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REVIEW

Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations

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Summary

Measurements of aerobic scope [the difference between minimum and maximum oxygen consumption rate ($\dot{M}_{O2,min}$ and $\dot{M}_{O2,max}$, respectively)] are increasing in prevalence as a tool to address questions relating to fish ecology and the effects of climate change. However, there are underlying issues regarding the array of methods used to measure aerobic scope across studies and species. In an attempt to enhance quality control before the diversity of issues becomes too great to remedy, this paper outlines common techniques and pitfalls associated with measurements of $\dot{M}_{O2,min}$, $\dot{M}_{O2,max}$ and aerobic scope across species and under different experimental conditions. Additionally, we provide a brief critique of the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis, a concept that is intricately dependent on aerobic scope measurements and is spreading wildly throughout the literature despite little evidence for its general applicability. It is the intention of this paper to encourage transparency and accuracy in future studies that measure the aerobic metabolism of fishes, and to highlight the fundamental issues with assuming broad relevance of the OCLTT hypothesis.

Key words: aerobic metabolism, excess post-exercise oxygen consumption, EPOC, global warming, oxygen- and capacity-limited thermal tolerance, oxygen consumption rate, oxygen uptake, specific dynamic action.

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Introduction

Measurements of aerobic metabolic rate (≈oxygen consumption rate, $\dot{M}_{O_2}$) are becoming increasingly popular to address questions relating to animal ecology owing to hypotheses like the metabolic theory of ecology (Brown et al., 2004; Whitfield, 2004; Price et al., 2012). In particular, measurements of $\dot{M}_{O_2}$ are proliferating in the context of fish biology and climate change, largely because of renewed interest and further developments in the hypothesis relating aerobic scope (the difference between minimum and maximum $\dot{M}_{O_2}$; $\dot{M}_{O_2,min}$ and $\dot{M}_{O_2,max}$, respectively) to whole-animal performance and fitness (Fry, 1947; Brett, 1971; Claireaux and Lefrançois, 2007; Pörtner and Knust, 2007; Farrell et al., 2008; Pörtner et al., 2008; Pörtner and Farrell, 2008; Munday et al., 2009; Nilsson et al., 2009; Pörtner, 2010; Pörtner and Peck, 2010; Clark et al., 2011; Eliason et al., 2011; Donelson et al., 2012; Munday et al., 2012; Pörtner, 2012). Termed oxygen- and capacity-limited thermal tolerance (OCLTT), the theoretical rationale for this hypothesis is that the biochemical and physiological capacities of aquatic ectotherms have evolved such that aerobic scope is maximised within a given temperature range (termed T_{optAS}) in order to optimise fitness-related performance (e.g. growth, reproduction and locomotion), while performance diminishes as aerobic scope decreases at higher and lower temperatures (Fig. 1A). Thus, it is implied in the hypothesis that critical performances such as growth, locomotion and reproduction are causally linked with aerobic scope and therefore animals should have optimal fitness when living at T_{optAS}.

The OCLTT hypothesis suggests that scientists must only measure $\dot{M}_{O2,min}$ and $\dot{M}_{O2,max}$ across a range of ecologically relevant temperatures to determine T_{optAS} and therefore provide insight into the optimal, fitness-maximising temperature of the species of interest. This idea has guided the interpretations of many researchers (e.g. Pörtner and Knust, 2007; Pörtner and Farrell, 2008; Nilsson et al., 2009; Gardiner et al., 2010; Donelson et al., 2011; Neuheimer et al., 2011; Miller et al., 2012; Comte and Grenouillet, 2013), as it promises an elegant, mechanistic approach to measuring animal performance, thermal tolerance and responses to a changing climate. Nevertheless, historic and recent data suggest that T_{optAS} provides little insight into the preferred temperature or performance of aquatic ectotherms, but rather aerobic scope continues to increase until temperature approaches lethal levels, beyond which aerobic scope declines rapidly as death ensues (Fig. 1B) [e.g. fig. 17 in Fry (Fry, 1947)] (Clark et al., 2011; Healy and Schulte, 2012).

Aside from the issues with using aerobic scope to inform the optimal temperature and fitness of different fish species, there is an underlying concern regarding the various methodologies used to measure aerobic scope. New technologies have made it possible to measure $\dot{M}_{O_2}$ relatively easily, but measurements can be crude and quality control is too often lacking. A growing number of studies have used unreliable techniques to measure the aerobic scope of fishes, and therefore the published data may be erroneous and misleading. Measurements of $\dot{M}_{O_2,min}$ and $\dot{M}_{O_2,max}$, and thus aerobic scope, require well-designed equipment and techniques that are suited to the species of interest. While some excellent previous publications exist on the technical aspects of aquatic respirometry (Steffensen, 1989;

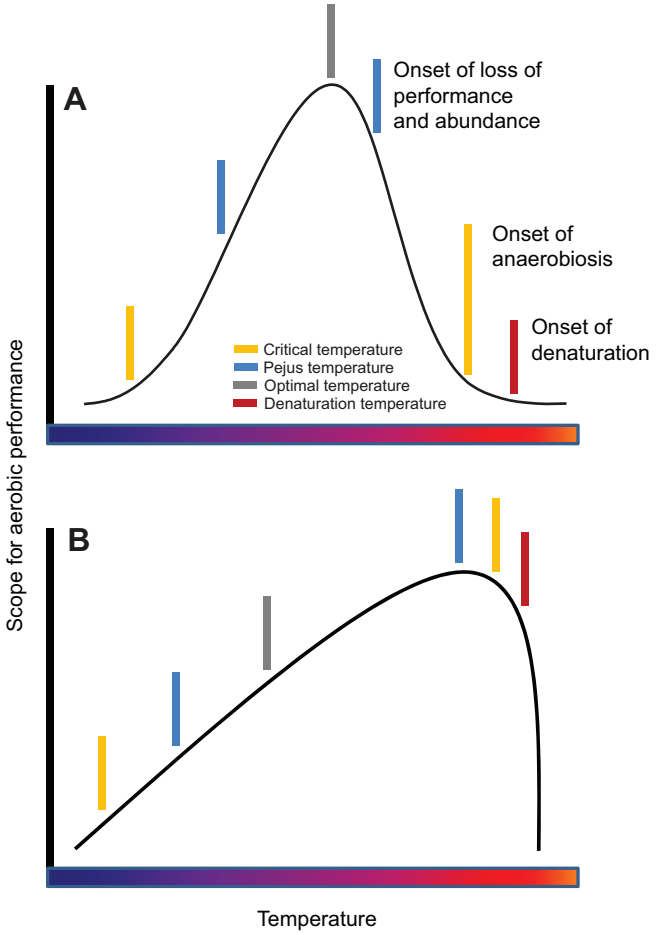


Fig. 1. Hypothetical curves depicting changes in aerobic performance (aerobic scope) of fishes with temperature, where A is redrawn from Pörtner and Farrell (Pörtner and Farrell, 2008) and B is an alternative explanation of how aerobic performance responds to temperature and interacts with animal performance [based on Clark et al. (Clark et al., 2011)]. Note the primary difference is that A assumes that the optimal (preferred) temperature of the species coincides with maximal aerobic scope, while B assumes the optimal temperature is below that which elicits maximal aerobic scope and instead aerobic scope increases until close to the upper critical temperature.

Cech, 1990), we felt a new review was timely given the relatively recent advent of new technologies and the renewed interest in metabolic measurements throughout the scientific community.

Rather than highlighting and critically assessing each of the studies that have used unreliable techniques and provided questionable interpretations, the purpose of the present paper is to (1) overview the common techniques and methodologies used to measure $\dot{M}_{O2,min}$ and $\dot{M}_{O2,max}$ in fishes, (2) highlight areas that require additional care and consideration by scientists, (3) provide some recommendations to ensure that future measurements of $\dot{M}_{O2}$ in fishes are necessarily rigorous and comparable between studies, (4) explore the relevance and usefulness of aerobic scope and T_{optAS}, and (5) call for caution when interpreting results of future studies so that the OCLTT hypothesis is tested rather than assumed to be widely applicable. While this paper focuses on fishes, much of the information is relevant to all vertebrates (e.g. amphibians) and invertebrates (e.g. corals, crustaceans, molluscs) that obtain oxygen from aquatic environments.

