Breathing new life into the critical oxygen partial pressure (P_{crit}): a new definition, interpretation and method of determination.

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Abstract: The critical oxygen partial pressure (P_{crit}) is most commonly defined as the oxygen partial pressure below which an animal's standard metabolic rate can no longer be maintained. It is widely interpreted as measure of hypoxia tolerance, which influences a species' aerobic scope and, thus, constrains biogeography. However, both the physiology underlying that interpretation and the methodology used to determine P_{crit} remain topics of active debate. The debate remains unresolved in part because P_{crit} , as defined above, is a purely descriptive metric that lacks a clear mechanistic basis. Here we redefine P_{crit} as the PO_2 at which physiological oxygen supply is maximized and refer to these values, thus determined, as $P_{crit-\alpha}$. The oxygen supply capacity (α) is a species- and temperature-specific coefficient that describes the slope of the relationship between the maximum achievable metabolic rate and PO_2 . This α is easily determined using respirometry and provides a precise and robust estimate of the minimum oxygen pressure required to sustain any metabolic rate. To determine α , it is not necessary for an individual animal to maintain a consistent metabolic rate throughout a trial (i.e. regulation) nor for the metabolic rate to show a clear break-point at low PO_2 . We show that $P_{crit-\alpha}$ can be determined at any metabolic rate as long as the organisms' oxygen supply machinery reaches its maximum capacity at some point during the trial. We reanalyze published representative P_{crit} trials for 40 species across five phyla, as well as complete datasets from six additional species, five of which have not previously been published. Values determined using the $P_{crit-\alpha}$ method are strongly correlated with P_{crit} values reported in the literature. Advantages of $P_{crit-\alpha}$ include: 1) $P_{crit-\alpha}$ is directly measured without the need for complex statistics that hinder measurement and interpretation; 2) it makes clear that P_{crit} is a measure of oxygen supply, which does not necessarily reflect hypoxia tolerance; 3) it alleviates many of the methodological constraints inherent in existing methods; 4) it provides a means of predicting the maximum metabolic rate achievable at any PO_2 ; 5) $P_{crit-\alpha}$ sheds light on the temperature- and size-dependence of oxygen supply and metabolic rate and 6) $P_{crit-\alpha}$ can be determined with greater precision than traditional P_{crit} .

Keywords: aerobic scope, hypoxia, metabolic rate, ocean deoxygenation, oxygen and capacity limit of thermal tolerance, oxygen supply, respirometry

Introduction

The relationship between metabolic rate and environmental oxygen has long been of interest due to its implications for human health, paleobiology, fisheries management, biogeography, species diversity and evolution (Tang, 1933; Hall, 1966; Fry and Hart, 1948; Seibel and Deutsch, 2020; Penn et al., 2018; Childress and Seibel, 1998; Deutsch et al., 2015; 2020; Ern et al., 2016; Rogers et al., 2016; Ultsch and Regan, 2019). This relationship has taken on special significance in light of anthropogenic ocean warming and deoxygenation (Seibel and Wishner, 2019; Breitbart et al., 2018). The critical oxygen partial pressure (critical PO_2 , P_{crit}) is a metric commonly 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). The ratio of environmental PO_2 to P_{crit} , normalized for temperature, provides a direct measure of aerobic scope that is believed to constrain the viability of current and future habitat (Deutsch et al., 2015; Deutsch et al., 2020). However, hypoxia and tolerance are subjective, time-dependent terms while P_{crit} is inconsistently defined and determined, resulting in debate about its meaning and significance (Wood, 2018; Regan et al., 2019).

An organism's P_{crit} is typically defined based on the physiological response to declining environmental PO_2 , at a clear decline (breakpoint) in the measured metric at the P_{crit} (Pörtner and Grieshaber, 1993; Richards, 2011; Hall, 1966; Ultsch and Regan, 2019; Childress and Seibel, 1998). Metrics employed to determine critical oxygen levels include cardiac output, anaerobic metabolite concentrations, gene expression, and behavioral impairment (e.g. loss of equilibrium) (Mandic et al., 2009; Ultsch and Regan, 2019; Farrell and Richards, 2009 for reviews). However, the most commonly measured response variable is the oxygen consumption (metabolic) rate, with the corresponding definition of P_{crit} as the minimum PO_2 at which a given metabolic rate can be sustained. Accordingly, the rate of oxygen consumption becomes dependent on PO_2 below P_{crit} . Because of the rate dependence of P_{crit} , as typically defined, recent protocols recommend measuring P_{crit} at the standard metabolic rate (SMR; Reemeyer and Rees, 2019). At P_{crit} for SMR, by definition, metabolism is oxygen limited and aerobic scope (the difference between resting and maximum rates) is abolished. This is what Fry and Hart (1948) referred to as the “oxygen level of no excess activity” or the “incipient lethal oxygen level”. In order to determine P_{crit} using this definition, the resting metabolic rate must be established and, preferably, maintained at high PO_2 (i.e. it must be “regulated”). A break-point

must be readily apparent between oxygen dependent and oxygen independent portions of the respiration curve. Alternatively, complex statistics may be used to identify an inflection point in the data (Muggeo, 2003).

At PO_2 levels above P_{crit} , a constant oxygen consumption rate (MO_2 or VO_2 in moles or volume of oxygen, respectively) is typically considered to be “regulated” and independent of PO_2 . Current P_{crit} methodology places a great deal of importance on this constancy and the degree of oxygen independence of the regulated rate at high PO_2 values. In fact, several methods have been developed to quantify oxyregulation, loosely defined as the overall 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 PO_2 (Marshall et al., 2013; Wood, 2019), although it is unclear whether analyzing the relationship between MO_2 and PO_2 at PO_2 greater than P_{crit} provides any additional information. More importantly, any information provided by P_{crit} is lost in mathematical descriptions of the entire trial. The term “regulation” has a specific implication that has injected a great deal of confusion into our collective understanding of P_{crit} , and, thus, how it should be measured. The concept of regulation, as applied to other physiological processes, implies that an organism strives for homeostasis by maintaining the constancy of internal conditions (e.g. acid-base or osmotic balance; Trowell, 1943). However, a metabolic rate is merely a measure of the energy demand at a point in time and, although there are upper and lower bounds on the rate of metabolism and the rate is under active control, it is not regulated to achieve homeostasis. There is no specific level of metabolic cost that must be maintained for proper function.

Unless the relationship between MO_2 and PO_2 is causal, there is no particular reason to describe it at all. At PO_2 above P_{crit} , MO_2 may correlate with a number of covariables, including time in captivity, time since feeding, accumulation of metabolic waste, diel cycles, light, 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 MO_2 above P_{crit} is supporting the same maintenance processes that comprise SMR at P_{crit} : “When PO_2 is reduced, there may be (and probably is) a reallocation of resources, including O_2 , towards a different

suite of processes (e.g. ventilation) that may nevertheless sum to a value of $\dot{M}O_2$ similar to normoxic SMR” (Ultsch and Regan, 2019). Thus, apparent oxyregulation is not necessarily oxygen independence and a lack of apparent regulation (i.e. oxyconformation) is not necessarily oxygen dependence. Rather, what we typically refer to as oxyregulation are physiological adjustments that provide additional oxygen as environmental PO_2 declines, regardless of the constancy or level of metabolic rate.

The plethora of incompatible and inconsistent definitions of, and methods for, describing the response of metabolism to declining PO_2 underscores an incomplete physiological understanding. All existing definitions focus on consequences, rather than causes, of physiological failure at low oxygen. The lack of a specific mechanistic basis limits the generality and utility of P_{crit} (Wood, 2018). Although oxygen supply limitation below a critical PO_2 is implied, oxygen supply itself is rarely determined. Here we redefine P_{crit} as the PO_2 at which physiological oxygen supply mechanisms are operating at maximum capacity (Deutsch et al., 2015; 2020; Seibel and Deutsch, 2020; Kielland et al., 2019). Values thus determined, designated $P_{crit-\alpha}$, are not specific to any particular metabolic rate (MR) and can be mathematically defined as $P_{crit-\alpha} = MR/\alpha$, where α is the maximum physiological capacity for oxygen supply (see detailed derivation below). This straightforward definition provides a strong theoretical justification for the physiological value 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 also provides some clarity to the concept and 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), but the ecological relevance of a particular P_{crit} value is often assumed. More troubling is that P_{crit} is often used to infer hypoxia tolerance in the absence of any measure of ambient PO_2 in the environment.

Seibel and Deutsch (2020) argued that P_{crit} is not a measure of hypoxia tolerance, but rather a measure of the oxygen supply capacity, which evolves to support maximum metabolic rate at the prevailing (highest persistently encountered) environmental PO_2 (Fig. 1). Because $P_{crit-\alpha}$ provides a measure of oxygen supply capacity, it can be used to calculate MMR and aerobic scope (Seibel and Deutsch, 2020; Deutsch et al., 2020; Howard et al., 2020). In other words, the ratio of $MR:PO_2$ should reach a maximum at $P_{crit-\alpha}$ regardless of the metabolic rate. This ratio provides a simple, direct, and quantitative method of determining α that does not require an obvious break-point in, nor a particular level of, metabolic rate.

Methods: Oxygen supply capacity and $P_{crit-\alpha}$ determination

For an aerobic organism to obtain sufficient energy for survival, its oxygen supply must meet its demand. Oxygen demand is the metabolic rate (MR), most commonly measured at rest (standard, SMR) or at maximum exertion (MMR, the maximum achievable rate for a given PO_2). The total amount of oxygen available for cellular respiration is a function of the ambient environmental PO_2 and the physiological oxygen provision, α_0 (Seibel and Deutsch, 2020; Deutsch et al., 2015). Physiological oxygen provision comprises each step in the oxygen cascade (Weibel et al., 1991) from ventilation and blood-oxygen binding to cardiac output and circulation. At a constant metabolic rate, physiological oxygen provision must increase as environmental PO_2 declines (Fig. 1). We define and calculate α_0 as the rate of oxygen consumption per unit of available environmental oxygen pressure ($\alpha_0 = MR/PO_2$; $\mu\text{mol O}_2 \text{ g}^{-1}\text{h}^{-1}\text{kPa}^{-1}$). The $P_{crit-\alpha}$ is reached when physiological oxygen provision is operating at its maximum capacity (α ; Fig. 1; Eq. 1). At capacity, these functional traits cannot be further upregulated and the metabolic rate is entirely dependent on, and directly proportional to, environmental PO_2 . Thus, the physiological oxygen supply capacity ($\alpha = MR/P_{crit-\alpha}$; Fig. 1) is set by attributes of the oxygen cascade that are specific to each species and to acclimation conditions. As a result, α describes the slope of the relationship between MMR and PO_2 and can be used to calculate $P_{crit-\alpha}$ at any metabolic rate ($P_{crit-\alpha} = MR/\alpha$). By rearranging this equation ($MMR = \alpha \cdot PO_2$), α can also be used to calculate MMR at any PO_2 up to $P_{crit-max}$ (the PO_2 above which no additional increase in MO_2 is possible, even in hyperoxia; Seibel and Deutsch, 2020). Thus, $P_{crit-\alpha}$ is defined by maximized oxygen supply, rather than the oxygen dependence of a particular metabolic rate.

$$\text{Eq. 1} \quad \alpha = MR_1/P_{crit1} = MR_2/P_{crit2}$$

To assist in the adoption of this new method, calculation of α and $P_{crit-\alpha}$ have been incorporated into the R package “respirometry” with an optional parameter available to define the metabolic rate of interest for P_{crit} estimation (Birk 2020). The “calc_pcrit” and “plot_pcrit” functions now return side-by-side comparisons of P_{crit} calculated using a broken-stick regression (Yeager and Ultsch 1989), nonlinear regression (Marshall et al 2013), LLO method (Reemeyer and Rees, 2019); sub-prediction interval method (Birk et al. 2019), and the $P_{crit-\alpha}$ method presented here. This software is freely available on CRAN at <https://cran.r-project.org/package=respirometry>.

The oxygen supply capacity (α) has been recently calculated from P_{crit} , determined using various historical methods, and from the metabolic rate at which it was determined (Deutsch et al., 2015; Seibel and Deutsch, 2020; Kielland et al., 2019). Here we show that α can be directly measured as the highest α_0 achieved during a respirometry trial and used to determine P_{crit} ($P_{crit-\alpha}$). $P_{crit-\alpha}$ is then calculated from *Eq. 1* for every metabolic rate bin measured during a trial, providing a straight line that identifies $P_{crit-\alpha}$ for *any* metabolic rate (Fig. 2). We used several approaches to test the applicability, generality and precision of this novel $P_{crit-\alpha}$ method. We first extracted and reevaluated available literature data for 40 species in five phyla (Table 1). Most studies that report P_{crit} provide only summarized data in the text but some studies included a “representative” trace showing the relationship between the oxygen consumption rate and PO_2 . Data were extracted from these respirometry curves using WebPlotDigitizer 4.2 (Rohatgi, 2019; <https://automeris.io/WebPlotDigitizer/index.html>). The oxygen supply capacity was determined and used to calculate $P_{crit-\alpha}$ across the range of measured metabolic rates. We suspect that these representative curves are likely the best of many trials, rather than being truly representative. Thus, they may not provide the most rigorous test of the new method. Accordingly, we also tested our method on existing datasets for zooplankton (Wishner et al., 2018), shrimp (Burns et al., unpubl.), squids (Birk and Seibel, unpubl.) and fishes (Slesinger et al., 2019; Andres et al., submitted). We tested inter-individual and temperature-related variances in α as well as the effect of duration bin size when calculating MO_2 .

Results

Table 1 presents $P_{crit-\alpha}$ and published P_{crit} for representative traces determined at the metabolic rate provided in the literature ($n = 40$). For comparative purposes, the rates and PO_2 values are

converted to common units in Table 1 and in the text. In the species analyzed here, α ranged from 0.09 $\mu\text{mol O}_2 \text{ g}^{-1}\text{h}^{-1}\text{kPa}^{-1}$ for the deep-sea anglerfish, *Melanocetus johnsoni*, at 5°C (Cowles and Childress, 1995), to 16.65 $\mu\text{mol O}_2 \text{ g}^{-1}\text{h}^{-1}\text{kPa}^{-1}$ at 28°C for larval zebrafish, *Danio rerio* (Mandic et al. 2020; Table 1). These values are within the previously reported range and are correlated with metabolic rate (Deutsch et al., 2020; Seibel and Deutsch, 2020; Kielland et al., 2019). $P_{\text{crit-}\alpha}$ was strongly correlated with published P_{crit} values that were determined using a variety of methods ($P_{\text{crit-}\alpha} = 0.14 + 0.80P_{\text{crit}}$; $R^2 = 0.83$; $n = 40$; Fig. 3). For a subset of the trials extracted from the literature, SMR and P_{crit} were determined as in Chabot and Claireaux (2008) and Reemeyer and Rees (2019), respectively. For these cases, $P_{\text{crit-}\alpha}$ and the reported P_{crit} were more tightly correlated because the metabolic rates used to determine P_{crit} were more tightly constrained ($y = 0.29 + 1X$; $R^2 = 0.99$; $n = 6$; Fig. 3A).

The physiological oxygen provision ($\alpha_0 = \text{MO}_2/\text{PO}_2$) was calculated for each MO_2 value from all available trials. In our dataset, α_0 responded to declining PO_2 in one of three distinct ways; a peak (with subsequent decline), a plateau, or a continuous increase. In most cases, α_0 increased to a peak or plateau as PO_2 decreased (Fig. 4). A peak in α_0 , with a subsequent decline at lower PO_2 , indicates that the individual is failing physiologically and MO_2 is declining faster than PO_2 below P_{crit} . A plateau in α_0 indicates maintenance of oxygen supply capacity as PO_2 declines linearly below P_{crit} toward anoxia. In either case, the highest resulting α_0 value is the oxygen supply capacity, α , which was used to calculate $P_{\text{crit-}\alpha}$ across the range of MO_2 values in each trial ($P_{\text{crit-}\alpha} = \text{MR}/\alpha$). A continuous increase in α_0 (Fig. 4C), with the highest value occurring at the lowest PO_2 , provides no clear peak or plateau and renders α more dubious. This represents either a decline in MO_2 before P_{crit} is reached or possible error in PO_2 measurement (see below). A small number of published representative trials (2 of 40) exhibited apparent “oxyconformation”, where MO_2 declined continuously with PO_2 with no obvious breakpoint (Fig. 5). For such trials, α_0 was either independent of PO_2 , suggesting that MO_2 is maximized throughout the experiment (Fig. 5A), or it increased continuously as PO_2 declined, never reaching a peak or plateau (Fig. 5B).

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- *Euphausia mucronata*, a midwater krill from the Eastern Tropical Pacific oxygen minimum zone (OMZ; Welsh and Seibel, unpublished), *Farfantepenaeus duorarum*, an estuarine pink shrimp (Burns et al., unpublished), the Atlantic Spiny Dogfish, *Squalus acanthias* (Andres et al., submitted), two oceanic squids, *Illex illecebrosus* and *Sthenoteuthis oualaniensis* (Birk, unpublished), and the Black Seabass, *Centripristis striata* (Slesinger et al., 2019), a coastal fish species. The mean values of α and $P_{crit-\alpha}$ and P_{crit} (LLO or breakpoint method) for these species are shown in Table 2. The values of α were highest in the squids, due to their high metabolic rates, and in the crustaceans, due to high rates at small sizes and, likely, adaptation to their hypoxic OMZ and estuarine habitats (Table 2). With the exception of crustaceans from hypoxic environments, $P_{crit-\alpha}$ was very consistent at a given temperature (ranging from 4.04 to 5.18 at ~20-23°C, n = 4 species). The α_0 response types (see Fig. 4) varied among individuals of each species, but typically displayed a clear peak or plateau, facilitating identification of a clear α . The length of respirometry measurement periods, or “bin sizes”, had an effect on α . Small bin sizes (< 5min) gave undue influence to outliers among the MO_2 measurements leading to unrealistically high α and low $P_{crit-\alpha}$. With increasing bin size, α declined and was consistent between bin sizes of 10, 15 or 20 min (Fig. 6). For krill, smaller bin sizes similarly resulted in a slight increase in α but did not have a significant effect on $P_{crit-\alpha}$. The pattern for krill was similar to the literature values mentioned above, with P_{crit} slightly, but consistently, higher than $P_{crit-\alpha}$. For Atlantic Spiny Dogfish and Black Seabass, $P_{crit-\alpha}$ matched P_{crit} determined using the LLO method (Reemeyer and Rees, 2019) across a range of temperatures and α increased with temperature in proportion to MMR (Fig. 6; Slesinger et al., 2019; Andres et al., in prep).

$P_{crit-\alpha}$ was typically slightly lower than the reported P_{crit} for an equivalent metabolic rate, in part because $P_{crit-\alpha}$ is based on the highest calculated oxygen supply, α_0 , which places $P_{crit-\alpha}$ (the oxygen supply limit; red lines in Fig 2E and Figs. 4-7) on or to the left of all MO_2 values. This precludes the possibility that PO_2 is underestimated due to measurement error, which, of course, is not a valid assumption. However, previous P_{crit} methods assume that PO_2 error is spread evenly around the PO_2 vs MO_2 regression line (the oxygen-dependent portion of the MO_2 trial) with a Gaussian distribution. This assumption is problematic if error is minimal (i.e. if scatter in the data is real variation in the organism’s oxygen consumption rate) because it suggests that

oxygen is sometimes consumed faster than it is supplied. Regardless, $P_{crit-\alpha}$ and P_{crit} derived from other methods were not significantly different (Fig. 3).

Although rare, a continuous increase in α_0 presents a challenge for $P_{crit-\alpha}$ determination because it is unclear whether oxygen supply had yet reached its capacity (i.e. whether $\alpha_0 = \alpha$) or whether the final α_0 represents measurement error. For most trials of this type, $P_{crit-\alpha}$ made visual sense and was not different from P_{crit} calculated using other methods (Fig. 4C). For a few others, α was unrealistically high, resulting in extremely low $P_{crit-\alpha}$. Some of these included positive MO_2 data at zero PO_2 or a positive y-intercept of the MO_2 vs PO_2 curve. In either case, this indicates continued oxygen consumption in the absence of oxygen. Because this is not physiologically possible, it must result from either PO_2 error, perhaps introduced during sensor calibration, or inappropriate bin sizes. Both of these have drastic effects on the calculation of α , especially at low PO_2 values where the ratio of error to true oxygen is magnified. If PO_2 is underestimated (e.g. measurements include negative PO_2 values due to a high zero calibration), α_0 has the potential to diverge towards infinity as PO_2 approaches zero. This occurs more often for species with very low P_{crit} , such as those living in oxygen minimum zones and in stratified ponds. For example, using $P_{crit-\alpha}$, we could not support the finding of lower P_{crit} in goldfish following slow hypoxic induction as reported by Regand Richards (2017), but the majority of their trials achieved α at the lowest PO_2 measured. Thus, we are less confident in our contrary finding.

We have developed a method to correct such PO_2 error that will be described in full elsewhere (Timpe et al., in prep). It is included in the R package referenced above (Birk, 2020). Briefly, the correction involves identifying the magnitude of any PO_2 error through incremental adjustments to PO_2 values until two equivalent MO_2 values are achieved for which $\alpha_0 = \alpha$. This method effectively provides a way to diagnose and correct for calibration error in respirometry experiments. It is applicable only in specific circumstances and the only corrected data presented here are from the worm, *Sipunculus nudus* (Fig. 5). For this species, the correction resulted in α and $P_{crit-\alpha}$ values that more closely adhered to the MO_2 vs PO_2 curve.

Discussion

We define $P_{crit-\alpha}$ as the PO_2 at which physiological oxygen provision, α_0 , reaches its maximum capacity (α). Oxygen supply capacity, although species- and temperature-specific, is independent of PO_2 or workload (i.e. metabolic rate). Thus, P_{crit} is a measure of α at any metabolic level from rest to maximum exertion and it is α , rather than P_{crit} *per se*, that contains relevant physiological information. Because α is not specific to a particular metabolic rate, $P_{crit-\alpha}$ need not be measured at SMR or any other specific metabolic rate. It is also not necessary for an individual animal to maintain a consistent metabolic rate throughout a trial or for an MO_2 vs PO_2 curve to have a clear break-point. $P_{crit-\alpha}$ can be calculated for any metabolic rate as long as oxygen supply reaches capacity at some point during the trial. $P_{crit-\alpha}$ is physiologically consistent and provides a simple, repeatable, and precise alternative to existing methods for P_{crit} determination. Moreover, this definition clarifies the physiological and evolutionary significance of P_{crit} .

$P_{crit-\alpha}$ is a measure of the oxygen supply capacity. Selection for both elevated metabolic capacity (i.e. athleticism) and for metabolic performance in persistent hypoxia may elevate oxygen supply capacity (Fig. 8A). The $P_{crit-max}$ (the PO_2 above which no further increase in metabolic rate is possible) must be known to distinguish between these selective pressures (Fig. 8A). A $P_{crit-max}$ less than air-saturation would suggest adaptation to persistent hypoxia. Most shallow-living aquatic or terrestrial species have a $P_{crit-max}$ near atmospheric PO_2 (21 kPa; Seibel and Deutsch, 2020). This means that additional environmental oxygen (hyperoxia) cannot elevate MMR. Among these “normoxic” species, most of the variation in P_{crit} reflects the differing temperature sensitivities of MMR and SMR and, thus, the factorial aerobic scope ($FAS = MMR/SMR$; Fig. 8B). Inserting MMR and SMR into Eq. 1 and rearranging shows that $FAS * P_{crit} = P_{crit-max}$. As such, the P_{crit} for normoxic species ($P_{crit-max} \sim 21$ kPa) is inversely correlated with FAS (Seibel and Deutsch, 2020), explaining 95% of the variation (from 2-12 kPa) in P_{crit} among diverse species (Seibel and Deutsch, 2020). None of the variation could be attributed to measured differences in environmental PO_2 . Thus, P_{crit} alone, without also knowing MMR, is not a useful measure of hypoxia tolerance. Relative hypoxia tolerance in normoxic species must be conferred by adaptations for metabolic suppression and anaerobic metabolism that permit extended survival below P_{crit} (Mandic et al., 2009; Seibel, 2011).

In contrast, adaptation to *persistent* hypoxia (that lasting longer than a diel or tidal cycle) results in a higher α for a given metabolic rate and P_{crit} is reduced at all metabolic levels, including MMR, relative to normoxic species. Thus, species that live in persistently hypoxic conditions have a lower P_{crit} and a lower $P_{crit-max}$ relative to similar species in higher oxygen environments (Childress and Seibel, 1998; