

COMMENTARY

Estimating maximum oxygen uptake of fishes during swimming and following exhaustive chase – different results, biological bases and applications

Bernard B. Rees^{1,*}, Jessica E. Reemeyer², Sandra A. Binning³, Samantha D. Brieske¹, Timothy D. Clark⁴, Jeremy De Bonville³, Rachel M. Eisenberg⁵, Graham D. Raby⁶, Dominique Roche⁷, Jodie L. Rummer⁸ and Yangfan Zhang⁹

ABSTRACT

The maximum rate at which animals take up oxygen from their environment ($\dot{M}_{O_2,max}$) is a crucial aspect of their physiology and ecology. In fishes, $\dot{M}_{O_2,max}$ is commonly quantified by measuring oxygen uptake either during incremental swimming tests or during recovery from an exhaustive chase. In this Commentary, we compile recent studies that apply both techniques to the same fish and show that the two methods typically yield different mean estimates of $\dot{M}_{O_2,max}$ for a group of individuals. Furthermore, within a group of fish, estimates of $\dot{M}_{O_2,max}$ determined during swimming are poorly correlated with estimates determined during recovery from chasing (i.e. an individual's $\dot{M}_{O_2,max}$ is not repeatable across methods). One explanation for the lack of agreement is that these methods measure different physiological states, each with their own behavioural, anatomical and biochemical determinants. We propose that these methods are not directly interchangeable but, rather, each is suited to address different questions in fish biology. We suggest that researchers select the method that reflects the biological contexts of their study, and we advocate for the use of accurate terminology that acknowledges the technique used to elevate \dot{M}_{O_2} (e.g. peak $\dot{M}_{O_2,swim}$ or peak $\dot{M}_{O_2,recovery}$). If the study's objective is to estimate the 'true' $\dot{M}_{O_2,max}$ of an individual or species, we recommend that pilot studies compare methods, preferably using repeated-measures designs. We hope that these recommendations contribute new insights into the causes and consequences of variation in $\dot{M}_{O_2,max}$ within and among fish species.

KEY WORDS: Respirometry, Aerobic metabolism, Swim tunnel, Exhaustive chase, Individual variation

Introduction

The maximum rate at which an aquatic animal can take up oxygen from its environment is its $\dot{M}_{O_2,max}$ (see Glossary). This rate reflects the upper limit for the extraction and delivery of oxygen to tissues to support aerobic metabolism and it is positively associated with the capacity for sustained aerobic performance (Claireaux et al., 2005; Norin and Clark, 2016). In aquatic ectotherms, especially fishes, $\dot{M}_{O_2,max}$ is commonly used as a proxy for maximum metabolic rate (MMR; see Glossary), specifically the aerobic component of MMR (Box 1). In addition, $\dot{M}_{O_2,max}$ sets the upper boundary of an animal's scope for metabolic activity (Fry, 1947) or aerobic scope (AS; see Glossary) (Clark et al., 2013). AS reflects the animal's capacity to aerobically fuel activities such as foraging, digestion, predator avoidance, migration, growth and reproduction (Fry, 1947; Priede, 1985; Claireaux and Lefrancois, 2007; Clark et al., 2013). A higher AS, indicating a greater capacity to support these activities, may enhance Darwinian fitness in natural populations (Claireaux and Lefrancois, 2007). Conversely, if AS is reduced by a decrease in $\dot{M}_{O_2,max}$ (or an increase in standard metabolic rate, SMR; see Glossary), energetic trade-offs among processes that 'use' AS could ensue (Killen et al., 2007; Farrell, 2009; Holt and Jørgensen, 2015; Eliason and Farrell, 2016; Farrell, 2016; Metcalfe et al., 2016). Although the time an animal spends at or near its $\dot{M}_{O_2,max}$ is likely to be short and species dependent (Priede, 1977; Farrell, 2016), its activities during this time (e.g. predator avoidance, migration or active foraging) might be disproportionately important to its survival. Hence, a high $\dot{M}_{O_2,max}$ and, by extension, an expanded AS are thought to benefit individuals, populations and species.

Methods to determine $\dot{M}_{O_2,max}$ in fishes

The Fick equation (see Glossary) states that \dot{M}_{O_2} (see Glossary) is a function of cardiac output and the difference in oxygen content of arterial and venous blood. Thus, methods to measure $\dot{M}_{O_2,max}$ should elicit rates of tissue oxygen consumption that reach the cardiorespiratory limits for oxygen extraction and delivery to the tissues (Jones and Randall, 1978; Rummer and Brauner, 2015; Scott and Dalziel, 2021; Rees et al., 2022). The two most common methods to estimate $\dot{M}_{O_2,max}$ in fishes are to use intermittent-flow respirometry (see Glossary) to measure \dot{M}_{O_2} either (1) as a fish swims at increasing speeds against an imposed current in a swim tunnel or (2) during recovery after an exhaustive chase (Clark et al., 2013; Rummer et al., 2016; Svendsen et al., 2016; Norin and Clark, 2016; Killen et al., 2017). Although we focus on these two methods, higher \dot{M}_{O_2} values can be attained under other conditions in certain species (see 'Matching method to biology', below).

Swim tunnel respirometry measures the \dot{M}_{O_2} required to support the elevated aerobic metabolism of vigorous, sustained swimming,

¹Department of Biological Sciences, University of New Orleans, New Orleans, LA 70148, USA. ²Department of Biology, McGill University, Montreal, QC, Canada H3A 1B1. ³Département de Sciences Biologiques, Université de Montréal, Montréal, QC, Canada H2V 0B3. ⁴School of Life and Environmental Science, Deakin University, Geelong, Victoria, Australia 3216. ⁵Department of Zoology, University of British Columbia, Vancouver, BC, Canada V6T 1Z4. ⁶Department of Biology, Trent University, Peterborough, ON, Canada K9L 0G2. ⁷Social Sciences and Humanities Research Council of Canada, Ottawa, ON, Canada K1R 0E3. ⁸College of Science and Engineering, James Cook University, Townsville, QLD 4811, Australia. ⁹Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA.

*Author for correspondence (brees@uno.edu)

© B.B.R., 0000-0001-5636-1700; J.E.R., 0000-0002-0081-2573; J.D.B., 0000-0002-6072-1833; D.R., 0000-0002-3326-864X; J.L.R., 0000-0001-6067-5892; Y.Z., 0000-0001-5625-6409

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution and reproduction in any medium provided that the original work is properly attributed.

Glossary

Aerobic scope (AS)

A metric that relates $\dot{M}_{O_2, \max}$ to SMR and is a measure of the capacity for aerobic activities above maintenance levels. Absolute aerobic scope is the difference between these two rates and has the same units as \dot{M}_{O_2} , whereas factorial aerobic scope is the ratio of $\dot{M}_{O_2, \max}$ to SMR and is unitless.

Fick equation

An equation stating that \dot{M}_{O_2} equals the product of cardiac output and the arteriovenous difference in oxygen content.

Intermittent-flow respirometry

A technique for determining \dot{M}_{O_2} in which the animal chamber alternates between a closed phase, during which the rate of oxygen decline in the chamber is measured, and an open phase, when the chamber is flushed with water to restore oxygen levels and remove metabolic wastes. This technique can be automated and multiplexed, allowing long-term \dot{M}_{O_2} measurements of several individuals simultaneously (Clark et al., 2013; Svendsen et al., 2016; Killen et al., 2021; Clark, 2022).

\dot{M}_{O_2}

The rate of oxygen uptake by an animal expressed in units of mass or moles of oxygen per unit time. This term is commonly used for aquatic animals, as opposed to the volume of oxygen taken up ($\dot{V}_{O_2, \max}$), which is more common for terrestrial animals.

$\dot{M}_{O_2, \max}$

The maximum rate of oxygen uptake, reflecting the maximum oxygen flux across the respiratory surfaces of an animal from its environment.

Maximum metabolic rate (MMR)

The theoretical maximum rate of energy expenditure by an animal. In most applications, MMR refers to the maximum aerobic metabolic rate, even though vigorous activity by animals generally relies upon anaerobic processes (e.g. glycolysis) to supplement ATP produced by oxidative phosphorylation (Nelson, 2016).

Peak \dot{M}_{O_2}

The highest \dot{M}_{O_2} determined using a specific protocol. For example, peak $\dot{M}_{O_2, \text{swim}}$ is the highest \dot{M}_{O_2} measured during an incremental swim test; whereas, peak $\dot{M}_{O_2, \text{recovery}}$ is the highest \dot{M}_{O_2} measured during recovery from exhaustion. This terminology can be extended to other physiological states of elevated \dot{M}_{O_2} (e.g. digestion; Steell et al., 2019).