Experimental setup

Oxygen measurements and $\dot{M}_{O2}$ calculations

Historically, rates of oxygen consumption from fishes were determined by intermittently sampling water from a sealed respirometer over time and measuring oxygen content of the samples on a bench-top analyser or using the Winkler method (Fry and Hart, 1948; Heath and Hughes, 1973; Butler and Taylor, 1975). By necessity, calculations of $\dot{M}_{O2}$ using these methods are often of low temporal resolution and typically it is not possible to identify where periods of spontaneous activity may have influenced the measurements (see Fig. 2). While the principles of respirometry have remained largely the same, technological advances have made it easier to obtain better quality and higher resolution data from undisturbed fish. Continuous measurements of the oxygen tension within the respirometer over time permit $\dot{M}_{O2}$ measurements over a broad temporal scale including during times when the experimenter is not present, and it is possible to identify periods of spontaneous activity that would have otherwise influenced data quality and interpretation (Fig. 2).

Galvanic oxygen electrodes rely on an electrochemical reaction between an anode and a cathode to measure dissolved oxygen in respirometer water. While these electrodes greatly enhanced respiration science in the past and are still used today, they have a number of limitations including the fact that (1) they are pressure and temperature sensitive, and (2) they are impractical for fine-scale measurements from small respirometers because the electrochemical reaction consumes oxygen during measurements.

Fibre-optic oxygen sensors (termed ‘optodes’) have been developed more recently, and rely on oxygen-dependent attenuation of emitted light from fluorescent indicator molecules (or ‘dynamic fluorescence quenching’). Fibre-optic sensors are typically much smaller than galvanic oxygen electrodes, they are not particularly pressure or temperature sensitive, and they do not consume oxygen during measurements. Consequently, they have opened the door to higher resolution $\dot{M}_{O2}$ measurements from small aquatic animals including larval fishes.

A recent development in oxygen sensing has been the advent of sensor spot material, which is essentially a thin sheet of material coated in relevant oxygen sensing chemicals (e.g. PyroScience^{TM}, PreSens^{TM}). A small amount of sensor spot material (e.g. 2 mm^2) can be attached on a transparent wall inside the respirometer, and oxygen measurements from within the respirometer can be made by focusing a fibre-optic sensor on the spot from outside the respirometer. Given that the oxygen measurements from this system can be sensitive to small changes in the position of the fibre-optic sensor in relation to the sensor spot material, it may be necessary to recalibrate the output from the sensor spot whenever the fibre-optic sensor has been moved. While this technology has some excellent applications in respiration science, it risks being used in a similar fashion to the historic method of measuring respirometer oxygen tension only intermittently (see above), subsequently leading to spurious $\dot{M}_{O2}$ data (Fig. 2).

It is possible to measure $\dot{M}_{O2}$ in flow-through systems according to:

$$\dot{M}_{O2} = \dot{V}_w \times (C_{wO2in} - C_{wO2out}) / M_f, \quad (1)$$

where $\dot{V}_w$ is the rate of water flow through the respirometer, C_{wO2in} and C_{wO2out} are the concentrations of oxygen in the incoming and outgoing water, respectively, and M_f is fish mass (gives a value of mass-specific $\dot{M}_{O2}$). This flow-through method suffers from several issues, a primary one being the issue of washout characteristics

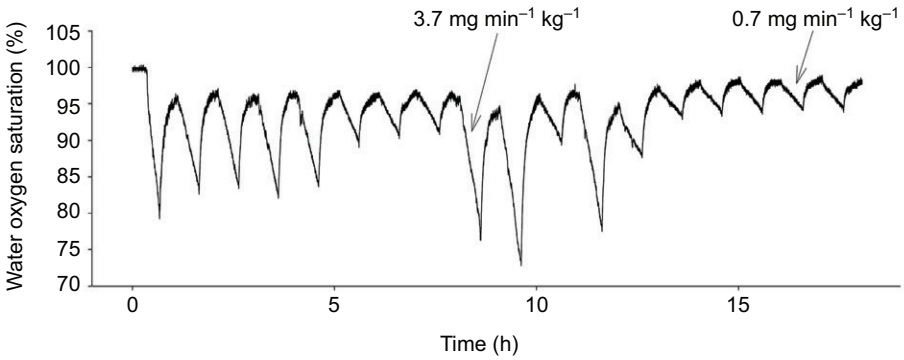


Fig. 2. Continuous raw trace of water oxygen saturation in a static intermittent-flow respirometer containing an adult coho salmon (*Oncorhynchus kisutch*) at 8°C. Negative slopes are due to the oxygen consumption of the fish, while positive slopes indicate when the flush pump intermittently switched on to replenish the respirometer with aerated water. The trace illustrates the danger of periodic rather than continuous sampling of oxygen when measuring $\dot{M}_{O_2}$. If the raw trace was not available and $\dot{M}_{O_2}$ was measured only once at 8 h after entry of the fish into the respirometer, a resting $\dot{M}_{O_2}$ of 3.7 mg min^{-1} kg^{-1} would be concluded. However, the continuous nature of the trace clearly shows that a single measurement of $\dot{M}_{O_2}$ at 8 h would yield a significantly elevated estimate of resting $\dot{M}_{O_2}$ (over five times the correct value of 0.7 mg min^{-1} kg^{-1} obtained after 16 h) due to spontaneous activity of the fish. Raw data taken from Clark et al. (Clark et al., 2012).

obscuring $\dot{M}_{O_2}$ measurements over short temporal scales (for a review, see Steffensen, 1989). Instead, a more suitable approach for measuring $\dot{M}_{O_2}$ of most water-breathing animals is intermittent-flow respirometry. In this situation, mass-specific $\dot{M}_{O_2}$ is calculated as:

$$\dot{M}_{O_2} = [(V_r - V_f) \times \Delta C_{wO_2}] / (\Delta t \times M_f), \quad (2)$$

where V_r is the respirometer volume, V_f is the fish volume (often assumed to be the same as M_f with 1 g of fish equivalent to 1 ml of water, but keeping in mind that the swim bladder can have a large volume but low mass), ΔC_{wO_2} is the change in oxygen concentration in the respirometer water, and Δt is the change in time during which ΔC_{wO_2} is measured. Note in both Eqns 1 and 2 that C_{wO_2} is the product of the partial pressure of oxygen in the water (P_{wO_2}) and the capacitance of oxygen in the water (β_{wO_2}), the latter being dependent on salinity and temperature. Given the widespread and preferred use of intermittent-flow respirometry, this will be the main technique examined herein.

Respirometer design and function

The most common respirometers are ‘swim tunnel’ respirometers and ‘static’ respirometers (the latter does not imply that the water within the respirometer remains static, but rather that the fish is not required to swim to maintain position). Swim tunnel respirometers are usually rounded-rectangular in shape and contain a propeller (or impeller) that circulates water in one direction, while the fish is restricted to one of the long compartments called the ‘working section’, where a laminar flow profile is desired (Fig. 3A) (Brett, 1964; Fry, 1971). Alternative designs exist, such as the Blazka swim tunnel (Blazka et al., 1960), but generally they are not as universally suitable across a broad range of fish sizes. The propeller or impeller in swim tunnels is typically driven by a variable-voltage motor such that a range of water speeds can be obtained, including high speeds to elicit $\dot{M}_{O_2,max}$. Static respirometers are usually cylindrical or rectangular chambers and they do not contain propellers to induce swimming of the fish (Fig. 3B,C). Thus, static respirometers are optimal for measuring $\dot{M}_{O_2,min}$ in fish species that are inactive under resting conditions.

The dimensions and volume of the respirometer are important first considerations when planning respirometry measurements. Clearly, the respirometer should be large enough to accommodate the length and width of the fish, bearing in mind that fish vary markedly in morphology from dorso-ventrally flattened rays and flatfishes, through laterally flattened cichlids, to elongated eels. In addition to ensuring that the respirometer is large enough to provide a suitable environment for the fish during measurements, there is another consideration relating to the aerobic capacity of the species and the requirement to maintain high levels of dissolved oxygen in the respirometer water. Respirometers should be equipped with a flush pump that may be switched on as necessary to refresh the respirometer water, at time intervals dependent on the fish species and the size of the respirometer (e.g. Forstner, 1983). The flush pump should be connected to the respirometer through a one-way flow valve to prevent any mixing of water between the respirometer and the surrounding water (e.g. reservoir bath), or the inlet pipe from the flush pump should be of sufficient length to provide the same function. An overflow pipe positioned on the respirometer should extend above the water surface and allow an outlet for the extra water being pumped into the respirometer by the flush pump. It is recommended that the flush pump is automated (at least when measurements of $\dot{M}_{O_2,min}$ are desired), such that $\dot{M}_{O_2}$ measurements are continuous and the experimenter need not be interacting with the respirometer and potentially disturbing the fish (see Steffensen et al., 1984) (Fig. 2). The flush pump may be triggered at a certain respirometer oxygen level or by a timer.

