Valid oxygen uptake measurements: using high $r^2$ values with good intentions can bias upward the determination of standard metabolic rate

Denis Chabot$^1$ | Yangfan Zhang$^2$ | Anthony P. Farrell$^2$

$^1$Fisheries & Oceans Canada, Institut Maurice-Lamontagne, Mont-Joli, Quebec, Canada
$^2$Faculty of Land and Food Systems, & Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada

Correspondence
Denis Chabot, Fisheries & Oceans Canada, Institut Maurice-Lamontagne, Mont-Joli, QC G5H 3Z4, Canada.
Email: denis.chabot@dfo-mpo.gc.ca

Funding information
Canada Excellence Research Chairs, Government of Canada; Fisheries and Oceans Canada

Abstract
This analysis shows good intentions in the selection of valid and precise oxygen uptake ($MO_2$) measurements by retaining only slopes of declining dissolved oxygen level in a respirometer that have very high values of the coefficient of determination, $r^2$, are not always successful at excluding nonlinear slopes. Much worse, by potentially removing linear slopes that have low $r^2$ only because of a low signal-to-noise ratio, this procedure can overestimate the calculation of standard metabolic rate (SMR) of the fish. To remedy this possibility, a few simple diagnostic tools are demonstrated to assess the appropriateness of a given minimum acceptable $r^2$, such as calculating the proportion of rejected $MO_2$ determinations, producing a histogram of the $r^2$ values and a plot of $r^2$ as a function of $MO_2$. The authors offer solutions for cases when many linear slopes have low $r^2$. The least satisfactory but easiest to implement is lowering the minimum acceptable $r^2$. More satisfactory solutions involve processing (smoothing) the raw signal of dissolved oxygen as a function of time to improve the signal-to-noise ratio and the $r^2$s.

KEYWORDS
intermittent flow respirometry, oxygen uptake, $r^2$, SMR, standard metabolic rate

1 INTRODUCTION

Although the metabolic rate is highly variable within individuals and across individuals and species of water breathing fishes, one particular rate, standard metabolic rate (SMR), is an especially useful benchmark. SMR is the maintenance level of energy expenditure, measured when a fish is calm, inactive and postprandial (Chabot et al., 2016b). SMR is widely used to compare the energy requirements of different fish species, to construct energy budgets and to assess the impact of abiotic variables, such as temperature, dissolved oxygen (DO), salinity or pH, on their physiology. The determination of metabolic rate in fishes is most easily accomplished by measuring their oxygen uptake ($MO_2$) (Nelson, 2016). Although flow-through respirometry is possible (Alcaraz & Kruesi, 2012; Cech & Brauner, 2011; Ullum & Kruesi, 1980), the most common technique used is intermittent flow respirometry (for reviews, see Steffensen, 1989; Svendsen et al., 2016b), which allows long-term (several days) measurements of oxygen uptake without degradation of water quality. It works well even when the rate of $MO_2$ varies, a situation for which flow-through respirometry is less well-suited (Cech Jr & Brauner, 2011; Steffensen, 1989).

Intermittent flow respirometry uses a closed respirometer to measure oxygen removal by the fish and a pump that is turned on at regular intervals to replenish the water inside the respirometer. During the closed phase, $MO_2$ is calculated from the rate of decline (slope) of DO using linear regression (Svendsen et al., 2016b). Poor mixing of water in the respirometer can produce nonlinear declines in DO, resulting in
unreliable determinations of \( \dot{M}_O_2 \) (Clark et al., 2013; Svendsen et al., 2016b). Leaks and changing behavioural state (calm, alert or stressed) of the fish can also create nonlinear declines in DO (Clark et al., 2013; Svendsen et al., 2016b).