Standard metabolic rate (SMR)

The minimum rate of energy expenditure by an animal required for maintenance at a given temperature. In fishes, SMR is generally estimated as the lowest stable \dot{M}_{O_2} of a quiescent, post-absorptive animal when measured over an extended period (18–48 h) and is sometimes referred to as $\dot{M}_{O_2, \min}$ (Chabot et al., 2016b).

primarily powered by contraction of red skeletal muscle and largely fuelled by oxidative phosphorylation. The rate of mitochondrial oxygen consumption, and therefore whole-animal \dot{M}_{O_2} (assuming mitochondrial and organismal oxygen uptake are in a steady state; Box 1), is largely to replenish ATP that is consumed by skeletal muscle cross-bridge cycling and calcium ion regulation (Hoppeler, 2018). In addition, heart rate and ventilation rate are elevated, although ram ventilation reduces the energetic costs of gill ventilation during high-speed swimming in some species (Steffensen, 1985). $\dot{M}_{O_2, \max}$ is estimated as the highest \dot{M}_{O_2} (peak \dot{M}_{O_2} ; see Glossary) as the fish swims (i.e. peak $\dot{M}_{O_2, \text{swim}}$) at increasing water speeds until exhaustion (U_{crit} test; Brett, 1964) or during more rapid and dynamic increases in water speed (U_{max} test; Clark et al., 2011; Raby et al., 2020). Forced locomotor activity was the first technique used to measure $\dot{M}_{O_2, \max}$ in fishes (Blazka et al., 1960; Brett, 1964; Steffensen et al., 1984), and it is still considered by many to be the ‘gold standard’ for determining $\dot{M}_{O_2, \max}$. However, swim tunnel respirometry is time consuming and requires continuous monitoring, which reduces experimental throughput. It is also cumbersome to deploy in remote field conditions, and some fishes either are poor sustained swimmers or cannot be coaxed to perform in a swim tunnel (Clark, 2022).

Box 1. Is $\dot{M}_{O_2, \max}$ equal to MMR?

$\dot{M}_{O_2, \max}$, the maximum rate at which animals take up oxygen from their environment, is frequently used as proxy of maximum metabolic rate (MMR). Doing so relies upon several seldom-evaluated assumptions (Nelson, 2016; Zhang and Gilbert, 2017), including the following.

- (1) Oxygen uptake at the respiratory surface is in steady state with oxygen transport to the tissues and oxygen consumption by the mitochondria. This assumption is probably valid during sustained activity but not necessarily during transitions between activity levels (Farrell and Clutterham, 2003). Hence, measurements of \dot{M}_{O_2} over brief intervals could fail to reflect tissue oxygen consumption.
- (2) Oxidative phosphorylation is the exclusive source of ATP production. At high activity levels, oxygen delivery to skeletal muscle does not match ATP demand, whereupon anaerobic metabolic pathways (e.g. glycolysis) are recruited to supplement oxidative phosphorylation (Burgetz et al., 1998), and \dot{M}_{O_2} underestimates metabolic rate.
- (3) The ratio of ATP production to oxygen consumed (P:O ratio) by mitochondrial oxidative phosphorylation is constant. Because metabolic rate ultimately reflects the rate of ATP turnover, changes in the P:O ratio would alter the relationship between \dot{M}_{O_2} and metabolic rate. The P:O ratio depends upon the substrate used for mitochondrial metabolism (Hinkle, 2005) and it varies according to physiological state and ecological conditions (Koch et al., 2021; Le Roy et al., 2021; Thorall et al., 2021; Voituron et al., 2022).

In the strictest sense, therefore, metabolic rates (including MMR), are most directly measured as the rate of heat production, i.e. by calorimetry (Regan et al., 2013, 2017). Currently, constraints of calorimeter design generally make this technique impractical for metabolic rate measurements of large, active animals, and it is especially challenging for aquatic animals because of the large heat capacity of water.

Because of these and other caveats, rather than equate $\dot{M}_{O_2, \max}$ with MMR, it may be more useful (and accurate) to use $\dot{M}_{O_2, \max}$ for what it is: the maximum rate of oxygen uptake (Nelson, 2016; Zhang and Gilbert, 2017).

Because of these challenges, $\dot{M}_{O_2, \max}$ has also been estimated as the highest \dot{M}_{O_2} during recovery from an exhaustive chase (hereafter, peak $\dot{M}_{O_2, \text{recovery}}$) (Soofiani and Priede, 1983; Reidy et al., 1995). Typically, fish are chased by the experimenter in a circular arena and, in some cases, held in air briefly (e.g. 1 min) prior to commencing intermittent-flow respirometry in a chamber with limited volume and minimal water movement (‘static’ chambers). The fish’s activity during the chase is supported by both oxidative phosphorylation and anaerobic metabolism (glycolysis and creatine phosphate hydrolysis). During recovery, very little \dot{M}_{O_2} is used for locomotion, but rather, \dot{M}_{O_2} remains elevated as a result of reoxygenation of internal oxygen stores (haemoglobin and myoglobin), persistently elevated cardiac activity and ventilation (without the benefit of ram ventilation), re-establishment of pH and ion balance and the clearance of anaerobic end-products (Wood, 1991; Moyes et al., 1992; Scarabello et al., 1992). Although \dot{M}_{O_2} is expected to decline exponentially after the chase, in some cases peak $\dot{M}_{O_2, \text{recovery}}$ is not achieved until hours later (Clark et al., 2012; Soofiani and Priede, 1983; Andersson et al., 2020; Brieske et al., 2024). Moreover, the extent to which \dot{M}_{O_2} is elevated depends upon how the chase is performed (Reidy et al., 1995; Roche et al., 2013; Zhang et al., 2018), presumably reflecting different degrees of metabolic and cellular disturbance. Nevertheless, the chase method is typically easier and quicker to conduct than swim tunnel respirometry, allowing several individuals to be tested in parallel, thereby increasing experimental throughput (Norin and Malte, 2011; Salin et al., 2016; Reemeyer and Rees, 2020). In addition, the

apparatus is more portable, facilitating experiments in remote locations (e.g. Little et al., 2020).

Rationale and dataset for this Commentary

Despite the differences in the biological processes measured and apparatus used by these two methods, a meta-analysis of data from 121 species of fishes differing in lifestyle (i.e. benthic, benthopelagic, pelagic) did not detect a systematic difference between peak $\dot{M}_{O_2,swim}$ and peak $\dot{M}_{O_2,recovery}$ (Killen et al., 2017). This conclusion was largely based on values of $\dot{M}_{O_2,swim}$ and $\dot{M}_{O_2,recovery}$ measured on different individuals and, in some cases, determined in different studies. This conclusion differs from those of a number of studies, many of which used both approaches on the same individuals, showing that peak $\dot{M}_{O_2,swim}$ can be substantially higher than peak $\dot{M}_{O_2,recovery}$ (Roche et al., 2013; Rummer et al., 2016; Hvas and Oppedal, 2019; Slesinger et al., 2019; Raby et al., 2020; Eisenberg et al., 2024; Brieske et al., 2024). In other species,

peak $\dot{M}_{O_2,swim}$ is markedly lower than peak $\dot{M}_{O_2,recovery}$ (Soofiani and Priede, 1983). Thus, the degree to which these methods yield similar estimates of $\dot{M}_{O_2,max}$ remains unclear.

Additionally, several studies have documented that an individual's peak \dot{M}_{O_2} is repeatable when determined in two or more trials of either swim tunnel respirometry or recovery from an exhaustive chase (Reidy et al., 2000; Marras et al., 2010; Norin and Malte, 2011; Killen et al., 2016; Norin et al., 2016; Reemeyer and Rees, 2020; Brieske et al., 2024). These results are important for two reasons. First, the repeatability of the technique is a measure of its precision. Second, repeatability of peak \dot{M}_{O_2} over time suggests that it is a stable feature of the individual, and thus potentially subject to evolution by natural selection (see Roche et al., 2016). However, whether an individual's peak \dot{M}_{O_2} is repeatable when measured by different techniques has received considerably less attention (but see Zhang et al., 2020; Brieske et al., 2024; Eisenberg et al., 2024).