Athletic species generally have higher routine $\dot{M}_{O_2}$ than more sedentary species, and therefore the former will deplete the respirometer oxygen faster than the latter. In this instance, a larger volume respirometer may be selected for the athletic species, or flushing of the respirometer water should occur more frequently. Similar considerations should be given to stressed or exercising fishes in comparison with resting individuals, and when measurements are taken at the high end of a species’ temperature range. In any event, researchers should aim to maintain dissolved oxygen levels in the respirometer above 80% air saturation at all times to minimise the chance of $\dot{M}_{O_2}$ measurements being influenced by hypoxia-induced metabolic adjustments (e.g. Hughes, 1973). However, as noise and drift are low in modern optode systems, there is little purpose in letting

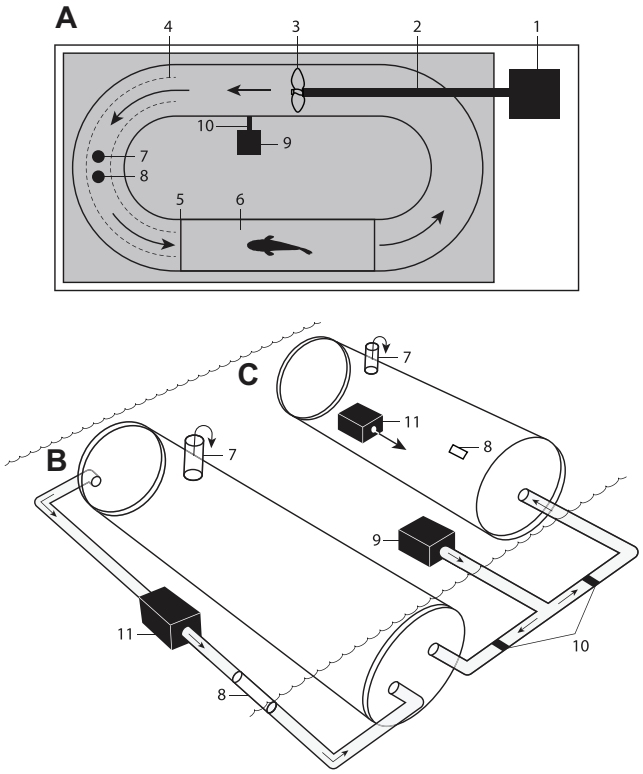


Fig. 3. Typical respirometers used for water-breathing organisms including fishes: (A) swim tunnel respirometer (view from above), (B) static respirometer with a closed-circuit recirculation loop, and (C) static respirometer with a recirculation pump within the main chamber. Numbers correspond to: (1) variable speed motor, (2) propeller shaft, (3) propeller, (4) baffles to assist with achieving laminar flow, (5) honeycomb grid to assist with laminar flow (should be as thick as feasible, at front and back of working section, and anywhere throughout the rest of the swim tunnel where space permits), (6) working section where fish is housed, (7) overflow pipe, which extends above water surface, (8) sealable port for oxygen sensor (A), or clear section to allow measurements of oxygen using fibre-optic sensors and spot material (B,C), (9) flush pump (water exits through 7), (10) one-way flow valve (alternatively, the lengths of hosing in B and C can be extended to perform the same function), and (11) recirculation pump. Grey shading depicts the water in A (1 remains dry, 7 extends above water surface), while everything is submerged in B and C except for the top of 7.

oxygen concentrations fall below approximately 90% air saturation. This will shorten measurement periods and thereby increase resolution of measurements and reduce the risk of spontaneous fish movement influencing $\dot{M}_{O_2}$ data.

While a large respirometer may enhance comfort of the fish and facilitate the maintenance of high dissolved oxygen levels, a respirometer that is too big can cause problems with oxygen stratification and insufficient mixing (Keys, 1930; Steffensen, 1989). In all respirometers, and especially in instances where a small fish is housed in an excessively large respirometer, the oxygen uptake of the respiring fish at one end of the respirometer may not be detected accurately at the opposite end unless there exists a perfectly stable diffusion gradient and the fish does not make any movements to disturb the gradient (even so, a significant lag time would exist). In these instances, $\dot{M}_{O_2}$ will be overestimated when using Eqn 2 if dissolved oxygen is measured close to the fish because oxygen tension is not homogeneous throughout the total water volume of the respirometer. Conversely, if dissolved oxygen is measured at the opposite end of the respirometer from where the fish is positioned, $\dot{M}_{O_2}$ will be underestimated for similar reasons. Often, this kind of situation will lead to erratic oxygen traces from the respirometer (due to pulses of water with different oxygen tensions coming into contact with the oxygen sensor) rather than a smooth decline in oxygen during the $\dot{M}_{O_2}$ measurement (see Fig. 4). To address these issues, respirometers should be constructed to an appropriate size [typical fish mass to water volume ratios (g:ml) are between 1:20 and 1:100 for static respirometers and some swim tunnel respirometers, and up to 1:350 for high-performance species exercising in swim tunnel respirometers] (e.g. Clark and Seymour, 2006; Blank et al., 2007; Steinhausen et al., 2008; Clark et al., 2011), and all respirometers should have appropriate mixing mechanisms to ensure that oxygen tension remains homogeneous throughout the entire water volume (Fig. 4). The latter can be accomplished, for example, by using a recirculation pump in a closed-circuit loop (Fig. 3B) (e.g. Clark et al., 2011) or within the respirometer chamber (Fig. 3C), or by using a magnetic stir bar at the bottom of the respirometer and placing the respirometer on a magnetic drive plate (e.g. Nilsson et al., 2007). It is often useful to have the oxygen sensor positioned in a closed-circuit recirculation loop (see Fig. 3B), as this removes the possibility of the sensor being damaged by the fish. If no recirculation loop is present and a recirculation pump is simply positioned within the main chamber of the respirometer (Fig. 3C), then the cleanest oxygen traces will be achieved by positioning the oxygen sensor within the stream of water produced by the recirculating pump (e.g. Fig. 4). Positioning the oxygen sensor sideways rather than facing downwards can help prevent bubbles from forming on the sensor and reduce the chance of spurious oxygen measurements. It can be useful to have a baffle at the inflow and outflow pipes within the respirometer to prevent a jet of water disturbing the fish and to ensure that fish do not get sucked against the outlet (the latter is of particular concern when working on small fish). Because swim tunnel respirometers are equipped with propellers or impellers to produce water current (Fig. 3A), the oxygen tension in these respirometers will usually remain homogeneous without any additional mixing devices. Whichever approach is taken, it is imperative that the method of mixing the respirometer water ensures homogeneous oxygen tension throughout the water volume and causes minimal disturbance to the fish. Data obtained from respirometers that lack a mixing mechanism should be treated with extreme caution as they rely on an unquantifiable level of mixing based on the movements of the fish.

The volume of the respirometer will also influence response times for $\dot{M}_{O_2}$ measurements. Even if appropriate oxygen mixing is accomplished, an excessively large respirometer (in relation to fish size and aerobic capacity) will yield lower resolution $\dot{M}_{O_2}$ measurements than a more appropriately sized respirometer. This is particularly important when examining $\dot{M}_{O_2,max}$, as this measurement often must be taken at a very specific time and at a resolution of only seconds to minutes. A large respirometer in relation to fish size will reduce response times and likely lead to an underestimation of $\dot{M}_{O_2,max}$.

Respiration that is not associated with the fish (e.g. oxygen consumption of bacteria, photosynthesis of algae) is often termed ‘background’ respiration and must be quantified and accounted for in any respirometry setup. Background respiration can be negligible in clean respirometers that have a relatively small

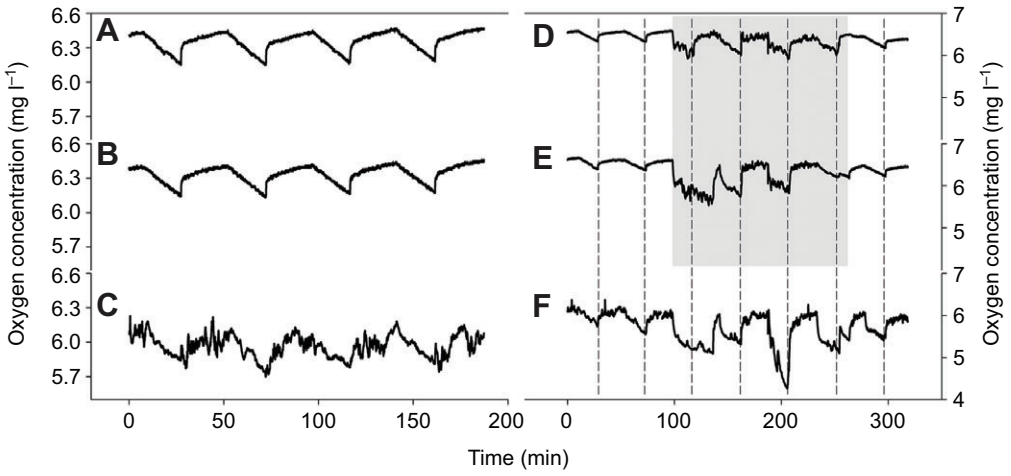


Fig. 4. Continuous raw traces of water oxygen concentration in three parallel, static intermittent-flow respirometers each being 27 l and containing a quiescent adult blue-spotted rock cod (*Cephalopholis cyanostigma*) at 28°C. Respirometers used for the traces shown in A and B are each equipped with a recirculation pump with the stream of water passing across the oxygen sensor, while the respirometer used for C does not contain a recirculation pump. The flush (~25 min) and seal (~20 min) cycles of the flush pump are clearly illustrated in A and B as positive and negative slopes, respectively, whereas the trends are not as clear in C due to stratification of oxygen throughout the respirometer in the absence of a mixing mechanism. Consequently, estimates of $\dot{M}_{O_2}$ across the four measurement cycles vary by a maximum of 6.0% for the fish in A, 9.7% for the fish in B, and 45.4% for the fish in C when using sections aligned identically. Panels D–F contain data from the same three fish as A–C with the only difference being that the recirculation pumps were switched off in D and E for the period indicated by the shaded box (respirometer in C and F was never equipped with a recirculation pump). Body masses: (A,D) 343 g; (B,E) 286 g; (C,F) 235 g. Vertical dashed lines are provided for clarity in D–F to indicate the periods when the flush pump automatically switched on. The mean ± s.e.m. estimate of $\dot{M}_{O_2}$ in D when the recirculation pump was on (three cycles) was 1.04±0.01 mg min^{-1} kg^{-1}, whereas when the recirculation pump was off (four cycles) the mean estimate was higher and more variable at 1.25±0.11 mg min^{-1} kg^{-1}. Corresponding values for E were 1.21±0.10 mg min^{-1} kg^{-1} (recirculation pump on) and 1.80±0.24 mg min^{-1} kg^{-1} (recirculation pump off). The mean ± s.e.m. estimate for F across all measurements (seven cycles) was higher and more variable at 3.66±1.06 mg min^{-1} kg^{-1}. Note that respirometers that lack a sufficient mixing mechanism yield $\dot{M}_{O_2}$ values with significant error and uncertainty, and any data obtained using such methods should be treated with extreme caution.

surface area to volume ratio and when the water contains few respiring microbes. In contrast, small systems with large surface area to volume ratios (e.g. a system to measure $\dot{M}_{O_2}$ of larval fishes) can rapidly build up background respiration rates that approach or exceed the respiration rates of the fish owing to microbial growth on the internal surfaces of the respirometer. Regardless of the size of the respirometer, it is good practice to test the background respiration prior to the introduction of the fish and again after removal of the fish at the conclusion of the experiment. If background respiration proves to be a concern in pilot experiments, the protocol should be refined accordingly to ensure that the respirometers are more regularly cleaned and sterilised (e.g. with bleach or ethanol). It is possible to maintain an empty respirometer alongside other respirometers in order to measure background respiration continuously throughout experiments, though this approach will underestimate background respiration if the simple presence of a fish in the respirometer (and associated waste products) enhances the build-up of microbes. Importantly, background respiration often changes in a non-linear (e.g. sigmoidal) fashion over time, and therefore accurately quantifying and accounting for background respiration can be vital to ensure accurate $\dot{M}_{O_2}$ measurements.

Oxygen leakage from the surrounding water into the respirometer can reduce the apparent $\dot{M}_{O_2}$ of the fish. This is particularly a problem when using a small fish biomass to water volume ratio, when $\dot{M}_{O_2}$ is very low (such as in cold water) or when $\dot{M}_{O_2}$ measurements are being conducted under hypoxic conditions. Leakage can occur through oxygen-permeable materials such as silicone tubing, or through chambers that are poorly sealed or have been constructed using inappropriate materials (e.g. Stevens, 1992). This should be tested and quantified prior to experiments by introducing hypoxic water to an empty respirometer with the mixing mechanism functional, the flush pump switched off, and the water in the external bath housing the respirometers maintained at full air saturation.

All of the abovementioned issues highlight the need to thoroughly test respirometry systems and protocols prior to embarking on experiments where the intention is to publish the data.

Aerobic scope measurements

Calculations of aerobic scope rely on two extreme measures of $\dot{M}_{O_2}$, namely $\dot{M}_{O_2,min}$ and $\dot{M}_{O_2,max}$. Although it would be optimal for comparative purposes if the techniques used for these two measures were consistent across studies, unfortunately that is not the case. While some of the variation between studies is an unavoidable consequence of working with different species and thus requiring different techniques, a significant part of the variation apparently stems from a lack of experience and quality control. Below, we outline some of the various techniques and conditions used in studies to estimate $\dot{M}_{O_2,min}$ and $\dot{M}_{O_2,max}$, with an aim to encourage transparency, enhance accuracy and maximise consistency of future studies.

Minimum oxygen consumption rate

Measurements of the low extreme of $\dot{M}_{O_2}$ (termed $\dot{M}_{O_2,min}$ in the present paper) vary substantially across studies. Preferably, $\dot{M}_{O_2,min}$ should be the equivalent of 'standard $\dot{M}_{O_2}$', which is the minimum $\dot{M}_{O_2}$ required to sustain life and has the specific requirements that the animal must be inactive, post-absorptive and not paying off any

oxygen debt associated with previous anaerobic exercise (Fry and Hart, 1948).

For many temperate and tropical species, it may often be sufficient to have a non-feeding period of 36–48 h prior to experiments to ensure sufficient time for the postprandial metabolic increment to subside [i.e. specific dynamic action (SDA)] (Jobling, 1981; Secor, 2009; Clark et al., 2010). However, the exact time will vary across species and temperatures, with cold-water species typically having longer SDA (Secor, 2009). For example, in Atlantic cod (*Gadus morhua*) fed to satiation the SDA lasts for approximately 85 h at 2°C (Perez-Casanova et al., 2010) or 72 h at 10°C (Behrens et al., 2012), while in Antarctic fishes living at subzero temperatures SDA durations of 9–16 days have been reported (Johnston and Battram, 1993; Boyce and Clarke, 1997). Thus, to be confident in reporting measurements of standard $\dot{M}_{O_2}$ in animals in a non-digesting state, it is necessary to conduct long-term and uninterrupted measurements of $\dot{M}_{O_2}$ in a subset of fish without disturbance following different periods of fasting.

As a rule of thumb when commencing research on a new species or under new conditions for a known species (e.g. temperature, hypoxia, CO_2), it is recommended that fish should remain in the respirometer for 24–48 h to provide a reasonable level of confidence that estimates of $\dot{M}_{O_2,min}$ are at least close to standard $\dot{M}_{O_2}$. Such experiments should allow an investigation of aspects such as metabolic recovery times following handling stress, declines in metabolism associated with prolonged fasting, and diurnal cycles in activity and $\dot{M}_{O_2}$. Once this knowledge has been obtained, experimental approaches and techniques can be refined to ensure the most accurate estimates of standard $\dot{M}_{O_2}$ and the most efficient use of time. Notably, some species settle in respirometers faster than others, and smaller fish often recover baseline metabolism faster than larger fish following a stressful event (Clark et al., 2012), hence these factors should be considered when determining the most appropriate respirometry techniques and protocols. Additionally, it is possible that measured values of $\dot{M}_{O_2,min}$ can decrease with subsequent, independent periods in the respirometer, as the fish may become progressively more comfortable with the handling and measurement procedures.

