TECHNICAL COMMENT

Maxed Out: Optimizing Accuracy, Precision, and Power for Field Measures of Maximum Metabolic Rate in Fishes

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ABSTRACT

Both laboratory and field respirometry are rapidly growing techniques to determine animal performance thresholds. However, replicating protocols to estimate maximum metabolic rate (MMR) between species, populations, and individuals can be difficult, especially in the field. We therefore evaluated seven different exercise treatments—four laboratory methods involving a swim tunnel (critical swim speed [U_{crit}], U_{crit} postswim fatigue, maximum swim speed [U_{max}], and U_{max} postswim fatigue) and three field-based chasing methods (3-min chase with 1-min air exposure, 3-min chase with no air exposure, and chase to exhaustion)—in adult coho salmon (*Oncorhynchus kisutch*) as a case study to determine best general practices for measuring and quantifying MMR in fish. We found that all seven methods were highly comparable and that chase treatments represent a valuable field alternative to swim tunnels. Moreover, we caution that the type of test and duration of measurement windows used to calculate MMR can have significant effects on estimates of MMR and statistical power for each approach.

Keywords: fish, metabolism, maximum metabolic rate, respirometry, oxygen consumption, salmon.

Introduction

Evolutionary fitness is notoriously difficult to measure, and as an alternative to lifetime fecundity, bioenergetics can provide a valuable snapshot for how individuals and populations are faring in their environments (Hall et al. 1992; Farrell et al. 2008; Sebens et al. 2018). To thrive, animals must engage in energetically demanding processes that include growth, development, feeding, digestion, predator evasion, reproductive development, and mating (Kleiber 1975). However, rates of energy production are finite, and eventually animals reach a state in which ATP production is constrained by mechanistic limitations (e.g., Pörtner 2010; Sokolova et al. 2012). This upper limit for the rate of energy production, which is termed "maximum metabolic rate" (MMR), is often estimated using respirometry and can help determine energy budgets and limitations for individuals and populations in response to changing environments (e.g., Lavaud et al. 2019). Aerobic scope (the absolute difference between standard aerobic metabolic rate and aerobic MMR) represents one of the best measures to quantify an animal's capacity to cope with energetic demands above standard metabolic processes. Aerobic scope, therefore, is one of the most widespread proxies for individual fitness and population-level health in ecophysiology (see Pörtner 2010; Clark et al. 2013; Rummer et al. 2014; Schulte 2015; Farrell 2016; Metcalfe et al. 2016). However, estimating metabolic rates, in particular MMR, can represent a difficult task, especially in field-based studies (Norin and Clark 2016).

Metabolism refers to the sum of all physiological reaction rates, and it is most accurately measured via calorimetry (Nelson 2016). Because physiological reactions rely both directly and indirectly on ATP (Pederson and Carifoli 1987), however,

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physiologists often rely on indirect calorimetry, where whole-animal rates of oxygen consumption are used to approximate metabolic rate (Ferrannini 1988). This approximation is possible because most of the energy produced by animal tissues is synthesized via oxidative phosphorylation, a mitochondrial pathway that couples the oxidation of food substrates to ATP production. Importantly, the main regulatory step of oxidative phosphorylation requires a constant supply of oxygen to drive ATP production (for review, see Little et al. 2017). Thus, rates of energy production strongly correlate with oxygen processing by animal tissues, where maximal rates of oxygen consumption (M_{O_2}) indicate upper limits for ATP production.

Although there has been major interest in MMR for many fish species during the last 70 years (Fry and Hart 1948; for review, see Norin and Clark 2016; Killen et al. 2017), there is surprising breadth in how MMR is both defined (Nelson 2016; Zhang and Gilbert 2017) and measured (Reidy et al. 1995; Kieffer 2000; Roche et al. 2013; Norin and Clark 2016; Rummer et al. 2016; Killen et al. 2017). It is generally agreed that the best way to elicit MMR in fish is via exhaustive exercise, and consequently, most experiments typically rely on critical swimming protocols (i.e., critical swimming speed [U_{crit}]), where fish are exercised in a swim flume with incrementally increasing speeds to the point of exhaustion. Depending on life-history strategies, however, MMR can occur during exhaustive exercise (e.g., tropical bridled monocle bream, *Scolopsis bilineata* [Roche et al. 2013]; coral reef fishes [Rummer et al. 2016]) or at time points from several minutes to more than an hour following exhaustion (e.g., pike, *Esox lucius* [Armstrong et al. 1992]; Atlantic cod, *Gadus morhua* [Soofiani and Priede 1985; Bushnell et al. 1994; Schurmann and Steffensen 1997]; barramundi, *Lates calcarifer* [Norin and Clark 2016]). In some cases, MMR may not be linked to exercise at all but may occur during less conspicuous processes, such as digestion (southern catfish, *Silurus meridionalis* [Fu et al. 2008]; Burmese python, *Python bivittatus* [Secor and Diamond 1998]). In cases where critical swimming protocols are not ideal (e.g., in field settings or for species with low aerobic capacities), "chase" protocols that elicit continuous burst swimming until exhaustion are also used (Soofiani and Priede 1985; Bushnell et al. 1994; Reidy et al. 1995; Svendsen et al. 2011; Casselman et al. 2012; Norin and Malte 2012; Clark et al. 2013; Norin and Clark 2016). However, it can be difficult to reconcile whether differences in values for aerobic MMR are ecologically based or result from differences in experimental techniques (for review, see Rummer et al. 2016; Norin and Clark 2016). Therefore, there is a need to develop best practices approaches to compare MMR both within and between species.

