

1 **Guidelines for reporting methods to estimate metabolic rates by aquatic intermittent-flow**  
2 **respirometry**

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47 **SUMMARY STATEMENT**

48 We present the first checklist of guidelines for reporting methods of intermittent-flow respirometry  
49 to enhance study replicability. We also show that methods-reporting for this technique has been  
50 inconsistent and incomplete in peer-reviewed articles.

51

52 **ABSTRACT**

53 Interest in the measurement of metabolic rates is growing rapidly, due to the relevance of  
54 metabolism for understanding organismal physiology, behaviour, evolution and responses to  
55 environmental change. The study of metabolism in aquatic animals is undergoing an especially  
56 pronounced expansion, with more researchers utilising intermittent-flow respirometry as a research  
57 tool than ever before. Aquatic respirometry measures the rate of oxygen uptake as a proxy for  
58 metabolic rates, and the intermittent-flow technique has numerous strengths for use with aquatic  
59 animals, allowing metabolic rate to be repeatedly estimated on individual animals over several hours  
60 or days and during exposure to various conditions or stimuli. There are, however, no published  
61 guidelines for the reporting of methodological details when using this method. Here, we provide the  
62 first guidelines for reporting intermittent-flow respirometry methods, in the form of a checklist of  
63 criteria that we consider to be the minimum required for the interpretation, evaluation and  
64 replication of experiments using intermittent-flow respirometry. Furthermore, using a survey of the  
65 existing literature, we show that there has been incomplete and inconsistent reporting of methods  
66 for intermittent-flow respirometry over the last several decades. Use of the provided checklist of  
67 required criteria by researchers when publishing their work should increase consistency of the  
68 reporting of methods for studies that use intermittent-flow respirometry. With the steep increase in  
69 studies using intermittent-flow respirometry, now is the ideal time to standardise reporting of  
70 methods, so that – in the future – data can be properly assessed by other scientists and  
71 conservationists.

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95 **Introduction**

96 Estimating metabolic rates of animals has been a core element of research in comparative  
97 physiology for decades (Kleiber, 1947; Rolfe and Brown, 1997). Metabolic rates have also been  
98 studied in the context of physiological and behavioural ecology (Killen et al., 2013; Mathot et al.,  
99 2019; Metcalfe et al., 2016), as well as in the examination of broad ecological phenomena across  
100 levels of biological organisation (Brown et al., 2004; Hatton et al., 2019). The study of metabolic  
101 rates has recently received even greater attention due to the need to understand plastic and  
102 evolutionary responses to environmental change, particularly in aquatic ecosystems (Jutfelt et al.,  
103 2018; Norin and Metcalfe, 2019; Pörtner et al., 2017). This increased interest has occurred alongside  
104 technological advances in methods of respirometry, which measure rates of gas exchange between  
105 an organism and their environment. In particular, the rate at which an organism takes up oxygen  
106 from its environment is expected to be related stoichiometrically to rates of ATP production by  
107 mitochondrial oxidative phosphorylation and, therefore, is considered a proxy for metabolic rate  
108 (Nelson, 2016). The rise of commercially available components has further facilitated the estimation  
109 of metabolic rates by respirometry in a variety of organisms. These factors have been particularly  
110 consequential for respirometry on animals that breathe water because, historically, this has been  
111 more difficult to conduct as compared to respirometry on air-breathers. As such, there are more  
112 scientists using aquatic respirometry as a research tool than ever before, with more than 60% of the  
113 papers in this field being generated in the last 10 years alone (Figure S1).

114 We begin this Commentary by describing methods of aquatic respirometry, particularly focusing on  
115 intermittent-flow respirometry, and go on to discuss the need to standardise the reporting of  
116 methods for studies using this research technique. We then provide a checklist of 53 essential  
117 methodological criteria that should be reported in all studies using intermittent-flow respirometry.  
118 We also present results of a literature survey, demonstrating the extent to which these various  
119 criteria have traditionally been inadequately reported. Finally, we provide a downloadable form  
120 (Table S1) that we encourage researchers to complete and include with future manuscripts for  
121 studies using intermittent-flow respirometry, to clearly and concisely summarise key methodological  
122 details.

123 **Intermittent-flow respirometry**

124 The most widely accepted method for measuring rates of oxygen uptake in water-breathing  
125 organisms is automated intermittent-flow respirometry (Steffensen, 1989; Svendsen et al., 2016a),  
126 also sometimes referred to as intermittent-closed respirometry (Norin and Gamperl, 2018).  
127 Although the technique has mainly been developed for use on fishes, it is suitable for almost any  
128 water-breathing organism (Figure 1). An animal is placed in a gas-impermeable respirometry  
129 chamber equipped with an oxygen sensor, and is then exposed to periodic, alternating, 'closed' and  
130 'flush' phases. During the closed phase, the respirometer is effectively sealed and there is a decline  
131 in dissolved oxygen in the water due to oxygen uptake by the animal. With traditional closed  
132 respirometry, the decline in oxygen concentration is measured while the animal is in a continually  
133 sealed chamber, and this containment can eventually cause hypoxia and accumulation of waste  
134 products in the respirometer, which can influence the resulting measurements of oxygen uptake.  
135 With intermittent-flow respirometry, however, this is avoided by the flush phase, during which the  
136 respirometer is flushed with clean, aerated water, which replaces oxygen and removes metabolic  
137 wastes.

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139 The alternation of the closed and flush phases means that real-time rates of oxygen uptake can be  
140 recorded in successive closed phases over extended periods, with animals left undisturbed. This can  
141 provide an accurate picture of dynamic changes in oxygen uptake over time. These changes might be  
142 due to factors such as initial handling stress, circadian rhythms and metabolic costs of digestion,

143 among others (Jourdan-Pineau et al., 2010; Steffensen, 1989). Using this technique can also reveal  
144 when the undisturbed animal is potentially functioning at basal rates of metabolism, denoted as  
145 standard metabolic rate (SMR) for ectotherms (Box 1), or when it is performing some defined level  
146 of activity or type of behaviour, often referred to as routine metabolic rate (RMR, Box 1). This ability  
147 to track changes in oxygen uptake rate in real time is a major improvement over the technique of  
148 flow-through respirometry (Ultsch et al., 1980), where there is a continuous flow of water through a  
149 respirometry chamber and oxygen uptake is measured by comparing the difference in oxygen  
150 concentration at inflow and outflow. This can result in large measurement error when the difference  
151 in dissolved oxygen at the inflow and outflow are small. Furthermore, with flow-through  
152 respirometry, changes in oxygen concentration at the outflow lag behind changes in metabolic  
153 activity of the animal due to a reservoir, or wash-out, effect. This lag depends upon the dilution  
154 factor, which is the ratio of respirometer volume to rate of water flow through it [see Steffensen  
155 (1989) for a detailed explanation]. The consequence is that flow-through respirometry can only be  
156 used to measure relatively steady physiological states (Steffensen, 1989).

157  
158 Intermittent-flow respirometry is, therefore, the best available method to estimate metabolic rate in  
159 water-breathing animals and should be utilised whenever possible. Notably, measures of SMR and  
160 RMR can be coupled with measures of aerobic maximum metabolic rate (MMR, Box 1) to estimate  
161 aerobic scope (AS, Box 1). Given this wide range of applications and the relative robustness of  
162 intermittent-flow respirometry, it has become an extremely popular choice of methodology among  
163 comparative physiologists, including researchers who are new to the estimation of metabolic rates  
164 *via* measurement of respiratory gas exchange.

#### 165 **A need for the standardisation of methods**

167 Despite its increasingly wide usage, there are no guidelines for reporting the methods used in  
168 intermittent-flow respirometry. There are several guides to best practice for measuring and  
169 analysing various types of metabolic rates (Chabot et al., 2016; Norin and Clark, 2016; Steffensen,  
170 1989; Svendsen et al., 2016a; Clark et al., 2013), but details can vary widely among researchers, and  
171 the quality and values of the data generated using intermittent-flow respirometry is completely  
172 dependent on a myriad of methodological decisions made throughout the experiment, from  
173 equipment setup to data collection and analysis (Steffensen, 1989; Svendsen et al., 2016a). Equally  
174 important is that the reporting of methods also differs greatly across peer-reviewed studies, with  
175 important details often not mentioned. A lack of methodological detail, or inaccurate and vague  
176 descriptions, are problematic because: (1) they make it difficult for readers to evaluate data  
177 reliability and judge the interpretation of results; (2) they can give a misleading impression of what  
178 was done; (3) they hinder replication of the experiments; and (4) data on metabolic rates are  
179 increasingly used in meta-analyses (Holtmann et al., 2017; Jerde et al., 2019; Killen et al., 2016), so  
180 proper methodological documentation would be useful to allow researchers to understand sources  
181 of residual variation across studies. In addition, a standard set of guidelines for reporting methods in  
182 intermittent-flow respirometry studies would make it easier for journal editors and reviewers to  
183 decide whether a given study warrants publication in the first place. Finally, a list of important  
184 methodological details would be extremely useful for students and researchers who are new to this  
185 field of research and are using this technique for the first time.