Failure to conduct long-term monitoring of $\dot{M}_{O_2}$ of the species of interest means that any hasty attempts to quantify $\dot{M}_{O_2,min}$ can, at best, yield measurements termed 'resting $\dot{M}_{O_2}$' or 'routine $\dot{M}_{O_2}$'. While these elevated estimates of $\dot{M}_{O_2,min}$ may not substantially affect subsequent calculations of aerobic scope (i.e. $\dot{M}_{O_2,max}$–$\dot{M}_{O_2,min}$) in species where $\dot{M}_{O_2,max}$ is high, they will have a significant effect on calculations of factorial aerobic scope (i.e. $\dot{M}_{O_2,max}/\dot{M}_{O_2,min}$) because of the marked influence of the denominator when calculating ratios (see discussion below). Indeed, factorial aerobic scope can vary widely between individuals if some fish do not reach a quiescent state during efforts to measure $\dot{M}_{O_2,min}$. This variability could be misinterpreted as biologically relevant differences in oxygen transport capacity, whereas in reality it is an artefact of inappropriate methodology. In any event, it is vital to keep protocols as consistent as possible within a study (e.g. time of day) if there is a desire to compare different treatment groups, because $\dot{M}_{O_2}$ can vary considerably over daily cycles even in starved fish (Brett and Zala, 1975; Steffensen, 1989). Furthermore, it is imperative that all studies be transparent about the methods used to measure the low extreme of $\dot{M}_{O_2}$, including a statement of the most appropriate term to describe this measurement in the context of the study and the species (e.g. standard, resting or routine $\dot{M}_{O_2}$). We recommend against using the ambiguous measurement of 'routine $\dot{M}_{O_2}$' in calculating aerobic scope, as the unquantifiable influence of fish activity can introduce substantial error.

In most published studies, the method for determining $\dot{M}_{O_2,min}$ is somewhat subjective. For example, it is not uncommon for researchers to take the single lowest $\dot{M}_{O_2}$ value in an entire data set and report it as $\dot{M}_{O_2,min}$, despite the fact that transient technical problems (e.g. temporarily aberrant measurements from the oxygen sensor) or physiological modifications of the fish (e.g. temporary bradycardia, hypoventilation or hypometabolism following a disturbance) can result in an abnormally low and incorrect measurement of $\dot{M}_{O_2,min}$. Some objective ways of calculating $\dot{M}_{O_2,min}$ are to use percentiles or frequency distributions to assess all collected data (Behrens and Steffensen, 2007; Dupont-Prinet et al., 2010; Nelson and Chabot, 2011). An example of an approach is illustrated in Fig. 5 using a histogram, where the mean of the lowest 10% of points from the entire data set is calculated and then outliers are excluded to produce the final $\dot{M}_{O_2,min}$. Outliers in this example are considered to be outside of the mean ± 2 s.d. of the lowest 10% of $\dot{M}_{O_2}$ values. All $\dot{M}_{O_2}$ measurements above the calculated $\dot{M}_{O_2,min}$ are assumed to be associated with spontaneous activity and they may provide insight into activity-related or routine $\dot{M}_{O_2}$. While specific techniques may have to be refined depending on the species and experiment, this kind of approach will facilitate some consistency across studies and will allow subsequent studies to replicate the experiments and analyses if desired.

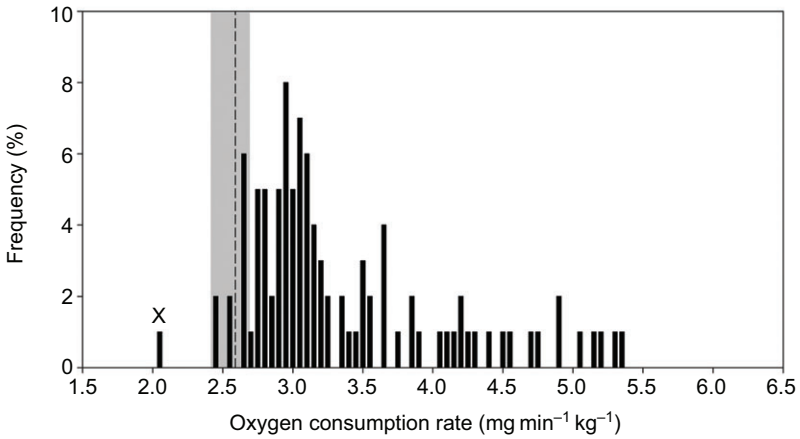


Fig. 5. Frequency histogram of the rates of oxygen consumption of an adult coral trout (*Plectropomus leopardus*) at 33°C while undisturbed in a static intermittent-flow respirometer ($\dot{M}_{O_2}$ measured for 6 min in every 10 min for a total of 18 h). Standard $\dot{M}_{O_2}$ was calculated by taking the lowest 10% of values and excluding outliers (outliers considered to be outside of the mean ± 2 s.d. of the lowest 10% of values, illustrated by 'X'). Shaded box indicates the points used in calculating standard $\dot{M}_{O_2}$, which had a mean (±s.d.) value of 2.59±0.09 mg min^{-1} kg^{-1} (dashed vertical line). Data have been binned into groups of 0.05 mg min^{-1} kg^{-1} for illustrative purposes, but in reality the lowest 10% of the raw (non-binned) data points should be used to calculate standard $\dot{M}_{O_2}$ after excluding outliers.

Maximum oxygen consumption rate

The goal of the $\dot{M}_{O2,max}$ measurement is to quantify the maximum rate at which oxygen can be consumed by the fish under any circumstance (typically ecologically relevant conditions are targeted). This is usually accomplished by maximally exercising the fish and measuring $\dot{M}_{O2}$ either during the exercise bout or some time shortly after. As with measurements of $\dot{M}_{O2,min}$, techniques for measuring $\dot{M}_{O2,max}$ can vary substantially and must be tailored to the species of interest.

A swim tunnel respirometer can be used for some species to challenge the fish with a maximum swimming speed whilst simultaneously measuring $\dot{M}_{O2}$. Of course, if $\dot{M}_{O2,max}$ is the desired measurement then the swim tunnel must be capable of producing water speeds that are in excess of the maximum swimming speed of the fish. This may not be problematic for small and/or non-athletic species, yet technical difficulties can arise when working with large, highly athletic species such as tunas and large salmonids that require water speeds of several metres per second in swim tunnels containing several hundred litres of water (Graham et al., 1990; Dewar and Graham, 1994; Farrell et al., 2003; Clark and Seymour, 2006; Blank et al., 2007; Steinhausen et al., 2008). The large, custom-built swim tunnels required for such experiments often require a substantial investment of funds. The swim tunnel method for measuring $\dot{M}_{O2,max}$ has an advantage in that fish can benefit from ram ventilation while swimming against high water flows, thus potentially producing a higher $\dot{M}_{O2,max}$ than would be attainable using static respirometry.

While swim tunnel respirometry may be the optimal method for obtaining measurements of $\dot{M}_{O2,max}$ in species that are accustomed to continuous swimming, there are many species that do not naturally swim for significant periods of time at high speed. Encouraging such species to exercise maximally in a swim tunnel can be difficult or impossible. In these circumstances, manually chasing the fish immediately prior to its introduction to a respirometer can achieve elevated levels of $\dot{M}_{O2}$ that are more likely to represent $\dot{M}_{O2,max}$ due to excess post-exercise oxygen consumption (see Reidy et al., 1995). The chase tank must be of sufficient size to allow burst-and-glide swimming, and circular tanks are most appropriate as they allow the fish to swim relatively unimpeded in circles. A chase time of 3–5 min is common because it is usually sufficient to render the fish unresponsive to touching its caudal fin, and several researchers have also added a short period of air exposure (e.g. 1 min) to the treatment to help ensure that the fish is maximally exhausted and most likely to elicit $\dot{M}_{O2,max}$ during the recovery period (e.g. Clark et al., 2012). While a short period of air exposure is common in standard fisheries practices for most species, some animal ethics committees may object to air-exposing fish for scientific purposes and therefore this should be considered before deciding on appropriate protocols. Species with higher stamina as well as those in cold water may require longer chase periods before becoming exhausted and unresponsive. Thus, when working with unfamiliar species or under new experimental conditions, relatively extensive pilot studies may be needed to verify that the treatment protocol used is capable of fully exhausting the fish and eliciting a maximum metabolic response. Furthermore, it can be very useful to formulate a 'performance' scoring system during pilot studies such that the level of effort of the fish during the chase protocol can be at least coarsely quantified.