We were specifically interested in how different experimental protocols to elicit MMR and analytical approaches to quantify MMR could be objectively evaluated in a comparative context. As a case study, we tested seven different exhaustive exercise treatments and a series of analytical methods to determine best general practices to quantify measurements of MMR. Adult Pacific salmon represent good candidates to elicit MMR via exercise because they undergo energetically demanding, long-distance migrations to return to their natal spawning grounds (see Dittman and Quinn 1996). We therefore compared aerobic MMR measurements from four common laboratory methods that require a swim tunnel respirometer (U_{crit}, U_{crit} postswim fatigue, maximum swim speed [U_{max}], and U_{max} postswim fatigue) and three field-based chasing methods (3-min chase with 1-min air exposure, 3-min chase with no air exposure, and chase to exhaustion) using coho salmon (*Oncorhynchus kisutch*). To optimize the relatively high frequencies of dissolved oxygen measurements possible with fiber-optic probes (as opposed to the more classic Clark-type electrodes), we assessed how altering the duration of measurement windows (the amount of time over which rates of oxygen consumption are calculated) affected both estimates of MMR and relative coefficients of determination (R^2). Here, we leverage this comparative approach not only to determine best methods to quantify MMR in Pacific salmon but to demonstrate methods to tailor a best practices approach to any study species. Given that environmental physiologists are increasingly using aerobic MMR to inform how performance is constrained by changing environments, it is crucial to address these technical issues so that data and subsequent conclusions are reliable.

Methods

Animal Collection and Holding

Adult coho salmon ($n = 48$; mean ± SEM: body mass, 2.05 ± 0.07 kg; fork length, 57.3 ± 0.59 cm) returning from the ocean were collected from the Chilliwack River Hatchery (British Columbia, Canada) and transported 23.7 km in a 1,250-L tank (8.2°–10.4°C; >90% air saturation) to the Fisheries and Oceans Canada Cultus Lake Research Laboratory (British Columbia, Canada). Approximately equal numbers of male and female fish were used to minimize potential effects of sex differences on MMR measurements. Fish were held in flow-through, UV-sterilized, sand-filtered freshwater under natural photoperiod in 8,000-L outdoor holding tanks (9°C; >90% air saturation; $n \leq 27$ fish per tank; mixed sex) for at least 36 h before experimentation to ensure recovery from handling stress. Fish were then transferred to 2,000-L acclimation tanks (9°C; >90% air saturation; $n \leq 6$ fish per tank; mixed sex) for at least 24 h before MMR tests. All experimental protocols were approved by the Animal Care Committee at the University of British Columbia in accordance with the Canadian Council on Animal Care (protocol A17-0160).

Chase Protocols

All tests were conducted outdoors under natural photoperiod. Eight intermittent-flow respirometers with volumes of 33.3 and 57.9 L were constructed using clear 8- and 10-in polyvinyl chloride (PVC) tubes, respectively. Each PVC tube was equipped with a PVC lid at both ends: one permanently attached and one detachable to load fish. A 600- or 1,200-L h^{-1} Eheim universal aquarium pump (Eheim, Germany) recirculated water through the chamber continually, while a 1,200-L h^{-1} Eheim universal aquarium pump flushed water from the surrounding tank through the chamber between cycles of M_{O_2} measurement to ensure that

fish never experienced hypoxic conditions. Four respirometers were placed in each of two temperature-controlled flow-through tanks (diameter = 181.6 cm; depth = 41.9 cm). Dissolved oxygen was measured continuously in each respirometer using a robust oxygen probe and FireSting optical oxygen meter (Pyroscience, Germany).

Fish were introduced to a "chase tank" (2,000 L; filled to ~660 L) in which they were subjected to only one of three chase treatments. For all treatments, four people manually elicited burst swimming by making quick movements with their hands under the water, often lightly touching the fish's caudal fin. This was meant to motivate the fish to burst continuously throughout the chase without allowing time for recovery, simulating predator evasion (Donaldson et al. 2010) or "catch-and-release" fisheries interactions (Gale et al. 2011). The first treatment was a 3-min chase followed by a 1-min air exposure in which the fish was held outside the water in a net for 1 min. The second treatment was a 3-min chase with no air exposure. The third treatment was a continuous chase until the fish ceased bursting for >10 s. These treatments will henceforth be referred to as AIR (n = 8; 4 male, 4 female), NO AIR (n = 9; 5 male, 4 female), and EXHAUST (n = 11; 6 male, 5 female), respectively. We selected these chase treatments because they represent commonly used techniques, especially in the field (Rummer et al. 2016; for review, see Norin and Clark 2016; Killen et al. 2017). No comparison to date has empirically evaluated how ostensibly subtle variations in technique (e.g., air vs. no air exposure or tailoring chase times to individual performance) influence measures of MMR. This type of comparison is especially intriguing because these variations in technique correspond to challenges associated with predation and catch-and-release angling (Gallagher et al. 2014; Cook et al. 2015).

Following the chase treatment, each fish was transferred to a respirometer, where dissolved oxygen recordings were initiated as soon as the respirometer lid was sealed and the chamber was flushed of all air bubbles (50–120 s after chase). Shade cloth was then placed over the holding tanks to minimize potential disturbance. $\dot{M}O_2$ was measured for 4 min followed by a 6-min flush period. This cycle continued for 90 min, yielding a total of nine $\dot{M}O_2$ measurement cycles per individual. These measurement/flush cycles ensured that oxygen levels remained above 70% air saturation at all times. Background respiration rates (blanks) were measured in each respirometer for 30 min immediately following MMR experiments and were determined to be negligible. Each individual was tested only once.