186  
187 Thus, we believe that researchers using intermittent-flow respirometry would benefit from a  
188 standardised, publicly available checklist of details that should be included when describing their  
189 methods, to prevent further under-reporting of important elements. The use of reporting guidelines  
190 for methods, in the form of checklists or flow-charts (Carp, 2012; Cowger et al., 2020; Michel et al.,  
191 2020), is widespread across the biological sciences, and is long overdue in comparative physiology  
192 and especially respirometry. With the steady increase in the number of published studies using

193 respirometry (Fig. S1), now is the ideal time to establish and institute such standard guidelines for  
194 accurate methodological reporting.

195

### 196 **A checklist for reporting methods using intermittent-flow respirometry**

197 Focusing on studies with fishes, here we provide a checklist of 53 criteria that are essential for  
198 understanding, interpreting and replicating experiments using aquatic intermittent-flow  
199 respirometry (Table 1; Figure 1). We aim to provide an explicit list of details that can be referenced  
200 when writing or evaluating research papers, or when planning new studies. Although the criteria are  
201 focussed on studies with fishes, most criteria could be applied to studies with any aquatic organism.  
202 In addition to being included as Table 1 of this article, we also provide a downloadable form that  
203 authors can use to easily list each criterion in a table format, which we suggest can be submitted as  
204 supplementary material with manuscripts to accompany contextual descriptions in the main text of  
205 published research papers (Table S1).

206

207 We have restricted our criteria to cover methods involved in the most common forms of aquatic  
208 intermittent-flow respirometry, namely, the measurement of SMR, RMR, MMR and AS (Box 1).  
209 Methods unique to other applications, such as protocols for measuring critical oxygen tension ( $P_{crit}$ ),  
210 are not specifically covered here (Claireaux and Chabot, 2016; Ultsch and Regan, 2019), but the  
211 checklist can still be used as a guide to ensure that the most basic criteria of respirometry are met  
212 when carrying out these more specialised procedures.

213

214 The criteria are divided into six categories, based on whether they describe the materials and  
215 conditions used in any given study, or are details of the various measurements that can be  
216 conducted using intermittent-flow respirometry. More detail on each of the categories in the  
217 checklist is given below.

218

#### 219 *Equipment, materials and setup*

220 It is necessary to provide adequate details of the specific equipment used in the study and the way  
221 these components are assembled to measure animal oxygen uptake. Equipment and setup details  
222 are important because there is a wide array of available oxygen sensors, data acquisition devices and  
223 logging software, as well as different options for respirometer construction and virtually every other  
224 component used in intermittent-flow respirometry. Each choice made when gathering equipment  
225 and setting up the apparatus can potentially have an impact on the results obtained. Consequently,  
226 depending on the exact setup, data reliability may be affected. Furthermore, details of the  
227 equipment choices are essential for attempts at replication.

228

#### 229 *Measurement conditions*

230 It is important to include details about conditions at the time at which measurements are made.  
231 These include details of exogenous factors such as temperature, oxygenation, lighting and any  
232 sensory stimulation, such as from visual interactions with experimenters or with conspecifics in  
233 adjacent respirometers. Such factors may directly affect rates of animal oxygen uptake (Claireaux  
234 and Chabot, 2016; Clarke and Johnston, 1999; Nadler et al., 2016). Other important conditions  
235 include endogenous factors, such as the feeding state of the experimental animal and their  
236 adjustment period to experimental conditions; these aspects can also influence oxygen uptake  
237 (Chabot et al., 2016).

238

#### 239 *Measurement of background oxygen uptake*

240 Over prolonged periods, throughout the alternating closed and flush phases, microbes may  
241 proliferate on the surfaces of respirometry systems. The magnitude of background microbial  
242 respiration can therefore be substantial and must be quantified and corrected for. There are several  
243 different methods that can be used, and the exact approach must be carefully documented.

244

#### 245 *Measurement of SMR (or RMR)*

246 There are several methodological details that are specific to the measurement of either SMR or RMR  
247 (Box 1). As for the choice of equipment and setup, there are a range of possibilities; experimental  
248 details that can vary across studies must be clearly communicated. After data are collected, there  
249 are various ways to calculate values for SMR and RMR (Box 1). The trait value may be affected by the  
250 specific method chosen, and some approaches may even be inappropriate for the experimental  
251 conditions used or the species under study.

252

#### 253 *Measurement of MMR*

254 As for the measurement of SMR and RMR, there are a variety of specific details that are unique to  
255 the measurement and processing of data for MMR (Box 1), most of which concern how increased  
256 rates of oxygen uptake are achieved. These details must be recorded to allow for accurate  
257 evaluation of the results and replicability of the experiment.

258

#### 259 *Data handling and analysis*

260 There are several additional factors regarding basic data handling and processing that may affect  
261 final values or statistical analyses and that must, therefore, be provided to ensure that the data are  
262 interpretable and replicable.

263

#### 264 **Survey of the existing literature**

265 Following the development of the checklist introduced above, we conducted a quantitative analysis  
266 of previous reporting of the checklist criteria among studies using aquatic intermittent-flow  
267 respirometry in fishes. Our aim was to highlight specific areas in which reporting of methodological  
268 details can be improved. For details of how this literature survey was conducted, please see  
269 Appendix 1.

270

271 Our analysis of 202 published papers (with  $n = 123$  of these including data for MMR in addition to  
272 SMR or RMR), from 1993-2021, shows that reporting of methods has been relatively poor and  
273 inconsistent, including in our own published articles. Reporting showed a slight negative correlation  
274 with journal impact factor (Clarivate Analytics 2020, where available for each journal; GLMM,  $p =$   
275  $0.031$ ), but there was tremendous variation around this relationship, indicating that problems with  
276 reporting persist throughout the published literature (Figure S2; Table S2). There was some evidence  
277 of an improvement in reporting over the last few decades (Figure 2; GLMM, effect of year,  $p =$   
278  $0.001$ ), but the reporting of methods for intermittent-flow respirometry generally remains  
279 inadequate, with extreme variation in reporting quality among recent studies (Figure 2). Although  
280 specific papers often scored highly within a particular category, all papers failed to report several  
281 criteria across categories. There was wide variation in reporting frequency of criteria within  
282 categories, with some specific criteria being consistently under-reported (Table 1; Figure 3). The lack  
283 of consistency across studies is undoubtedly due to the lack of any established guidelines for  
284 reporting the methodological details of intermittent-flow respirometry.

285

286 In addressing specific methodological details, it is important for researchers to be as clear and  
287 explicit as possible, to eliminate any chance of misinterpretation. Another common problem is that

288 articles often refer to multiple prior studies for methodological details, but these references would  
289 contain inconsistent or contradictory information. The use of inaccurate or vague phrasing can also  
290 cause confusion, misunderstanding of what methods were performed and, potentially, the spread of  
291 incorrect information and terminology. Of course, although the use of a checklist for methodological  
292 details should improve the reporting of methods for intermittent-flow respirometry, it is ultimately  
293 dependent upon researchers to use clear and unambiguous language when describing methods. Our  
294 findings on the reporting of various aspects of respirometry measurements across the literature are  
295 discussed in more detail below.

296

#### 297 *Equipment, materials and setup*

298 In our survey, only 71% of articles clearly specified the body mass of animals at the time of  
299 respirometry, as opposed to upon arrival at the lab or during holding conditions. Body size affects  
300 both minimum and maximum metabolic rates (Jerde et al., 2019; Killen et al., 2016) and temporal  
301 variation in body mass will affect metabolic rate estimation. This information is also required when  
302 assessing the ratio between respirometer volume and animal mass, a measure that was only  
303 explicitly provided in 12% of studies (criterion 4).

304

305 There were several other criteria that were consistently underreported. For example, only 48% of  
306 papers surveyed provided any mention of whether mixing was performed inside the respirometers  
307 and how it was accomplished (criterion 3). Proper mixing of the water in respirometers is critical to  
308 homogenise oxygen concentrations throughout chambers, and accurately measuring oxygen uptake  
309 is simply not possible without effective mixing. Criteria relating to mixing circuit tubing (criteria 4, 5,  
310 6 and 7) were only mentioned in 12–21% of papers, despite this being a key component of  
311 intermittent-flow respirometry methods that use an external mixing circuit (Rodgers et al., 2016;  
312 Svendsen et al., 2016a). In some respirometer designs, mixing may be achieved by using an impellor  
313 or stir-bar, but in situations where an external pump is used for mixing, any tubing used in a mixing  
314 circuit needs to be as clean as possible, as short as possible and made of relatively gas impermeable  
315 material. Any respiration from microbes adhering to the surface, or gas exchange across the tubing,  
316 could have confounding effects that need to be corrected for or avoided. Moreover, the volume of  
317 the mixing circuit must be included in the calculation of respirometer volume and, therefore, animal  
318 oxygen uptake rate (criterion 7).

