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RESEARCH ARTICLE

Oxygen supply capacity breathes new life into critical oxygen partial pressure (P_{crit})

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ABSTRACT

The critical oxygen partial pressure (P_{crit}), typically defined as the P_{O_2} below which an animal's metabolic rate (MR) is unsustainable, is widely interpreted as a measure of hypoxia tolerance. Here, P_{crit} is defined as the P_{O_2} at which physiological oxygen supply (α_0) reaches its maximum capacity (α; μmol O_2 g^{-1} h^{-1} kPa^{-1}). α is a species- and temperature-specific constant describing the oxygen dependency of the maximum metabolic rate (MMR=P_{O_2}×α) or, equivalently, the MR dependence of P_{crit} (P_{crit}=MR/α). We describe the α-method, in which the MR is monitored as oxygen declines and, for each measurement period, is divided by the corresponding P_{O_2} to provide the concurrent oxygen supply (α_0=MR/P_{O_2}). The highest α_0 value (or, more conservatively, the mean of the three highest values) is designated as α. The same value of α is reached at P_{crit} for any MR regardless of previous or subsequent metabolic activity. The MR need not be constant (regulated), standardized or exhibit a clear breakpoint at P_{crit} for accurate determination of α. The α-method has several advantages over P_{crit} determination and non-linear analyses, including: (1) less ambiguity and greater accuracy, (2) fewer constraints in respirometry methodology and analysis, and (3) greater predictive power and ecological and physiological insight. Across the species evaluated here, α values are correlated with MR, but not P_{crit}. Rather than an index of hypoxia tolerance, P_{crit} is a reflection of α, which evolves to support maximum energy demands and aerobic scope at the prevailing temperature and oxygen level.

KEY WORDS: Aerobic scope, Hypoxia, Metabolic rate, Ocean deoxygenation, Oxygen and capacity limited thermal tolerance, Oxygen supply, Respirometry

INTRODUCTION

The relationship between metabolic rate (MR; Box 1) and environmental oxygen has long been of interest because of its implications for human health, fisheries management, biogeography, species diversity and evolution (Tang, 1933; Lindroth, 1942; Hall, 1966; Fry and Hart, 1948; Childress, 1968; Weibel, et al., 1991; Suarez, 1998; Ern et al., 2016; Rogers et al., 2016; Ultsch and Regan, 2019; Slesinger et al., 2019). Although this relationship has taken on special significance in light of anthropogenic ocean warming and deoxygenation (Wishner et al., 2018; Breitburg et al., 2018; Claireaux and Chabot, 2019; Pörtner et al., 2017; Rubalcaba et al., 2020; Howard et al., 2020; Deutsch et al., 2020), there is no consensus on how it should be measured. A commonly employed metric is the critical oxygen partial pressure (P_{crit}), which is typically interpreted as a measure of hypoxia tolerance, with a lower P_{crit} indicating greater tolerance for low oxygen (e.g. Rogers et al., 2016; Seibel, 2011). However, as 'hypoxia' and 'tolerance' are subjective, time-dependent terms, and P_{crit} is inconsistently defined and measured, there is active debate about the meaning and significance of the metric (Wood, 2018; Regan et al., 2019).

Typically, P_{crit} is defined as the minimum P_{O_2} at which a given MR can be sustained and is identified by a clear decline (breakpoint) in the MR in response to declining P_{O_2} (Farrell and Richards, 2011; Pörtner and Grieshaber, 1993; Richards, 2011; Hall, 1966; Ultsch and Regan, 2019; Childress and Seibel, 1998). Intraspecifically, P_{crit} is dependent on the MR, which is itself dependent on temperature and activity levels. For comparison among species, recent protocols (Claireaux and Chabot, 2016; Reemeyer and Rees, 2019) therefore recommend measuring P_{crit} at the standard metabolic rate (SMR), the lowest 'sustainable' MR measured in a fasted and resting state. By definition, aerobic metabolism is oxygen limited at P_{crit} for SMR ($P_{crit\text{-}SMR}$) and aerobic scope [the difference between standard and maximum (MMR) rates] is nil. This is what early researchers referred to as the 'oxygen level of no excess activity' (Fry and Hart, 1948; Lindroth, 1942).

Ultsch and Regan (2019) recently argued that $P_{crit\text{-}SMR}$ is the most appropriate benchmark because it is truly 'critical' for the animal's survival, whereas P_{crit} for other rates is not a lethal oxygen level. Accordingly, they defined $P_{crit\text{-}SMR}$ as the P_{O_2} below which life cannot be sustained. However, survival is also time limited at, or even at some factor above, $P_{crit\text{-}SMR}$, because aerobic scope (and thus feeding, growth and reproduction) may be oxygen limited. Furthermore, many species rarely (or never) experience P_{O_2} levels near their $P_{crit\text{-}SMR}$ (Deutsch et al., 2020) and, among those that do, the hypoxia is often temporary, intermittent or cyclic. Survival below $P_{crit\text{-}SMR}$ is dependent on the capacity to temporarily suppress metabolic demands to match limited oxygen availability and to increase anaerobic ATP production (Seibel et al., 2014, 2018; Storey, 2015).

Lindroth (1942) argued that P_{crit} should be determined at the lowest P_{O_2} at which an organism is indefinitely viable, i.e. that for which development and reproduction are undisturbed and 'continued prosperity' is supported. Recent work suggests P_{O_2} must exceed $P_{crit\text{-}SMR}$ by a factor of ∼3 to meet this benchmark. That is, a factorial aerobic scope (FAS; MMR/SMR=P_{O_2}/P_{crit}; see Box 1; Seibel and Deutsch, 2020) of ∼3 corresponds to a biogeographic limit for many species (Deutsch et al., 2015, 2020). However, because, intraspecifically, P_{crit} is typically temperature sensitive, a FAS of 3 may be met or exceeded at low temperature even in relative hypoxia, while, at warmer temperatures, even atmospheric P_{O_2} may not provide

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Box 1. Definitions

Physiological oxygen supply capacity (α)

The maximum amount of oxygen that can be supplied per unit time and oxygen pressure, here presented mass-specifically (μmol O_2 g^{-1} h^{-1} kPa^{-1}). The amount of oxygen being taken up at any given point (α_0=MR/P_{O_2}) increases as oxygen declines and/or metabolic rate (MR) increases until it reaches its maximum capacity at P_{crit} (α=MR/P_{crit}). The α is a species- and temperature-specific constant defining the α-line, which quantifies the oxygen dependency of maximum metabolic rate (MMR=α×P_{O_2}, at P_{O_2}>$P_{crit\text{-}max}$, where $P_{crit\text{-}max}$ is the critical oxygen partial pressure at MMR) or, equivalently, the rate dependence of P_{crit} (P_{crit}=MR/α). The equations describing the α-line have slope α, and they intercept the origin.

Metabolic rate (MR)

The rate of aerobic energy usage, estimated from oxygen consumption. MMR is the highest achievable MR, typically measured during or following exercise protocols. At P_{O_2} less than $P_{crit\text{-}max}$, MMR is directly oxygen dependent (MMR=P_{O_2}×α). Standard metabolic rate (SMR) refers to the fasted, resting metabolic rate at a specified temperature.

Factorial aerobic scope (FAS)

The factorial difference between MMR and SMR (FAS=MMR/SMR). It is equivalently expressed in terms of environmental oxygen availability relative to physiological oxygen supply capacity (FAS=P_{O_2}×α/SMR at P_{O_2}<$P_{crit\text{-}max}$). FAS is a measure of the aerobic capacity to support activities (e.g. growth, reproduction, locomotion) beyond basic maintenance.

Critical P_{O_2} (P_{crit})

The P_{O_2} at which oxygen supply reaches its maximum capacity (α) for any given MR ($P_{crit\text{-}MR}$=MR/α). P_{crit} is any rate-specific point on the α-line. Equivalently, P_{crit} is the P_{O_2} below which its corresponding MR becomes oxygen limited. Throughout this paper, P_{crit} specified for a particular MR is indicated by a subscript (e.g. $P_{crit\text{-}SMR}$ or $P_{crit\text{-}max}$, for SMR and MMR, respectively).

sufficient metabolic scope. Thus, the ecological significance of P_{crit} remains unclear, regardless of the rate at which it is measured, and there is no consensus on the best way to determine P_{crit}.