The coefficient of determination, \( r^2 \), measures the proportion of the variance in DO explained by the linear relationship. It is not a measure of linearity, but \( r^2 \) values close to 1 are possible only when the DO declines linearly. A high \( r^2 \) is also associated with high precision of the DO determination (Svendsen et al., 2016a). Nonetheless, low signal-to-noise ratios, such as when using a large respirometer for a small fish, can lower \( r^2 \) even if the decline is linear (Svendsen et al., 2016a). Ideally, preliminary experiments make it possible to design a respirometry system that uses an appropriate fish to water volume ratio and results in high \( r^2 \) for most or all slopes (e.g., Svendsen et al., 2014; Tirsgaard et al., 2015a, > 0.95). Consequently, DO slopes with \( r^2 \) lower than a pre-selected threshold or minimum acceptable \( r^2 \), called \( r_{min}^2 \) hereafter, are typically rejected by researchers, with \( r_{min}^2 \) being set between 0.90 (McKenzie et al., 2000) (Behrens & Steffensen, 2007) and 0.98 (Schurmann & Steffensen, 1997). Yet, a justification for the choice of \( r_{min}^2 \) is rare, as are other quality assurance analyses.

One reason for the absence of other quality assurance measures is convenience: the software that automatically controls the flush pump(s) also calculates the slopes and \( r^2 \), e.g., AutoResp™ (Loligo Systems, Denmark) and AquaResp (www.aquaresp.com). Thus, the practitioners see \( M_O_2 \) in real time and do not need to deal with the hundreds of raw DO slopes. Practitioners set \( r_{min}^2 \) to a high value with the good intention of retaining only precise \( M_O_2 \) determinations. The justification for a given \( r_{min}^2 \) is rarely based on notes taken during the experiment or on plots of individual slopes of the raw time series of DO values. The drawback of this strategy is that a high \( r_{min}^2 \) may remove linear slopes corresponding to very low \( M_O_2 \) values that should be retained to calculate SMR, but may still retain nonlinear slopes. In this study, the authors propose tools to diagnose when a given \( r_{min}^2 \) is very high and offer solutions when an experiment is plagued by numerous low \( r^2 \) values.

## 2 | MATERIALS AND METHODS

Results from an intermittent flow respirometry experiment involving a 33 g Atlantic salmon (\( S_a_l_mo_s_l_o_r \) L.) from Zhang et al. (2016) are analysed. The respirometer (2.1 l) was placed in a tank filled with aerated fresh water kept at a temperature of 12.0 ± 0.5 °C. After 72 h of fasting to reach a postabsorptive state, the fish was chased to exhaustion in a 10 l bucket for 10 min followed by a 2 min air exposure, to ensure that the fish has excess post-exercise oxygen consumption, and placed into the respirometer. A computer software (AquaResp version 2) controlled respirometry cycles: flushing for 120 s (Compact 600 pump, EHEIM, Germany) with water from the surrounding tank, 80 s stabilization and 400 s measurement of DO decline. A recirculation loop ensured good mixing of water in the respirometer by removing water at one end of the respirometer and returning it to the other end. The recirculation loop consisted of a second pump (Universal 300, Eheim, Germany), vinyl tubing and an optical oxygen probe (Robust Oxygen Probe OXROB3, Pyroscience, Germany), which was calibrated before the beginning of the experiment (0% sat. using sodium sulphite—saturated water and 100% sat. using fully aerated water) and sampled DO at c. 1 Hz. The value of \( r_{min}^2 \) was initially set to 0.90. \( M_O_2 \) was calculated according to Svendsen et al. (2018). Background respiration, obtained from measurements without fish and after the experiment, was negligible. SMR was calculated as the quantile (\( P = 0.2 \)) of all \( M_O_2 \) values recorded after the 6 h acclimation period (Chabot et al., 2016b), up to when the fish was removed from the respirometer at 64 h.

Four diagnostic tools for the possible overestimation of SMR are demonstrated: calculating the proportion of \( M_O_2 \) values that are rejected because \( r^2 \) is below \( r_{min}^2 \), the histogram of \( r^2 \) values, a scatter plot of \( r^2 \) as a function of \( M_O_2 \), and plots showing SMR and the proportion of rejected slopes as a function of \( r_{min}^2 \) for a wide range of possible \( r_{min}^2 \) values.