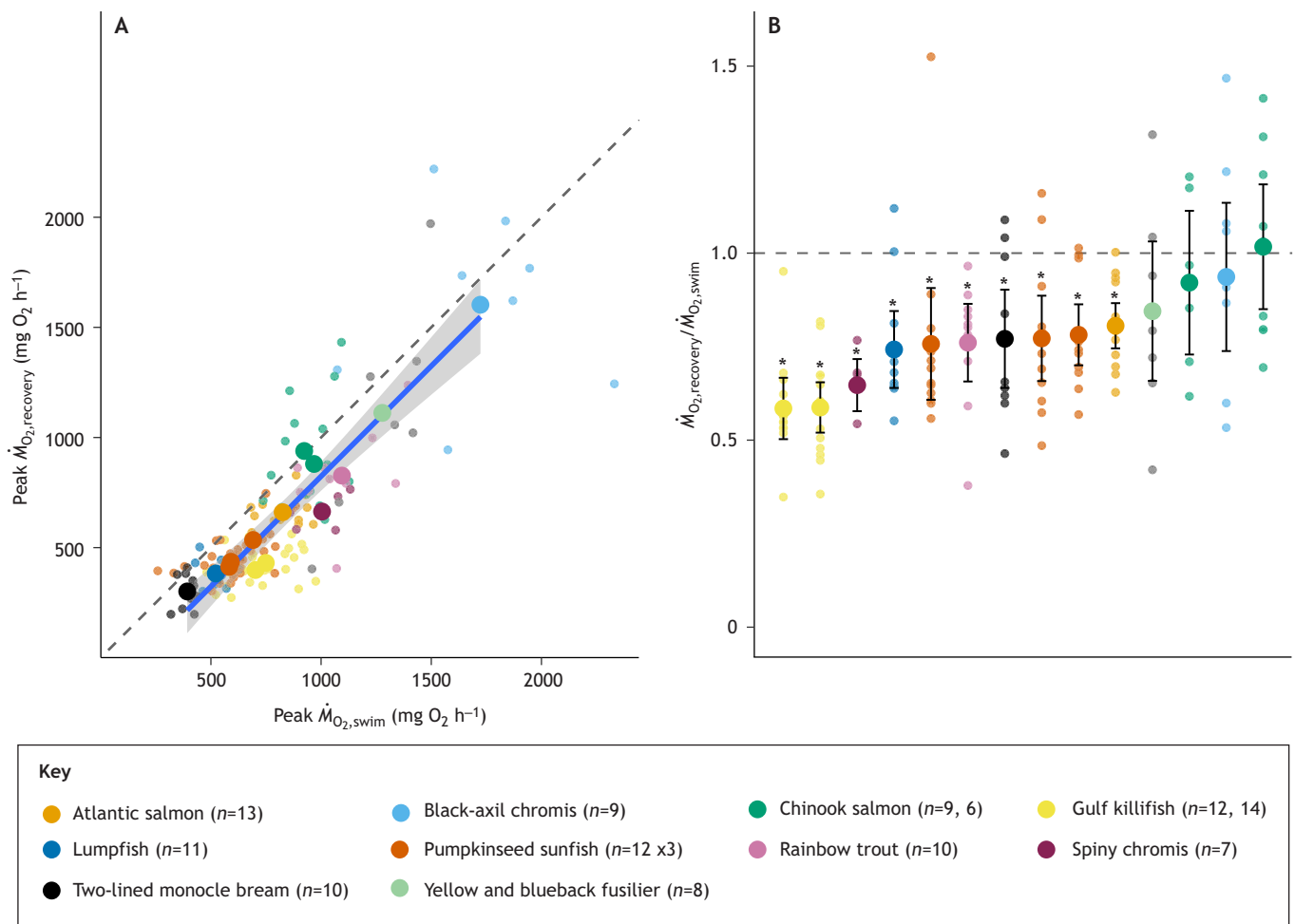


Fig. 1. Agreement between peak rate of oxygen uptake of fishes determined during swim tunnel respirometry ($\dot{M}_{O_2,swim}$) and during recovery from an exhaustive chase ($\dot{M}_{O_2,recovery}$). (A) A scatterplot of peak $\dot{M}_{O_2,recovery}$ and $\dot{M}_{O_2,swim}$ determined in 14 repeated measures trials with 10 species (the key shows sample sizes per trial; multiple trials with a single species are indicated with corresponding sample sizes). \dot{M}_{O_2} was normalized to a common temperature (20°C) and body mass (1.0 kg) as in Killen et al. (2017), except that the relationship between \dot{M}_{O_2} , mass and temperature for the current dataset was described by the equation $\log_{10} \dot{M}_{O_2} = -0.755 + 1.071 \times \log_{10} \text{body mass} + 0.0176 \times \text{temperature}$. Large coloured circles correspond to the mean for that trial and smaller coloured circles show values for individual fish. The blue line represents the line of best fit (least-squares linear regression) and the grey shaded area indicates the 95% confidence interval (CI). The equation of the line is $\dot{M}_{O_2,recovery} = 1.00(\dot{M}_{O_2,swim}) - 172$. The 95% CI of the slope is 0.82 to 1.17, and the 95% CI of the intercept is -332 to -11. $r^2 = 0.93$. The dashed line shows the line of equality. (B) The ratio of $\dot{M}_{O_2,recovery}$ to $\dot{M}_{O_2,swim}$. Large coloured circles correspond to the mean for that trial and smaller coloured circles show values for individual fish. The dashed line at 1.0 indicates $\dot{M}_{O_2,recovery} = \dot{M}_{O_2,swim}$. Experimental trials are displayed along the x-axis in order of ascending values of this ratio. Error bars show the 95% CI of the mean ratio and asterisks indicate significant differences between $\dot{M}_{O_2,recovery}$ and $\dot{M}_{O_2,swim}$ ($P < 0.05$, paired t -tests; see Table S1 for individual P -values). See Table S1 for references.

In this Commentary, therefore, we directly compare estimates of peak \dot{M}_{O_2} when the same individuals were used both in swim tunnel respirometry and during recovery from an exhaustive chase (i.e. using repeated-measures protocols). We asked (1) whether mean estimates of peak \dot{M}_{O_2} determined by these two approaches were the same, and (2) whether an individual's peak \dot{M}_{O_2} determined in swim tunnel respirometry was correlated with its peak \dot{M}_{O_2} after an exhaustive chase (i.e. repeatable across techniques). We extracted data from 10 species representing various fish lineages and habitats, used in 14 experimental trials, conducted over a range of experimental conditions (temperature, salinity and apparatus) (Table S1). All data were published previously (references in Table S1), except for those for pumpkinseed sunfish (*Lepomis gibbosus*). Sunfish were collected, housed and acclimated for 4 weeks to one of three temperatures (20, 25 and 30°C; $n=12$ each) as described in De Bonville et al. (2024). At each temperature, peak $\dot{M}_{O_2,swim}$ and peak $\dot{M}_{O_2,recovery}$ were determined essentially as described in Binning et al. (2013) and Guitard et al. (2022), respectively. These experiments were approved by Université de Montréal's animal care committee (Comité de déontologie de l'expérimentation sur les animaux; certificate number 22-025).

Paired comparisons show differences in mean and individual peak \dot{M}_{O_2} estimates

Mean values for peak $\dot{M}_{O_2,recovery}$ are plotted against peak $\dot{M}_{O_2,swim}$ in Fig. 1A. For this comparison, \dot{M}_{O_2} was normalized to a common body size (1 kg) and temperature (20°C) (Killen et al., 2017). Although the slope of the relationship was not significantly different from 1.0 (95% confidence intervals, 0.82 to 1.17), the intercept differed from zero (95% confidence intervals, -332 to -11 mg O₂ h⁻¹) and the large majority of individual \dot{M}_{O_2} measurements fell below the line of unity, suggesting that peak $\dot{M}_{O_2,recovery}$ was less than peak $\dot{M}_{O_2,swim}$. This suggestion was reinforced when each individual's peak $\dot{M}_{O_2,recovery}$ was expressed as a proportion of its peak $\dot{M}_{O_2,swim}$ (Fig. 1B). The mean ratios of peak $\dot{M}_{O_2,recovery}$ to peak $\dot{M}_{O_2,swim}$ ranged from 0.57 to 1.01 (median 0.765), and for 10 of 14 experimental trials the 95% confidence intervals for this ratio did not overlap one, meaning that peak $\dot{M}_{O_2,recovery}$ was significantly less than peak $\dot{M}_{O_2,swim}$ ($P<0.05$, paired t -tests; see Table S1 for individual P -values). Thus, for most species and experimental conditions examined here, peak $\dot{M}_{O_2,recovery}$ was substantially lower

than peak $\dot{M}_{O_2,swim}$ (up to ~40% lower; median 23.5%). Because these comparisons were paired (i.e. made on the same individuals under defined conditions), this conclusion is not influenced by differences in body size, temperature or other experimental variables.

When peak $\dot{M}_{O_2,swim}$ and peak $\dot{M}_{O_2,recovery}$ were measured in the same individuals, they were generally weakly related or unrelated to one another (Fig. S1). Values for Pearson's correlation coefficients comparing these two metrics within an experiment ranged from -0.25 to 0.93 (median 0.32), and in only two of 14 experimental trials were the correlations between peak $\dot{M}_{O_2,swim}$ and peak $\dot{M}_{O_2,recovery}$ significant (Fig. 2A; see Table S1 for individual P -values). The same pattern arose when each individual's rank within the group was assessed. Spearman's rank order correlation coefficients (ρ) ranged from -0.10 to 0.88 (median 0.29), and in only one experimental trial was the correlation between individual ranks statistically significant (Fig. 2B; see Table S1 for individual P -values). Perhaps peak \dot{M}_{O_2} is simply poorly repeatable for the species included in these analyses. This possibility was directly addressed by Brieske et al. (2024), who found that the repeatability of peak $\dot{M}_{O_2,swim}$ of Gulf killifish, *Fundulus grandis*, was high in two trials of swim tunnel respirometry (Pearson's $r=0.67$, $P<0.05$), as was the repeatability of peak $\dot{M}_{O_2,recovery}$ in replicate trials of recovery after an exhaustive chase (Pearson's $r=0.79$, $P<0.01$). For the same fish, however, peak $\dot{M}_{O_2,swim}$ was unrelated to peak $\dot{M}_{O_2,recovery}$ ($r<0.30$, $P>0.25$; Fig. 2A; Table S1, Fig. S1). This study clearly showed that peak \dot{M}_{O_2} may be consistent when assessed by a given method yet be unrelated across methods. Thus, for the species and contexts studied here, an individual's peak $\dot{M}_{O_2,swim}$ has little to no bearing on the same individual's peak $\dot{M}_{O_2,recovery}$.

