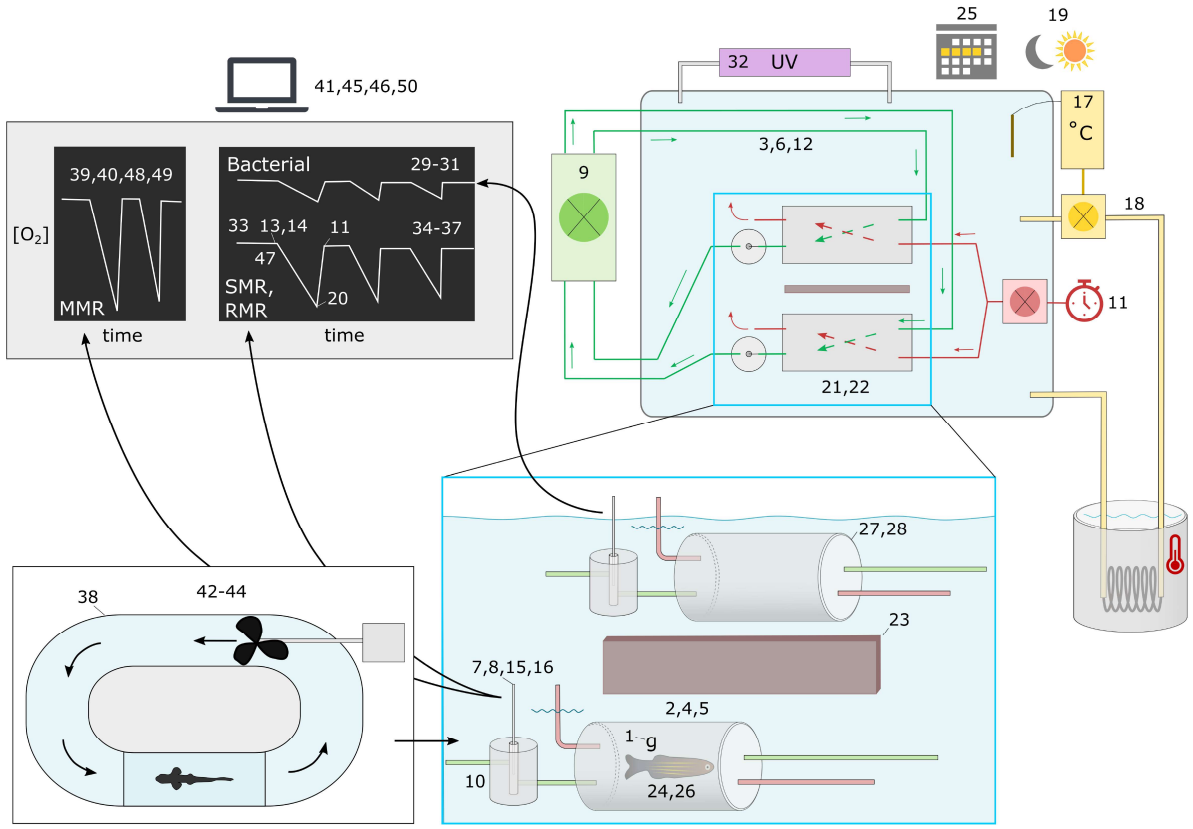

FIGURE 2. Schematic of a typical intermittent-closed respirometry setup. Numbers correspond to the criteria listed in Table 1 and show the general location of each criteria within the setup. Red items are those used for periodically flushing the respirometer with clean, aerated water from the surrounding bath. Green represents the mixing circuit. Note that in this scheme, mixing is performed with a multichannel pump, but mixing can also be achieved with a single-channel pump or stir-bar, depending on the size and shape of the respirometers. Yellow represents elements associated with temperature control; here temperature is maintained using a thermostat that controls a pump to direct water through a heat exchanger within a heated reservoir whenever temperature within the bath drops below the setpoint. Refer to Svendsen et al. (2016) for more information on setup components and overall system functioning. RMR = routine metabolic rate; SMR = standard metabolic rate; MMR = maximum metabolic rate; UV = ultraviolet.