Immediately post-exercise, the fish can be placed into a swim tunnel respirometer at a low water speed (to ensure appropriate oxygen mixing and allow ram ventilation), or a static respirometer may be used. So as not to inhibit ram ventilation in the static respirometer, flow can be increased on the closed-circuit recirculation loop (see above) to provide a significant flow of water into which the fish can orientate. This method of measuring $\dot{M}_{O2,max}$ following an exhaustive treatment has the potential to prolong the recovery duration when compared with a standard swim tunnel test because the oxygen uptake during recovery comprises the tissue oxygen consumption as well as the additional oxygen required to regain homeostasis (e.g. re-establish high energy phosphates and glycogen, and reverse biochemical, ionic and osmotic imbalances) and restore body oxygen stores that were depleted during the treatment protocol (e.g. oxygen associated with haemoglobin and myoglobin). While this represents a valid theoretical possibility, we are not aware of any studies that have thoroughly investigated this idea.

Another potential caveat that must be considered when using exhaustive chase protocols to elicit $\dot{M}_{O2,max}$ is that the maximum response does not necessarily occur immediately after the chase, but may instead appear several minutes or hours later. As an extreme example, a study by Clark et al. (Clark et al., 2012) reported that the maximum metabolic response following exhaustive chasing at 7°C was delayed by 6–8 h in sexually mature coho salmon (*Oncorhynchus kisutch*). Emphasising the variability that can exist between species and ecotypes, the same study on coho salmon showed that the lag time until the maximum metabolic response was only 5 h in smaller sexually mature male coho salmon ('jacks'). In 20–70 g juvenile brown trout (*Salmo trutta*) at 15°C, the first $\dot{M}_{O2}$ measurement (commencing <10 s post-exercise) is always the highest, indicating that metabolic recovery commences immediately (Norin and Malte, 2011). Such rapid recovery in small fishes is likely to have compromised estimates of $\dot{M}_{O2,max}$ in a study of 1–9 g juvenile Atlantic salmon (*Salmo salar*), where $\dot{M}_{O2}$ measurements did not commence until 20 min post-exercise because of the limitations with using a flow-through respirometer system and associated washout characteristics (Cutts et al., 2002) (see above). Thus, unless continuous and automated $\dot{M}_{O2}$ measurements commence rapidly during the post-chase recovery period, differences in metabolic responses might not be detected and the true $\dot{M}_{O2,max}$ may be underestimated. Previous researchers have kept fish in respirometers until complete metabolic recovery has occurred, at which point $\dot{M}_{O2,min}$ has been obtained (e.g. Clark et al., 2012). While this enhances the efficiency of measuring aerobic scope by removing the need for independent trials to achieve each of $\dot{M}_{O2,min}$ and $\dot{M}_{O2,max}$, comparative studies should be conducted to compare $\dot{M}_{O2,min}$ both before and after an exhaustive exercise protocol.

It is notable that the metabolic increment associated with SDA in some species can combine with the elevated $\dot{M}_{O2}$ associated with exercise to produce a higher $\dot{M}_{O2,max}$ than attainable in the same animal in a fasted state (Dupont-Prinet et al., 2009; Fu et al., 2009b; Jourdan-Pineau et al., 2010; Li et al., 2010). Other species (e.g. salmonids), however, do not appear to have the capacity to increase $\dot{M}_{O2,max}$ further after feeding and thus the SDA comes at the expense of a reduced maximum aerobic swimming speed (U_{crit}) (Alsop and Wood, 1997; Thorarensen and Farrell, 2006). The summation of metabolic demands and how it differs across environmental conditions (e.g. temperature, hypoxia, hypercapnia) requires further exploration across species, not least because it has obvious implications for defining and measuring $\dot{M}_{O2,max}$.

Despite a great range of publications using vastly different techniques (Fry and Hart, 1948; Blazka et al., 1960; Brett, 1965; Farrell et al., 2003; Nilsson et al., 2007; Clark et al., 2012), very little attention has been focused on trialling different methodologies

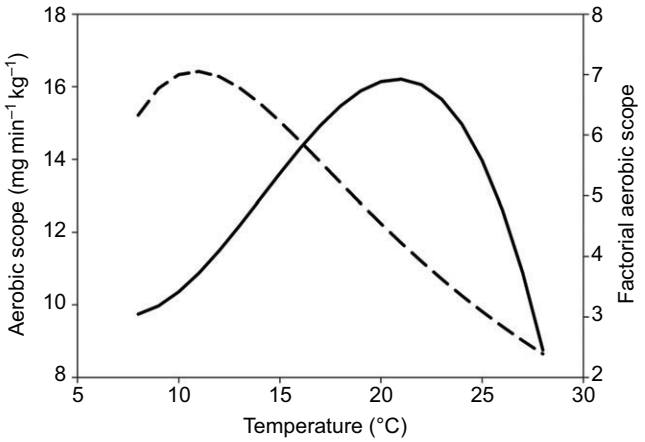


Fig. 6. Aerobic scope (solid line) and factorial aerobic scope (dashed line) as a function of temperature in adult female pink salmon (*Oncorhynchus gorbuscha*), depicting the opposing conclusions that can be drawn from the same data set depending on how the data are analysed and visualised. Modified from Clark et al. (Clark et al., 2011).

to determine which approaches are most suitable to elicit $\dot{M}_{O_2,max}$ in species of interest. For example, some studies of Atlantic cod report significantly higher $\dot{M}_{O_2}$ values following a chase protocol than during swimming at U_{crit} (Soofiani and Priede, 1985; Reidy et al., 1995), while another study on the same species failed to find a difference (Sylvestre et al., 2007). While these contrasting findings may result from subtle differences in experimental protocols or population-specific differences, they clearly have implications for the quantification of $\dot{M}_{O_2,max}$ and aerobic scope, and point to a requirement for further investigation into the methods used to maximally challenge the oxygen transport system of fishes.

Aerobic scope *versus* factorial aerobic scope

Aerobic scope can be expressed in absolute terms (i.e. $\dot{M}_{O_2,max}$–$\dot{M}_{O_2,min}$) or as factorial aerobic scope (i.e. $\dot{M}_{O_2,max}$/$\dot{M}_{O_2,min}$). These metrics provide an indication of the absolute and proportional increases, respectively, in oxygen consumption rate that an animal can achieve above baseline levels. Notably, these two metrics can run counter to each other when calculated using the same data (Fig. 6). Because there is no clear standard concerning which metric is most appropriate, many previous studies have based their conclusion on one metric while the other metric would have yielded a different, and often opposite, conclusion. For example, factorial aerobic scope was used to form the conclusion that larval and juvenile marine fishes may be compromised by a low capacity for oxygen transport in comparison with adults of the same species (Killen et al., 2007), yet examining the data in absolute terms reveals that aerobic scope is actually higher in the early life history stages (reworked data from Killen et al., 2007). The rationale for choosing factorial aerobic scope in that study was the assumption that smaller fish consume more oxygen per unit body mass than larger fish for a given activity such as swimming. While this is a valid possibility, our general understanding of this assumption is limited and conflicting data highlight the need for further research (e.g. White et al., 2006; Clark et al., 2012). In another study, T_{optAS} was reported as 21°C for adult pink salmon (*Oncorhynchus gorbuscha*), yet if factorial aerobic scope was used the T_{optAS} would be 11°C (Clark et al., 2011) (Fig. 6). Similar examples are widespread in the scientific literature (e.g. Cutts et al., 2002; Clark et al., 2005; Donelson et al., 2012), and the different outcomes have clear implications for data interpretation in an ecological context.

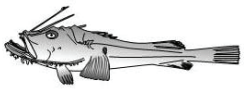
Given that it takes a specific amount of oxygen to perform a given activity (rather than a specific proportional increase above baseline metabolism), and that factorial aerobic scope can vary greatly with relatively minor changes in the denominator (i.e. $\dot{M}_{O_2,min}$), we believe that absolute aerobic scope is more informative and robust than factorial aerobic scope in most instances. Moreover, absolute rather than factorial aerobic scope is more informative when comparing across species, as emphasised when contrasting the aerobic scope (16.1 mg min^{-1} kg^{-1}) and factorial aerobic scope (3.9) of the athletic pink salmon at 22°C [data for female fish (Clark et al., 2011)] with the same metrics of the sedentary Murray cod (*Maccullochella peelii peelii*) at the same temperature (2.0 mg min^{-1} kg^{-1} and 3.8, respectively) (Clark et al., 2005). A comparison of the factorial aerobic scope of these species yields very little insight into their greatly contrasting lifestyles, while the absolute aerobic scope provides a good indication of their respective aerobic performances. In any event, we recommend that future studies report both absolute and factorial aerobic scope, and provide thorough justification if factorial rather than absolute aerobic scope is chosen to be discussed. This will provide transparency and will hopefully lead to a more balanced and comprehensive assessment of metabolic data sets.

Effects of temperature

Many studies seek to quantify aerobic scope at a wide range of temperatures and subsequently determine the temperature at which aerobic scope is maximal (T_{optAS}), as it has been proposed that processes such as growth and reproductive output are maximised at this temperature (Brett, 1971; Pörtner and Knust, 2007; Pörtner and Farrell, 2008; Pörtner, 2010). There are a variety of techniques that have been used to examine the influence of temperature on the aerobic scope of different species. For example, some studies have used different individuals at each temperature (Fry and Hart, 1948; Brett, 1972; Lee et al., 2003; Eliason et al., 2011), while others have used the same individuals at a range of temperatures to allow repeated-measures analyses (e.g. Clark et al., 2011). In the latter case, it is important to repeat $\dot{M}_{O_2}$ measurements after returning to particular temperatures (e.g. the starting temperature) to ensure that repeated bouts of maximal exercise do not negatively influence subsequent $\dot{M}_{O_2,max}$ measurements. The outcome of these repeated $\dot{M}_{O_2}$ measurements is likely to be influenced by the recovery time provided between exercise bouts, the rate of temperature change, and the aerobic capacity of the study species.

Few studies have allowed for any potential thermal acclimation of metabolism by examining the effects of chronic thermal exposure (weeks to years) on aerobic scope (e.g. Fry and Hart, 1948; Brett, 1972; Duthie, 1982; Healy and Schulte, 2012), although this level of acclimation is presumably much more relevant from a climate change perspective, especially for open-water species that do not encounter rapid and extreme daily fluctuations in temperature. It has been suggested that metabolic acclimation to warmer or cooler temperatures typically occurs within 1 or 3 weeks, respectively [see Barrionuevo and Fernandes, (Barrioneuvo and Fernandes, 1998) and references within], yet the physiological mechanisms and temporal dynamics of thermal acclimation in modulating aerobic scope are not well understood (Cossins and Bowler, 1987; Wang and Overgaard, 2007; Franklin and Seebacher, 2009; Healy and Schulte, 2012).

Table 1. A range of traits that determine aerobic scope and its relevance compared between two opposing lifestyles

Trait	 Pelagic piscivore	 Benthic ambush predator
Swimming style	Continuous aerobic swimmer	Burst anaerobic swimmer
Sustained aerobic capacity	High	Low
White muscle mass (relative to M_b)	High	Low
Red muscle mass (relative to M_b)	High	Low
Gill area (relative to M_b)	High	Low
$\dot{M}_{O_2,min}$ (inter-species comparison)	High	Low
$\dot{M}_{O_2,max}$ (inter-species comparison)	High	Low to medium
SDA (% increase)	Low	High
Swim capacity when in SDA	High	Low
When $\dot{M}_{O_2,max}$ achieved	Burst swim and recovery	SDA and exhaustion recovery
Aerobic scope importance for locomotion	High	Low
Aerobic scope importance for digestion	Low to medium	High
$\dot{M}_{O_2,max}$ measurement technique	Swim tunnel respirometry	Exhaustion recovery (+SDA)
Ecological relevance of aerobic scope	Vital for locomotion and simultaneous demands	Reduces time required for digestion and burst exercise recovery

Measurements of aerobic scope from these two types of fishes can yield vastly different information, and direct comparison of aerobic scope between them is of limited value. Most fishes exist along the axis between these two extremes. When designing experiments to obtain $\dot{M}_{O_2,max}$ measurements from a new species, it is important to understand where the species sits on the axis between aerobic athlete and ambush predator. Furthermore, the interpretation of the aerobic scope results will be highly dependent on the type of $\dot{M}_{O_2,max}$ measurements performed. The ecological relevance of aerobic scope in ambush predators may relate mainly to reducing the time required for digestion or to recover from burst swimming, in order to be ready to strike should a new prey item appear. In contrast, pelagic swimmers use a large proportion of their oxygen uptake to support locomotion in a way that more closely reflects how aerobic scope is commonly regarded.

M_b, body mass; $\dot{M}_{O_2,max}$, maximum oxygen consumption rate; $\dot{M}_{O_2,min}$, minimum oxygen consumption rate; SDA, specific dynamic action.

Whichever approaches are taken, the thermal treatment of the fish must be defensible in an ecologically relevant context if the goal is to apply the results to the natural environment. For the purpose of examining physiological mechanisms, particularly under forecasted climate change scenarios, it can be useful to go beyond what the fish currently experiences in nature.

Ecological relevance of aerobic scope and T_{optAS}

Aerobic scope should set the limit for the magnitude of oxygen-demanding processes that can be performed simultaneously, and therefore it is likely to be an important mechanism contributing to the ecology of fishes and other animals. Nevertheless, there are few studies that have investigated how aerobic scope is partitioned between activities in the natural environment, whether animals routinely seek out T_{optAS} to maximise their aerobic scope, and indeed whether (and how often) the total aerobic scope of an animal is ever used in daily and seasonal activities.

A key aspect that must be considered when interpreting the relevance of aerobic scope in fishes is the fact that species with different behaviours and foraging strategies (e.g. pelagic active predators *versus* benthic ambush predators) use their available aerobic scope for different purposes (Table 1). In less active species, higher $\dot{M}_{O_2,max}$ values may be attained during digestion alone than during aerobic swimming or following exhaustive burst swimming challenges (Fu et al., 2009a). Thus, the maximum oxygen transport capacity in these species may have evolved to accommodate the metabolic requirements during digestion of large meals while remaining relatively inactive, rather than to maintain a high aerobic scope during continuous swimming (Table 1). A standard swim challenge or chase protocol may therefore fail to elicit the maximum metabolic response in such species, and thus great care must be taken when interpreting the ecological consequences of aerobic scope estimates.

There is a tendency in the scientific literature to refer to T_{optAS} as simply 'T_{opt}'. This implies that T_{optAS} is the overall optimal temperature for the species of interest, and therefore the species should seek out this temperature in order to optimise all fitness-related processes. This terminology has undoubtedly contributed to the confusion and largely unchallenged acceptance of the OCLTT concept throughout much of the scientific community. In reality, there is a range of different optimal temperatures for different processes and life history attributes, and often these other optimal temperatures are markedly different from T_{optAS}. For example, T_{optAS} of adult pink salmon occurs at 21°C and aerobic scope decreases with decreasing temperature (Clark et al., 2011), yet if reproduction was attempted at 21°C it would fail spectacularly because the optimal temperature for spawning in Pacific salmonids is typically <14°C (Richter and Kolmes, 2005). Moreover, it is improbable that pink salmon would spend any more than a few hours or days at 21°C in their entire ~2-year life cycle, although very little is known of how T_{optAS} may be modulated at different points in the life history. Whether relevant or simply coincidental, it is notable that pink salmon spend a significant portion of their lives at temperatures around 11°C, which is the temperature that elicits maximum factorial aerobic scope in mature individuals (Fig. 6). Even in Atlantic cod (*G. morhua*), a species that has been used extensively to strengthen support for the OCLTT concept (e.g. Pörtner et al., 2008), aerobic scope has been found to increase continuously with temperature throughout the normal range rather than being maximal at an intermediate temperature and declining slowly as temperatures warm or cool (Claireaux et al., 2000) (see Fig. 1). This suggests that T_{optAS} has little relevance in the life history of this species across its geographic range.