The traditional brokenstick method (Yeager and Ultsch, 1989) depends on a relatively constant MR as P_{O_2} declines (i.e. it must be 'regulated') and a discontinuity in the measured rate must be readily apparent at P_{crit}, where the rate begins to conform to available P_{O_2}. In the brokenstick method, the intersection of two regressions, through the oxygen-limited and oxygen-independent portions of the trial, is taken as P_{crit} (Yeager and Ultsch, 1989). This methodology imparts a great deal of importance to the constancy or degree of oxygen independence of the regulated rate at high P_{O_2} values as variation in slope will affect the determined P_{crit} value. A more consistent method uses only a single regression (MR versus P_{O_2}) through the oxygen-limited portion of the curve and solves for SMR, which must be established at higher P_{O_2}. This limiting low oxygen (LLO) method is not influenced by variation in rate throughout the trial (Reemeyer and Rees, 2019). However, SMR estimates may vary with respirometry or statistical methodology (Chabot et al., 2021) and other variables, which will influence P_{crit} determination (e.g. Negrete and Esbaugh, 2019; Regan and Richards, 2017). What constitutes SMR is also unclear for the majority of species and many measurements to date may be more appropriately described as routine metabolic rate (RMR), for which activity and feeding history are unknown. Additionally, P_{O_2} below $P_{crit\text{-}SMR}$ is in the realm of 'incipient lethal oxygen levels' (Claireaux and Chabot, 2016), where MR does not necessarily decline in direct proportion to P_{O_2}. In that case, the slope of that portion of the trial will vary unpredictably, resulting in variable estimates for $P_{crit\text{-}SMR}$. Some studies have forced the oxygen-limited regression through the origin, increasing the consistency of $P_{crit\text{-}SMR}$ determination, but without a stated physiological justification (McArley et al., 2019).

Others have recommended statistical approaches to identify an inflection point in the data that represents P_{crit} (Muggeo, 2003) or to statistically describe the entire relationship between MR and P_{O_2}, without implication of any physiological mechanism or evidence that oxygen is driving the apparent relationship (non-linear regression, regulation index, or Michaelis–Menten analysis; Mueller and Seymour, 2011; Cobbs and Alexander, 2018; Wood, 2018). However, P_{crit} is effectively lost in such mathematical descriptions of the entire respirometry trial and it is unclear what information can be extracted from a description of the entire trial, much of which may not have any functional relationship to available oxygen.

For an individual organism, activity elevates MR and a higher rate will become oxygen limited (i.e. reach its P_{crit} for that MR) at higher oxygen pressures (Fry and Hart, 1948; Claireaux and Chabot, 2016). The relationship between MR and its corresponding P_{crit} is often assumed to be curvilinear between $P_{crit\text{-}SMR}$ and $P_{crit\text{-}max}$ (the P_{crit} for the maximum metabolic rate, MMR). Claireaux and Chabot (2016) described this relationship schematically as the limiting oxygen level (LOL) curve. They note that, for any rate lower than MMR, ventilatory rates and cardiac output increase as environmental P_{O_2} declines until the LOL curve is reached. Further decline in P_{O_2} requires that extraneous metabolic costs (e.g. activity) be reduced. While this description is physiologically consistent, the authors did not explain or justify the curvilinear shape of the LOL curve and their direct measurements do not unambiguously support the assumed shape. Unfortunately, this assumption masks the quantifiable oxygen dependency of MR described by Seibel and Deutsch (2020) and reinforces the idea that $P_{crit\text{-}SMR}$ is an independent metric that reflects environmental hypoxia tolerance.

Here, we define P_{crit} as the P_{O_2} at which physiological oxygen supply mechanisms are operating at maximum capacity (α, see Box 1; Deutsch et al., 2015, 2020; Seibel and Deutsch, 2020; Kielland et al., 2019; Lindroth, 1942). P_{crit} values are a MR-specific measure of the oxygen supply capacity, α, that can be mathematically defined as P_{crit}=MR/α (see detailed derivation below and definitions in Box 1). α is a species- and temperature-specific constant that describes the linear dependency of P_{crit} on MR and, equivalently, the oxygen dependency of the MMR (MMR=P_{O_2}×α; Seibel and Deutsch, 2020). We show here that α can be determined directly without an obvious breakpoint in, nor a standardized level of, MR.

This definition provides a strong theoretical justification for the concept of P_{crit} and eliminates most, if not all, of the recently described pitfalls associated with its measurement (Wood, 2018; Reemeyer and Rees, 2019; Ultsch and Regan, 2019; Regan et al., 2019). This new definition calls into question widely held beliefs about the ecological and evolutionary significance of P_{crit}. As stated by Fry and Hart (1948), 'the worth of such data to the ecologist must ultimately depend on proof that they have real significance as values limiting the activity of the organism in nature'. Many decades later, such proof is still not readily available and most evidence is merely correlative. Strong comparative approaches have been employed to demonstrate that P_{crit} sometimes reflects physiological adaptations to low oxygen (Regan et al., 2019; Wishner et al., 2018; Childress and Seibel, 1998; Mandic et al., 2009). However, a comparatively

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low P_{crit} value is often assumed to reflect adaptation to environmental hypoxia, even where oxygen levels never approach P_{crit} or in the absence of direct environmental P_{O_2} measurements. We argue here that P_{crit} is not a measure of hypoxia tolerance, per se, but rather an indirect way of measuring the oxygen supply capacity, itself adapted to supply oxygen for species-specific metabolic demands and aerobic scope across temperatures and at the prevailing oxygen pressure.

MATERIALS AND METHODS

Oxygen supply capacity determination

For an aerobic organism to obtain sufficient energy for survival, oxygen supply must meet oxygen demand. Oxygen demand is the aerobic MR, most commonly measured at rest (SMR) or at maximum exertion (MMR). The total amount of oxygen available for cellular respiration at any given time is a function of the ambient environmental P_{O_2} and the physiological oxygen supply (α_0; Seibel and Deutsch, 2020; Deutsch et al., 2015). Physiological oxygen supply, α_0, comprises each step in the oxygen cascade (Weibel et al., 1991) from ventilation and blood–oxygen binding to cardiac output and circulation. We define and calculate α_0 as the rate of oxygen consumption (MR) per unit of available environmental oxygen pressure (α_0=MR/P_{O_2}; μmol O_2 g^{-1} h^{-1} kPa^{-1}). When an individual has maximized these physiological mechanisms of oxygen delivery, the physiological oxygen supply, α_0, has reached the physiological oxygen supply capacity, α, and MR=MMR at that P_{O_2}.

Thus, α_0 must increase toward α, via upregulated oxygen extraction and transport, as environmental P_{O_2} declines toward P_{crit} and/or as MR increases toward MMR (Fig. 1). The P_{crit} for a given MR is reached when α_0=α and the oxygen cascade cannot be further upregulated. At P_{O_2} below P_{crit}, MR necessarily declines. Below $P_{crit\text{-}SMR}$, MR may be suppressed or maintained anaerobically or death may occur. The physiological oxygen supply capacity is set by species- and acclimation-specific attributes of the oxygen cascade and is constant across MR (Eqn 1; Fig. 1). P_{crit} is defined by maximized oxygen supply regardless of MR, rather than by the oxygen dependence of a particular MR (see Box 1 for definition of terms):

$$\alpha = SMR/P_{crit\text{-}SMR} = RMR/P_{crit\text{-}RMR} = MMR/P_{crit\text{-}max}. \quad (1)$$

To determine α, the rate of oxygen consumption (MR) is monitored using established respirometry techniques as P_{O_2} declines. Each MR measurement period within a trial is divided by the corresponding P_{O_2} to provide a value for the rate of oxygen supply (α_0) at that point in the trial. For any given MR, oxygen supply increases toward its maximum capacity (α) as P_{O_2} declines and the highest α_0 value is taken as the maximum capacity, α. To be more conservative, here we designated the mean of the three highest α_0 values as α. The α-line (MMR=P_{O_2}×α) defines the limiting P_{O_2} for any MR between SMR and MMR. The oxygen supply capacity is conserved across MR (i.e. the α-line is linear, with slope, α, and intercept at the origin), as was demonstrated previously (Seibel and Deutsch, 2020) and is further supported here.

When MR is maintained at SMR throughout a trial, as is often the goal in traditional P_{crit} trials, α_0 will gradually increase throughout the run and may only reach α briefly at $P_{crit\text{-}SMR}$. Thus, α may be represented by few points before α_0 declines again as the subject either suppresses metabolism or experiences physiological failure. A decline in α_0 below $P_{crit\text{-}SMR}$ is frequently observed, which diminishes the accuracy of traditional methods, such as the LLO or the brokenstick method, that rely on the slope of the that portion of the curve (Fig. 2). If, in contrast, the MR is directly proportional to P_{O_2} below a given P_{crit}, then α_0 will plateau when it reaches capacity, α. In this case, multiple α_0 values can be averaged to determine α (see lines 1 and 2 in Fig. 1A,B). A final consideration is that α_0 may increase following the brief plateau if substantial error exists in P_{O_2} measurement (i.e. sensor calibration error; see Fig. 1). This occurs because P_{O_2} measurement error leads to a measurable rate of oxygen consumption (the numerator) even as P_{O_2} (the denominator) apparently, but erroneously, approaches anoxia. This is sometimes apparent as a positive *y*-intercept in the MR versus P_{O_2} trace. This is recognizable (Fig. 1) using the α-method but would represent undiagnosed error using traditional techniques. Once diagnosed,

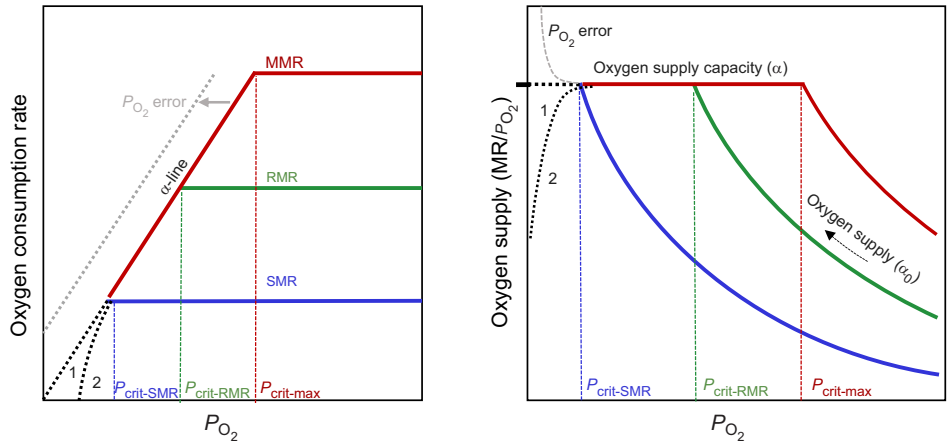

Fig. 1. Schematic diagram showing the oxygen dependency of metabolic rate and physiological oxygen supply (α_0). (A) Maximum (MMR, red), routine (RMR, green) and standard (SMR, blue) metabolic rate (MR) as a function of P_{O_2}. (B) The corresponding oxygen supply (α_0=MR/P_{O_2}) increases as P_{O_2} decreases, reaching its maximum capacity, α, at the critical P_{O_2} (P_{crit}). Thus, α=MR/P_{crit} and the α-line (red, P_{O_2} between $P_{crit\text{-}SMR}$ and $P_{crit\text{-}max}$) defines the rate dependence of P_{crit} and, equivalently, the oxygen dependence of MMR. The α-line is described by the following equivalent equations: MR=α×P_{crit}; MMR=α×P_{O_2}. Black dotted lines in each panel represent two common patterns of MR decline below $P_{crit\text{-}SMR}$. (1) Oxygen supply capacity is maintained and the MR conforms to P_{O_2} and (2) the MR declines faster than P_{O_2}, indicating physiological failure or death. Patterns represented by gray dotted lines are rarely seen, but indicate possible error in P_{O_2} calibration. Each panel in Figs 3 and 5 presents similar side-by-side plots of oxygen consumption rate and oxygen supply as a function of P_{O_2} for different species.

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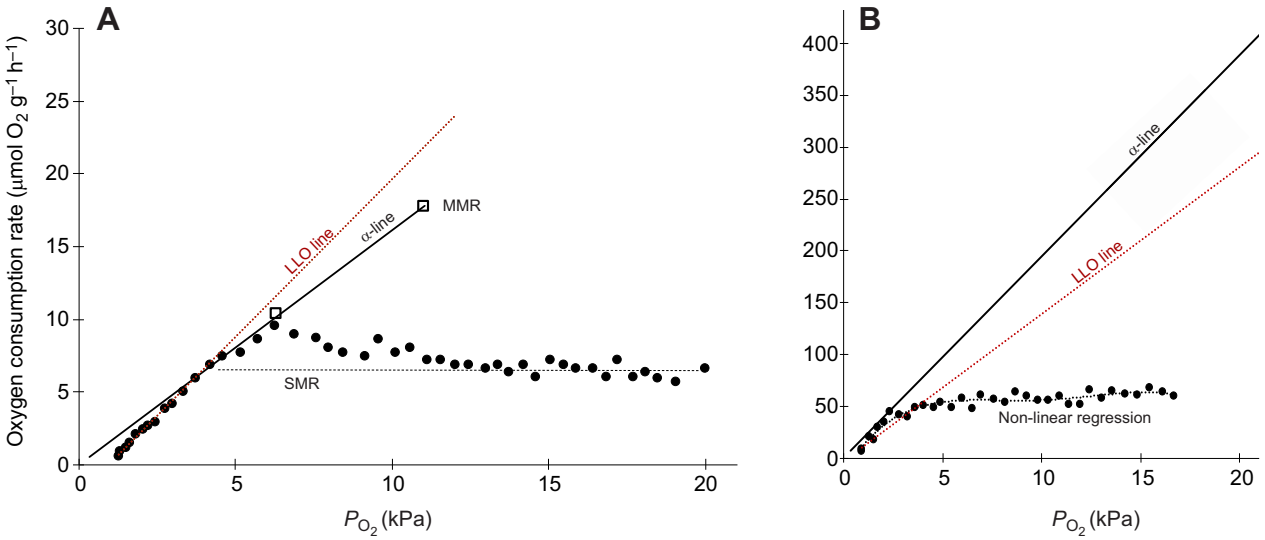

Fig. 2. Representative respirometry trials comparing respirometry analysis methods. Trials are presented for (A) *Fundulus grandis* (Reemeyer and Rees, 2019) and (B) *Crinia georgiani* (Marshall et al., 2013) showing the linear low oxygen line (LLO, red dashed line; Reemeyer and Rees, 2019), non-linear regression (dotted black line in B only; Marshall et al., 2013) and the α-line (solid black line; MMR=α×P_{O_2}). The LLO curve is extrapolated out to the estimated $P_{crit\text{-}max}$ for each case (Seibel and Deutsch, 2020) for comparison with the α-line. For each α-line, α is the mean of the highest three α_0 values, where α_0=MR/P_{O_2} for each measured point. The α-line, but not the LLO line, for *F. grandis* accurately predicts MMR (open squares), both at its estimated $P_{crit\text{-}max}$ and in hypoxia (Reemeyer and Rees, 2020). The LLO method relies on regression of P_{O_2} values with MR below the SMR, the slope of which may reflect metabolic suppression, physiological failure (i.e. dying) or ambiguity in SMR determination rather than oxygen dependence. As a result, the LLO curve does not typically intercept the origin and its slope may be higher (as in A) or lower (as in B) than the α-line. The LLO line may accurately predict $P_{crit\text{-}SMR}$ (as in A) or not (as in B). Non-linear regressions and the regulation index (Mueller and Seymour, 2011) are purely descriptive and the relationship between MR and P_{O_2} that they describe may not be causal. As such, they cannot be used to predict rates under conditions beyond those at which they were originally measured.

P_{O_2} measurement error may be corrected. No correction has been applied to any of the data in the present manuscript.

Specific recommendations

We make the following specific recommendations for use of the α-method. α should be determined during exercise when possible because α_0 will be maintained near capacity ($\alpha_0 \approx \alpha$) for a greater proportion of the trial and/or α may be reached multiple times throughout the trial (Fig. 1). If measured at MMR, α_0 will be near α for the entire trial, in which case, α may be determined as the mean of all α_0 values. A further advantage of measurement in an active state is that α can be determined at higher P_{O_2}, well above potentially lethal oxygen levels. If α is measured near SMR, we recommend averaging no more than the three the highest α_0 values to determine α. This diminishes the underestimating effect of averaging sub-maximal oxygen supply values above and below $P_{crit\text{-}SMR}$.