The authors of this study propose three solutions to generate a more reliable estimate of SMR, which are explained and justified below. The first solution does not involve reanalysing the raw data and consist in selecting a lower value of \( r_{min}^2 \), with the objective of rejecting a low proportion of the slopes. The other solutions involve reanalysing the raw DO time series. The series can be broken into short bins (20 s bins are used here), retaining only the median for each bin. Alternatively, a moving average can be applied on the time series of DO values to improve the signal-to-noise ratio. The authors used a 29 point window width to illustrate this approach. After each of these two approaches, the linear regression of DO as a function of time must be recalculated for each cycle in the experiment. Both AquaResp and AutoResp let the user set a “wait” period after the respirometer is closed and before data are used to calculate the regression for a given cycle. With the binning approach, the same measurement period used by these applications is used to recaleculate the slopes. Nonetheless, when a running average is used, the measurement period must be shortened at each end by half the window width minus 1, or 14 points for a 29 point window width, to remove any influence of the wait or flush periods on the slope (Svendsen et al., 2016b).

## 3 | RESULTS AND DISCUSSION

In the example of this study, the water to fish volume ratio was 62.6, and DO decreased by c. 1% sat. during the 400 s closed respirometer measurement period once the fish had repaid its oxygen debt and acclimated to the respirometer (after 6 h). Without inspecting rejected values, a typical plot of \( M_O_2 \) as a function of time might not have raised any suspicion concerning \( r_{min}^2 \) and the calculated SMR. Nonetheless, rejected \( r^2 \) values clearly corresponded mostly to very low \( M_O_2 \) values, that is, the very values that might correspond to SMR (Figure 1a). This is because a lower slope with the same noise (scatter about the regression line) as a steeper slope must have a lower \( r^2 \) (Barrett, 1974; Cornell & Berger, 1987), a problem of low signal-to-noise ratio. Consequently, the \( M_O_2 \) values at SMR will have proportionately more low \( r^2 \) values and risk rejection despite being linear when \( r_{min}^2 \) is
FIGURE 1  Oxygen uptake, calculated SMR and diagnostic plots for a 33 g salmon in a relatively large (62.6 water to fish ratio), setting min\(^2\) to 0.9. (a) \(M_O_2\) as a function of time spent in the respirometer. Blue circles were past the acclimation period and had an \(r^2 \geq \text{min} r^2\) and were used to calculate SMR; orange squares were rejected (\(r^2 < \text{min} r^2\)) and these values are not usually shown on this type of plot; black triangles were recorded during the acclimation period and were not used to calculate SMR. (b) Histogram of \(r^2\) values for the \(M_O_2\) values shown in A, with \(\text{min} r^2\) shown as a vertical line. (c) Relationship between \(r^2\) and \(M_O_2\), with \(\text{min} r^2\) and SMR shown as horizontal and vertical lines, respectively. (d) Relationship between possible \(\text{min} r^2\) values between 0.80 and 0.99 and both SMR and rejection rate, the vertical line is the \(\text{min} r^2\) set for this fish. (e) Example of a linear slope corresponding to a low value of \(M_O_2\) that was rejected (\(r^2 < \text{min} r^2\)). (f) Example of a nonlinear slope that failed to be rejected (\(r^2 \geq \text{min} r^2\)).
FIGURE 2  Oxygen uptake, calculated SMR and diagnostic plots for the fish shown in Figure 1, after splitting the original time series of DO values into 20 s bins, retaining the median DO of each bin to recalculate the slopes (△) acclimation period (○) $R^2 \geq \text{min}_R^2$ (□) $R^2 < \text{min}_R^2$ (—) SMR (—) Proportion of rejected values. (a)-(f) as in Figure 1
FIGURE 3  Oxygen uptake, calculated SMR and diagnostic plots for the fish shown in Figure 1, after applying a 29 s running mean on the original time series of DO values and recalculating the slopes (a) acclimation period (.) $R^2 \geq \min R^2$ (.) $R^2 < \min R^2$ ( – – – ) SMR (– – – ) Proportion of rejected values. (a)–(f) as in Figure 1
arbitrarily set very high. With a $\min^2$ of 0.9, SMR was 74 mg O$_2$ h$^{-1}$ kg$^{-1}$ (Figure 1a), a potential overestimation because 22.1% of the slopes were rejected. Cases with rejection rates above ~5–10%, especially if mostly low values of $\dot{M}$O$_2$ are rejected, should be further examined.