Experimental design does not explain these differences

Several experimental design considerations affect the accuracy, precision and temporal resolution of \dot{M}_{O_2} measurements by intermittent-flow respirometry (Clark et al., 2013; Svendsen et al., 2016). Accordingly, we tabulated several features of the experimental designs used in the studies compiled here (Table S1), and asked whether these features were related to either the agreement between mean values or the repeatability of an individual's peak \dot{M}_{O_2} when assessed by these two approaches (Fig. S2).

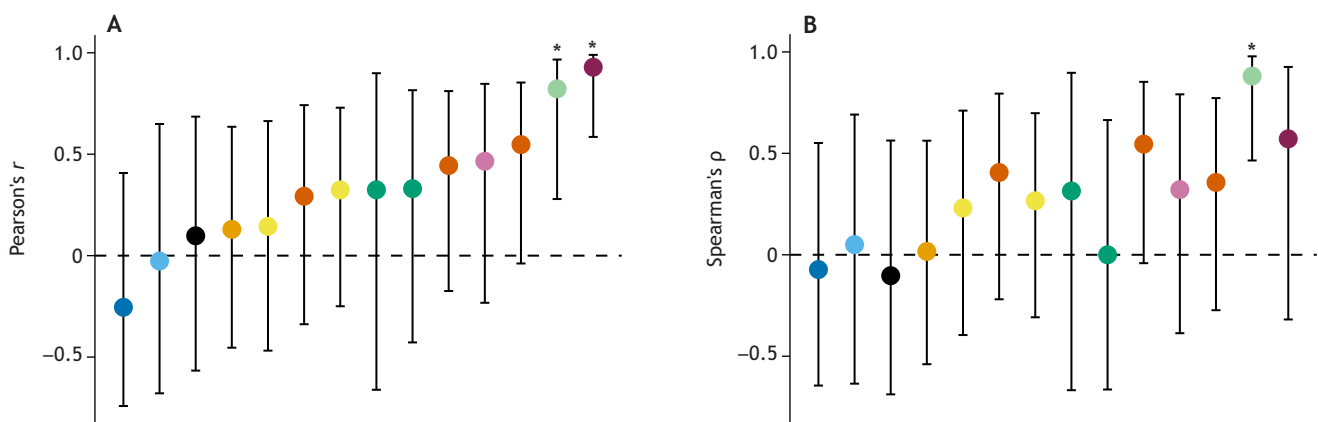
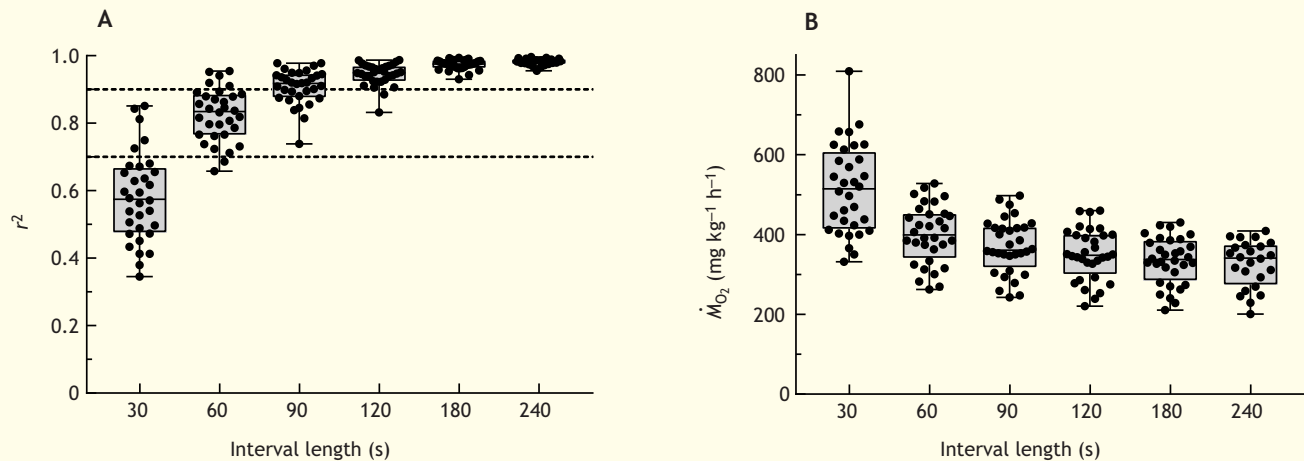


Fig. 2. Repeatability of peak \dot{M}_{O_2} estimates for individual fish determined during swim tunnel respirometry ($\dot{M}_{O_2,swim}$) and during recovery from an exhaustive chase ($\dot{M}_{O_2,recovery}$). (A) Pearson's correlation coefficients (r) for the relationship between peak $\dot{M}_{O_2,swim}$ and peak $\dot{M}_{O_2,recovery}$. Experimental trials are displayed along the x-axis in order of ascending r . (B) Spearman's rank order correlation coefficients (ρ) in the same species order as in A. Error bars represent the 95% CI of the coefficients and asterisks indicate significant correlations ($P<0.05$; see Table S1 for individual P -values). See Fig. 1 for species colour codes and trial sample sizes. See Table S1 for references.

Box 2. Increasing the temporal resolution of \dot{M}_{O_2} determination

The time required to accurately determine \dot{M}_{O_2} during intermittent-flow respirometry depends upon the rate of oxygen uptake, the sensitivity of the measurement device and respirometer design (Svendsen et al., 2016). Commonly, \dot{M}_{O_2} is measured over intervals of 3–20 min, which results in an average rate over the entire interval. However, \dot{M}_{O_2} is dynamic during swimming or recovery from an exhaustive chase, and accurate estimates of peak \dot{M}_{O_2} require a technique that confidently captures the highest rate. In 'sliding window' or 'rolling' regression, the decline in oxygen is determined over the shortest sampling window that achieves an adequate level of precision (Zhang et al., 2019; 2020; Little et al., 2020; Prinzing et al., 2021). This is done by fitting linear regressions to intervals of increasing duration, and for each duration, the slopes over all possible intervals are determined (i.e. each advancing by the sampling frequency of the sensor) (<https://github.com/boennecd/rollRegres>).



In the example shown above, dissolved oxygen concentrations were recorded during recovery from an exhaustive chase of the Gulf killifish, *Fundulus grandis* (two trials, $n=16$ each) (Brieske et al., 2024). The rate of oxygen decline was determined over all possible intervals ranging from 30 to 240 s, each advancing by 1 s (e.g. 211×30 s intervals; 1–30, 2–31...211–240). For each interval length, the single highest slope was used to determine \dot{M}_{O_2} . A 90 s sampling window had a median $r^2 > 0.90$ (see figure, panel A, upper dotted line) and all trials had $r^2 > 0.70$ (panel A, lower dotted line). The mean \dot{M}_{O_2} measured over 90 s was 14% higher than that determined over the entire 4 min period (panel B). Shorter intervals yielded higher estimates of \dot{M}_{O_2} , but they were less precise. At very short intervals (≤ 30 s), the range of \dot{M}_{O_2} estimates may include negative values (Zhang et al., 2020), clearly indicating that shorter measurement intervals can yield spurious estimates. Ultimately, the choice of the appropriate sampling interval strikes a balance between precision and the ability to capture transiently elevated \dot{M}_{O_2} .

Of four attributes related to the species employed in these studies (taxonomic group, habitat, life stage and swimming ability) (Fig. S2A–D), only taxonomic group was related to how well mean peak \dot{M}_{O_2} estimates agreed between techniques. The four salmonid species, on average, had a higher ratio of peak $\dot{M}_{O_{2, \text{recovery}}}$ to peak $\dot{M}_{O_{2, \text{swim}}}$ than non-salmonids ($P=0.047$, Mann–Whitney U -test; Fig. S2A). This observation is consistent with Little et al. (2020) who found that swim tunnel respirometry and exhaustive chase generally produced similar estimates of peak \dot{M}_{O_2} in Coho salmon (*Oncorhynchus kisutch*). While the salmonids studied here had somewhat better agreement between peak \dot{M}_{O_2} determined by these methods than non-salmonids, it is important to note that peak $\dot{M}_{O_{2, \text{recovery}}}$ was, nevertheless, statistically lower than peak $\dot{M}_{O_{2, \text{swim}}}$ in two of the four trials with salmonids (Fig. 1; Table S1). Also, the repeatability of peak \dot{M}_{O_2} across these methods was poor for salmonids and non-salmonids alike (Fig. S2A).