As with traditional measures of MR and P_{crit}, values of α may be influenced by the precision of the measurement. However, because α is not dependent on the MR, it is not dependent on the behavior of the animal in the respiration chamber, the technique used to measure the rate (e.g. closed versus intermittent-flow respirometry) or the duration of the trial (Fig. 3). In some cases, extended trial duration could result in physiological acclimation of, or impairment to, the oxygen supply cascade in response to declining oxygen or accumulated metabolic waste, respectively, resulting in variation in α relative to shorter trials (Reemeyer and Rees, 2020; Regan and Richards, 2017; Snyder et al., 2016; Sollid et al., 2005). However, differences in methodology between studies that only influence the MR will not affect the oxygen supply capacity.

Data selection

We used several approaches to test the applicability, generality and precision of the α-method. We first extracted and re-evaluated available literature data for 40 species in five phyla (Table S1) using studies that provided a 'representative' trace showing the relationship between the oxygen consumption rate and P_{O_2}. Data were extracted from these respirometry curves using WebPlotDigitizer 4.2 (https://automeris.io/WebPlotDigitizer/index.html). The oxygen supply capacity was determined as described above and compared with α calculated using literature values of P_{crit} and the corresponding MR.

Many of the 'representative' curves provided in the literature may be the best of many trials, rather than being truly representative. Thus, they may not provide the most rigorous test of this method. Accordingly, we also tested full datasets (Table S2) for zooplankton (Wishner et al., 2018), shrimp (A.L.B. unpublished), squid (M.A.B. and B.A.S., unpublished) and fishes (Slesinger et al., 2019; A.A., unpubllished) to assess inter-individual variance in α. Oxygen consumption for each species was measured using published methods, based on accepted practices (Clark et al., 2013; Chabot et al., 2016) using chambers of appropriate size and type for each species (see references in Table S2). All experiments were carried out with IACUC approval (protocol # W IS00007992, W IS00004975).

To assist in the adoption of this new method, calculation of α has been incorporated into the R package 'respirometry' with an optional parameter available to define the MR of interest for P_{crit} estimation (http://cran.r-project.org/package=respirometry). The 'calc_pcrit' and 'plot_pcrit' functions now return side-by-side comparisons of P_{crit} calculated using a brokenstick regression (Yeager and Ultsch, 1989), non-linear regression (Marshall et al., 2013), the LLO method (Reemeyer and Rees, 2019), the sub-prediction interval method (Birk et al., 2019), and the α-method presented here. Additionally, the function 'calc_alpha' determines α when the user inputs MR and oxygen data. This software is freely available on CRAN.

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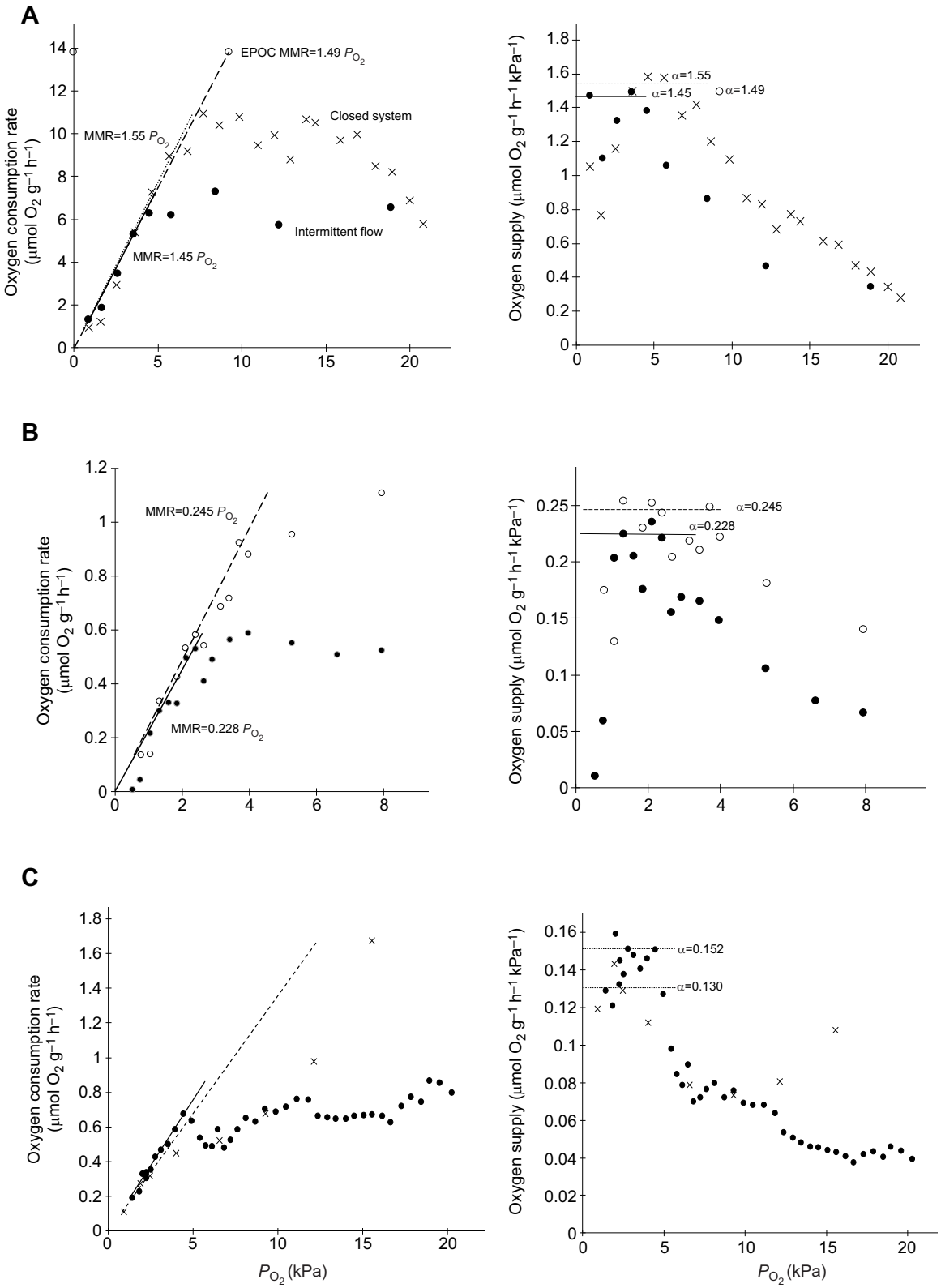

Fig. 3. Oxygen supply capacity resulting from different respirometry methods. Different MRs result in different P_{crit} values but the same oxygen supply capacity, α. The α-line (left panels) describes the oxygen dependency of the MMR (MMR=P_{O_2}×α; equations shown on plots) or, equivalently, the rate dependency of P_{crit} (P_{crit}=MR/α). The slope of these lines is α, the mean of the highest three α_0 values calculated as MR/P_{O_2} for each point on the graph (right panels). (A) Red drum (*Sciaenops ocellatus*): measurements made at rest using intermittent flow (filled circles) or closed respirometry (crosses; Negrete and Ern, 2019) and following exhaustive exercise in hypoxia (open circle, excess post-exercise oxygen consumption, EPOC, at 9 kPa; Ern et al., 2016). (B) *Octopus bimaculoides*: the effect of experiment duration on MR and critical P_{O_2}. Open circles represent measurements made with a trial duration of 4 h, while filled symbols were measured over 24 h with sufficient acclimation to achieve SMR (Seibel and Childress, 2000). (C) *Nautilus pompilious*: surgery-induced stress may have caused elevated MR and apparent oxyconformation (crosses; Boutilier et al., 1996) compared with acclimated specimens (filled symbols; Staples et al., 2000).

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RESULTS

Published MR and P_{crit}, as well as the directly determined and calculated α values, are presented in Table S1 for 40 species of diverse aquatic and terrestrial animals. The individual trials used are presented pictorially in Table S1. The MR, P_{crit} and α values were converted to common units (μmol O_2 g^{-1} h^{-1} and kPa) for ease of comparison. The physiological oxygen supply capacity, α, was determined using the α-method and calculated as the published MR divided by its corresponding P_{crit} (α=MR/P_{crit}). The α values determined using these two distinct methods are correlated (Fig. 4; y=1.96x−0.10; Pearson's r=0.96, P<0.001, n=40). However, the α-method resulted in higher values on average (mean difference=4.4%), which reflects its dependence on the highest α_0 values, rather than a regression through all values as in other P_{crit} methods. In the species analyzed here, α ranged from 0.09 μmol O_2 g^{-1} h^{-1} kPa^{-1} for the deep-sea anglerfish, *Melanocetus johnsoni*, at 5°C (Cowles and Childress, 1995), to 4.11 μmol O_2 g^{-1} h^{-1} kPa^{-1} at 28°C for larval zebrafish, *Danio rerio* (Mandic et al., 2020; Table S1). These values are within the range previously reported for a similar diversity of species and are correlated with the MR at which they were determined (α=0.195×SMR+0.317; Pearson's r=0.98, P<0.001; Fig. 4; Deutsch et al., 2020; Seibel and Deutsch, 2020). The oxygen supply capacity is not significantly correlated with P_{crit} (Pearson's r=−0.10, P=1) but it is elevated, relative to oxygen demand, in the few species included here that inhabit persistently hypoxic environments, such as pronounced oxygen minimum zones (OMZs; Tables S1 and S2).

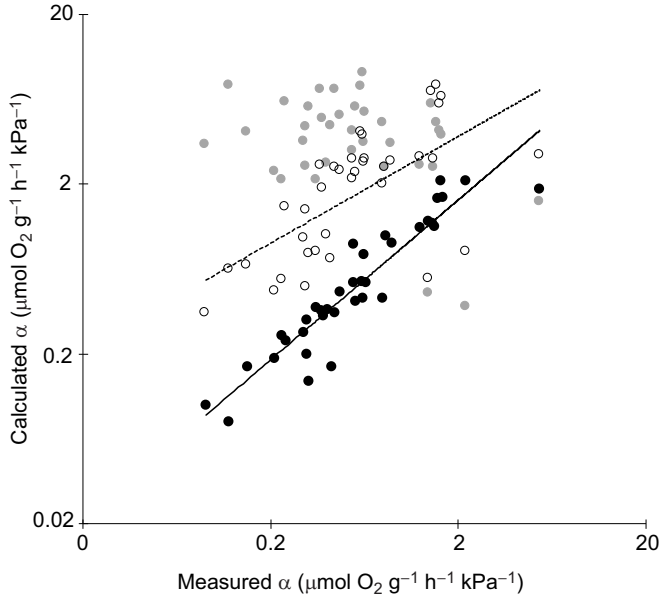

Fig. 4. The measured (α-method) and calculated (published MR/P_{crit}) oxygen supply capacity (α). Values are presented in Table S1. The measured α (x-axis) is strongly correlated with that determined as MR/P_{crit} (y-axis) using reported values (black symbols, y=1.07x−0.10; Pearson's R=0.96, P<0.001). Oxygen supply capacity is significantly correlated with MR (α=0.195×SMR+0.317; Pearson's r=0.98, P<0.001; open symbols), but not the reported P_{crit} value (Pearson's r=−0.10, P=1; gray symbols). Data from Behrens and Steffensen, 2007; Birk et al., 2018; Birk et al., 2019; Brill et al., 2015; Burggren et al., 2019a,b; Caposella 2012; Childress, 1975; Childress and Seibel, 1998; Christensen and Colacino 2000; Claireaux and Chabot 2016; Collins et al., 2013; Cowles and Chidress, 1995; Crear and Forteath, 2000; Dall 1986; Ern et al., 2016; Fu et al., 2011; Lefevre et al., 2015; Mandic et al., 2020; Negrete and Esbaugh, 2019; Nillson and Ostlund-Nilsson, 2004; Onthank et al., 2021; Paschke et al., 2010; Reemeyer and Rees, 2019; Rutherford and Thuesen, 2005; Schurmann and Steffenson, 1997; Seibel and Childress, 2000; Slesinger et al., 2019; Snyder et al., 2016; Speers-Roesch, 2012; Staples et al., 2000; Stoffels 2015; Thuy et al., 2010; Tuong et al, 2018; Ultsch et al., 1981; Wishner et al., 2018.

In our dataset, α_0 increased in response to declining P_{O_2} in all cases and peaked or reached a plateau in all but two cases (see Table S1). As P_{O_2} continued to decline below $P_{crit\text{-}SMR}$, α_0 either was maintained at capacity (α_0=α), with MR conforming directly to available P_{O_2} for the remainder of the trial (line 1; Fig. 1), or declined, indicating metabolic suppression or physiological failure below $P_{crit\text{-}SMR}$ (line 2; Fig. 1). In all cases, the mean of the highest three resulting α_0 values was designated as the oxygen supply capacity, α (Table S1), which was used to generate the α-line (see Box 1). In two cases (see Table S1), a continuous increase in α_0, with the highest value occurring at the lowest P_{O_2}, provided no clear peak or plateau, which likely indicates P_{O_2} measurement error. For these two cases, the highest α_0 was substantially higher than the mean of the highest three values. Unlike when using traditional P_{crit} methods, these potential P_{O_2} measurement errors are identifiable using the α-method. The highest α_0 for the remaining species was only slightly higher than, and directly correlated with, the mean of the three highest values (y=1.1x−0.03; R^2=0.99, n=40).

The oxygen supply capacity, α, was insensitive to differences in MR that resulted from differences in respirometry methods, such as closed versus intermittent flow respirometry, trial duration or organismal stress (Fig. 3). However, within a species, higher temperature typically results in higher oxygen supply capacity (see *Chitala ornata* and *Lates calcarifer*; Table S2). This occurs because α supports maximum oxygen demand, which is elevated by temperature. Maximum or active MRs have been measured during declining P_{O_2} for several species. Below $P_{crit\text{-}max}$, the decline in MMR with P_{O_2} was described well by the α-line (MMR=P_{O_2}×α) and MMR and $P_{crit\text{-}SMR}$ trials provided similar α values (Fig. 5).

To assess intraspecific variability and to ensure that our analysis was not biased by the use of idealized representative curves from the literature, we also analyzed several existing respirometry datasets (Table S2): *Euphausia mucronata*, a midwater krill from the Eastern Tropical Pacific OMZ (C.J.W. and B.A.S., unpublished), *Farfantepenaeus duorarum*, an estuarine pink shrimp (A.L.B. unpublished), the Atlantic spiny dogfish, *Squalus acanthias* (A.A., unpublished), and two oceanic squids, *Illex illecebrosus* and *Sthenoteuthis oualaniensis* (M.A.B., unpublished). Among these species, α varied from 3.35±2.64 in *E. mucronata* to 0.41±0.13 in *S. acanthias*. The α_0 response types (described above) varied among individuals of each species, but typically displayed a clear peak or plateau, facilitating unambiguous identification of α. The α values were similar for each species whether derived using the α-method or from P_{crit} and SMR determined using the LLO method (Reemeyer and Rees, 2019).

DISCUSSION

P_{crit}, regardless of how it is determined, is a rate-specific measure of α and it is α, rather than P_{crit} per se, that provides relevant physiological information. Because α is not specific to a particular MR, P_{crit} need not be measured at SMR or any other specific MR. It is also not necessary for an individual animal to maintain a consistent MR throughout a trial or for a MR versus P_{O_2} curve to have a clear breakpoint. The oxygen supply capacity, whether directly determined or extracted from a P_{crit} value, defines the α-line that describes the oxygen limit for every MR from SMR to MMR. This definition of P_{crit} is consistent with current physiological theory and the α-method provides a simple, repeatable and precise

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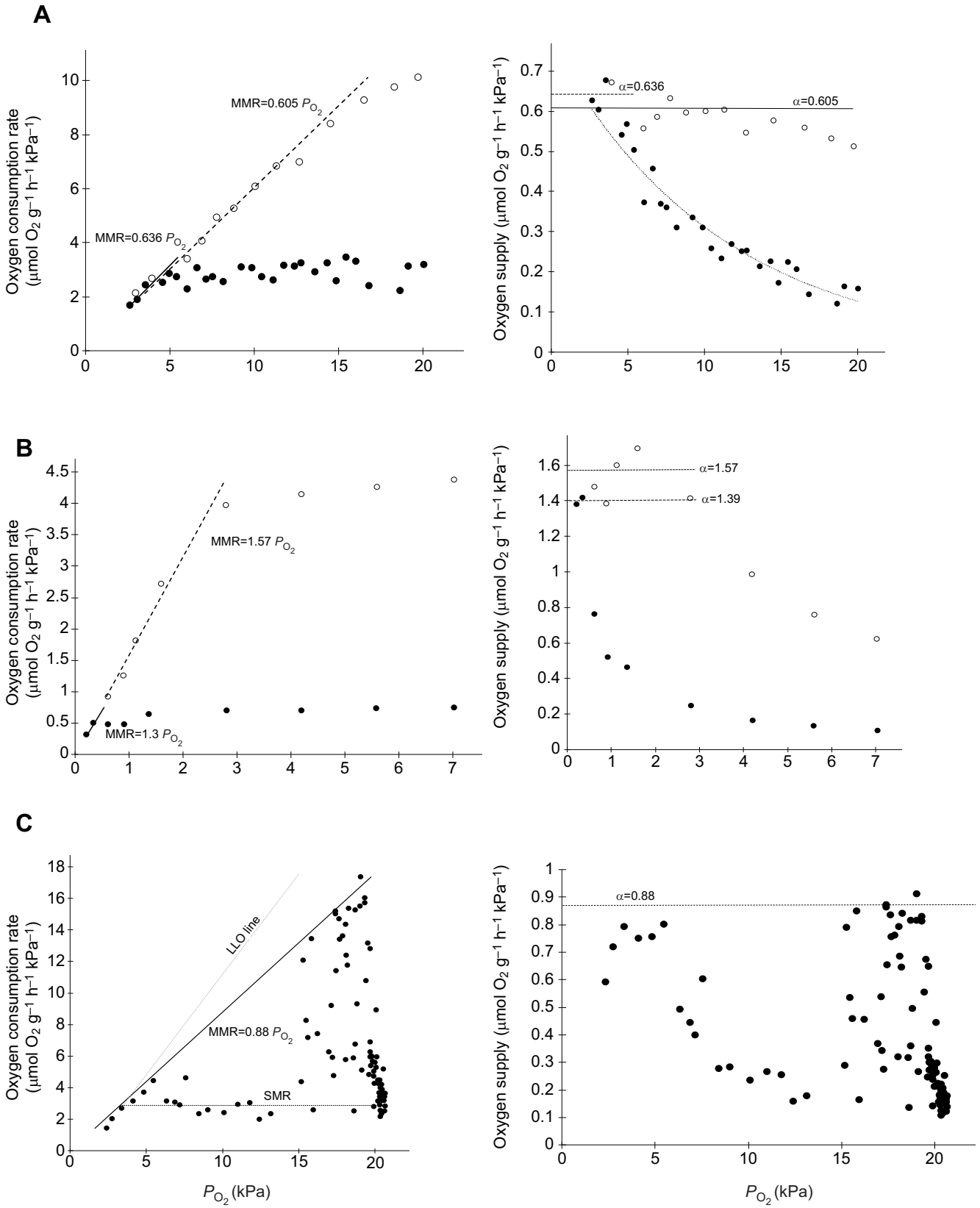

Fig. 5. Oxygen supply capacity determined for MRs between standard and maximum. Left: the oxygen dependence of the maximum achievable oxygen consumption (metabolic) rate (MMR, open symbols) as described by the α-lines and their equations (MMR=α×P_{O_2}). SMR (filled symbols) are also shown for some species. Right: the oxygen supply capacity, α, is identified as the mean of the three highest oxygen supply values (α_0) recorded. (A) *Penaeus esculentus* (Dall, 1986); α determined as the mean of all α_0 values between $P_{crit\text{-}SMR}$ and $P_{crit\text{-}max}$. (B) *Gnathophausia ingens* (deep-sea lophogastrid shrimp; Childress and Seibel, 1998). Hyperoxia (P_{O_2}>$P_{crit\text{-}max}$) does not permit higher MMR. (C) *Salvelinus fontinalis* (brook trout; Claireaux and Chabot, 2016). The LLO line (Reemeyer and Rees, 2019) would not accurately predict MMR.

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alternative to existing methods of determining P_{crit}. Moreover, this definition, with its emphasis on oxygen supply capacity, clarifies the physiological and evolutionary significance of P_{crit}.

What does P_{crit} tell us?

P_{crit} is a measure of α, from which the maximum metabolic rate achievable at any P_{O_2} can be determined. Seibel and Deutsch (2020) showed that, for most shallow, coastal species, α is nearly identical whether determined as SMR/$P_{crit\text{-}SMR}$ or MMR/21 kPa. Interestingly, when using the α-method, α is often slightly (mean 4.4%) higher than that determined as SMR/$P_{crit\text{-}SMR}$ (Table S1). A higher α predicts a higher MMR, suggesting that some previous MMR measurements may have been slight underestimates. MMR trials typically start at P_{O_2} near air saturation and allow oxygen to decline by as much as 5–10%. In fact, Clark et al. (2013) specifically recommended maintaining oxygen saturation above 80%. However, if, as we suggest, MMR declines linearly with P_{O_2} below $P_{crit\text{-}max}$, MMR values will be underestimated in proportion to the measurement P_{O_2}. Thus, the reported match between α values determined in active and resting states may reflect off-setting discrepancies in both MMR and $P_{crit\text{-}SMR}$ determination. We suggest that future studies should maintain P_{O_2} at or above air saturation during MMR measurement.

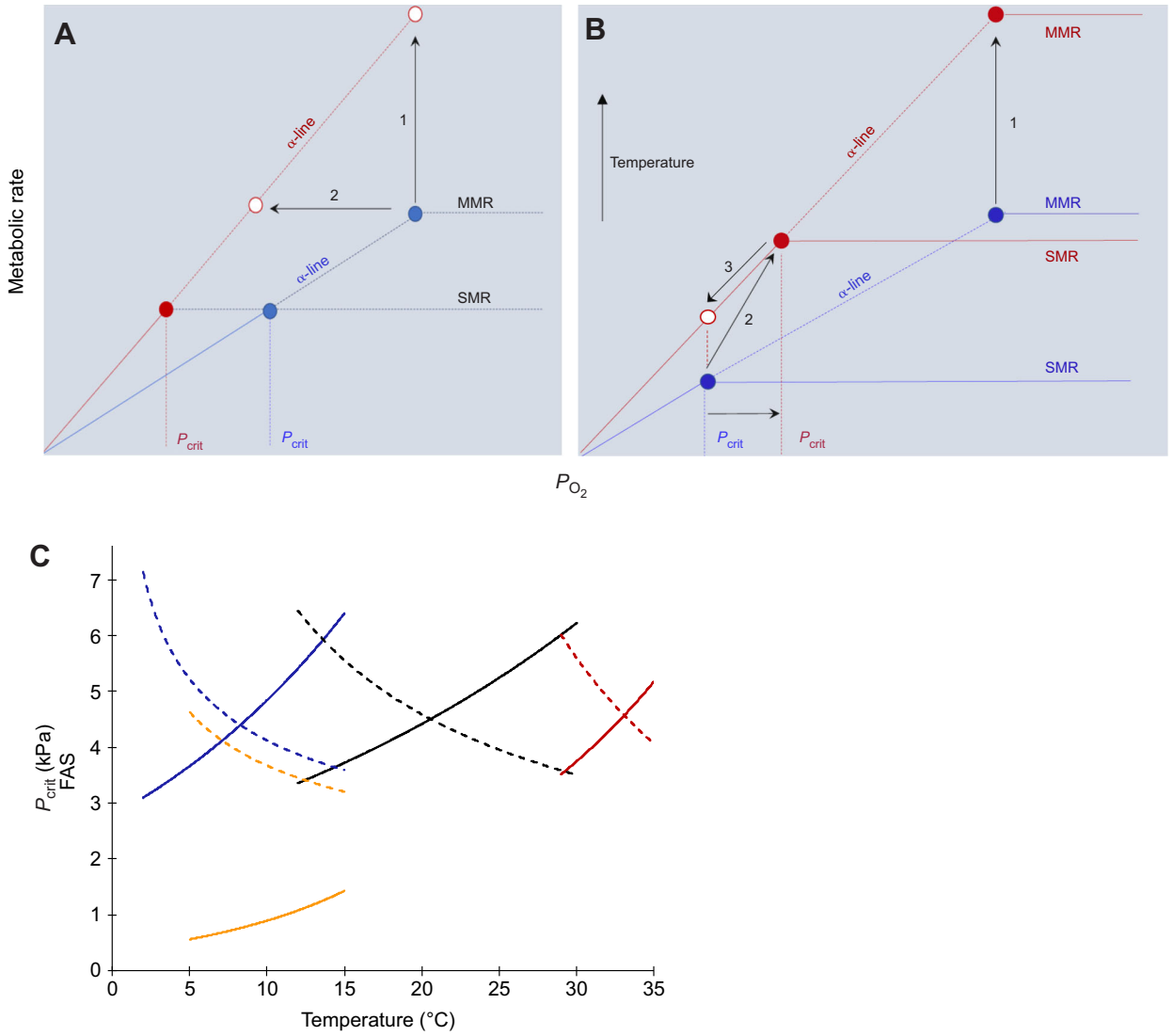

Fig. 6. Schematic diagrams showing the effect of potential selective forces acting on oxygen supply capacity. Variation in P_{crit} reflects variation in both MR and oxygen supply capacity, that results from adaptations for athleticism, hypoxia tolerance or aerobic scope at different temperatures. (A) Two species with identical SMR (blue, red). The red species has a lower P_{crit} and, thus, a higher α (SMR/P_{crit}; greater slope of the α-line), which may support one of two strategies: (1) greater athleticism (a higher MMR) in normoxia for the red species (arrow 1) or (2) hypoxia tolerance (lower $P_{crit\text{-}max}$; arrow 2). MMR and $P_{crit\text{-}max}$ must be known to be able to distinguish between these two alternatives. (B) A single individual or species measured at two temperatures (warm, red; cold, blue). Black numbered arrows represent the change in each metric (SMR, MMR and P_{crit}) with temperature. Arrow 1: temperature drives an increase in MMR and oxygen supply capacity. Arrow 2: SMR typically (but not always) increases faster with temperature than does MMR, resulting in an increase in P_{crit}. Arrow 3: chronic exposure to warmer temperatures results in adaptation or acclimation that reduces SMR, increasing aerobic scope and lowering P_{crit}. (C) Data compiled in Seibel and Deutsch (2020) for the subpolar Atlantic cod (blue), temperate black seabass (black), tropical coral reef damselfish (red) and the freshwater goldfish (orange). P_{crit} (solid lines) increases with acute temperature exposure (intraspecifically) but decreases as a result of chronic exposure (intraspecific acclimation or, in this case, interspecific adaptation) to high temperature. Factorial aerobic scope (FAS; dashed lines) decreases with a slope that is identical in magnitude but opposite in sign to P_{crit} because FAS=MMR/SMR=$P_{crit\text{-}max}$/P_{crit} (Seibel and Deutsch, 2020). For the goldfish, $P_{crit\text{-}max}$ is low, reflecting adaptation of the oxygen supply capacity to maintain FAS in its hypoxic habitat.

Selection for elevated metabolic capacity (i.e. athleticism), increased aerobic scope or metabolic performance in persistent hypoxia may elevate the oxygen supply capacity (Fig. 6A,B). In order to distinguish between these selective pressures, $P_{crit\text{-}max}$ (the P_{O_2} above which no further increase in metabolism is possible) must be determined (Fig. 6). A $P_{crit\text{-}max}$ less than air saturation suggests adaptation to persistent hypoxia. Most terrestrial or shallow-living aquatic species have a $P_{crit\text{-}max}$ near atmospheric P_{O_2} (21 kPa; Seibel and Deutsch, 2020). For such species, additional environmental oxygen (hyperoxia) should not elevate metabolism. Among these 'normoxic' species, most of the variation in P_{crit} reflects the differing temperature sensitivities of MMR and SMR and, thus, the FAS (Fig. 6C).

Inserting MMR and SMR into Eqn 1 and rearranging shows that FAS×$P_{crit\text{-}SMR}$=$P_{crit\text{-}max}$, meaning that, for species with similar $P_{crit\text{-}max}$, $P_{crit\text{-}SMR}$ is inversely correlated with FAS (Fig. 6C; Seibel and Deutsch, 2020). FAS explained 95% of the variation in $P_{crit\text{-}SMR}$ among 39 taxonomically diverse, normoxic species analyzed by Seibel and Deutsch (2020). No variation could be attributed to measured differences in environmental P_{O_2}. Thus, $P_{crit\text{-}max}$, rather than $P_{crit\text{-}SMR}$, provides a useful measure of hypoxia tolerance, while hypoxic effects, as noted by Ern et al. (2016), are evident at any P_{O_2} less than $P_{crit\text{-}max}$ (air saturation for most coastal species). Below $P_{crit\text{-}max}$, MMR and aerobic scope are diminished in all species and the decrement is quantifiable using the α-line. The precise decrement in aerobic scope that results in reduced fitness is unknown, but recent work suggests that a FAS of ∼3 defines biogeographical limits for many species (Deutsch et al., 2020) and may provide the hypoxic benchmark above which species are 'indefinitely viable' as prescribed by Lindroth (1942). Although a lower $P_{crit\text{-}SMR}$ allows SMR to be sustained to that lower P_{O_2}, it is rare for most species to experience hypoxia less than about 3 times $P_{crit\text{-}SMR}$ (Deutsch et al., 2020). For those that do, hypoxia is typically intermittent or cyclic and selects for the ability to temporarily suppress metabolism rather than, or in addition to, enhanced oxygen supply capacity (Seibel, 2011; Mandic et al., 2009; McArley et al., 2019; Storey, 2015).

In contrast, adaptation to persistent hypoxia (longer than a diel or tidal cycle) results in a relatively high α for a given MR, which improves active (not resting) performance in low oxygen (Seibel and Deutsch, 2020). As a result, P_{crit} is reduced at all metabolic levels and both P_{crit} and $P_{crit\text{-}max}$ are low relative to those of similar species adapted to higher oxygen environments (Childress and Seibel, 1998; Seibel, 2011; Wishner et al., 2018; Mandic et al., 2009; Richards, 2011; Regan et al., 2019). For example, for *Gnathophausia ingens*, a deep-sea lophogastrid crustacean living permanently in the OMZ, α is ∼1.39 µmol g^{-1} h^{-1} kPa^{-1}, which is among the highest values measured here and its $P_{crit\text{-}max}$ is extremely low (∼2.5 kPa; Fig. 5; Childress, 1968). *Gnathophausia ingens* lives persistently below 600 m in the California Current OMZ and rarely, if ever, experiences oxygen levels higher than its $P_{crit\text{-}max}$.

To understand whether variation in P_{crit} and α reflects adaptation for metabolic capacity, aerobic scope or hypoxia, $P_{crit\text{-}max}$ must also be known (Fig. 6A). In the absence of such data, one can tentatively infer the adaptive value of α if it is assumed that FAS for the species of interest falls within the typical range (∼3 to 6; Seibel and Deutsch, 2020; Killen et al., 2016; Peterson et al., 1989). If true, $P_{crit\text{-}max}$ will be 3–6 times $P_{crit\text{-}SMR}$, which can be calculated as SMR/α. An estimated $P_{crit\text{-}max}$ value substantially less than 21 kPa suggests that oxygen supply mechanisms may be adapted for hypoxia. For *G. ingens*, mentioned above, $P_{crit\text{-}max}$, estimated as 6×SMR/α, is only 2.42 kPa (Table S1), which is very near its independently measured $P_{crit\text{-}max}$ (Fig. 5). In contrast, an assumed $P_{crit\text{-}max}$ of 21 kPa would provide an unlikely FAS of 57. Thus, *G. ingens* high oxygen supply capacity facilitates high FAS (∼6) in extreme hypoxia (<2.5 kPa) (Childress and Seibel, 1998). Higher oxygen values (>2.5 kPa) will not enable higher MR or aerobic scope in *G. ingens*. The difference between $P_{crit\text{-}max}$ and $P_{crit\text{-}SMR}$ is less than 2 kPa for *G. ingens*, consistent with the very low and narrow oxygen range experienced in its deep-sea habitat (Childress and Seibel, 1998). Similarly, the goldfish maintains high FAS across a wide temperature range and in hypoxia by elevating α and reducing both P_{crit} and $P_{crit\text{-}max}$ (Fig. 6C).

How should we determine the oxygen supply capacity?

The α-method results in estimates of α that are similar to those determined from P_{crit} using other methods. This is especially true for the LLO (SMR extension) method (Reemeyer and Rees, 2019) because the variation in the MR for which P_{crit} is being determined is reduced or eliminated. The LLO method is further improved if the oxygen-limited portion of the curve is forced through the origin (McArley et al., 2019). If α is determined from a P_{crit} that is measured by the intersection of curves above and below $P_{crit\text{-}SMR}$, large errors may occur if the low-P_{O_2} portion of the curve does not extend through the origin (Fig. 2). Below $P_{crit\text{-}SMR}$, oxygen transport or oxidative metabolism may be failing or shutting down. Thus, the low-P_{O_2} portion of the curve cannot be informative of performance in a non-lethal oxygen range. For ∼30% of the trials in Table S1, MR is not directly proportional (does not conform) to P_{O_2} below $P_{crit\text{-}SMR}$ as evidenced by a decline in α_0 following a peak at α. Using the α-method, MR measurements below $P_{crit\text{-}SMR}$ are not diagnostic and, thus, are not relevant. However, P_{crit} is often defined as the transition between oxyconformation (below P_{crit}) and oxyregulation (above P_{crit}) (e.g. Rogers et al., 2016). The definitions of P_{crit} and α in the present study require reassessment of the concepts of oxygen consumption regulation and conformation (Gnaiger, 1993; Pörtner and Grieshaber, 1993).

Several methods have been developed to quantify the degree of regulation, loosely defined as the ability to maintain a constant MR across a range of oxygen levels, from the respiratory response of organisms over the complete range of measured oxygen pressures (e.g. regulation index: Mueller and Seymour, 2011; Tang, 1933; non-linear regression: Marshall et al., 2013; and best-fit approaches: Cobbs and Alexander, 2018; Muggeo, 2003). Proponents of these methods argue that a great deal of information is lost by distilling a respirometry trial down to a single critical P_{O_2} (Marshall et al., 2013; Wood, 2018). However, the oxygen supply capacity, which we argue is the important information provided by P_{crit}, is lost in mathematical descriptions of the entire trial and it is unclear what information is gained from such analyses. Unless the relationship between MR and P_{O_2} above the α-line is causal, there is no particular reason to describe it at all. At P_{O_2} above the α-line, MR may correlate with a number of covariables, including time in captivity, time since feeding, accumulation of metabolic waste, diel cycles, stress and activity. Some of these variables may be controlled for during experiments, but even so, as Ultsch and Regan (2019) point out, it is impossible to know whether MR above P_{crit} is supporting the same maintenance processes that comprise a MR at its P_{crit} (Ultsch and Regan, 2019). For example, vision is energetically expensive and it may be diminished at oxygen values between $P_{crit\text{-}max}$ and $P_{crit\text{-}SMR}$ (McCormick et al., 2019). Thus, apparent oxyregulation is not necessarily oxygen independence and a lack of apparent regulation (i.e. conformation) is not necessarily oxygen dependence. Rather, what is typically referred to as regulation are the physiological adjustments that provide additional oxygen to meet the concurrent metabolic demands as environmental P_{O_2} declines,

regardless of the constancy or level of those demands. Only at maximum exertion for a given P_{O_2} is oxygen supply operating at capacity and, as a result, MMR conforms linearly to environmental oxygen availability between $P_{crit\text{-}SMR}$ and $P_{crit\text{-}max}$ (Fig. 5).

True conformation (a continuous decline in MR in direct proportion to P_{O_2} throughout a respirometry trial), in contrast, implies that an individual is operating continuously at its maximum oxygen supply capacity or that it completely lacks aerobic scope (i.e. MMR equals SMR regardless of oxygen availability). For example, *Nautilus pompilius* was described as an oxyconformer (Boutilier et al., 1996), but Fig. 3C shows that α_0 increases as P_{O_2} declines, suggesting some level of active regulation. Staples et al. (2000) later showed that *N. pompilius* does in fact regulate and that the previously reported elevated rate and oxyconformation may have been due to surgery-induced stress. Regardless, the α we determined from the two *N. pompilius* studies is similar (Fig. 3C). True conformation is seemingly rare (Ultsch and Regan, 2019), even among animals that lack complex circulatory systems (Rutherford and Thuesen, 2005).

An additional benefit of measuring α is that it can be measured at MRs higher than SMR and at oxygen pressures well above lethal limits. Measuring $P_{crit\text{-}SMR}$ using any traditional method requires exposing animals to potentially lethal oxygen levels below $P_{crit\text{-}SMR}$. If measured in an active state, as recommended here, an organism can simply lower oxygen demand by reducing activity as oxygen becomes limiting. If desired, the SMR can be measured independently at high P_{O_2} to determine aerobic scope or to calculate $P_{crit\text{-}SMR}$. Thus, the α-method, applied to MRs above SMR, as recommended here, will alleviate the effects of measurement on animal welfare.

Thus, direct determination of α has several advantages over P_{crit} and non-linear descriptions of respirometry data. (1) The α-method provides a direct and unambiguous measure of oxygen supply capacity. Using the α-method, any two independent researchers will arrive at the same α value from the same dataset. The ambiguity in other P_{crit} methods arises because they are influenced by which MR measurement periods are included in the oxyconforming regression (below P_{crit}) and how the MR itself is calculated. (2) The value of α obtained using the α-method is more accurate than other P_{crit} methods as evidenced by the ability to predict limiting oxygen levels for any MR or to predict the maximum achievable MR at any P_{O_2}. The accuracy derives from the fact that the α-line runs through the origin and that α is consistently defined as the highest α_0 value (or mean of highest 3). (3) The α-method does not depend on respirometry methodology to the extent that other methods do, which means that any two researchers are likely to arrive at similar values for the same species even if using very different respirometry methods.

Conclusions

P_{crit} is a rate-specific measure of the oxygen supply capacity (α), rather than hypoxia tolerance. Variation in $P_{crit\text{-}SMR}$ reflects variation in FAS, while $P_{crit\text{-}max}$ indicates the P_{O_2} below which that aerobic scope becomes oxygen limited. We suggest that α is the important physiological information provided by traditional P_{crit} measurements and that this information is lost in non-linear analyses. We describe a method to directly determine α, which has several advantages over traditional P_{crit} determination, including (1) less ambiguity and greater accuracy, (2) fewer constraints on respirometry methods and the analysis of respirometry data, and (3) greater predictive power and ecological and physiological insight. The oxygen supply capacity enables testing of previously obscured hypotheses regarding aerobic scope and its response to environmental change.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: B.A.S.; Methodology: B.A.S., A.A., M.A.B., A.L.B.; Validation: B.A.S., A.A., M.A.B., A.L.B., C.T.S., A.W.T., C.J.W.; Formal analysis: B.A.S., C.T.S., A.W.T., C.J.W.; Data curation: B.A.S., A.A., M.A.B., A.L.B., C.T.S., A.W.T., C.J.W.; Writing - original draft: B.A.S.; Writing - review & editing: B.A.S., A.A., M.A.B., A.L.B., C.T.S., A.W.T., C.J.W.; Supervision: B.A.S.; Funding acquisition: B.A.S.

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Table S1. Oxygen supply capacity (α) in 38 species of animals from across the tree of life.

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Table S2. Oxygen supply capacity (α) and ($P_{crit\text{-}SMR}$) determined using the LLO method (Reemeyer and Rees, 2019) and the α-method for five previously unpublished datasets to assess intraspecific variability.

Species	taxa	T	n	MO_2 (µmol $g^{-1}h^{-1}$)	$P_{crit\text{-}SMR}$ (kPa) (LLO method)	$P_{crit\text{-}SMR}$ (kPa) (α-*method*)	Measured α (µmol g^{-1} h^{-1} kPa^{-1})	Calculated α (MO_2/P_{crit})	Data Reference	Respirometry Methods
Euphausia mucronata	Krill	10	2 2	5.68 ± 2.63	2.27 ± 1.11	1.95 ± 1.44	4.08 ± 2.69	2.50	Welsh unpubl.	Closed respirometry (Wishner et al., 2018)
Farfantepenaeus duorarum	Pink Shrimp	23	5	4.59 ± 0.86	n.a.	2.71 ± 0.27	1.67 ± 0.17		Burns, unpubl.	Closed respirometry (Burns, 2021, after Birk et al., 2019)
Squalus acanthias	Dogfish shark	13	7	1.25 ± 0.41	4.48 ± 0.89	4.04 ± 0.93	0.41 ± 0.13	0.28	Andres et al., submitted.	Annular Respirometer (Andres, 2021)
		21	8	2.03 ± 0.27	5.57 ± 0.89	3.02 ± 0.10	0.67 ± 0.10	0.36		
Illex illecebrosus	Short-fin Squid	20	3	13.95 ± 2.24	5.28 ± 0.56	5.18 ± 0.18	2.69 ± 0.37	2.64	Birk, unpubl.	Intermittent Flow (Birk et al., 2018)
Sthenoteuthis oualaniensis	Purple-back Squid	23	3	12.35 ± 1.47	6.38 ± 1.99	4.04 ± 0.67	3.16 ± 0.87	1.94	Birk, unpubl.	Intermittent Flow (Birk et al., 2018)