Three visual tools can help assess if $\min^2$ is set too high for any given fish. First, the frequency distribution of $r^2$ values, with the chosen $\min^2$ displayed as a vertical line, shows the range and proportion of rejected $r^2$ values (Figure 1b). For this fish, $r^2$ values of 0.85–0.89 were common and rejected, which is clearly a concern.

Second, Figure 1c, a scatter plot of $r^2$ as a function of $\dot{M}$O$_2$, with $\min^2$ and SMR shown as horizontal and vertical lines, respectively, proved even more informative. Ideally, no or few values should appear in the lower left quadrant, and rejected values, if any, should be distributed across the entire range of $\dot{M}$O$_2$ values. This would indicate that nonlinearity, but not low signal-to-noise ratios, caused the rejection of these $\dot{M}$O$_2$ values. In the example of this study, over half of the rejected $\dot{M}$O$_2$ values were lower than the calculated SMR, a clear problem that can result in the overestimation of SMR, independent of the calculation method for estimating SMR (quantile, where proportions are important, lowest frequency distribution or averaging minimum values, Chabot et al., 2016b).

Figure 1d raised further suspicion about the calculated value of SMR by showing that even small changes in $\min^2$ around 0.9 caused shifts in SMR and in the proportion of rejection. Not only did the chosen $\min^2$ reject many linear slopes (Figure 1e, $r^2$ of 0.87), jeopardizing the calculation of SMR, but it did not achieve the objective of rejecting nonlinear slopes (Figure 1f, $r^2 = 0.92$). With long measurement periods, a brief bout of activity will cause nonlinearity and possibly increase $r^2$ compared with periods when the fish is always calm, because of the larger DO decline overall due to the bout of activity. The most common approaches of calculating SMR (Chabot et al., 2016b; Steffensen et al., 1994) are robust against a moderate proportion of nonlinear slopes, which should nevertheless be avoided.

The high $r^2$ rejection rate shown in Figure 1 would normally lead a researcher to remove the fish from further analysis. Nonetheless, other analytical solutions exist. An arbitrarily lower $\min^2$ value would reject fewer slopes. For instance, using Figure 1d, a $\min^2$ of 0.87 rejects only 4.4% rather than 22.1% of slopes, yielding a 6% lower SMR (69.8 vs. 74.0 mg O$_2$ h$^{-1}$ kg$^{-1}$). However, this approach is unlikely to reject nonlinear slopes. Less arbitrary and better approaches require processing the raw data, the DO time series, using a programming language, such as R (R Core Team, 2020) to improve the signal-to-noise ratio. Many processing methods can be used, separately or together, and just two examples are offered in this study.

Figure 2 shows how the same DO time series used for the slopes of Figure 1 was broken into 20 s data bins, and the median DO for each bin was retained to calculate slopes. This processing removed the high frequency noise oscillations in DO values and greatly improved $r^2$ values (all >0.90 and a $\min^2$ of 0.95 rejected only 6% of the slopes; Figure 2b,c). Moreover, SMR became insensitive to the choice of $\min^2$ between 0.80 and 0.97 (Figure 2d). Thus, with the processed DO data, low $\dot{M}$O$_2$ values were no longer preferentially rejected (Figure 2a,c), and SMR was 7% lower (68.7 vs. 74.0 mg O$_2$ h$^{-1}$ kg$^{-1}$). Furthermore, this processing method benefitted linear slopes more than the nonlinear ones (Figure 2e,f).

The second processing method of this study (Figure 3) applies a 29 s running average to the original DO time series. Once again, the $r^2$ values improved considerably (Figure 3b) and more so for linear (Figure 3e) than the nonlinear slopes (Figure 3f). Again, rejected slopes were not associated with low values of $\dot{M}$O$_2$ (Figure 3c), and SMR was not strongly related to $\min^2$ (Figure 3d), which facilitates the selection of $\min^2$. A value of 0.95 yielded a rejection rate of 4.6%, and SMR was identical to that obtained by the previous method. Window width for the running average depends on the signal-to-noise ratio: a wider window may be required with noisier data, but the calculation of SMR with the quantile method of Chabot et al. (2016b) is not very sensitive to the choice of window width. For this fish, varying window width from 9 to 149 points, in 20 point increments, and $\min^2$ from 0.94 to 0.98 to keep rejection rate within 4–7%, had little impact on SMR (range = 68.1–68.9, mean = 68.49, S.D. = 0.275, Table A1, Figures A1 and A2).