Of six attributes related to experimental design (Fig. S2E–J), only the ratio of respirometer chamber volume to fish mass during the chase method was related to the agreement between peak \dot{M}_{O_2} estimates. When the static chamber used in intermittent-flow respirometry was larger relative to the size of the fish (i.e. chamber volume to fish mass ratio > 50), peak $\dot{M}_{O_{2, \text{recovery}}}$ and peak $\dot{M}_{O_{2, \text{swim}}}$ agreed better than when smaller chambers were used ($P=0.047$, Mann–Whitney U -test; Fig. S2I). It could be that larger static chambers allow greater room for fish locomotion, thus elevating peak $\dot{M}_{O_{2, \text{recovery}}}$ (Peake and Farrell, 2004). However, this comparison is tempered by three considerations. First, three of four trials with salmonids used larger respirometer chambers, thus

confounding chamber volume with taxonomic group (see above). Second, chambers with volume to fish mass ratios < 50 are generally recommended for intermittent-flow respirometry because of better temporal resolution and signal to noise ratios (Svendsen et al., 2016). Third, the repeatability of peak \dot{M}_{O_2} across these methods was equally poor regardless of the chamber volume to fish mass ratio (Fig. S2I).

Other experimental design features reported to influence peak $\dot{M}_{O_{2, \text{recovery}}}$ [e.g. time elapsed between the chase and the start of respirometry (Fig. S2G) and whether fish are exposed to air (Fig. S2H)] failed to explain the differences noted in either the ratio of peak $\dot{M}_{O_{2, \text{recovery}}}$ to peak $\dot{M}_{O_{2, \text{swim}}}$ or the repeatability of an individual's peak \dot{M}_{O_2} . Finally, in nine of 14 experiments, the two methods were applied in a random order (Table S1), suggesting that these results were not biased by factors such as training, fatigue or duration of laboratory maintenance. Therefore, differences in the mean and repeatability of peak \dot{M}_{O_2} determined by swim tunnel respirometry compared with exhaustive chase were not obviously related to the species or experimental conditions employed in the studies examined here. Rather, we attribute these differences to the fact that peak $\dot{M}_{O_{2, \text{swim}}}$ and peak $\dot{M}_{O_{2, \text{recovery}}}$ reflect different physiological states, each with their own underlying determinants and ranges of variation among individuals.

Improving methods to measure peak \dot{M}_{O_2}

Because peak \dot{M}_{O_2} is dynamic and context dependent, devices and analytical techniques must be able to capture transiently elevated rates. Given the high sampling frequency of oxygen sensors and

Box 3. Recommendations for studying peak \dot{M}_{O_2} in fishes

We offer the following recommendations to consider when designing or interpreting studies of elevated aerobic metabolism in fishes.

- (1) Different methods of elevating a fish's \dot{M}_{O_2} will likely yield different estimates of peak \dot{M}_{O_2} and a different order of individual \dot{M}_{O_2} values within a group. Thus, one should use the method for determining peak \dot{M}_{O_2} that best suits the biology of the organism and the question of interest.
- (2) If the goal of a study is to estimate the 'true' $\dot{M}_{O_2,max}$ for an individual or a species, we recommend comparing peak \dot{M}_{O_2} determined by different methods. Ideally, such comparisons would employ repeated measurements on the same individuals, randomized trial order and adequate sample sizes (ideally $n \geq 20$) to robustly discriminate among methods.
- (3) When relating the peak \dot{M}_{O_2} to other traits measured on the same individuals or species, these relationships may depend upon the method used to determine peak \dot{M}_{O_2} (Lawrence et al., 2023; Brieske et al., 2024). If peak $\dot{M}_{O_2,swim}$ and $\dot{M}_{O_2,recovery}$ reflect different physiological states, then the strength of their correlation to other behavioural, anatomical, biochemical and genetic traits will almost certainly differ.
- (4) Follow current recommendations for designing and conducting respirometry experiments (e.g. Clark et al., 2013; Svendsen et al., 2016; Killen et al., 2021; Clark, 2022), and include 'rolling' regression with a conservative minimum sampling window to estimate dynamic changes in peak \dot{M}_{O_2} (Box 2; Zhang et al., 2020).
- (5) Finally, employ consistent terminology that accurately reflects the method used to elevate \dot{M}_{O_2} (e.g. peak $\dot{M}_{O_2,swim}$, peak $\dot{M}_{O_2,recovery}$).

the development of 'rolling' or 'sliding-window' regressions in analytical software, it is now possible to evaluate multiple measurement intervals and select the shortest interval that captures the highest \dot{M}_{O_2} without sacrificing precision (Box 2; Zhang et al., 2019, 2020; Little et al., 2020; Prinzing et al., 2021). Thus, 'rolling' regression is more likely to capture transiently elevated \dot{M}_{O_2} than determinations made over longer measurement intervals, and it should be incorporated into analyses whose goal is to estimate peak \dot{M}_{O_2} .

Another innovation is to modify a static respirometer chamber by introducing a chasing device, allowing the fish to be motivated to swim vigorously while \dot{M}_{O_2} is simultaneously recorded (Norin and Clark, 2016; Zhang et al., 2019, 2020). Such modification allows determination of peak \dot{M}_{O_2} during the chase itself ($\dot{M}_{O_2,chase}$). When this modification was combined with 'rolling' regression, peak $\dot{M}_{O_2,chase}$ of juvenile rainbow trout (*Oncorhynchus mykiss*) was higher than $\dot{M}_{O_2,recovery}$ but not different from peak $\dot{M}_{O_2,swim}$ of the same individuals (Zhang et al., 2020). Moreover, peak $\dot{M}_{O_2,chase}$ and peak $\dot{M}_{O_2,swim}$ of individual fish were correlated (i.e. repeatable; Pearson's r and Spearman's $\rho \geq 0.77$; $P < 0.05$; Zhang et al., 2020), whereas neither was significantly correlated with $\dot{M}_{O_2,recovery}$. In contrast, a study on juvenile barramundi (*Lates calcarifer*) showed that $\dot{M}_{O_2,chase}$ was not as high as $\dot{M}_{O_2,recovery}$ measured immediately post-chase (Norin and Clark, 2016, 2017). Clearly, more validation is required, but using a modified 'chase respirometer' might offer researchers a tool that is higher throughput, lower cost and more portable than traditional swim tunnel respirometry, while also providing estimates of peak \dot{M}_{O_2} that may be similar to $\dot{M}_{O_2,swim}$ in mean and repeatability.

Matching method to biology

Because swim tunnel respirometry and exhaustive chase protocols measure different physiological states, it follows that they are not equivalent methods to estimate $\dot{M}_{O_2,max}$ in fishes. Rather, each

method is useful to explore a different set of biological questions and, potentially, better suited for a given species.

For questions related to the aerobic costs of locomotion, swim tunnel respirometry is the obvious choice. Swim tunnels and swimming protocols can be modified to accommodate fishes with different swimming styles and abilities (Priede and Holliday, 1980; Van den Thillart et al., 2004; Clark et al., 2011; Rummer et al., 2016). However, some species are not strong, sustained swimmers, and using swim tunnel respirometry to determine the $\dot{M}_{O_2,max}$ of such species might not be appropriate. We also acknowledge that even for strong, sustained swimmers, the dimensions of swim tunnels might constrain activity and underestimate a fish's swimming capacity in more naturalistic settings (Peake and Farrell, 2006; Kern et al., 2017).

An exhaustive chase protocol could help define the metabolic costs of recovering from vigorous activity such as predator avoidance or capture. This could be more ecologically relevant than the cost of sustained swimming in certain conservation or fisheries studies. For example, following the chase with a period of air exposure was originally developed as a way of mimicking the stress and handling of catch-and-release sport fishing (Donaldson et al., 2010; Clark et al., 2012).

It is also possible that $\dot{M}_{O_2,max}$ is reached during states other than sustained swimming or recovery from an exhaustive chase. Ingestion of a meal brings about an increase in \dot{M}_{O_2} , which is thought to reflect the cost of food handling, breakdown, assimilation and somatic growth (Brett and Groves, 1979; Goodrich et al., 2024). The magnitude of the post-prandial \dot{M}_{O_2} depends upon the species of fish, the quantity and quality of food consumed, and other factors (e.g. temperature; Chabot et al., 2016a). In some circumstances, the post-prandial \dot{M}_{O_2} may approach, or even exceed, the \dot{M}_{O_2} measured during swimming or after an exhaustive chase, especially for poor-swimming predatory fishes that consume large meals (i.e. ambush predators; Soofiani and Hawkins, 1982; Fu et al., 2009; Steell et al., 2019). Yet other species might achieve their highest \dot{M}_{O_2} during spontaneous activity as a result of light changes associated with photoperiod (Andersson et al., 2020).