319

#### 320 *Measurement conditions*

321 Numerous criteria pertaining to conditions during measurement were reported infrequently.  
322 Temperature or photoperiod during holding conditions were often given without explicit reference  
323 to conditions during respirometry, or how temperature conditions were maintained. Metabolic rates  
324 of ectothermic animals are profoundly influenced by temperature (Clarke and Johnston, 1999;  
325 Schulte, 2015), and photoperiod may affect animal oxygen uptake (Biswas and Takeuchi, 2002);  
326 temperature and photoperiod may also interact so that diurnal patterns in oxygen uptake are  
327 different at different temperatures (Speers-Roesch et al., 2018). Although many studies measure  
328 multiple animals simultaneously during respirometry, with each animal within its own chamber, only  
329 15% of studies mention whether the animals were visually shielded from each other (criterion 25).  
330 This could have various impacts on activity and metabolic rates that could differ among species,  
331 depending on their level of sociability or aggression (Killen et al., 2014; Nadler et al., 2016; Ros et al.,  
332 2006). Only 19% of papers reported the total time taken to measure all animals in a study, from the  
333 start of the study to the end of the study (criterion 27). This criterion may be especially important for  
334 studies with large sample sizes, leading to overlap with breeding seasons or significant changes in  
335 mass of small, rapidly growing animals. Only 31% of studies mentioned the lowest water oxygen

336 concentration that animals were exposed to during respirometer closed phases (criterion 22). If  
337 oxygen depletion by the animal actually causes hypoxia during closed phases, this may cause  
338 repeated reliance upon anaerobic pathways to meet energy requirements of metabolism, which  
339 would then interfere with estimates of metabolic rate that use oxygen uptake as a proxy (Snyder et  
340 al., 2016). Additionally, repeated hypoxia may elicit an endocrine stress response or stimulate  
341 swimming activity and increase ventilation frequency, also affecting metabolism and rates of oxygen  
342 uptake due to physical activity (Aboagye and Allen, 2014; Killen et al., 2012).

#### 343 344 *Background microbial respiration*

345 Overall, criteria associated with measuring background microbial (e.g. bacterial) oxygen uptake were  
346 the most inconsistently reported among studies (GLMM, effect of category,  $p < 0.0001$ ). In fact,  
347 more than 30% of papers surveyed did not mention whether any form of background microbial  
348 respiration measurement was performed or accounted for. This is a critical oversight because the  
349 amount of background respiration and the exact way it is measured, or incorrectly accounting for  
350 rates of background oxygen uptake, can greatly impact estimates of animal metabolic rates (Rodgers  
351 et al., 2016; Svendsen et al., 2016a).

352  
353 A large proportion of remaining papers failed to describe how background respiration was controlled  
354 (e.g. by cleaning of respirometry chambers and setup), precisely how it was measured and  
355 accounted for, or the proportion of animal metabolism that it represented. Simply measuring  
356 background respiration prior to respirometry trials does not account for how it might increase with  
357 time of residence of animals in a respirometer. Background respiration should either be measured in  
358 parallel to animal respiration, using empty chambers, or should be measured at the start and finish  
359 of a trial, with a model decided upon for how it may have changed (increased) over time. In fact,  
360 parallel measurements of background respiration in empty chambers should ideally be combined  
361 with measurements at the start and finish of a trial in all chambers, as the temporal development of  
362 microbial respiration in chambers with animals may differ from that without animals. This is  
363 especially likely in studies where animals have been fed in the respirometers or before being placed  
364 in respirometers (e.g. studies that estimate the metabolic costs incurred during digestion and  
365 assimilation of nutrients, so called “specific-dynamic action” (McLean et al., 2018)). Without  
366 appropriate details on background respiration, it is extremely difficult to assess data validity. This is,  
367 therefore, a methodological element that researchers must perform properly and report clearly.

#### 368 369 *Estimating SMR/RMR and MMR*

370 There are several criteria unique to the estimation of either SMR/RMR or MMR that were often not  
371 reported. Regarding SMR/RMR, the total number of oxygen uptake rate measurements (i.e. number  
372 of closed phases) used in the derivation of the metabolic rate estimate (criterion 37) was reported in  
373 only 34% of studies. Methods for statistically estimating SMR, for example, including the use of  
374 quantiles or frequency distributions, require a large number of repeated measures and so the total  
375 number of slopes used in their derivation should be provided, as should any slopes that were  
376 disregarded during acclimation to the respirometer or periods of increased activity (criterion 38;  
377 including whether such periods were included in quantile- or frequency distribution-based methods  
378 of calculating SMR; Chabot et al., 2016). Note that there are excellent R packages available that are  
379 specifically designed for calculation of oxygen uptake rates using data obtained through  
380 intermittent-flow respirometry (*respR*: Harianto et al., 2019; *FishResp*: Morozov et al., 2020). These  
381 packages include various options for estimation of SMR, RMR, and MMR, as well as corrections for  
382 background respiration. Wider use of these packages would help facilitate replication and  
383 reproducibility.



384

385 Although reporting for MMR was relatively good when compared to the other criteria categories  
386 (appearing in a mean of 61% of papers across criteria), there were still important details that were  
387 often neglected. For example, when measuring oxygen uptake immediately after exhaustion, many  
388 studies did not report whether the animal was exposed to air before placement in the chamber  
389 (criterion 45). The time taken to initiate measurements of oxygen uptake (e.g. in seconds, after the  
390 cessation of exercise) was also often not provided (criterion 46). Finally, only 21% of papers  
391 described the specific method of slope estimation for determining MMR. This is important because  
392 data processing procedures can bias estimates of MMR (Zhang et al., 2020), including the duration of  
393 the slope used to estimate MMR and the specific method of determining the maximum rate of  
394 oxygen uptake during recovery after exercise (criterion 48). Our survey revealed that both criteria  
395 were relatively underreported but, given emerging awareness of their importance, it is vital that  
396 authors provide these details going forward.

397

#### 398 *Data handling*

399 Additional basic information about data processing was also frequently under-reported. For  
400 example, while 85% of papers clearly reported study sample size, several did not provide this  
401 fundamental information or did so in a way that was unclear (i.e. provided total animals used in  
402 study but not specific treatments). Only 54% of papers specifically mentioned that animal volume  
403 was subtracted from total respirometer volume, a step in data processing which is required to obtain  
404 accurate rates of oxygen uptake. Finally, only 62% of papers explicitly stated whether they used any  
405 form of body size adjustment or correction for rates of oxygen uptake in analyses or described their  
406 methods for doing so. Due to the strong correlation between body size and whole-animal metabolic  
407 rate (and corresponding oxygen uptake), this information is key for ensuring data interpretability.

408

#### 409 **Conclusions**

410 As discussed above, the reporting of methods for intermittent-flow respirometry has generally been  
411 inconsistent and insufficient. However, we hope the development of guidelines and the availability  
412 of a reporting checklist will facilitate systematically clear and accurate reporting of methods.  
413 Reporting needs to be improved for all the general areas we examined and for specific criteria, but  
414 especially when considering measurement of background respiration and details of the mixing  
415 circuit. Although we suggest that intermittent-flow respirometry should be the method of choice  
416 whenever possible, elements of the checklist related to methodological details, measuring  
417 conditions and background respiration are also relevant to the general use of other forms of  
418 respirometry to estimate metabolic rate in aquatic animals.

419

420 As authors that frequently use intermittent-flow respirometry, we appreciate the challenges in  
421 reporting the numerous details required for adequate replication and interpretation of data  
422 collected using this technique. We acknowledge that our own work has been prone to the same  
423 reporting deficiencies we have described in this Commentary and, indeed, many of our own papers  
424 are contained within our literature survey. We suggest that authors use our downloadable checklist  
425 form (Table S1) to concisely address all the criteria outlined in the current paper and that authors  
426 make a filled-in version of this table available as a supplement in future published papers. This  
427 checklist can also be used to help carefully plan important details when designing setups for the  
428 collection of oxygen uptake data.

429

430 It is more important than ever to ensure the collection and reporting of reliable and replicable data,  
431 as metabolic rates are increasingly becoming a focus for understanding the ability of animals to cope

432 with environmental and climate change. Accurate reporting of methodologies is particularly  
433 important in cases where data may be used to inform conservation efforts. The availability of a  
434 checklist of important methodological details should also be useful to new researchers entering this  
435 rapidly developing field, and we hope that our checklist will be a valuable resource to both new and  
436 experienced researchers in this area.

437

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441

#### 442 **COMPETING INTERESTS**

443 The authors declare no competing interests.

444

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458

#### 459 **DATA AVAILABILITY**

460 Data and scripts are available at Mendeley Data ([https://doi.org/ 10.17632/fky5n2nt9x.3](https://doi.org/10.17632/fky5n2nt9x.3)).

461

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600

### 601 **Box 1. Metabolic traits that can be estimated using intermittent-flow respirometry**

602 **Standard metabolic rate (SMR):** This is the minimum rate of ATP use required to sustain life, in the  
603 absence of voluntary muscular movements and digestion/absorption of nutrients (Chabot et al.,  
604 2016). With intermittent-flow respirometry, SMR is estimated by collecting measurements of oxygen  
605 uptake over an extended period on an undisturbed animal, after acclimation to the respirometer,  
606 and then extracting a value for SMR using one of a number of statistical methods (Chabot et al.,  
607 2016). In ectotherms, SMR is especially likely to change with environmental temperature (Chabot et  
608 al., 2016; Schulte, 2015), so temperature must be reported.

609 **Routine metabolic rate (RMR):** This is the average oxygen uptake rate of a post-absorptive animal,  
610 where spontaneous activity contributes to ATP use and, therefore, oxygen demand (Chabot et al.,  
611 2016). It is typically measured as the average of the oxygen uptake rate measurements that are  
612 collected to estimate SMR, although some portions of the dataset may not be considered; for  
613 example, high rates of oxygen uptake when the animal is stressed by handling for placement in the  
614 respirometer (Chabot et al., 2016; Steffensen, 1989). The RMR can, in theory, lie anywhere between  
615 SMR and MMR, but it is expected to be closer to SMR if animals are undisturbed (Chabot et al.,  
616 2016). RMR is often used to infer a metabolic response to a stimulus or stressor (e.g. perceived  
617 predator threat) (Hall and Clark, 2016; Palacios et al., 2016). Also note that the abbreviation 'RMR' is  
618 sometimes used to refer to 'resting metabolic rate', a term often used as a less strict equivalent of  
619 SMR.