These solutions increase signal-to-noise ratio for each slope and typically increase $r^2$ more for linear than nonlinear slopes. On the contrary, values of $\dot{M}$O$_2$ remain largely unchanged (Figure A3). In cases where large respirometers are used with small fish and very long measurement periods are necessary, the probability of changes in fish behaviour or state increases, and it is possible that a large proportion of slopes are nonlinear for this reason. Removing them may leave fewer linear slopes for the determination of SMR, especially that long measurement periods involve fewer $\dot{M}$O$_2$ determinations, unless experiment duration is increased. In such cases, the rolling regression algorithm proposed by Zhang et al. (2019) to measure maximum metabolic rate could be adapted for the determination of SMR. The algorithm examines the dynamic nature of active $\dot{M}$O$_2$ in a large volume respirometer when fish metabolic state is more likely to change during a long measurement period. The shortest duration of the period used for each $\dot{M}$O$_2$ measurement is determined using a more objective examination of the variance and $r^2$ using the background $\dot{M}$O$_2$ as a function of measurement duration. Linear regressions are then applied to blocks of this duration, either sequentially or moving one point at a time.

In conclusion, a pilot study or possibly past experiments with the species of interest will help select a combination of respirometer volume and duration of the measurement period that ensures that most or all DO slopes have good $r^2$, even when the animal is quiescent in the respirometer. The range of $r^2$ values and the rejection rate should be mentioned in SMR experiments using intermittent flow respirometry. Although $\min^2$ is typically stated, only a few published studies reported rejection of no (e.g., Svendsen et al., 2015; Tirsgaard et al., 2015b) or a small proportion (Chabot et al., 2016a) of slopes. The vast majority of studies using intermittent flow respirometry to measure SMR do not mention the rejection rate, making impossible an assessment of inadvertently rejected, but valid low values of $\dot{M}$O$_2$ on the validity of SMR.

Sometimes one has to deal with already-collected data that have low values of $r^2$. The first priority is to inspect if the selected $\min^2$ rejects a high proportion of the slopes for the fish in the study. Fish
yielding large rejection rates, say >5%, should be flagged and investigated with the diagnostic tools discussed earlier (Figure 1a–d), retaining the remainder for statistical analysis without further work.

If few fish are flagged, especially when low values of \( \dot{M}O_2 \) are selectively affected, one solution is to remove them from the study, reporting their number and the justification. If these fish are important and the main cause of the low \( r^2 \) appears to be low signal-to-noise ratio, a quick and dirty method to avoid overestimation of SMR is to lower min\(^2\) (Zhang et al., 2016). The authors acknowledge that the more satisfactory solutions, although more time-consuming, are to process the original time series of DO data to improve the signal-to-noise ratio and then recompute the slopes.

ACKNOWLEDGEMENTS

The authors acknowledge G. Claireaux for early discussions of the ideas used in this paper and G. Gusceli for reviewing a draft before submission. This work was funded by an NSERC Discovery Grant held by A.P.F., who also holds a Canada Research Chair Tier I. Y.Z. holds Elizabeth R Howland Fellowship & George Weston Ltd. Doctoral Fellowship. D.C. was funded by Fisheries and Oceans Canada (Aquatic Climate Change Adaptation Services Program and Strategic Program for Ecosystem-Based Research and Advice).

AUTHOR CONTRIBUTIONS

D.C. had the initial idea that selecting high values of min\(^2\) removed data that were necessary to estimate SMR. He analysed the data, produced the results and wrote the original draft. Y.Z. provided the original data. Y.Z. and A.F.P. had numerous discussions with D.C. to refine the initial idea and made essential contributions to the final manuscript.