Conclusions and recommendations for future studies

Using paired comparisons of \dot{M}_{O_2} determined for a diverse group of fishes measured under an array of experimental conditions, we show that peak \dot{M}_{O_2} depends upon the method used. This is true for mean values of peak \dot{M}_{O_2} determined for a group of individuals, as well as for the repeatability of an individual's peak \dot{M}_{O_2} . These results reinforce the need to carefully consider the biological context of experiments that measure peak \dot{M}_{O_2} (Roche et al., 2013; Clark et al., 2013; Norin and Clark, 2016; Farrell, 2016; Rummer et al., 2016; Raby et al., 2020) and lead to several recommendations for studies of elevated aerobic metabolism in fishes (Box 3).

We hope that these recommendations will be integrated into the determination of peak \dot{M}_{O_2} in future studies of fish physiology, behavioural ecology, conservation and management. Such studies might explore how certain biotic or abiotic variables differentially affect peak \dot{M}_{O_2} measured by diverse methods. For example, exposure to elevated temperature might have different effects on peak $\dot{M}_{O_2,swim}$ and peak $\dot{M}_{O_2,recovery}$ or the ranking of individual \dot{M}_{O_2} determined by these two methods. Such outcomes would suggest that the underlying physiological processes differ in their thermal sensitivities, which could have implications for a fish's capacity for sustained swimming versus recovery from burst swimming in the context of climate warming (Clark et al., 2017; Johansen et al., 2021). Furthermore, parasites that impair sustained

swimming (e.g. Palstra et al., 2007) might alter the group mean and individual variation of peak $\dot{M}_{O_2,swim}$ but not peak $\dot{M}_{O_2,recovery}$. It would also be valuable to compare the repeatability of peak $\dot{M}_{O_2,swim}$ and peak $\dot{M}_{O_2,recovery}$ over the lifespan of individuals to assess the influences of ontogeny (e.g. Downie et al., 2023) and acclimation to changing environments (e.g. Auer et al., 2018; Reemeyer and Rees, 2020).

By appreciating that these methods measure different biological processes and address different biological questions, we hope to enhance our understanding of both the biology of fishes and the impacts of human-induced changes to aquatic habitats.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization – B.B.R., J.E.R. Formal Analysis – B.B.R., J.E.R. Investigation – S.D.B., J.D.B., R.M.E., G.D.R., D.R., J.L.R., Y.Z. Data Curation – J.E.R. Writing (Original Draft) – All Authors. Writing (Reviewing and Editing) – All Authors. Visualization – B.B.R., J.E.R. Project Administration – B.B.R. Funding Acquisition – B.B.R., S.A.B., T.D.C., J.D.B., G.D.R., Y.Z.

Funding

The following sources of funding are acknowledged: the Greater New Orleans Foundation (B.B.R.); the Natural Sciences and Engineering Research Council of Canada (G.D.R., S.A.B.); Australian Research Council Future Fellowship (FT180100154) funded by the Australian Government (T.D.C.); Postdoctoral Fellowship of the Natural Sciences and Engineering Research Council of Canada (557785-2021), Banting Postdoctoral Fellowship (202309BPF-510048-BNE-295921) of the Natural Sciences and Engineering Research Council of Canada and Canadian Institutes of Health Research (Y.Z.); and a Fonds de Recherche du Québec – Nature et Technologies 3rd cycle scholarship (J.D.B.). Deposited in PMC for immediate release.

Data availability

The data and analysis code for this study are publicly available from figshare: <https://doi.org/10.6084/m9.figshare.24964491>.

ECR Spotlight

This article has an associated ECR Spotlight interview with Jessica Reemeyer.

References

- Andersson, M., Sundberg, F. and Eklöv, P. (2020). Chasing away accurate results: exhaustive chase protocols underestimate maximum metabolic rate estimates in European perch *Perca fluviatilis*. *J. Fish Biol.* **97**, 1644–1650. doi:10.1111/jfb.14519
- Auer, S. K., Salin, K., Anderson, G. J. and Metcalfe, N. B. (2018). Individuals exhibit consistent differences in their metabolic rates across changing thermal conditions. *Comp. Biochem. Physiol. A* **217**, 1–6. doi:10.1016/j.cbpa.2017.11.021
- Binning, S. A., Roche, D. G. and Layton, C. (2013). Ectoparasites increase swimming costs in a coral reef fish. *Biol. Lett.* **9**, 20120927. doi:10.1098/rsbl.2012.0927
- Blazka, P., Volf, M. and Cepala, M. (1960). A new type of respirometer for the determination of the metabolism of fish in an active state. *Physiol. Bohemoslov.* **9**, 553–558.
- Brett, J. R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Board Can.* **21**, 1183–1226. doi:10.1139/f64-103
- Brett, J. R. and Groves, T. D. D. (1979). Physiological energetics. In *Fish Physiology*, Vol. 8 (ed. W. S. Hoar and D. J. Randall), pp. 279–352. New York, NY: Academic Press, Inc.
- Brieske, S. D., Mullen, S. C. and Rees, B. R. (2024). Method dependency of maximum oxygen uptake rate and its repeatability in the Gulf killifish, *Fundulus grandis*. *J. Fish Biol.* 1–11. doi:10.1111/jfb.15692
- Burgetz, I. J., Rojas-Vargas, A., Hinch, S. G. and Randall, D. J. (1998). Initial recruitment of anaerobic metabolism during sub-maximal swimming in rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* **201**, 2711–2721. doi:10.1242/jeb.201.19.2711
- Chabot, D., Koenker, R. and Farrell, A. P. (2016a). The measurement of specific dynamic action in fishes. *J. Fish Biol.* **88**, 152–172. doi:10.1111/jfb.12836
- Chabot, D., Steffensen, J. F. and Farrell, A. P. (2016b). The determination of standard metabolic rate in fishes. *J. Fish Biol.* **88**, 81–121. doi:10.1111/jfb.12845
- Claireaux, G. and Lefrançois, C. (2007). Linking environmental variability and fish performance: integration through the concept of scope for activity. *Philos. Trans. R. Soc. B Biol. Sci.* **362**, 2031–2041. doi:10.1098/rstb.2007.2099
- Claireaux, G., McKenzie, D. J., Genge, A. G., Chatelier, A., Aubin, J. and Farrell, A. P. (2005). Linking swimming performance, cardiac pumping ability and cardiac anatomy in rainbow trout. *J. Exp. Biol.* **208**, 1775–1784. doi:10.1242/jeb.01587
- Clark, T. D. (2022). Chapter 8: Respirometry. In *Methods for Fish Biology*, Vol. 2 (ed. S. Midway, C. T. Hasler and P. Chakrabarty), pp. 247–274. American Fisheries Society.
- Clark, T. D., Jeffries, K. M., Hinch, S. G. and Farrell, A. P. (2011). Exceptional aerobic scope and cardiovascular performance of pink salmon (*Oncorhynchus gorbuscha*) may underlie resilience in a warming climate. *J. Exp. Biol.* **214**, 3074–3081.
- Clark, T. J., Donaldson, M. R., Pieperhoff, S., Drenner, S. M., Lotto, A. J., Cooke, S. J., Hinch, S. G., Patterson, D. A. and Farrell, A. P. (2012). Physiological benefits of being small in a changing world: responses of coho salmon (*Oncorhynchus kisutch*) to an acute thermal challenge and a simulated capture event. *PLoS ONE* **7**, e39079. doi:10.1371/journal.pone.0039079
- Clark, T. J., Sandblom, E. and Jutfelt, F. (2013). Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *J. Exp. Biol.* **216**, 2771–2782. doi:10.1242/jeb.084251
- Clark, T. D., Messmer, V., Tobin, A. J., Hoey, A. S. and Pratchett, M. S. (2017). Rising temperatures may drive fishing-induced selection of low-performance phenotypes. *Sci. Rep.* **7**, 40571. doi:10.1038/srep40571
- De Bonville, J., Côté, A. and Binning, S. A. (2024). Thermal tolerance and survival are modulated by a natural gradient of infection in differentially acclimated hosts. *Conserv. Physiol.* **12**, coae015. doi:10.1093/conphys/coae015
- Donaldson, M. R., Clark, T. D., Hinch, S. G., Cooke, S. J., Patterson, D. A., Gale, M. K., Frappell, P. B. and Farrell, A. P. (2010). Physiological responses of free swimming adult coho salmon to simulated predator and fisheries encounters. *Physiol. Biochem. Zool.* **83**, 973–983.
- Downie, A. T., Lefevre, S., Iling, B., Harris, J., Jarrold, M. D., McCormick, M. I., Nilsson, G. E. and Rummer, J. L. (2023). Rapid physiological and transcriptomic changes associated with oxygen delivery in larval anemonefish suggest a role in adaptation to life on hypoxic coral reefs. *PLoS Biol.* **21**, e3002102. doi:10.1371/journal.pbio.3002102
- Eisenberg, R. M., Sandrelli, R. M. and Gamperl, A. K. (2024). Comparing methods for determining the metabolic capacity of lumpfish (*Cyclopterus lumpus* Linnaeus 1758). *J. Fish Biol.* 1–11. doi:10.1111/jfb.15716
- Eliason, E. J. and Farrell, A. P. (2016). Oxygen uptake in Pacific salmon *Oncorhynchus* spp.: when ecology and physiology meet. *J. Fish Biol.* **88**, 359–388.
- Farrell, A. P. (2009). Environment, antecedents and climate change: lessons from the study of temperature physiology and river migration of salmonids. *J. Exp. Biol.* **212**, 3771–3780.
- Farrell, A. P. (2016). Pragmatic perspective on aerobic scope: peaking, plummeting, pejus and apportioning. *J. Fish Biol.* **88**, 322–343. doi:10.1111/jfb.12789
- Farrell, A. P. and Clutterham, S. M. (2003). On-line venous oxygen tensions in rainbow trout during graded exercise at two acclimation temperatures. *J. Exp. Biol.* **206**, 487–496. doi:10.1242/jeb.00100
- Fry, F. E. (1947). Effects of the environment on animal activity. *Ontario Fisheries Research Laboratory Publication, Biol. Ser.* **55**, 1–62.
- Fu, S., Zeng, L., Li, X., Pang, X., Cao, Z., Peng, J. and Wang, Y. (2009). The behavioural, digestive and metabolic characteristics of fishes with different foraging strategies. *J. Exp. Biol.* **212**, 2296–2302. doi:10.1242/jeb.027102
- Goodrich, H. R., Wood, C. M., Wilson, R. W., Clark, T. D., Last, K. B. and Wang, T. (2024). Specific dynamic action: the energy cost of digestion or growth? *J. Exp. Biol.* **227**, jeb246722. doi:10.1242/jeb.246722
- Guitard, J. J., Chrétien, E., De Bonville, J., Roche, D. G., Boisclair, D. and Binning, S. A. (2022). Increased parasite load is associated with reduced metabolic rates and escape responsiveness in pumpkinseed sunfish. *J. Exp. Biol.* **225**, 243160. doi:10.1242/jeb.243160
- Hinkle, P. C. (2005). P/O ratios of mitochondrial oxidative phosphorylation. *Biochim. Biophys. Acta* **1706**, 1–11.
- Holt, R. E. and Jørgensen, C. (2015). Climate change in fish: effects of respiratory constraints on optimal life history and behaviour. *Biol. Lett.* **11**, 20141032. doi:10.1098/rsbl.2014.1032
- Hoppeler, H. (2018). Deciphering $\dot{V}_{O_{2,max}}$: limits of the genetic approach. *J. Exp. Biol.* **221**, jeb164327. doi:10.1242/jeb.164327
- Hvas, M. and Oppedal, F. (2019). Influence of experimental set-up and methodology for measurements of metabolic rates and critical swimming speed in Atlantic salmon *Salmo salar*. *J. Fish Biol.* **95**, 893–902. doi:10.1111/jfb.14087
- Johansen, J. L., Nadler, L. E., Habary, A., Bowden, A. J. and Rummer, J. L. (2021). Thermal acclimation of tropical reef fishes to global heat waves. *eLife* **10**, e59162. doi:10.7554/eLife.59162
- Jones, D. R. and Randall, D. J. (1978). The respiratory and circulatory systems during exercise. In *Fish Physiology*, Vol. VII (ed. W. S. Hoar and D. J. Randall), pp. 425–501. New York: Academic Press.
- Kern, P., Cramp, R. L., Gordos, M. A., Watson, J. R. and Franklin, C. E. (2017). Measuring U_{crit} and endurance: equipment choice influences