620 **Maximum metabolic rate (MMR):** This is the maximum rate of oxygen uptake that an animal can  
621 achieve to create ATP aerobically (Norin and Clark, 2016). Two main methods are used to estimate  
622 MMR in fishes (Killen et al., 2017; Norin and Clark, 2016): they can be exposed to incremental swim  
623 speeds in a swim-tunnel respirometer, with MMR taken as the highest rate of oxygen uptake before  
624 fatigue, or they can be chased to exhaustion in a tank and then placed in a respirometer chamber,  
625 with MMR taken as the highest rate of oxygen uptake during recovery. There is no consensus on  
626 how best to measure MMR (Killen et al., 2017; Norin and Clark, 2016; Zhang et al., 2020).

627 **Aerobic metabolic scope (AS):** This is the maximum capacity to supply oxygen to sustain metabolic  
628 activities beyond SMR (Fry 1971). Absolute AS is calculated as MMR minus SMR, whereas factorial AS  
629 is MMR divided by SMR; the choice of which is more appropriate may depend on the research  
630 question of interest (Halsey et al., 2018).

631 **TABLE 1.** Checklist of criteria that should be reported when using aquatic intermittent-flow respirometry to estimate SMR/RMR or MMR, along with  
 632 detailed descriptions of each criterion. Also shown is the prevalence of each criterion in the existing literature.

Number	Criterion and category	Prevalence (% of papers)	Description	References
<b>EQUIPMENT, MATERIALS, AND SETUP</b>				
1	Provide body mass of animals at time of respirometry	71.2	Metabolic rate is strongly correlated with body mass in animals. Studies should specify that mass was measured immediately before or after respirometry and not simply state mass upon arrival to the laboratory or time of capture.	(Clarke and Johnston, 1999; Jerde et al., 2019)
2	Provide volume of empty respirometer	86.1	Chamber volume can affect factors such as confinement stress and the time taken to measure a decrease in water dissolved oxygen. See criterion 4 (below).	(Svendsen et al., 2016b)
3	Describe how chamber mixing was achieved	47.6	Chamber mixing is crucial for homogenising dissolved oxygen within the system. This is often achieved with an external in-line pump, peristaltic pump or stir-bar. Relying on animal activity to mix the water is not sufficient.	(Clark et al., 2013; Rodgers et al., 2016)
4	Provide ratio of net respirometer volume (plus any associated tubing in mixing circuit) to animal body mass	12.0	This ratio should be explicitly stated, ideally as a range, because it will vary among fish and respirometers of different sizes. A chamber that is too small may increase animal stress due to confinement. Larger respirometer sizes will require longer closed cycles to provide a measurable decline in oxygen, and may also reduce the ability to accurately measure maximum metabolic rate due to a lag between when the fish consumes oxygen and when that decline is detected by the system.	(Svendsen et al., 2016b)
5	Provide material of tubing used in any mixing circuit	15.5	Some materials are gas permeable or absorb or release oxygen. Silicone is particularly permeable and should be avoided as the mixing (recirculation) circuit.	(Stevens, 1992)

6	Provide volume of tubing in any mixing circuit	20.9	The volume of tubing in any mixing circuit should be minimised, to avoid adding unnecessary volume and providing surfaces for microbial adherence.	(Svendsen et al., 2016a)
7	Confirm volume of tubing in any mixing circuit was included in calculations of oxygen uptake rates	14.5	Tubing volume should always be added to the total volume of the respirometer system when calculating rates of oxygen uptake.	(Svendsen et al., 2016a)
8	Provide material of respirometer (e.g. glass, acrylic, etc.)	57.2	Some materials are gas permeable or absorb or release oxygen.	(Stevens, 1992)
9	Provide type of oxygen probe and data recording	89.4	Different probe types have different response times, pressure sensitivity and signal quality (level of noise). Some probe types (e.g. galvanic) have their own oxygen consumption that should be corrected for.	(Klimant et al., 1995)
10	Provide sampling frequency of water-dissolved oxygen	48.6	The number of readings per unit time may affect estimates and $r^2$ values of the slopes for the decrease in dissolved oxygen over time that are used to calculate oxygen uptake rates.	(Chabot et al., 2016; Clark et al., 2013)
11	Describe placement of oxygen probe (in mixing circuit or directly in chamber)	56.7	Probe placement may affect lag time between animal oxygen uptake and detection, the sensitivity to mixing and, depending on the type of probe, sensitivity to pressure changes in the respirometer. If the probe is placed within the chamber, the animal may touch it and thus affect recordings, or even become disturbed. For probes that are directly inside respirometers, it should be stated whether they are small sensor spots or full-sized probes.	(Clark et al., 2013)
12	Provide flow rate during flushing and recirculation, or confirm that chamber returned to normoxia during flushing	30.0	Knowledge of flow rates in relation to respirometer volume is essential to evaluate whether flow was sufficient to replenish water oxygen levels (flush) or mix adequately (recirculation) without being so high as to disturb the animal. As a rule-of-thumb, it will take 5 min to fully (>99%) replace the water in a respirometer when the flow rate is one respirometer volume per minute.	(Steffensen, 1989)



13	Provide timing of flush/closed cycles	85.1	Knowing the flush/closed timing allows one to evaluate whether oxygen level returned to normoxia during flushing, and whether declines in oxygen level during the closed cycle could produce a stable linear decrease for determination of oxygen uptake rate. Intervals that are too long may also make it difficult to detect and account for spontaneous activity during SMR determination. If cycle timing is based on an oxygen threshold, this should be specified.	(Steffensen, 1989)
14	Provide wait (delay) time excluded from closed measurement cycles	53.8	Linear decrease in oxygen content is not immediately achieved during the onset of the closed cycle due to mixing of water within the respirometer and the response time of the oxygen sensor.	(Steffensen et al., 1984; Svendsen et al., 2016a)
15	Describe frequency and method of probe calibration (for both 0 and 100% calibrations)	22.1	The calibration of the probe can drift over time, especially for those sensitive to pressure.	
16	State whether software temperature compensation was used during recording of water oxygen concentration	18.3	Some oxygen probes are more sensitive than others to temperature fluctuations (e.g. optical sensors are very sensitive), meaning that oxygen recordings may not be accurate if temperature changes during respirometry.	
<b>MEASUREMENT CONDITIONS</b>				
17	Provide temperature during respirometry	86.1	Metabolic rate is strongly affected by environmental temperature in ectotherms.	(Schulte, 2015)
18	State how temperature was controlled	44.7	Different methods of temperature control may generate different levels of variability around the desired thermal setpoint.	
19	Provide photoperiod during respirometry	29.0	Metabolic rate fluctuates over the course of a 24-h day and can be affected by photoperiod. Switching on the light in the experimental room can result in abruptly increased rates of oxygen uptake.	(Speers-Roesch et al., 2018)
20	Describe if (and how) the ambient water bath was cleaned and aerated during measurement of oxygen uptake (e.g. filtration, periodic or continuous water changes)	38.5	Aeration of the ambient bath ensures oxygen in respirometers is replaced during flushing and removes metabolic CO <sub>2</sub> produced by the animal. Replacement of water in the ambient bath ensures metabolic wastes such as ammonia do not accumulate.	(Snyder et al., 2016)

21	Provide total volume of ambient water bath and any associated reservoirs	40.4	This can inform about the likelihood of accumulation of wastes, and potential difficulties in flushing chambers back to normoxia.	
22	Provide minimum water oxygen level reached during closed phases	30.9	Reduced oxygen availability (hypoxia) does not affect standard metabolic rate until near-lethal levels (at the so-called critical oxygen tension), but reduces maximum and eventually routine rates of oxygen uptake, especially if it alters animal activity.	(Claireaux and Chabot, 2016; Utsch and Regan, 2019)
23	State whether chambers were visually shielded from external disturbance	45.4	External disturbance may cause agitation and stress, potentially altering rates of oxygen uptake.	
24	State how many animals were measured during a given respirometry trial (i.e. how many animals were in the same water bath)	63.9	This provides an idea of the biological load in the respirometry setup and potential interdependence of animals measured in a single block of measurements.	
25	If multiple animals were measured simultaneously, state whether they were able to see each other during measurements	14.9	Visual or olfactory cues of con- or hetero-specifics can influence behaviour and rates of oxygen uptake. Species may differ in these responses.	(Hall and Clark, 2016; Nadler et al., 2016)
26	Provide duration of animal fasting before placement in respirometer	79.8	Processes of digestion and assimilation of food (so-called specific dynamic action) raises rates of oxygen uptake. SMR is, by definition, measured on fasted animals.	(Chabot et al., 2016)
27	Provide duration of all trials combined (number of days to measure all animals in the study)	18.8	Should ideally be reported as the start and end dates of data collection for the study. Animals may habituate or acclimate to the laboratory setting, which could affect behaviour and metabolic rate. Body mass or life-stage may also change over time.	(White et al., 2013)
28	Provide acclimation time to the laboratory (or time since capture for field studies) before respirometry measurements	73.1	A change of environment may cause endocrine responses with unknown consequences for rates of oxygen uptake. Changes in holding temperature may require time for acclimation to be complete. Various behavioural changes with lab adjustment (e.g. establishment of social hierarchies) may also affect metabolic rates. If animals were bred in the lab this should be stated.	(Killen et al., 2014; Sidell et al., 1973; Sloman et al., 2000)