Swimming Protocols

A Brett-type swim tunnel respirometer (diameter = 25.4 cm; volume = 450 L; Brett 1964; Farrell et al. 2003; Lee et al. 2003*b*) was used to obtain either U_{crit} or U_{max} for each fish. For U_{crit}, fish were transferred to the tunnel by net in <30 s to minimize air exposure and handling stress. Fish were then left to acclimate for 1 h at ~0.25 body lengths (BL) s^{-1} water velocity. We chose 1 h to acclimate fish to their swim tunnel respirometer because even after our most intense chase protocols, individuals began to reach low and stable metabolic rates around the 30-min mark (see fig. A1). Following tunnel acclimation, flow was increased in 5-min intervals of approximately 0.26 BL s^{-1} until ~1.50 BL s^{-1} was reached. Thereafter, water velocity was increased by 0.26 BL s^{-1} every 20 min until fish were unable to maintain their position in the water column (i.e., fish rested against the back grid for >30 s). $\dot{M}O_2$ was measured during the last 10 min of each speed interval using an optical oxygen probe attached to a FireSting optical oxygen meter. When fish began to near U_{crit}, $\dot{M}O_2$ was measured intermittently (broken up by flush cycles to ensure dissolved oxygen >70% air saturation) throughout the 20-min intervals to obtain a U_{crit} MMR measurement. Upon fatigue, flow was immediately decreased to ~0.25 BL s^{-1}, and fish were allowed to recover for 1 h. Oxygen consumption rates were measured continuously, with the occasional interruption for a manual flush cycle, during this 1-h recovery to obtain a U_{crit} fatigue MMR measurement. For U_{max}, fish were transferred to the swim tunnel respirometer and acclimated for 1 h at a flow rate of ~0.25 BL s^{-1}. The velocity in the tunnel was then increased every minute by ~0.26 BL s^{-1} until fish were unable to maintain position in the water column (i.e., fish rested against the back grid for >30 s). The velocity was then decreased back to ~0.25 BL s^{-1}, and fish were allowed to recover for 1 h. $\dot{M}O_2$ was measured intermittently while the fish was swimming but continuously as it neared exhaustion to measure U_{max} MMR. $\dot{M}O_2$ was continuously measured during the 1-h recovery (broken up by occasional manual flush cycles) to obtain a U_{max} fatigue MMR measurement. Thus, U_{crit} fatigue and U_{max} fatigue refer to $\dot{M}O_2$ measurements taken when the fish were no longer actively swimming but fatigued and recovering from exhaustive exercise. Background respiration rates were measured after each fish was removed from the tunnel and were determined to be negligible. Each individual was tested only once.

Data Analyses

Respirometer-dissolved oxygen content was plotted over time for each 4-min measurement cycle. All measurement traces were plotted and visually assessed for linearity. $\dot{M}O_2$ (mg O_2 kg^{-1} min^{-1}) was then calculated using the slope of each line (mg O_2 L^{-1} min^{-1}). To account for water displacement by the fish, we subtracted the volume of the fish from the volume of the respirometer, approximating that 1 kg of fish was equivalent to 1 L of water. We then normalized this rate of oxygen consumption to the respective body mass of the fish. Thus, $\dot{M}O_2$ was calculated using the formula $\dot{M}O_2 = \text{slope} \times (v_R - v_F) \times m^{-1}$, where v_R represents the volume of the respirometer (L), v_F represents the volume of the fish (L), and m represents the mass of the fish (kg). Although MMR has been shown to scale isometrically with body size in Pacific salmon (Brett and Glass 1973), we actively collected fish of approximately 2 kg to minimize the potentially confounding effects of scaling on metabolic rates and swimming speeds. MMR was defined as the highest $\dot{M}O_2$ value calculated across all measurement cycles. For our postchase respirometry tests (AIR, NO AIR, EXHAUST), this always occurred in the first 4-min measurement period after the

fish was sealed in the respirometer. For the swim tests, MMR always occurred in the measurement period within the last two test increments for U_{crit} and near exhaustion for U_{max}. MMR values for U_{crit} fatigue and U_{max} fatigue always occurred in the first 10-min measurement cycle after exhaustion.

In calculating MMR, it is common practice to use the slope of the steepest $\dot{M}O_2$ measurement in its entirety, although there is considerable variation in measurement durations across studies. Additionally, some studies have used the slope of a shorter window within a longer measurement period to calculate MMR (e.g., Rummer et al. 2016 used the steepest 1-min slope within their 5-min measurement period). We therefore wanted to test the effects of variable measurement durations on estimates of MMR. For all tests, we first calculated MMR using the slope of the entire measurement cycle, followed by sliding-window-type analyses to identify the steepest slopes over any 180-, 120-, 90-, 60-, 30-, 20-, and 10-s time interval (i.e., window). Specifically, each sliding window began at the start of the measurement period and was shifted in 1-s increments. Using a 90-s measurement window, for instance, we calculated respective slopes from 0–90, 1–91, 2–92 s, and so on until we reached the end of the measurement period (i.e., 150–240 s). For each measurement window duration, MMR represented the sliding window that produced the steepest slope where all fish had $R^2 > 0.85$. We also calculated these slopes using measurements from the blank respirometer runs to test for potential artifacts from noise within the chambers.

Statistical Analysis

All data were analyzed with mixed effects linear models using the free and open software jamovi (ver. 0.9; GAMLj module; https://www.jamovi.org). We used a significance threshold of $\alpha = 0.05$ for all statistical tests. Individuals were included as a random effect to account for repeated measures between swim and fatigue protocols and repeated subsampling within oxygen consumption curves for the sliding-window analysis. Main effects represented the treatment (i.e., swim or chase type, U_{crit}, U_{max}, U_{crit} fatigue, or U_{max} fatigue) and sliding-window duration (i.e., Tukey post hoc tests were conducted on the expected means). Power analysis (Rosner 2015) was conducted using mean MMR and standard deviation to estimate minimum theoretical sample sizes required to detect 10%, 15%, and 20% changes in mean $\dot{M}O_2$ using each treatment (power = 0.8).

Results and Discussion

There is no one-size-fits-all approach for animal respirometry. We opted to use Pacific salmon for this case study because they undergo long, energetically demanding migrations and have been the focus of much respirometry work. Owing to their unique life history, however, our more specific findings are not necessarily applicable to other species nor do we intend them to be. Our broad intent was to use this system as a case study to demonstrate universal approaches and considerations that will optimize accuracy and precision for aerobic MMR measurements across taxa, both in the laboratory and in the field.

Optimal Measurement Window Duration

Aerobic MMR is typically calculated from entire measurement cycles, which can exceed 10–20 min in duration (Soofiani and Priede 1985; Tang et al. 1994; Reidy et al. 1995; Farrell et al. 2003; Wagner et al. 2005; MacNutt et al. 2006; Jordan and Steffensen 2007; Eliason et al. 2011; Clark et al. 2012; Roche et al. 2013). Many animals, however, are unlikely to sustain MMR for periods of this length. In determining optimal measurement window durations, our goal was to maximize measures for MMR while (1) maintaining a sufficiently high coefficient of determination for the curve and (2) minimizing experimental error. We found a significant interaction between treatment type and measurement window duration, where shortening the measurement window increased observed MMR more in some treatments than in others (fig. 1*A*; see table 1 for statistics). For example, reducing the measurement window to 60 s almost doubled MMR in the U_{crit} fatigue treatment but increased MMR by only ~25% in the U_{crit} treatment. Thus, strictly in terms of maximizing values for MMR, shorter measurement windows were better.