- estimates of fish swimming performance. *J. Fish Biol.* **92**, 237-247. doi:10.1111/jfb.13514
- Killen, S. S., Brown, J. A. and Gamperl, A. K.** (2007). The effect of prey density on foraging mode selection in juvenile lumpfish: balancing food intake with the metabolic cost of foraging. *J. Anim. Ecol.* **76**, 814-825.
- Killen, S. S., Glazier, D. S., Rezende, E. L., Clark, T. D., Atkinson, D., Willener, A. S. T. and Halsey, L. G.** (2016). Ecological influences and morphological correlates of resting and maximal metabolic rates across teleost fish species. *Am. Nat.* **187**, 592-606. doi:10.1086/685893
- Killen, S. S., Norin, T. and Halsey, L. G.** (2017). Do method and species lifestyle affect measures of maximum metabolic rate in fishes? *J. Fish Biol.* **90**, 1037-1046. doi:10.1111/jfb.13195
- Killen, S. S., Christensen, E., Cortese, D., Závorka, L., Norin, T., Cotgrove, L., Crespel, A., Munson, A., Nati, J. J. H., Papatheodoulou, M. et al.** (2021). Guidelines for reporting methods to estimate metabolic rates by aquatic intermittent-flow respirometry. *J. Exp. Biol.* **224**, jeb242522. doi:10.1242/jeb.242522
- Koch, R. E., Buchanan, K. L., Casagrande, S., Crino, O., Dowling, D. K., Hill, G. E., Hood, W. R., McKenzie, M., Mariette, M. M., Noble, D. W. A. et al.** (2021). Integrating mitochondrial aerobic metabolism into ecology and evolution. *Trends Ecol. Evol.* **36**, 321-332. doi:10.1016/j.tree.2020.12.006
- Lawrence, M. J., Scheuffele, H., Beever, S. B., Holder, P. E., Garroway, C. J., Cooke, S. J. and Clark, T. D.** (2023). The role of metabolic phenotype in the capacity to balance competing energetic demands. *Physiol. Biochem. Zool.* **96**, 106-118.
- Le Roy, A., Mazué, G. P. F., Metcalfe, N. B. and Seebacher, F.** (2021). Diet and temperature modify the relationship between energy use and ATP production to influence behavior in zebrafish (*Danio rerio*). *Ecol. Evol.* **11**, 9791-9803. doi:10.1002/ece3.7806
- Little, A. G., Dressler, T. L., Kraskura, K., Hardison, E. A., Hendriks, B., Prystay, T. S., Farrell, A. P., Cooke, S. J., Patterson, D. A., Hinch, S. G. et al.** (2020). Maxed out: optimizing accuracy, precision, and power for field measures of maximum metabolic rate in fishes. *Physiol. Biochem. Zool.* **93**, 243-254. doi:10.1086/708673
- Marras, S., Claireaux, G., McKenzie, D. R. and Nelson, J. M.** (2010). Individual variation and repeatability in aerobic and anaerobic swimming performance of European sea bass, *Dicentrarchus labrax*. *J. Exp. Biol.* **213**, 26-32. doi:10.1242/jeb.032136
- Metcalfe, N. B., Van Leeuwen, T. E. and Killen, S. S.** (2016). Does individual variation in metabolic phenotype predict fish behaviour and performance? *J. Fish Biol.* **88**, 298-321. doi:10.1111/jfb.12699
- Moyes, C. D., Schulte, P. M. and Hochachka, P. W.** (1992). Recovery metabolism of trout white muscle: role of mitochondria. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **262**, R295-R304. doi:10.1152/ajpregu.1992.262.2.R295
- Nelson, J. M.** (2016). Oxygen consumption rate v. rate of energy utilization of fishes: a comparison and brief history of the two measurements. *J. Fish Biol.* **88**, 10-25. doi:10.1111/jfb.12824
- Norin, T. and Clark, T. J.** (2016). Measurement and relevance of maximum metabolic rate in fishes. *J. Fish Biol.* **88**, 122-151. doi:10.1111/jfb.12796
- Norin, T. and Clark, T. D.** (2017). Reply to Zhang & Gilbert (2017): Comment on 'Measurement and relevance of maximum metabolic rate in fishes by Norin & Clark (2016)'. *J. Fish Biol.* **91**, 403-408.
- Norin, T. and Malte, H.** (2011). Repeatability of standard metabolic rate, active metabolic rate and aerobic scope in young brown trout during a period of moderate food availability. *J. Exp. Biol.* **214**, 1668-1675. doi:10.1242/jeb.054205
- Norin, T., Malte, H. and Clark, T. D.** (2016). Differential plasticity of metabolic rate phenotypes in a tropical fish facing environmental change. *Funct. Ecol.* **30**, 369-378.
- Palstra, A. P., Heppener, D. F. M., van Ginneken, V. J. T., Székely, C. and van den Thillart, G. E. J. M.** (2007). Swimming performance of silver eels is severely impaired by the swim-bladder parasite *Anguillicola crassus*. *J. Exp. Mar. Biol. Ecol.* **352**, 244-256. doi:10.1016/j.jembe.2007.08.003
- Peake, S. J. and Farrell, A. P.** (2004). Locomotory behaviour and post-exercise physiology in relation to swimming speed, gait transition and metabolism in free-swimming smallmouth bass (*Micropterus dolomieu*). *J. Exp. Biol.* **207**, 1563-1575. doi:10.1242/jeb.00927
- Peake, S. J. and Farrell, A. P.** (2006). Fatigue is a behavioral response in respirometer confined small mouth bass. *J. Fish Biol.* **68**, 1742-1755. doi:10.1111/j.0022-1112.2006.01052.x
- Priede, I. G.** (1977). Natural selection for energetic efficiency and relationship between activity level and mortality. *Nature* **267**, 610-611.
- Priede, I. G.** (1985). Metabolic scope in fishes. In *Fish Energetics* (ed. P. Tytler and P. Calow), pp. 33-64. Dordrecht: Springer.
- Priede, I. G. and Holliday, F. G. T.** (1980). The use of a new tilting tunnel respirometer to investigate some aspects of metabolism and swimming activity of the plaice (*Pleuronectes platessa* L.). *J. Exp. Biol.* **85**, 295-309. doi:10.1242/jeb.85.1.295
- Prinzinger, T. S., Zhang, Y., Wegner, N. C. and Dulvy, N. K.** (2021). Analytical methods matter too: Establishing a framework for estimating maximum metabolic rate for fishes. *Ecol. Evol.* **11**, 9987-10003.
- Raby, G. D., Doherty, C., Mokdad, A., Pitcher, T. E. and Fisk, A. T.** (2020). Post-exercise respirometry underestimates maximum metabolic rate in juvenile salmon. *Conserv. Physiol.* **8**, coaa063. doi:10.1093/conphys/coaa063
- Reemeyer, J. E. and Rees, B. B.** (2020). Plasticity, repeatability, and phenotypic correlations of aerobic metabolic traits in a small estuarine fish. *J. Exp. Biol.* **223**, jeb228098. doi:10.1242/jeb.228098
- Rees, B. B., Reemeyer, J. E. and Irving, B. A.** (2022). Interindividual variation in maximum aerobic metabolism varies with gill morphology and myocardial bioenergetics in Gulf killifish. *J. Exp. Biol.* **225**, jeb243680. doi:10.1242/jeb.243680
- Regan, M. D., Gosline, J. M. and Richards, J. G.** (2013). A simple and affordable calorimeter for assessing the metabolic rates of fishes. *J. Exp. Biol.* **216**, 4507-4513.
- Regan, M. D., Gill, I. S. and Richards, J. G.** (2017). Calorespirometry reveals that goldfish prioritize aerobic metabolism over metabolic rate depression in all but near-anoxic environments. *J. Exp. Biol.* **220**, 564-572. doi:10.1242/jeb.145169
- Reidy, S. P., Kerr, S. R. and Nelson, J. M.** (2000). Aerobic and anaerobic swimming performance of individual Atlantic cod. *J. Exp. Biol.* **203**, 347-357. doi:10.1242/jeb.203.2.347
- Reidy, S. P., Nelson, J. M., Tang, Y. and Kerr, S. R.** (1995). Post-exercise metabolic rate in Atlantic cod and its dependence upon the method of exhaustion. *J. Fish Biol.* **47**, 377-386. doi:10.1111/j.1095-8649.1995.tb01907.x
- Roche, D. G., Binning, S. A., Bosiger, Y., Johansen, J. L. and Rummer, J. L.** (2013). Finding the best estimates of metabolic rates in a coral reef fish. *J. Exp. Biol.* **216**, 2103-2110. doi:10.1242/jeb.082925
- Roche, D. G., Careau, V. and Binning, S. A.** (2016). Demystifying animal 'personality' (or not): why individual variation matters to experimental biologists. *J. Exp. Biol.* **219**, 3832-3843. doi:10.1242/jeb.146712
- Rummer, J. L. and Brauner, C. J.** (2015). Root effect haemoglobins in fish may greatly enhance general oxygen delivery relative to other vertebrates. *PLoS ONE* **10**, e0139477. doi:10.1371/journal.pone.0139477
- Rummer, J. L., Binning, S. A., Roche, D. G. and Johansen, J. L.** (2016). Methods matter: considering locomotory mode and respirometry technique when estimating metabolic rates of fishes. *Conserv. Physiol.* **4**, cow008. doi:10.1093/conphys/cow008
- Salin, K., Auer, S. K., Rudolf, A. M., Anderson, G. J., Selman, C. and Metcalfe, N. B.** (2016). Variation in metabolic rate among individuals is related to tissue-specific differences in mitochondrial leak respiration. *Physiol. Biochem. Zool.* **89**, 511-523. doi:10.1086/688769
- Scarabello, M., Heigenhauser, G. J. F. and Wood, C. M.** (1992). Gas exchange, metabolite status and excess post-exercise oxygen consumption after repetitive bouts of exhaustive exercise in juvenile rainbow trout. *J. Exp. Biol.* **167**, 155-169. doi:10.1242/jeb.167.1.155
- Scott, G. R. and Dalziel, A. C.** (2021). Physiological insight into the evolution of complex phenotypes: aerobic performance and the O₂ transport pathway of vertebrates. *J. Exp. Biol.* **224**, jeb210849. doi:10.1242/jeb.210849
- Slesinger, E., Andres, A., Young, R., Seibel, B. A., Saba, V. S., Phelan, B., Rosendale, J. D., Wiczorek, D. and Saba, G.** (2019). The effect of ocean warming on black sea bass (*Centropristis striata*) aerobic scope and hypoxia tolerance. *PLoS ONE* **14**, e0218390. doi:10.1371/journal.pone.0218390
- Soofiani, N. M. and Hawkins, A. D.** (1982). Energetic costs at different levels of feeding in juvenile cod, *Gadus morhua* L. *J. Fish Biol.* **21**, 577-592. doi:10.1111/j.1095-8649.1982.tb02861.x
- Soofiani, N. M. and Priede, I. G.** (1983). Aerobic metabolic scope and swimming performance in juvenile cod, *Gadus morhua* L. *J. Fish Biol.* **26**, 127-138. doi:10.1111/j.1095-8649.1985.tb04249.x
- Stell, S. C., Van Leeuwen, T. E., Brownscombe, J. W., Cooke, S. J. and Eliason, E. J.** (2019). An appetite for invasion: digestive physiology, thermal performance, and food intake in lionfish (*Pterois* spp.). *J. Exp. Biol.* **222**, jeb209437. doi:10.1242/jeb.209437
- Steffensen, J. F.** (1985). The transition between branchial pumping and ram ventilation in fishes: energetic consequences and dependence on water oxygen tensions. *J. Exp. Biol.* **114**, 141-150. doi:10.1242/jeb.114.1.141
- Steffensen, J. F., Johansen, K. and Bushnell, P. G.** (1984). An automated swimming respirometer. *Comp. Biochem. Physiol.* **79A**, 437-440.
- Svendsen, M. B. S., Bushnell, P. G. and Steffensen, J. P.** (2016). Design and setup of intermittent flow respirometry system for aquatic organisms. *J. Fish Biol.* **88**, 26-50. doi:10.1111/jfb.12797
- Thoral, E., Queiros, Q., Roussel, D., Dutto, G., Gasset, E., McKenzie, D. J., Romestaing, C., Fromentin, J. M., Saroux, C. and Teulier, L.** (2021). Changes in foraging mode caused by a decline in prey size have major bioenergetic consequences for a small pelagic fish. *J. Anim. Ecol.* **90**, 2289-2301. doi:10.1111/1365-2656.13535
- Van Den Thillart, G., Van Ginneken, V., Körner, F., Heijmans, R., Van Der Linden, R. and Gluvers, A.** (2004). Endurance swimming of European eel. *J. Fish Biol.* **65**, 312-318. doi:10.1111/j.0022-1112.2004.00447.x