## BACKGROUND RESPIRATION

29	State whether background microbial respiration was measured and accounted for, and if so, method used (e.g. parallel measures with empty respirometer, measurements before and after for all chambers while empty, both)	67.8	Background microbial respiration can be substantial, especially in small chambers that have a large surface area to volume ratio, when water temperature is warm, or when food or faeces are present in the respirometer. Background respiration must, therefore, be measured and accounted for. In studies which estimate specific dynamic action, it should be mentioned whether excess food and faeces in respirometers were removed.	(Svendsen et al., 2016a)
30	If background respiration was measured at beginning and/or end, state how many slopes and for what duration	33.7	If background respiration is low, long measurement durations may be needed to evaluate it accurately. Measuring background respiration at the beginning and end of a trial is required to properly account for gradual increases in microbial activity over time.	(Svendsen et al., 2016a)
31	State how changes in background respiration were modelled over time (e.g. linear, exponential, parallel measures)	36.5	If background respiration changes over time (for example increasing while fish are held in a chamber), assumptions are required for the form of such an increase.	(Rodgers et al., 2016; Svendsen et al., 2016a)
32	Provide level of background respiration (e.g. as a percentage of SMR)	22.6	This permits evaluation of potential problems with validity of estimates of metabolic traits.	(Svendsen et al., 2016a)
33	State method and frequency of system cleaning (e.g. system bleached between each trial, UV lamp)	28.4	Regular cleaning can help minimise background respiration, especially in warm water systems. This provides an idea of water quality and the amount of background respiration that could be expected, if this is not performed.	
<b>STANDARD OR ROUTINE METABOLIC RATE</b>				
34	Provide acclimation time after transfer to chamber, or alternatively, time to reach beginning of metabolic rate measurements after introduction to chamber	62.1	At the outset of measures, rates of oxygen uptake will be affected by handling and possibly confinement stress.	(Chabot et al., 2016; Steffensen, 1989)
35	Provide time period, within a trial, over which oxygen uptake was measured (e.g. number of hours)	84.1	SMR may not be reached if the period used to estimate metabolic rate is too short, for example due to initial handling stress. Periods of spontaneous activity may also influence metabolic rate estimates if only a few measurements of oxygen uptake are taken, regardless of the time the fish are in the respirometer.	(Chabot et al., 2016)

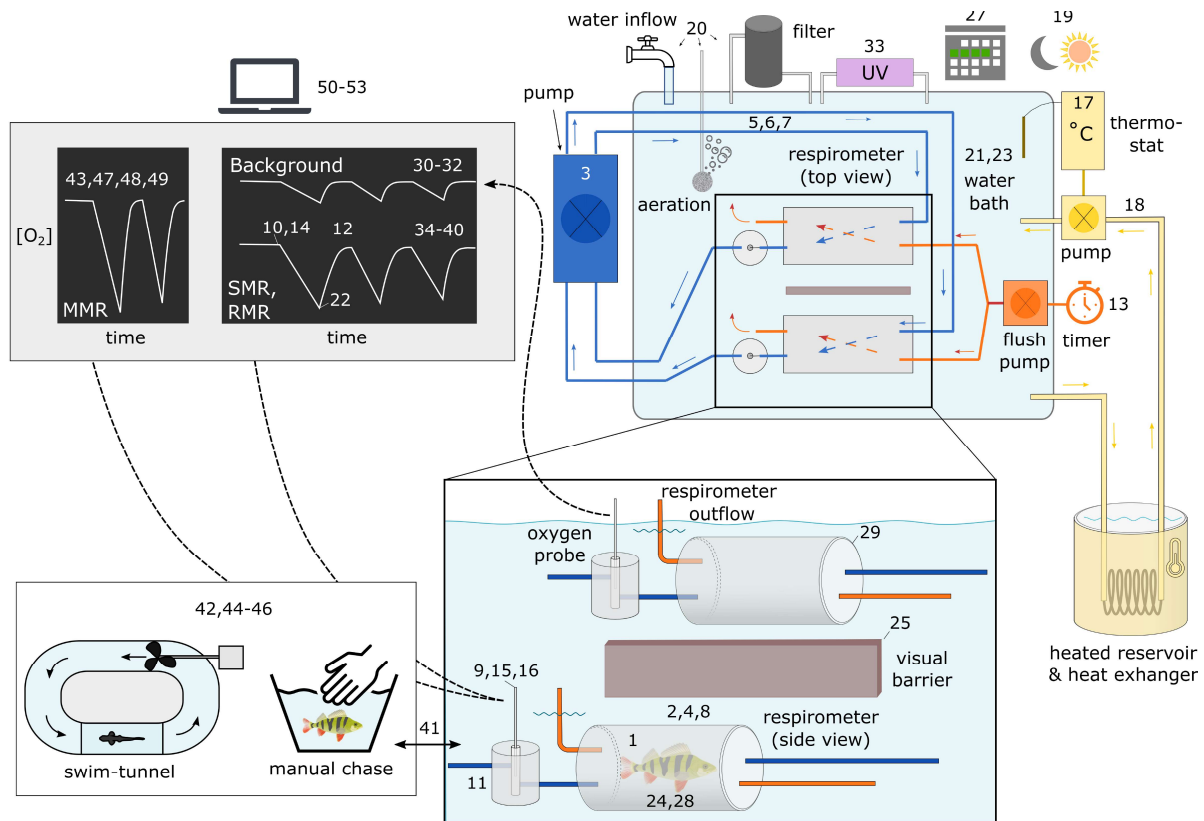
36	State what value was taken as SMR/RMR (e.g. quantile, mean of lowest 10 percent, mean of all values)	82.5	There is no universally accepted method to statistically estimate SMR, and the exact value considered as SMR may vary with the method chosen.	(Chabot et al., 2016; Harianto et al., 2019; Morozov et al., 2020)
37	Provide total number of slopes measured and used to derive metabolic rate (e.g. how much data were used to calculate quantiles)	33.8	Ideally reported as a range of slope numbers across individuals, this permits evaluation of how accurately SMR might be estimated.	(Chabot et al., 2016)
38	State whether any time periods were removed from calculations of SMR/RMR [e.g. data during acclimation, periods of high activity (e.g. daytime)]	57.9	Periods when animals were affected by recent handling, or when they exhibit spontaneous increased activity, may affect estimation of SMR.	(Chabot et al., 2016)
39	Provide $r^2$ threshold for slopes used for SMR/RMR (or mean $r^2$ )	33.8	By convention, $r^2$ should be above 0.9. This convention has been questioned, so this threshold should be reported. This provides an indication of the quality of the recorded data, since low and more variable $r^2$ (that would be sorted out as outliers) indicate poor mixing and/or too large a respirometer-to-animal-volume ratio.	(Chabot et al., 2021; Steffensen, 1989; Svendsen et al., 2016a)
40	Provide proportion of data removed due to being outliers below $r^2$ threshold	8.8		(Chabot et al., 2021)
<b>MAXIMUM METABOLIC RATE</b>				
41	State when MMR was measured in relation to SMR (i.e. before or after)	80.8	This could be significant for any carry-over effects (for example if inadequate recovery time was given when measuring SMR after MMR).	
42	State method used (e.g. critical swimming speed respirometry, swim to exhaustion in swim tunnel, or chase to exhaustion in tank or respirometer)	87.5	The method used may affect estimation of MMR.	(Killen et al., 2017; Norin and Clark, 2016; Zhang et al., 2020)
43	State what value was taken as MMR (e.g. the highest oxygen uptake rate value after transfer, average of highest values)	78.9	The value chosen may affect the estimated magnitude of MMR.	(Andersson et al., 2020; Harianto et al., 2019; Morozov et al., 2020)
44	State length of activity challenge used for estimating MMR (e.g. duration and water velocity increment of swim test, duration of chase in minutes or until exhaustion, etc.)	81.5	Important to ensure that MMR was achieved.	(Roche et al., 2013)
45	If MMR was measured post-exhaustion, state whether further air-exposure was added after exercise	48.7	Air-exposure has been proposed to ensure that MMR is achieved when measured as excess post-exercise oxygen consumption (EPOC).	(Roche et al., 2013)