The OCLTT hypothesis states that at a species' upper pejus temperature (where the aerobic scope starts to drop), the performance decreases because of reduced aerobic scope, which in turn leads to reduced tissue oxygen levels (tissue hypoxia) (Pörtner and Farrell, 2008). However, tissue hypoxia should not appear until temperatures where the aerobic scope is next to zero, and where the fish have very little scope for increasing their oxygen uptake. Thus,

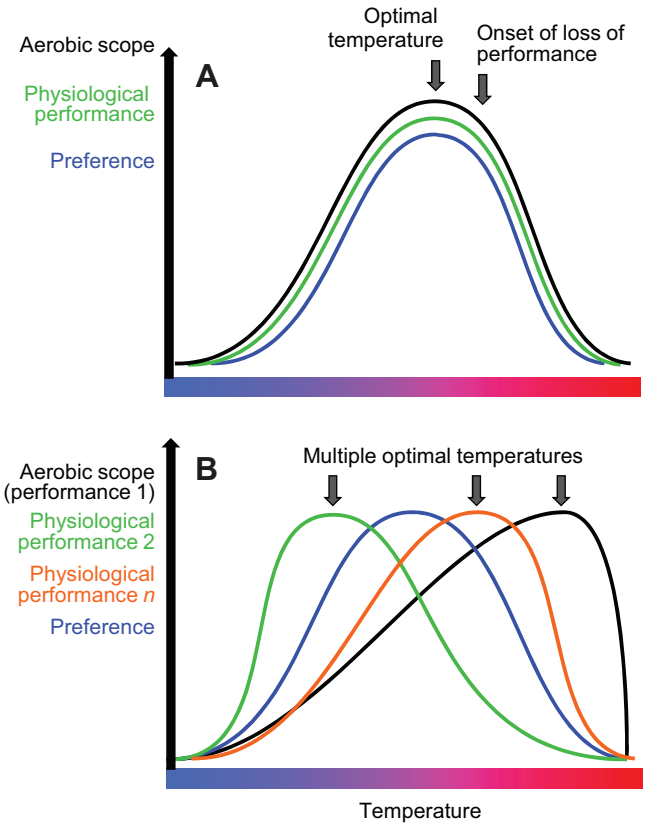


Fig. 7. Two contrasting ideas that aim to describe the thermal tolerance and preference of fishes. (A) The oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis assumes that aerobic scope is the fundamental physiological process driving overall physiological performance, thermal preference/tolerance and fitness. (B) In contrast, the idea of 'multiple performances – multiple optima' (MPMO) assumes that different physiological processes have different optimal temperatures, and therefore thermal preference/tolerance and fitness are governed by multiple physiological parameters that can shift in relative importance between species, life stage and the nature of the thermal challenge. It is notable that preference temperatures may be determined partly by ecological and environmental factors in addition to physiological requirements.

simultaneous reductions in performance and aerobic scope merely correlate with temperature. For example, the aerobic scope of two species of coral reef fishes tended to decline as temperature increased from 29 to 32°C, yet the fish maintained a high aerobic scope (>70% of maximal) at 32°C despite being only marginally below their estimated lethal temperature of around 33°C (Munday et al., 2009). This suggests that oxygen limitation only appears at exactly lethal temperature, which contradicts the fundamental principles of the OCLTT hypothesis. To establish causality requires showing that tissue hypoxia occurs at the onset of other adverse effects of temperature and that experimentally manipulating tissue hypoxia levels (e.g. through experimental hyperoxia) improves thermal tolerance in fishes (Weatherley, 1970; Rutledge and Beitinger, 1989).

While processes such as growth and reproductive output are suggested, as a unifying principle, to be directly determined by the available aerobic scope (Pörtner and Knust, 2007; Pörtner and Farrell, 2008), studies linking such fundamental processes with aerobic scope under chronic thermal exposures remain scarce. In fact, recent data have shown that specific growth rate was negative in a population of killifish (*Fundulus heteroclitus*; 'northern population') at acclimation temperatures where aerobic scope was highest (25–30°C), indicating that growth was restricted by mechanisms other than oxygen transport capacity (Healy and Schulte, 2012). Furthermore, in females of this killifish population the gonadosomatic index, an estimate of reproductive output, was highest at acclimation temperatures of 10–15°C despite the fact that T_{optAS} was reported as 25–30°C (Healy and Schulte, 2012). The 'southern population' in that study showed varying responses, but still any suggested link with OCLTT would be tenuous.

The OCLTT hypothesis suggests that aerobic scope can be viewed as an overarching physiological process that governs most other performance attributes (e.g. growth, digestion, reproduction, immune function, muscular activity, behaviour), yet there are alternative and perhaps more plausible explanations. The OCLTT concept suggests that fishes at T_{optAS} will maximise all physiological performances and ultimately optimise fitness. An alternative view is the idea of 'multiple performances – multiple optima' (MPMO), where different physiological functions have different optimal temperatures (Fig. 7). In this view, aerobic scope is regarded as just one of many physiological functions, and the different functions are placed in no hierarchical order. This idea of MPMO acknowledges that any physiological function can be the limiting factor in a thermal challenge, and that this likely differs between species, life stage and the nature of the thermal challenge (e.g. acute *versus* global warming). The preference temperature may be determined partly by ecological and environmental factors, as well as being modulated by physiological aspects such as nutritional status and health. While this does not necessarily provide a specific framework that can be tested, it does provide a timely reminder that the OCLTT hypothesis is not universally applicable and there are other performance metrics that may work independently or synergistically with attributes such as aerobic scope to govern the thermal preferences and fitness of water-breathing organisms. A fruitful direction for future research is to investigate which performance metric(s) becomes progressively limited as ectothermic animals are shifted away from their preferred temperature (i.e. the temperature they select when given the opportunity).

Conclusions

Measurements of aerobic scope in fishes have increased in popularity in recent years, particularly in disciplines outside of classic respiratory physiology. While this trend is exciting to see and will likely continue into the future, there is a need to implement a higher level of quality control before the diversity of issues becomes too great to remedy. Although it is not possible to cover all of the technical aspects that must be considered when conducting respirometry experiments on fishes, we have endeavoured in the present paper to outline a range of fundamental issues that must be considered by scientists before embarking on future studies.

Beyond methodological aspects, this paper highlights the need to use caution when interpreting metabolic data in the framework of the OCLTT concept. The latter is a hypothesis, and like all hypotheses it should be tested rather than assumed to be a law (see Hilborn, 2006). Indeed, a concerning observation in publications supporting OCLTT is the general lack of reference to the large number of previous studies that have quantified aerobic scope across temperature and found no support for OCLTT. There is no doubting the importance of aerobic metabolism in daily activities,

but currently there is little understanding of the ecological relevance of T_{optAS}, how aerobic scope and T_{optAS} are modulated throughout the lifecycle, and how much of maximal aerobic scope is needed to maintain optimal fitness. The relevance of aerobic scope to overall fitness has yet to be conclusively demonstrated. One thing is clear, the OCLTT concept does not apply generally across fish species. We rely on future studies on different species by a range of scientists to better determine the applicability and usefulness of the OCLTT concept in governing the thermal tolerance of fishes. Indeed, many other plausible factors could be responsible for governing temperature tolerance, including oxidative stress, cell damage, protein denaturation, disturbed neural function, effects on membrane fluidity, thermal inactivation of enzymes at rates that exceed rates of formation, and different temperature effects in interdependent metabolic reactions (Cossins and Bowler, 1987; Schmidt-Nielsen, 1990; Lushchak and Bagnyukova, 2006; Overgaard et al., 2012). Our aspiration is to encourage uninhibited interpretation of future results with a goal to better understand environmental impacts on animal performance. Energy metabolism is fundamental to lifetime fitness; we encourage greater consistency, accuracy and scrutiny within and across studies to ensure that measurements of aerobic metabolism continue to provide insight into the physiology and ecology of water-breathing animals.

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Author contributions

T.D.C., E.S. and F.J. developed the ideas in this paper and contributed to writing the manuscript.

Competing interests

No competing interests declared.

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