Short measurement windows as brief as 30 s have been used (Ern et al. 2017). However, a trade-off with these shorter measurement windows is that random effects begin to represent more of the overall signal. In this study, we found a significant interaction between treatment type and measurement window duration on the coefficient of determination, R^2 (fig. 1*B*; see table 1 for statistics). Specifically, shorter measurement windows resulted in significantly lower R^2. This occurs because shorter sampling windows have smaller sample sizes and generating a model line is less accurate, which results in larger residuals. However, reducing measurement window lengths had a greater effect in the U_{crit} and U_{max} swim and postswim fatigue tests than in the postchase treatments. This variation is likely attributed to the different ratios of respirometer to fish size that are required by the different tests. It is recommended that respirometry chambers are 20–50 times larger than the volume of the fish for static respirometry and 50–150 times larger than the volume of the fish for swim flume respirometers (Svendsen et al. 2016). This means we would expect faster decreases in dissolved oxygen and therefore steeper slopes in the postchase respirometers (33.3 or 57.9 L) relative to the much larger swim flume respirometer (450 L). Thus, signal-to-noise ratios were likely much higher in the static postchase respirometers. In addition to decreasing R^2, shorter measurement windows also increased experimental error (fig. 1*C*). In the blank (no-fish control) treatment, we observed an exponential increase in observed MMR as an artifact of shortening the measurement window below 60 s. As measurement windows shorten, background noise from the fiber-optic oxygen probe represents an increasing proportion of the overall signal. Thus, in addition to quantifying rates of potential background respiration, blank controls should also be analyzed to reach a balance between maximizing measures for MMR and minimizing measurement error. The increased resolution associated with shorter measurement windows can also result in detection of irregular fluctuations in dissolved oxygen levels as a result of poor mixing. Our respirometers were equipped with continuous recirculation pumps to