46	If MMR was measured post-exhaustion, provide time until transfer to chamber after exhaustion and time to start of oxygen uptake recording	37.5	Important to evaluate whether fish may have recovered some EPOC prior to measurement	(Zhang et al., 2020)
47	Provide duration of slopes used to calculate MMR (e.g. 1 min, 5 min, etc.)	49.6	Important for evaluating whether MMR may have been under- (or over-) estimated (e.g. if the animal has started recovering after an exhaustive chase or a swim to exhaustion).	(Norin and Clark, 2016; Zhang et al., 2020)
48	State slope estimation method for MMR (e.g. rolling regression, sequential discrete time frames)	21.4	Method of estimation can affect the magnitude of MMR.	(Harianto et al., 2019; Morozov et al., 2020; Zhang et al., 2019)
49	State how absolute aerobic scope and/or factorial aerobic scope is calculated (i.e. using raw SMR and MMR, allometrically mass-adjusted SMR and MMR, or allometrically mass-adjusting aerobic scope itself)	61.9	This can affect the magnitude of aerobic scope, and whether the measure is adjusted for body mass.	
<b>DATA HANDLING AND STATISTICS</b>				
50	Provide sample size	85.1	Important for evaluating the robustness of reported effects.	
51	State how oxygen uptake rates were calculated (software or script, equation, units, etc.)	77.4	Important for replication of the study. Not subtracting animal volume will lead to inaccurate estimates of metabolic rates, since the actual volume of water from which the animals were taking up oxygen would be incorrect. For convenience, animal volume is often assumed to be equal to its body mass (i.e. the animal having a density of 1 g/mL).	
52	Confirm that volume (or mass) of the animal was subtracted from respirometer volume when calculating oxygen uptake rates	53.8	Important for comparing reported estimates of metabolic rates among treatment groups, other studies, and in general, animals of different sizes. Note that reporting mass-specific metabolic rate (i.e. dividing oxygen uptake of the animal by its mass) usually does not correct for variation in size, because with a log-log plot, the relationship between metabolic rate (or oxygen uptake) and body mass will have a slope of $b - 1$ , with $b$ being the allometric exponent for whole-animal metabolic rate.	(Schmidt-Nielsen, 1975)
53	Specify whether variation in body mass was accounted for in analyses and describe any allometric body-mass-correction or adjustment	61.8		

Thus, unless  $b = 1.0$ , mass-specific metabolic will remain dependent on body mass.

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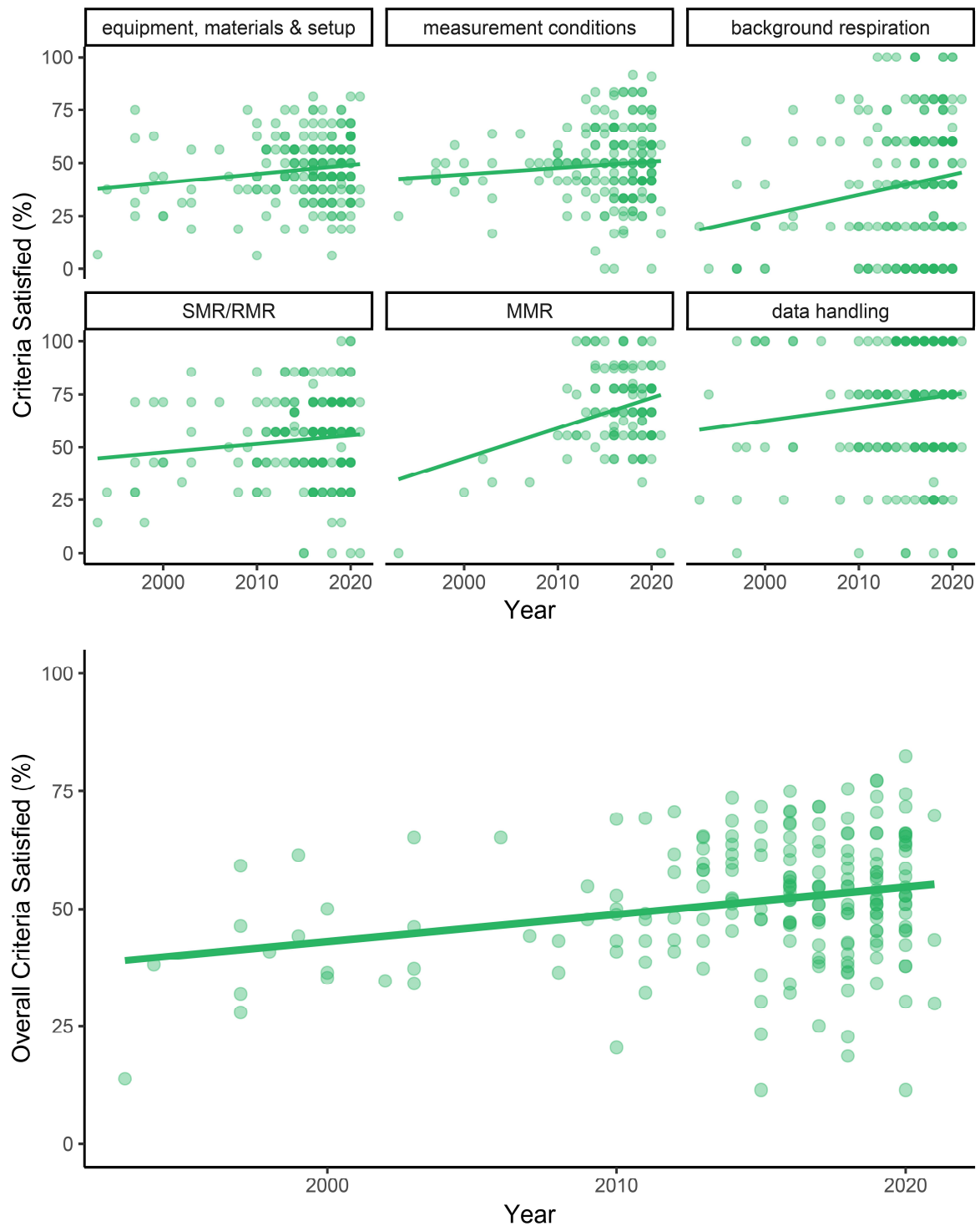
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637 **FIGURE 1.** Schematic of a typical intermittent-flow respirometry setup. Numbers correspond to the  
 638 criteria listed in Table 1 and show the general location of each criterion within the setup. The top  
 639 right depicts a top-down view of the setup; the enlarged box below presents a more detailed side-  
 640 view of two respirometers (one containing a fish and the other being an empty chamber for  
 641 measuring background respiration). Orange items (excluding the sun in the top corner, which  
 642 represents photoperiod) are those used for periodically flushing the respirometer with clean,  
 643 aerated water from the surrounding bath, with orange lines representing tubing in this flushing  
 644 circuit. Dark blue represents the mixing circuit and associated tubing. Note that in this scheme,  
 645 mixing is performed with a multi-channel peristaltic pump, but mixing can also be achieved with a  
 646 single-channel pump or stir-bar, depending on the size and shape of the respirometers. Yellow  
 647 represents elements associated with temperature control; here, temperature is maintained using a  
 648 thermostat that controls a pump to direct water through a heat exchanger within a heated reservoir  
 649 whenever temperature within the bath drops below the setpoint. The box to the lower left depicts  
 650 methods for exercising fish for estimates of maximum metabolic rate. The top left box represents  
 651 computer-based data collection and analyses. Dashed black arrows represent transmission of data  
 652 from oxygen probes to computer for analyses. Refer to Svendsen et al. (2016) for more information  
 653 on setup components and overall system functioning. SMR, standard metabolic rate; RMR, routine  
 654 metabolic rate; MMR, maximum metabolic rate; UV, ultraviolet.

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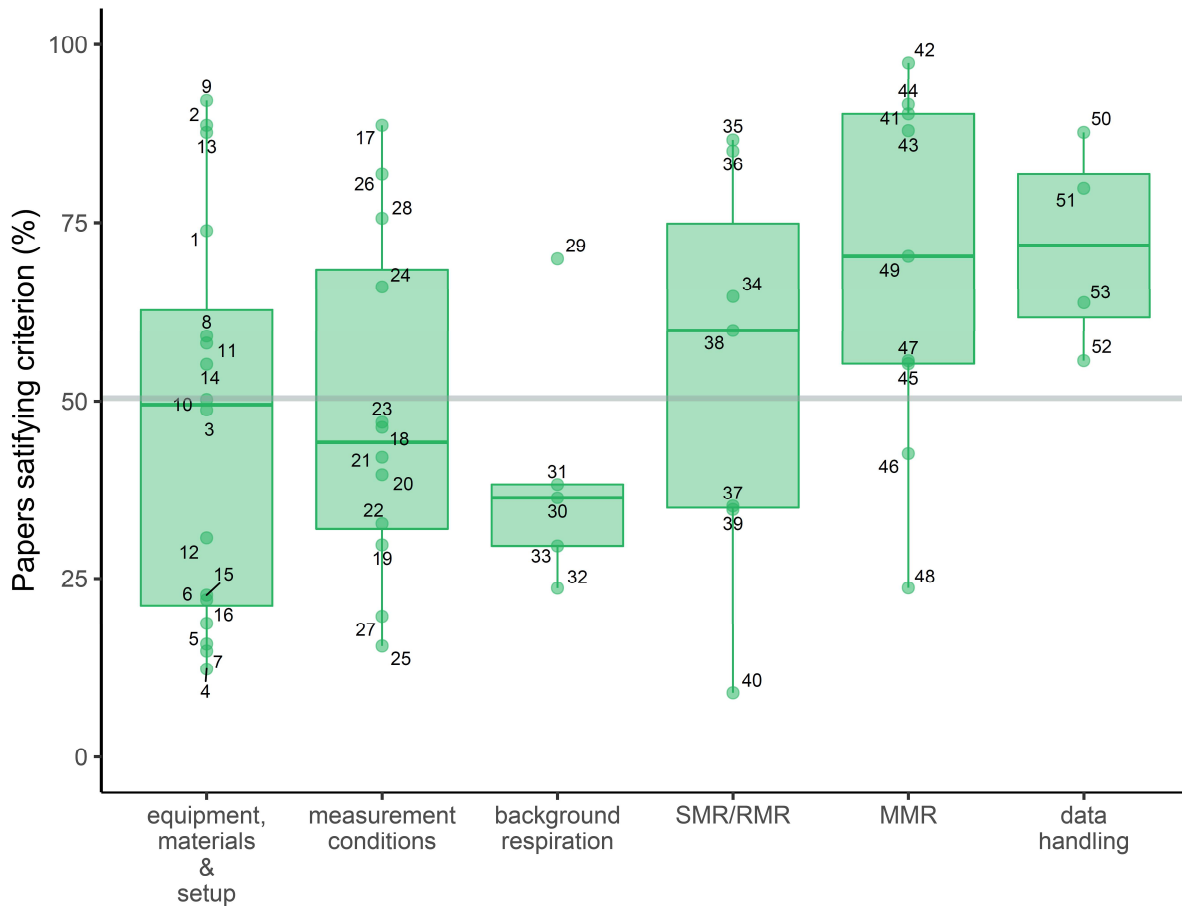
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 658 **FIGURE 2.** The percentage of criteria listed in Table 1 that were satisfied in the surveyed papers.  
 659 Each point represents one paper; solid lines are linear regressions with publication year on the x-axis  
 660 (see Table S2 for model summary and parameter estimates). Points are partially transparent and so  
 661 darker shades of green indicate greater numbers of overlapping data points. The top faceted panels  
 662 show the criteria sub-divided according to category; the bottom panel shows the overall percentage  
 663 of criteria satisfied. The number of studies in each panel is  $n = 202$ , except for the panel for  
 664 maximum metabolic rate (many papers did not contain data for maximum metabolic rate, see  
 665 Appendix 1), where  $n = 123$ . Regression equations and p-values for effect of year are as follows:  
 666 Equipment, materials and setup:  $y = -797.23 + 0.419(\text{year})$ ,  $p = 0.0232$ ; Measurement conditions:  $y =$