- Voituron, Y., Roussel, D., Teulier, L., Vagner, M., Ternon, Q., Romestaing, C., Dubillot, E. and Lefrançois, C.** (2022). Warm acclimation increases mitochondrial efficiency in fish: a compensatory mechanism to reduce the demand for oxygen. *Physiol. Biochem. Zool.* **95**, 15-21.
- Wood, C. M.** (1991). Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. *J. Exp. Biol.* **160**, 285-308. doi:10.1242/jeb.160.1.285
- Zhang, Y. and Gilbert, M. J. H.** (2017). Comment on 'Measurement and relevance of maximum metabolic rate in fishes by Norin & Clark (2016)'. *J. Fish Biol.* **91**, 397-402.
- Zhang, Y., Claireaux, G., Takle, H., Jørgensen, S. M. and Farrell, A. P.** (2018). A three-phase excess post-exercise oxygen consumption in Atlantic salmon *Salmo salar* and its response to exercise training. *J. Fish Biol.* **92**, 1385-1403.
- Zhang, Y., Gilbert, M. J. and Farrell, A. P.** (2019). Finding the peak of dynamic oxygen uptake during fatiguing exercise in fish. *J. Exp. Biol.* **222**, jeb196568. doi:10.1242/jeb.196568
- Zhang, Y., Gilbert, M. J. and Farrell, A. P.** (2020). Measuring maximum oxygen uptake with an incremental swimming test and by chasing rainbow trout to exhaustion inside a respirometry chamber yields the same results. *J. Fish Biol.* **97**, 28-38. doi:10.1111/jfb.14311