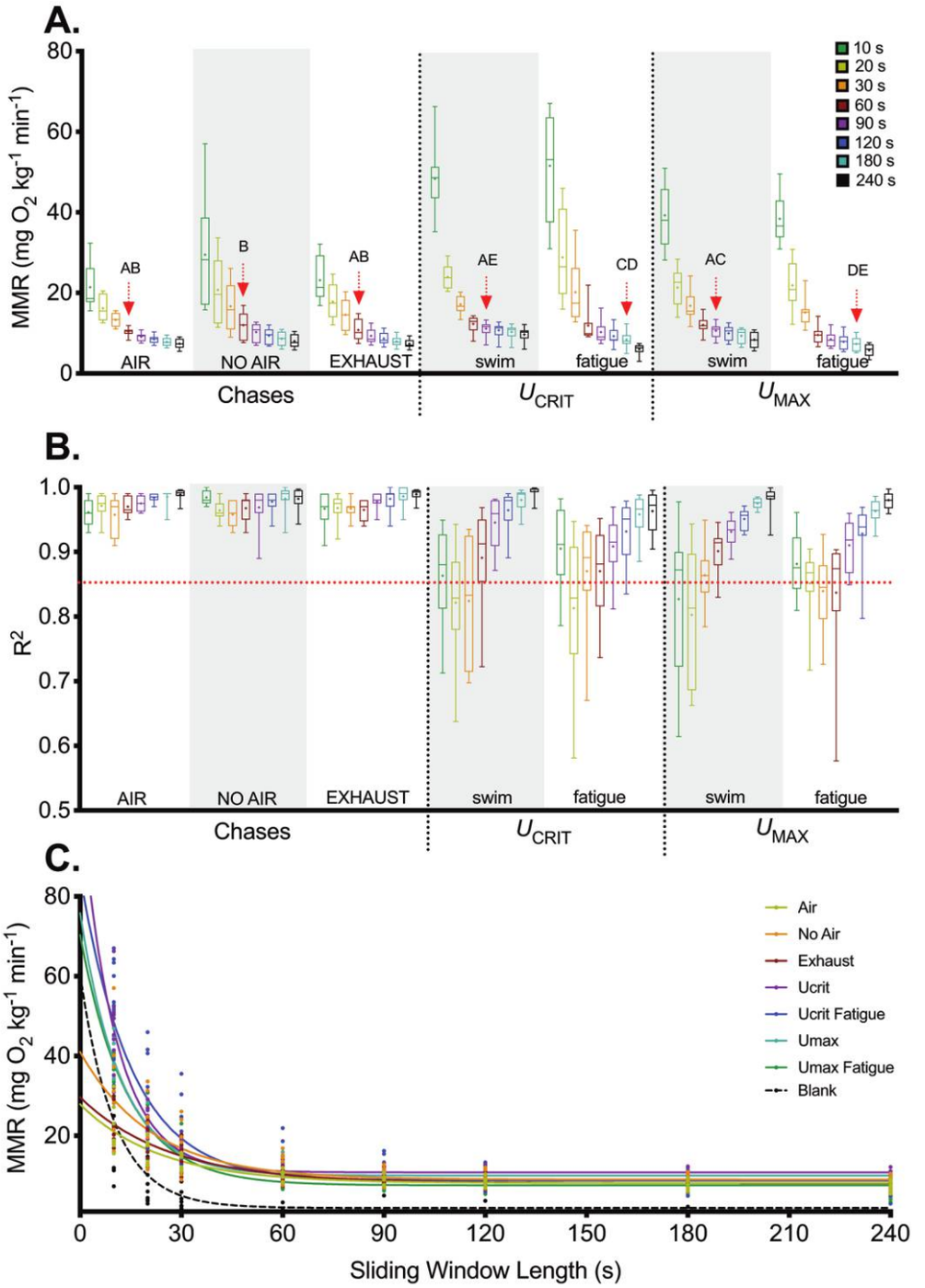

Figure 1. Effect of respirometry treatment type and sliding-window duration on measures of maximum metabolic rate (MMR; *A*), coefficients of determination (R^2; *B*), and measurement error in MMR (*C*). R^2 values (*B*) were derived from the slopes of the raw oxygen consumption curves over the respective sliding-window duration. For boxplots (*A*, *B*), the middle lines inside the boxes represent the medians, while the lower and upper box boundaries represent the first and third quartiles, respectively. The lower and upper whiskers represent the minimum and maximum values, respectively, and the plus signs represent the means. Different colors represent different sliding-window durations (*A*, *B*) or respirometry treatment types (*C*). The red arrows (*A*) indicate best mean estimates of MMR accounting for our R^2 threshold of 0.85, indicated by the red dashed line (*B*), and background noise, indicated by the black dashed line (*C*), where measurement errors in the estimation of MMR were determined in the absence of a fish. U_{crit} = critical swim speed; U_{max} = maximum swim speed.

Table 1: Mean maximum metabolic rate (MMR; mg O_2 kg^{-1} min^{-1}) and R^2 for chase treatments (AIR, NO AIR, EXHAUST)

	AIR ($n = 8$)		NO AIR ($n = 9$)		EXHAUST ($n = 11$)		U_{crit} ($n = 10$)		U_{crit} fat. ($n = 10$)		U_{max} ($n = 9$)		U_{max} fat. ($n = 9$)	
W (s)	MMR	SEM	MMR	SEM	MMR	SEM	MMR	SEM	MMR	SEM	MMR	SEM	MMR	SEM
10	21.38	2.04	29.49	4.56	23.15	1.68	48.34	2.58	51.55	4.16	39.26	2.59	21.38	2.04
20	16.15	1.20	20.73	2.73	17.82	1.37	23.96	.93	28.85	3.48	21.32	1.59	16.15	1.20
30	13.32	.66	16.67	1.99	14.57	1.26	17.14	.68	20.19	2.47	16.81	1.22	13.32	.66
60	10.35[a]	.37	11.97[a]	1.13	10.78[a]	.84	12.29	.63	11.87	1.43	12.04	.69	10.35	.37
90	9.09	.37	10.23	.76	9.32	.60	11.12[a]	.57	10.23	.97	10.58[a]	.59	9.09	.37
120	8.51	.37	9.36	.65	8.67	.49	10.70	.58	9.26	.74	9.97	.68	8.51	.37
180	7.83	.39	8.62	.58	7.96	.40	10.27	.57	8.40[a]	.62	9.33	.67	7.83[a]	.39
240	7.40	.44	7.98	.54	7.34	.33	9.67	.57	6.02	.41	8.38	.67	7.40	.44
	R^2	SEM	R^2	SEM	R^2	SEM	R^2	SEM	R^2	SEM	R^2	SEM	R^2	SEM
10	.96	.01	.98	.00	.97	.01	.86	.02	.90	.02	.83	.04	.88	.02
20	.97	.01	.96	.01	.97	.01	.82	.03	.81	.04	.80	.03	.85	.02
30	.96	.01	.96	.01	.97	<.01	.82	.03	.87	.03	.86	.02	.84	.02
60	.97	.01	.97	.01	.97	.01	.89	.03	.87	.02	.90	.01	.84	.04
90	.98	<.01	.97	.01	.98	<.01	.95	.01	.91	.02	.93	.01	.91	.01
120	.98	<.01	.98	.01	.98	.01	.96	.01	.93	.01	.95	.01	.93	.02
180	.99	.01	.98	.01	.99	<.01	.98	.01	.96	.01	.98	<.01	.96	.01
240	.99	<.01	.98	.01	.99	<.01	.99	<.01	.96	.01	.98	.01	.98	<.01

Note. Statistical values for mixed effects linear models looking at the effects of treatment (T), measurement window duration (W), and their interaction (T × W) on mean MMR: for T, $F = 23.6$, df $= 7$, $P < 0.001$; for W, $F = 40.0$, df $= 6$, $P < 0.001$; for T × W, $F = 4.1$, df $= 42$, $P < 0.001$. Statistical values for mixed effects linear models looking at the effects on coefficients of determination (R^2): for T, $F = 411.8$, df $= 7$, $P < 0.001$; for W, $F = 5.9$, df $= 6$, $P < 0.001$; for T × W, $F = 10.6$, df $= 42$, $P < 0.001$. All P values were significant at <0.05. U_{crit} = critical swim speed; U_{max} = maximum swim speed; fat. = fatigue.

[a]Best mean estimate of MMR based on our analyses.

ensure adequate mixing over time. However, very short measurement windows (e.g., 10 s) would be more likely to capture artificial jumps in dissolved oxygen concentrations as a result of momentary disruptions in flow, potentially as the fish repositions itself within the chamber. In any case, measurement windows of 10, 20, and 30 s were too brief to yield reliable data using both the swim tunnel and the static respirometers that we employed.

Using an R^2 threshold >0.85 to maintain goodness of fit and disregarding measurement window durations <60 s to minimize measurement error, we found that a 60-s measurement window provided the highest estimates of MMR for the three chase treatments, a 90-s measurement window provided the highest estimates of MMR for U_{crit} and U_{max}, and a 180-s measurement window provided the highest estimates of MMR for U_{crit} fatigue and U_{max} fatigue (fig. 1*A*, red arrows). Thus, optimal measurement window durations can differ with experimental parameters and ratios of fish to respirometer size. For instance, because swim flume respirometers can be more than seven times the volume of static respirometers for a given size fish (Svendsen et al. 2016), our data suggest that swim flume respirometers require longer measurement windows. The biological question of interest must also be taken into careful consideration when selecting experimental and analytical methods. Theoretically, a reliable measurement window of <1 s may represent an interesting data point from a purely physiological standpoint (e.g., to assess maximal limits of whole-animal energy turnover) but may be meaningless in terms of ecological relevance (i.e., most fitness-related activities would require MMR to be sustained for more than 1 s). Because of the physiological and ecological importance of aerobic scope during Pacific salmon migration (Eliason et al. 2011), the 60-s measurement window used here arguably represents a more relevant measure for MMR than longer measurement windows. However, another risk of shorter measurement windows is that hypoxia induced by exercise or air exposure can create a disequilibrium between whole-animal oxygen uptake and tissue-level oxygen processing (Farrell 2016; Killen et al. 2017; Zhang and Gilbert 2017), which theoretically leads to overestimates of MMR. This appears unlikely to be a factor in our analyses because time points for individual MMR were spread throughout the first measurement cycle (fig. A2) rather than concentrated in the beginning, as might be expected for quick replenishment of oxygen stores following exercise- and air-induced hypoxia.