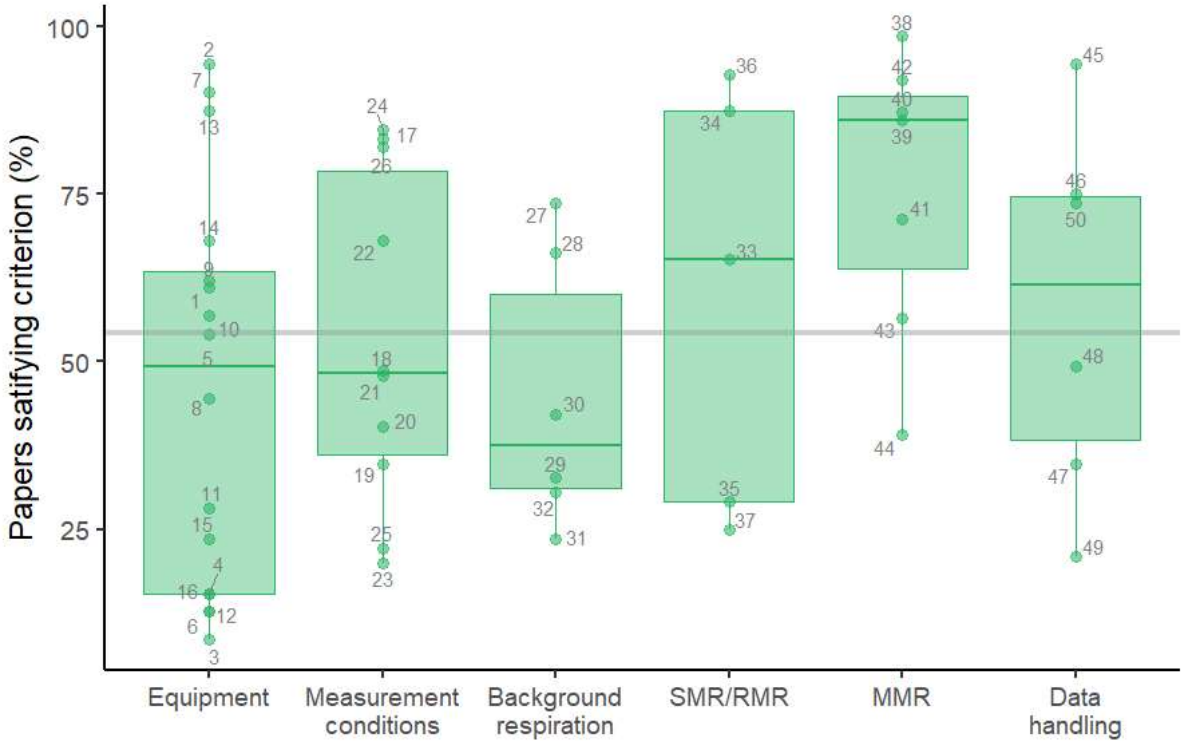

FIGURE 3. The percentage of papers that referred to the specific criteria listed in Table 1. Each point represents one criterion; grey numbers correspond to criteria numbering in Table 1. The grey line is the overall average across papers. Boxplot lower and upper hinges represent the 25th and 75th percentiles, respectively; the horizontal line within the box represents the median; the length of whiskers represents the range data points between each hinge and 1.5× the difference between the 25th and 75th percentiles.