667  $-551.42 + 0.298(\text{year})$ ,  $p = 0.154$ ; Background respiration:  $y = -1916.42 + 0.971(\text{year})$ ,  $p = 0.00867$ ;  
 668  $\text{SMR/RMR} = -758.29 + 0.403(\text{year})$ ,  $p = 0.098$ ;  $\text{MMR}: y = -2819.76 + 1.43(\text{year})$ ,  $p = 0.0007$ ; Data  
 669 handling:  $y = -1165.27 + 0.614(\text{year})$ ,  $p = 0.079$ ; Overall:  $y = -1113.06 + 0.578(\text{year})$ ,  $p = 0.0003$ . SMR,  
 670 standard metabolic rate; RMR, routine metabolic rate; MMR, maximum metabolic rate.

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674 **FIGURE 3.** The percentage of papers that referred to the specific criteria listed in Table 1. Each point  
 675 represents one criterion; numbered labels correspond to criteria numbering in Table 1. Criteria for  
 676 each category were scored across  $n = 202$  studies, except for the maximum metabolic rate category,  
 677 where  $n = 123$  (several papers did not include data for maximum metabolic rate, see Appendix 1).

678 The grey line is the overall average across all criteria. Boxplot lower and upper hinges represent the  
 679 25<sup>th</sup> and 75<sup>th</sup> percentiles, respectively; the horizontal line within the box represents the median; the  
 680 length of whiskers represents the range of data points between either the upper or lower hinge and  
 681 1.5<sup>th</sup> the difference between the 25<sup>th</sup> and 75<sup>th</sup> percentiles. SMR, standard metabolic rate; RMR,  
 682 routine metabolic rate; MMR, maximum metabolic rate.

683 **APPENDIX 1. Methods for the literature survey and scoring of criteria**

684

685 *Literature survey and criteria scoring*

686 Focussing on studies with fish, we performed a survey of the literature to determine variation in the  
687 reporting of methods and the extent to which various criteria are (or are not) reported. Using Web  
688 of Science and Google Scholar in April 2021, we used the topic search terms: (1) ‘fish AND  
689 respirometry AND intermittent’; and (2) ‘[fish AND ("standard metabolic rate" OR "resting metabolic  
690 rate" OR "routine metabolic rate") AND "maxim\* metabolic rate"]’. This survey was not meant to be  
691 exhaustive but was meant to be representative of the methodological reporting across research  
692 using fish intermittent-flow respirometry as a whole. Articles were excluded from further analysis if  
693 they were review articles, meta-analyses or any other study that did not estimate metabolic rates of  
694 fish using intermittent-flow respirometry. Studies that estimated metabolic rates while the animal  
695 was in a swim-tunnel were also not included in the survey (i.e., we only included studies where  
696 measurements of oxygen uptake were performed in static respirometry chambers). In total, 202  
697 studies from 71 journals were assessed from between the years 1993 and 2021 (data are available at  
698 Mendeley Data: <https://doi.org/10.17632/fky5n2nt9x.2>; and are also included with this submission).  
699 This consisted of 123 studies that measured both SMR (or RMR) and MMR, and 79 studies that  
700 measured or SMR (or RMR) only, without MMR.

701

702 Each study was scored for whether they satisfied each criterion in the checklist. Studies were  
703 awarded a point for a given criterion if they gave a clear, unambiguous description of that  
704 methodological detail, without the need for reader assumptions or calculations. Importantly, scores  
705 were not based on the quality of a methodology itself – they were simply based on whether a given  
706 detail was provided. For example, if a paper had stated that the respirometer was made of Swiss  
707 cheese, the criterion “provide material of respirometer” (criterion 8; Table 1) would be considered  
708 satisfied and a point would be awarded, without judgement of whether Swiss cheese is an  
709 appropriate material for respirometer construction. Methodological details for specific criteria were  
710 considered present if they were provided in the main article text, figures, tables or supplementary  
711 material, or in references to previously published work. When there were references to multiple  
712 prior studies for a given criterion, a point was not given if those prior sources provided inconsistent  
713 or contradictory descriptions. In some cases, the absence of a specific criterion made it impossible to  
714 assess other associated criteria, in which case a value of NA was assigned to criteria that were  
715 unable to be scored, and those instances were not included in calculating the mean score for that  
716 paper or in calculating the mean prevalence of that criteria across papers. Although most studies  
717 were evaluated by one scorer, sixteen studies were initially evaluated by two scorers each, ensuring  
718 consistency across scorers and allowing refinement of criteria phrasing to minimise ambiguity. For  
719 each article, we also recorded the title, year of publication and journal (paper titles have been  
720 anonymised in the data file at Mendeley Data: <https://doi.org/10.17632/fky5n2nt9x.3>).

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722 *Statistical analysis*

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

730 package *lme4* (Bates et al., 2015). All R scripts are available at Mendeley Data:  
731 <https://doi.org/10.17632/fky5n2nt9x.3>.

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778 **TABLE S1.** The checklist of essential criteria for the reporting of methods for aquatic intermittent-  
 779 flow respirometry. This table can be copied by authors and included as supplemental information  
 780 when submitting manuscripts and publishing papers. This will facilitate clear and concise means for  
 781 reporting all important methodological details, with the article main text being used to provide  
 782 additional information and context.

Number	Criterion and Category	Response	Value (where required)	Units
<b>EQUIPMENT, MATERIALS, AND SETUP</b>				
1	Body mass of animals at time of respirometry			
2	Volume of empty respirometers			
3	How chamber mixing was achieved			
4	Ratio of net respirometer volume (plus any associated tubing in mixing circuit) to animal body mass			
5	Material of tubing used in any mixing circuit			
6	Volume of tubing in any mixing circuit			
7	Confirm volume of tubing in any mixing circuit was included in calculations of oxygen uptake			
8	Material of respirometer (e.g. glass, acrylic, etc.)			
9	Type of oxygen probe and data recording			
10	Sampling frequency of water dissolved oxygen			
11	Describe placement of oxygen probe (in mixing circuit or directly in chamber)			
12	Flow rate during flushing and recirculation, or confirm that chamber returned to normoxia during flushing			
13	Timing of flush/closed cycles			
14	Wait (delay) time excluded from closed measurement cycles			
15	Frequency and method of probe calibration (for both 0 and 100% calibrations)			
16	State whether software temperature compensation was used during recording of water oxygen concentration			
<b>MEASUREMENT CONDITIONS</b>				
17	Temperature during respirometry			
18	How temperature was controlled			
19	Photoperiod during respirometry			
20	If (and how) ambient water bath was cleaned and aerated during measurement of oxygen uptake (e.g. filtration, periodic or continuous water changes)			
21	Total volume of ambient water bath and any associated reservoirs			

- 22 Minimum water oxygen dissolved oxygen reached during closed phases
- 23 State whether chambers were visually shielded from external disturbance
- 24 How many animals were measured during a given respirometry trial (i.e. how many animals were in the same water bath)
- 25 If multiple animals were measured simultaneously, state whether they were able to see each other during measurements
- 26 Duration of animal fasting before placement in respirometer
- 27 Duration of all trials combined (number of days to measure all animals in the study)
- 28 Acclimation time to the laboratory (or time since capture for field studies) before respirometry measurements