Next, we wanted to compare whether the measurement window durations that we determined to be optimal provided better estimates of MMR than using the entire measurement cycle, which represents a common analytical approach (Soofiani and Priede 1985; Tang et al. 1994; Reidy et al. 1995; Farrell et al. 2003; Wagner et al. 2005; MacNutt et al. 2006; Jordan and Steffensen 2007; Eliason et al. 2011; Clark et al. 2012; Roche et al. 2013). We found a significant interaction between treatment type and measurement length ($F = 5.58$, df $= 6$, $P < 0.001$). Using the entire measurement cycle, we significantly underestimated MMR in the chase treatments but not in the U_{crit}, U_{max}, U_{crit} fatigue, or

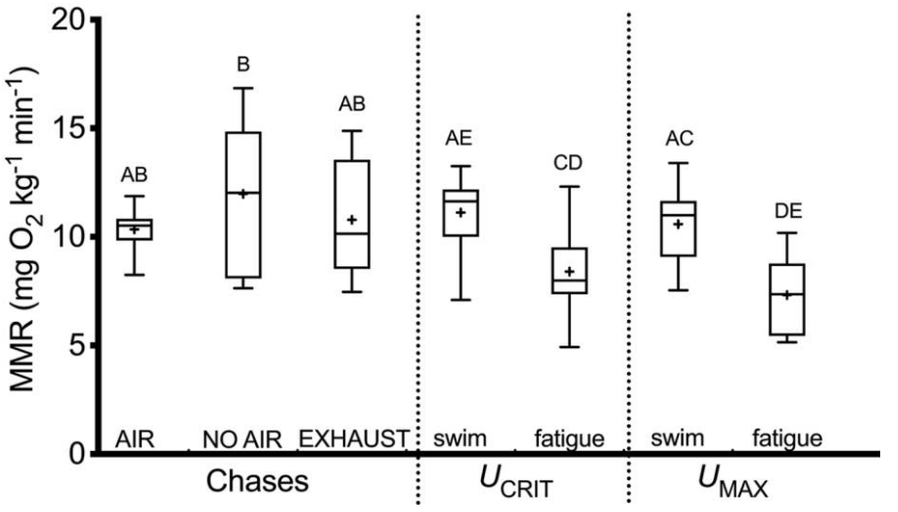

Figure 2. Best measures for maximum metabolic rate (MMR) based on respirometry treatment types. The middle lines inside the boxes represent the medians, while the lower and upper box boundaries represent the first and third quartiles, respectively. The lower and upper whiskers represent the minimum and maximum values, respectively, and the plus signs represent the means. Different capital letters represent statistical significance ($P < 0.05$). U_{crit} = critical swim speed; U_{max} = maximum swim speed.

U_{max} fatigue treatments (see table A1 for post hoc values). This difference between treatments was likely because postchase measurements represent rapid recovery from exercise and longer measurement windows would intrinsically incorporate more of the recovery profile. It may not be surprising that the optimal measurement windows for U_{crit} fatigue and U_{max} fatigue, which also represent recovery from exercise, did not result in significantly higher MMR, given that they were nearly as long as the total measurement cycle. Alternatively, we may not have seen a difference between optimal measurement window duration and full measurement cycle in measures taken during U_{crit} and U_{max} because these treatments represent active aerobic swimming, during which the fish is likely to be in steady state. In addition to specific experimental parameters and the biological question of interest, the physiological underpinnings of the technique used to elicit MMR (e.g., whether the fish is in steady state) also represent an important consideration when fine-tuning measurement windows.

According to these best practices for determining MMR, the population of Chilliwack River coho salmon used here had a mean MMR between 10.1 and 11.0 mg O_2 kg^{-1} min^{-1} (depending on the treatment used; table 1). These values are similar to those from Raby et al. (2016), who found a mean MMR of 9.9 mg O_2 kg^{-1} min^{-1} in Chilliwack River coho salmon at 10°C using a U_{max} swim protocol and a 5-min measurement window. Farrell et al. (2003) and Lee et al. (2003*a*) found a marginally lower MMR of 8.6–9.9 mg O_2 kg^{-1} min^{-1} at 8°–9.8°C using a U_{crit} swim protocol with a nearby population of fall-run coho salmon (Chehalis River). In contrast, Clark et al. (2012) found a lower mean MMR of ~6 mg O_2 kg^{-1} min^{-1} in the Chehalis River population at 7°C using a 3-min chase with 1-min air exposure protocol and a 5-min measurement window. For reference, we found a mean MMR of 7.4 mg O_2 kg^{-1} min^{-1} using a suboptimal 4-min measurement window. According to our findings, the long measurement window associated with the chase treatment may in part account for the lower estimate of MMR in the Clark et al. (2012) study.

Optimal Treatment Type

Our study found no significant difference in MMR between the three chase treatments and the U_{crit} and U_{max} swim protocols except that the NO AIR treatment resulted in significantly higher MMR than U_{crit} and U_{max} (fig. 2; table 1; see table A1 for post hoc values) when estimated using the respective optimal window lengths. U_{crit} fatigue and U_{max} fatigue resulted in significantly lower measures of MMR than their active-swimming counterparts (U_{crit} and U_{max}) and all three chase treatments. Importantly, chase treatments are generally more anaerobic in nature than U_{crit} and U_{max} treatments. This means that high aerobic MMRs in the chase treatments likely reflect greater investments in restoring glycolytic capacity via excessive postexercise oxygen consumption. Taken together, these findings are largely consistent with a recent meta-analysis (Killen et al. 2017) that found little variability in MMR between swimming and postexercise respirometry across 14 species of fishes. Killen et al. (2017) concluded that subtle differences in technique are more likely to introduce variation in MMR results than the overall method used. In our work, we were able to pinpoint differences in measurement window durations as a potential source of this inconsistency. Different studies, on the other hand, have found that the respirometry protocol (i.e., chase vs. active swimming vs. postswimming fatigue) does matter. For instance, Rummer et al. (2016) found M_{O_2} values up to 38% higher using U_{crit} rather than chase treatments in four species of coral reef fishes (Marr's fusilier, *Pterocaesio marri*; yellow and blueback fusilier, *Caesio teres*; spiny chromis,

Acanthochromis polyacanthus; black-axil chromis, *Chromis atripectoralis*). Similarly, Roche et al. (2013) found that U_{crit} in bridled monocle bream produced mean $\dot{M}O_2$ levels that were 23% higher than an AIR-type chase and 36% higher than a 15-min exhaustive chase. In contrast, Reidy et al. (1995) elicited up to 35% higher $\dot{M}O_2$ values chasing Atlantic cod to exhaustion compared with both U_{crit} and U_{max} treatments. These discrepancies highlight the importance of tailoring respirometry techniques to the study species. Additionally, the wide range of measurement windows used across studies (i.e., up to 10 min, where reported) may have also contributed to endorsement for one MMR treatment over another.

Although we found only a few differences in the observed mean MMR between the exhaustive exercise treatments, we show that the AIR treatment conferred the greatest statistical power and thereby required the smallest theoretical sample size (table 2). This is because individual variation in performance varied considerably across the different tests. For example, although mean MMR was virtually identical between groups, the standard deviation for the NO AIR treatment was nearly double that for the AIR treatment. Accordingly, the number of individuals necessary to identify population-level differences at a given resolution (or minimum detectable difference) more than doubles depending on the method selected to elicit MMR. We were not able to test for differences in the repeatability of MMR in this experiment because adult migrating salmon rapidly deteriorate in condition over time because of senescent processes. However, both interindividual variability and repeatability of various MMR protocols represent an important consideration when selecting an experimental approach. Repeatability for critical swimming speeds and the associated MMR tends to be high in Pacific salmon (e.g., Lee et al. 2003*b*; Eliason et al. 2013) and other fishes (e.g., Kolok 1999; Oufiero et al. 2009; Nelson et al. 2015; Auer et al. 2018). This appears to suggest that U_{crit}, U_{max}, and respective fatigue measures for MMR would also have high repeatability. Likewise, evidence from brown trout (*Salmo trutta*; Norin and Malte 2011) and lake sturgeon (*Acipenser fulvescens*; Svendsen et al. 2014) suggest repeatability of MMR as elicited via chase-type tests is high, although this has received far less attention.

The discrepancy in variance associated with the different chase tests is intriguing. For instance, whether you expose the fish to air following a chase means the difference between a sample size of 16 and 57 individuals (AIR and NO AIR, respectively) to detect a 15% difference in MMR (table 2). Although work in brook trout (*Salvelinus fontinalis*) suggests air exposures under 60 s likely do not affect exercise performance (Schreer et al. 2005), they represent acute bouts of hypoxia, causing increased lactate production, acidosis, osmoregulatory disruption, and tachycardia (elevated heart rate) upon reentry to the water (Ferguson and Tufts 1992; Cooke et al. 2001, 2015). The EXHAUST treatment also introduced more experimental variation than the AIR treatment, likely because techniques for visually assessing exhaustion during acute exercise are largely subjective and difficult to standardize between individuals. There were only minor differences in mean MMR between treatments, but the lower theoretical sample sizes required by the AIR treatment would favor experimental costs, time, labor, and animal welfare and regulatory commitments to reduce animal use (Curzer et al. 2016).

Conclusions

Field respirometry represents a rapidly growing technique to determine performance limits in wild species and populations in their natural habitat (e.g., Mochnacz et al. 2017). But common protocols that are staples to elicit MMR in the laboratory (e.g., critical swim tests) are often logistically too difficult to perform in the field. Here, we clearly demonstrate that chase treatments represent powerful alternatives to traditional swim tunnel respirometry. We caution, however, that the specific research question, life history of the focal species, chase type, and measurement window duration must remain careful considerations when selecting an experimental protocol. Going beyond simple comparisons of MMR, we show that methodology matters, particularly in terms of enhancing statistical power and reducing animal numbers. Specifically, we show that a 3-min chase with 1-min air exposure represented the best experimental protocol to elicit MMR in this particular system, requiring an even lower theoretical sample size than the traditional U_{crit} treatment. It should be noted, however, that Pacific salmon at this life stage represent unique physiological specimens, and while our more specific findings (i.e., optimal chase type, measurement window length, etc.) will not necessarily extend to other species, these parameters should be considered more generally. More importantly, though, this work suggests that metabolism may become more canalized with maximal sympathetic activation, regardless of the specific stimulus involved (e.g., exhaustive chase vs. exhaustive swim). Our findings thereby lend support to the growing use of post-chase respirometry to evaluate bioenergetics in species and populations, both in laboratory and in field settings.

Table 2: Estimates for theoretical sample size required to detect 10%, 15%, and 20% changes in mean maximum metabolic rate (MMR) using a power of 0.8 according to the optimal MMR value for each respective treatment

Minimum detectable difference (%)	U_{crit}	U_{crit} fat.	U_{max}	U_{max} fat.	AIR	NO AIR	EXHAUST
10	49	136	58	46	35	128	67
15	22	61	26	21	16	57	30
20	13	34	15	12	9	32	17

Note. U_{crit} = critical swim speed; U_{max} = maximum swim speed; fat. = fatigue.