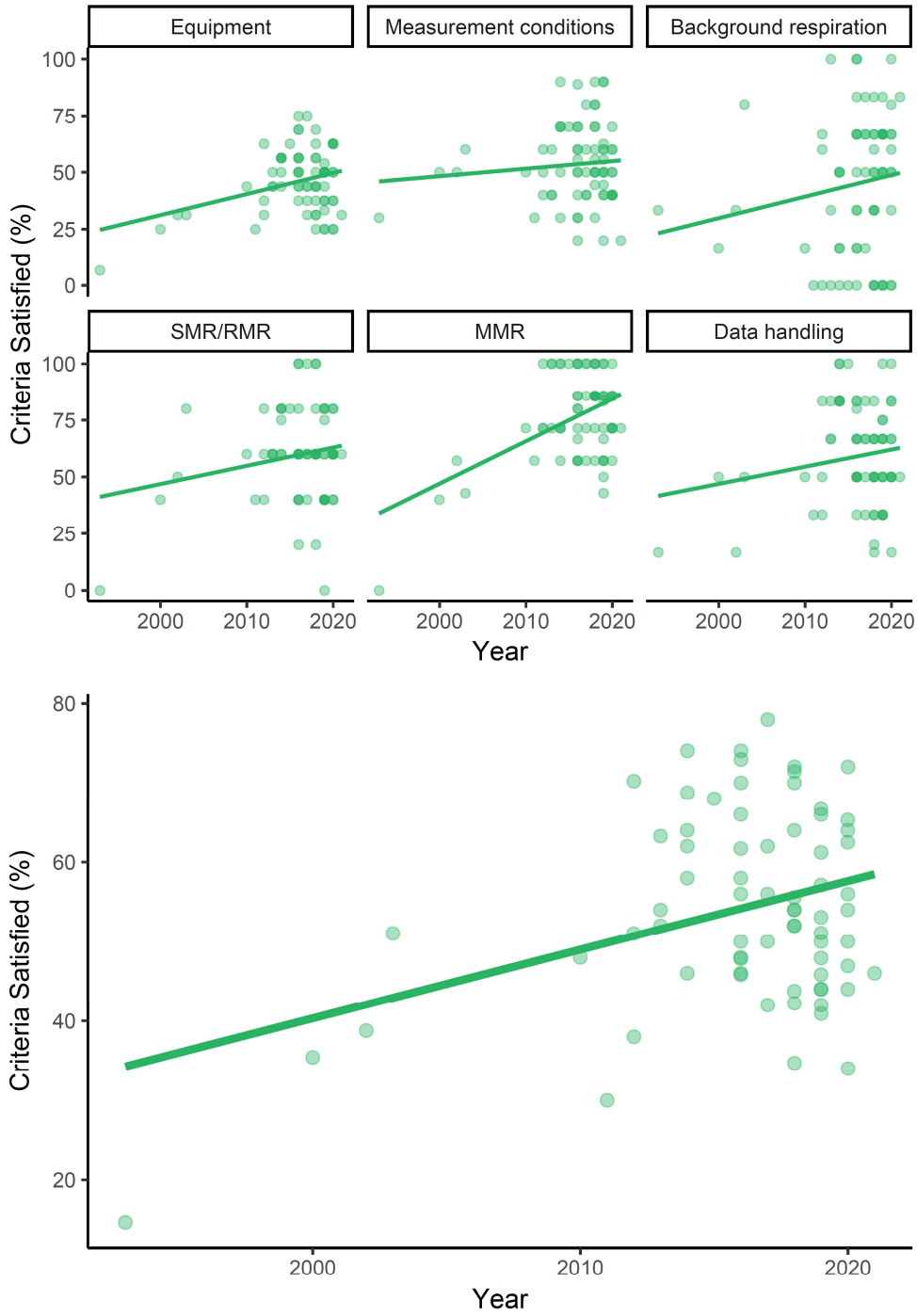

FIGURE 4. The percentage of criteria listed in Table 1 that were satisfied in the surveyed papers. Each point represents one paper; solids lines are linear regressions with publication year on the x-axis. The top faceted panels show the criteria sub-divided according to category; the bottom panel shows the overall percentage of criteria satisfied.