#### **BACKGROUND RESPIRATION**

- 29 Whether background microbial respiration was measured and accounted for, and if so, method used (e.g. parallel measures with empty respirometry chamber, measurements before and after for all chambers while empty, both)
- 30 If background respiration was measured at beginning and/or end, state how many slopes and for what duration
- 31 How changes in background respiration were modelled over time (e.g. linear, exponential, parallel measures)
- 32 Level of background respiration (e.g. as a percentage of SMR)
- 33 Method and frequency of system cleaning (e.g. system bleached between each trial, UV lamp)

#### **STANDARD OR ROUTINE METABOLIC RATE**

- 34 Acclimation time after transfer to chamber, or alternatively, time to reach beginning of metabolic rate measurements after introduction to chamber
- 35 Time period, within a trial, over which oxygen uptake was measured (e.g. number of hours)
- 36 Value taken as SMR/RMR (e.g. quantile, mean of lowest 10 percent, mean of all values)
- 37 Total number of slopes measured and used to derive metabolic rate (e.g. how much data were used to calculate quantiles)

Whether any time periods were removed from calculations of SMR/RMR (e.g. data during acclimation, periods of high activity [e.g. daytime])

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39  $r^2$  threshold for slopes used for SMR/RMR (or mean)

40 Proportion of data removed due to being outliers below r-squared threshold

#### **MAXIMUM METABOLIC RATE**

41 When MMR was measured in relation to SMR (i.e. before or after)

42 Method used (e.g. critical swimming speed respirometry, swim to exhaustion in swim tunnel, or chase to exhaustion)

43 Value taken as MMR (e.g. the highest rate of oxygen uptake value after transfer, average of highest values)

44 If MMR measured post-exhaustion, length of activity challenge or chase (e.g. 2 min, until exhaustion, etc.)

45 If MMR measured post-exhaustion, state whether further air-exposure was added after exercise

46 If MMR measured post-exhaustion, time until transfer to chamber after exhaustion or time to start of oxygen uptake recording

47 Duration of slopes used to calculate MMR (e.g. 1 min, 5 min, etc.)

48 Slope estimation method for MMR (e.g. rolling regression, sequential discrete time frames)

49 How absolute aerobic scope and/or factorial aerobic scope is calculated (i.e. using raw SMR and MMR, allometrically mass-adjusted SMR and MMR, or allometrically mass-adjusting aerobic scope itself)

#### **DATA HANDLING AND STATISTICS**

50 Sample size

51 How oxygen uptake rates were calculated (software or script, equation, units, etc.)

52 Confirm that volume (mass) of animal was subtracted from respirometer volume when calculating oxygen uptake rates

53 State whether analyses accounted for variation in body mass and describe any allometric mass-corrections or adjustments

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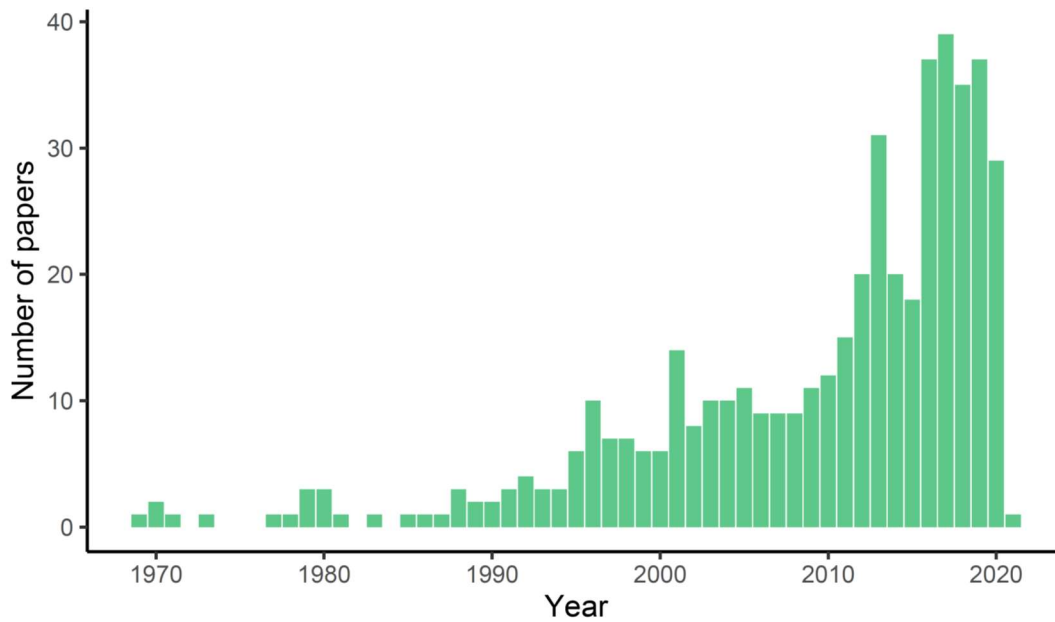
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786 **TABLE S2.** Summary for the generalised linear mixed model with a binomial distribution (logit link),  
 787 constructed to examine factors affecting methods reporting across published papers. The score for  
 788 each criterion per paper (0 or 1) was used as the response variable, and criteria category, year,  
 789 journal impact factor, and all interactions among these variables were initially included as  
 790 explanatory variables. Year and impact factor were scaled. Paper ID (coded anonymously by title)  
 791 and scorer were included as random effects. For the term 'category', background respiration is the  
 792 reference level.

793 <b>term</b>	<b>estimate</b>	<b>s.e.</b>	<b>z</b>	<b>p</b>
794 intercept	-0.432	0.093	-4.627	< 0.0001
795 year	0.130	0.041	3.186	0.0014
796 impact factor	-0.083	0.038	-2.155	0.031
797 category				
798 Equipment and Setup	0.322	0.078	4.127	< 0.0001
799 Measurement conditions	0.435	0.081	5.368	< 0.0001
800 SMR/RMR	0.610	0.089	6.870	< 0.0001
801 MMR	1.212	0.099	12.129	< 0.0001
802 Data handling	1.398	0.108	13.100	< 0.0001

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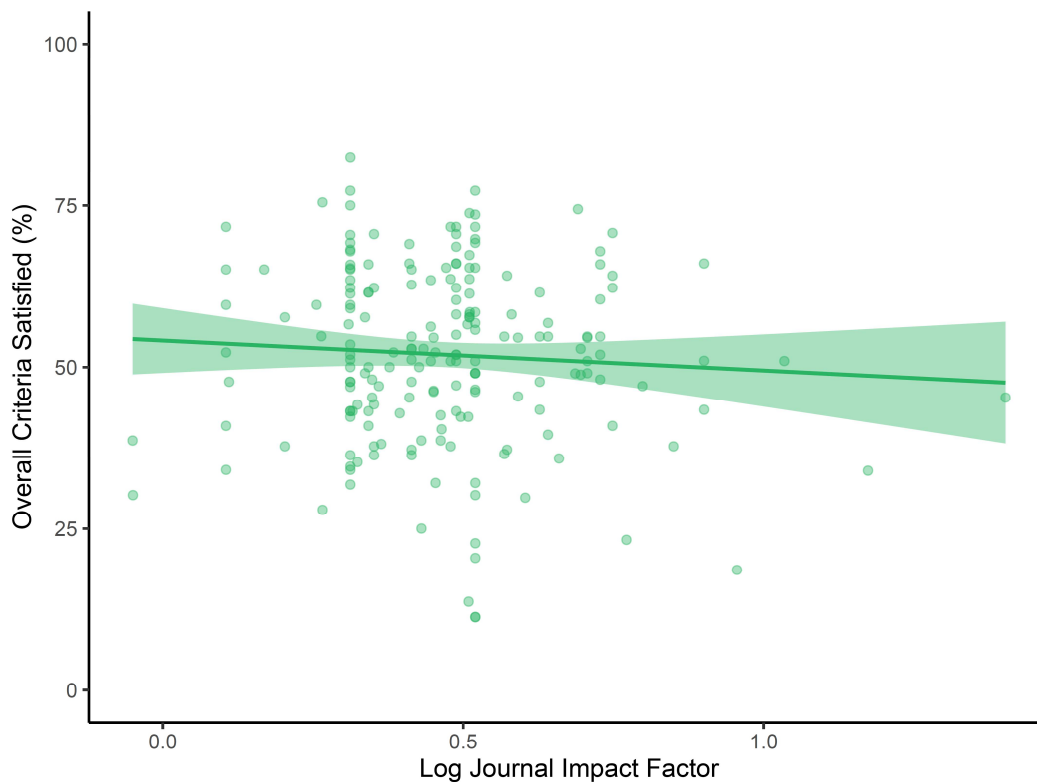


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835 **FIGURE S1.** Research in aquatic respirometry has increased steeply over the last several decades.  
 836 The graph shows the number of papers per year, returned by the topic search [aquatic AND  
 837 respirometry] on Web of Science (February 2021).

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841 **FIGURE S2.** The percentage of criteria listed in Table 1 that were satisfied in papers, in relation to the  
 842 impact factor of the journal each paper was published in. Each point represents one paper (n = 202);  
 843 the solid line is a linear regression with log journal impact factor on the x-axis and the shaded area  
 844 represents 95% CIs (see Table S2 for model summary and parameter estimates). Darker shades of  
 845 green indicate greater numbers of overlapping data points.