ORCID

Denis Chabot https://orcid.org/0000-0002-4199-0915

REFERENCES

Alcaraz, G., & Kruesi, K. (2012). Exploring the phenotypic plasticity of standard metabolic rate and its inter-individual consistency in the hermit crab *Calcinus californiensis*. *Journal of Experimental Marine Biology and Ecology*, 412, 20–26.

Barrett, J. P. (1974). The coefficient of determination—some limitations. *The American Statistician*, 28(1), 19–20.

Behrens, J. W., & Steffensen, J. F. (2007). The effect of hypoxia on behavioural and physiological aspects of lesser sandeel, Ammodytes tobianus (Linnaeus, 1785). *Marine Biology*, 150(6), 1365–1377.

Cech, J. J., Jr., & Brauner, C. J. (2011). Techniques in whole animal respiratory physiology. In A. P. Farrell (Ed.), *Encyclopedia of fish physiology: From genome to environment* (pp. 846–853). San Diego, CA: Academic Press.

Chabot, D., Koenker, R., & Farrell, A. P. (2016a). The measurement of specific dynamic action in fishes. *Journal of Fish Biology*, 88, 152–172.

Chabot, D., Steffensen, J. F., & Farrell, A. P. (2016b). The determination of the standard metabolic rate in fishes. *Journal of Fish Biology*, 88, 81–121.

Clark, T. D., Sandblom, E., & Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: Respirometry relevance and recommendations. *Journal of Experimental Biology*, 216(Pt 15), 2771–2782.

Cornell, J. A., & Berger, R. D. (1987). Factors that influence the value of the coefficient of determination in simple linear and nonlinear regression models. *Phytopathology*, 77(1), 63–70.

McKenzie, D. J., Piraccini, G., Piccolella, M., Steffensen, J. F., Bolis, C. L., & Taylor, E. W. (2000). Effects of dietary fatty acid composition on metabolic rate and responses to hypoxia in the European eel (*Anguilla anguilla*). *Fish Physiology and Biochemistry*, 22(4), 281–296.

Nelson, J. (2016). Oxygen consumption: surrogate for energy utilization or it’s own measurement? *Journal of Fish Biology*, 88, 10–25.

R Core Team. (2020). *R: A language and environment for statistical computing* v4.0.2. Vienna, Austria: R Foundation for Statistical Computing.

Schurmann, H., & Steffensen, J. F. (1997). Effects of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. *Journal of Fish Biology*, 50(6), 1166–1180.

Steffensen, J. F. (1989). Some errors in respirometry of aquatic breathers: how to avoid and correct for them. *Fish Physiology and Biochemistry*, 6(1), 49–59.

Steffensen, J. F., Bushnell, P. G., & Schurmann, H. (1994). Oxygen consumption in four species of teleosts from Greenland: No evidence of metabolic cold adaptation. *Polar Biology*, 14(1), 49–54.

Svendsen, J. C., Genz, J., Anderson, W. G., Stol, J. A., Watkinson, D. A., & Enders, E. C. (2014). Evidence of circadian rhythm, oxygen regulation capacity, metabolic repeatability and positive correlations between forced and spontaneous maximal metabolic rates in lake sturgeon *Acipenser fulvescens*. *PloS One*, 9(4), e94693.

Svendsen, J. C., Tirsgaard, B., Cordero, G. A., & Steffensen, J. F. (2015). Intraspecific variation in aerobic and anaerobic locomotion: gillhead sea bream (*Sparus aurata*) and Trinidadian guppy (*Poecilia reticulata*) do not exhibit a trade-off between maximum sustained swimming speed and minimum cost of transport. *Frontiers in Physiology*, 6, 43.

Svendsen, M. B. S., Bushnell, P. G., Christensen, E. A. F., & Steffensen, J. F. (2016a). Sources of variation in oxygen consumption of aquatic animals demonstrated by simulated constant oxygen consumption and different size respirometers. *Journal of Fish Biology*, 88, 51–64.

Svendsen, M. B. S., Bushnell, P. G., & Steffensen, J. F. (2016b). Design and setup of an intermittent-flow respirometry system for aquatic organisms. *Journal of Fish Biology*, 88, 26–50.

Svendsen, M. B. S., Andersen, N. R., Hansen, P. J., & Steffensen, J. F. (2018). Effects of harmful algal blooms on fish: insights from *Pseudonitzschia* parvus. *Fishes*, 3(1), 11.

Tirsgaard, B., Behrens, J. W., & Steffensen, J. F. (2015a). The effect of temperature and body size on metabolic scope of activity in juvenile Atlantic cod *Gadus morhua* L. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 179, 89–94.

Tirsgaard, B., Mølendesn, J. C., & Steffensen, J. F. (2015b). Effects of temperature on specific dynamic action in Atlantic cod *Gadus morhua*. *Fish Physiology and Biochemistry*, 41(1), 41–50.

Ultsch, G. R., Ott, M. E., & Heisler, N. (1980). Standard metabolic rate, critical oxygen tension, and aerobic scope for spontaneous activity of trout (*Salmo gairdneri*) and carp (*Cyprinus carpio*) in acidified water. *Comparative Biochemistry and Physiology Part A: Physiology*, 67(3), 329–335.

Zhang, Y., Gilbert, M. J. H., & Farrell, A. P. (2019). Finding the peak of aerobic scope with environmental correlates. *Ecology*, 100, 1–11.

Zhang, Y., Tyler, R. J., & Farrell, A. P. (2016). Design and validation of the high-resolution respirometry system for determination of standard metabolic rate. *Journal of Experimental Biology*, 219(17), 2491–2498.
APPENDIX A.

TABLE A1  SMR of the fish illustrated in Figure 1 after processing the time series of DO in the respirometer to improve the signal-to-noise ratio. Techniques used were splitting the signal into 20 s bins and keeping only the median from each bin, using a running mean with window width varying from 9 to 149 s, and using a running mean with 29 s window width followed by splitting into 20 s bins to extract the median from each bin. With all processing methods, choosing a min$^2$ that rejected 4–7% of the slopes allowed rejection of slope 215 (Figure 1f) and similar nonlinear slopes, but retained slope 214 (Figure 1e).

| Processing of DO time series | Window width (s) | Binning width (s) | min$^2$ | % rejection | SMR  |
|------------------------------|------------------|-------------------|---------|-------------|------|
| Binning + median             |                  | 20                | 0.95    | 6.3         | 68.7 |
| Running mean                 | 9                |                   | 0.94    | 5.7         | 68.9 |
| Running mean                 | 29               |                   | 0.95    | 4.2         | 68.7 |
| Running mean                 | 49               |                   | 0.96    | 5.2         | 68.7 |
| Running mean                 | 69               |                   | 0.96    | 3.6         | 68.5 |
| Running mean                 | 89               |                   | 0.97    | 5.7         | 68.3 |
| Running mean                 | 109              |                   | 0.97    | 3.9         | 68.2 |
| Running mean                 | 129              |                   | 0.98    | 7.3         | 68.1 |
| Running mean                 | 149              |                   | 0.98    | 4.4         | 68.5 |
| Running mean + binning + median | 29            | 20                | 0.95    | 4.4         | 68.7 |
FIGURE A1  Oxygen uptake, calculated SMR and diagnostic plots for the fish shown in Figure 1, after applying a 9 s running mean on the original time series of DO values and recalculating the slopes. (a)–(f) as in Figure 1.
FIGURE A2  Oxygen uptake, calculated SMR and diagnostic plots for the fish shown in Figure 1, after applying a 149 s running mean on the original time series of DO values and recalculating the slopes. (a)–(f) as in Figure 1
FIGURE A3  Regression between processed and original $\dot{M}_O_2$ values for the 33 g salmon of this study (red line); black line: identity line (1:1). (a) Original DO trace was split into 20 s bins, and the median of each bin was used to calculate $\dot{M}_O_2$ values; (b) a 9 s running average was applied to the original DO trace and $\dot{M}_O_2$ values were recalculated; (c) like B, with a 29 s running average; like B, with a 149 s running average. Only in (d) is the regression line slightly different from the identity line.