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APPENDIX

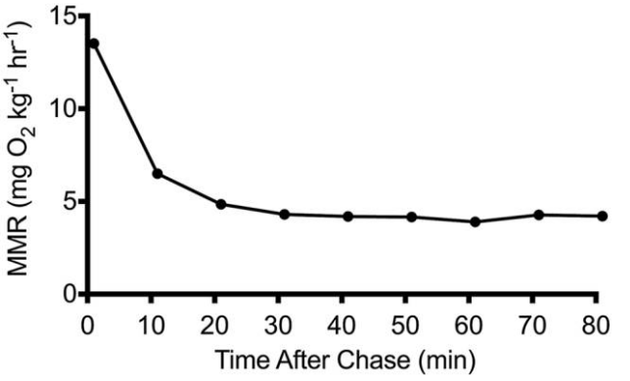

Figure A1. Representative postchase (AIR) Mo_2 trace for a 1.275-kg female. MMR = maximum metabolic rate.

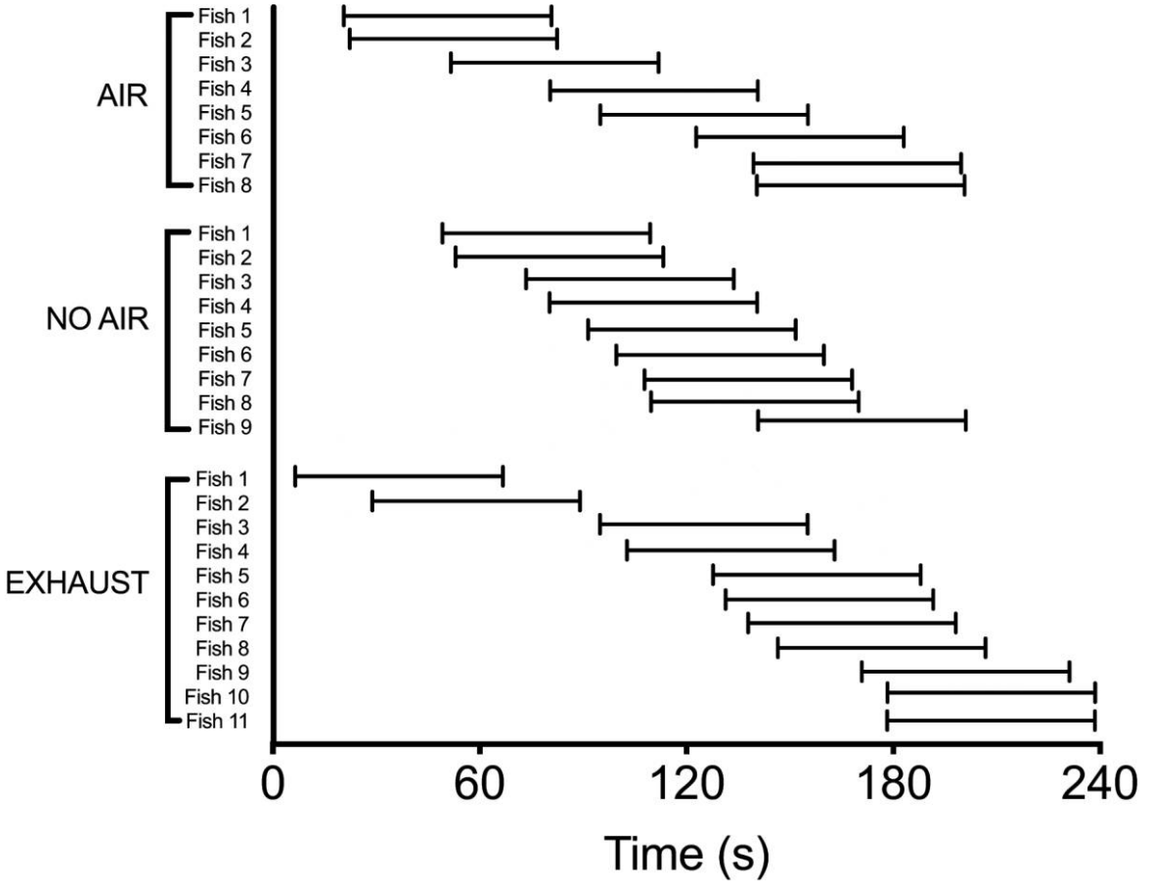

Figure A2. Time points that maximum metabolic rate was identified within the 4-min measurement period for each fish in the chase treatments. The steepest 60-s measurement windows (black lines) within each treatment occur throughout the total measurement period (i.e., 240 s) rather than at the beginning, which would be expected for quick equilibration of oxygen stores following exercise- and air-induced hypoxia. Note that the fish identification number does not reflect the order in which they were swum.

Table A1: Post hoc statistical values for mixed effects linear models using treatment and measurement window length (optimal or full) as fixed factors

Treatment 1	Window	Treatment 2	Window	$P_{\text{Bonferoni}}$
AIR	Optimal	AIR	Full	<.001
NO AIR	Optimal	NO AIR	Full	<.001
EXHAUST	Optimal	EXHAUST	Full	<.001
U_{crit}	Optimal	U_{crit}	Full	1.000
U_{crit} fat.	Optimal	U_{crit} fat.	Full	.163
U_{max}	Optimal	U_{max}	Full	.737
U_{max} fat.	Optimal	U_{max} fat.	Full	1.000
AIR	Optimal	NO AIR	Full	.630
		EXHAUST	Optimal	1.000
		U_{crit}	Optimal	1.000
		U_{crit} fat.	Optimal	.006
		U_{max}	Optimal	1.000
		U_{max} fat.	Optimal	<.001
NO AIR	Optimal	EXHAUST	Optimal	1.000
		U_{crit}	Optimal	<.001
		U_{crit} fat.	Optimal	<.001
		U_{max}	Optimal	<.001
		U_{max} fat.	Optimal	<.001
EXHAUST	Optimal	U_{crit}	Optimal	.214
		U_{crit} fat.	Optimal	<.001
		U_{max}	Optimal	.073
		U_{max} fat.	Optimal	<.001
U_{crit}	Optimal	U_{crit} fat.	Optimal	.036
		U_{max}	Optimal	1.000
		U_{max} fat.	Optimal	.089
U_{crit} fat.	Optimal	U_{max}	Optimal	1.000
		U_{max} fat.	Optimal	1.000
U_{max}	Optimal	U_{max} fat.	Optimal	<.001

Note. U_{crit} = critical swim speed; U_{max} = maximum swim speed; fat. = fatigue.

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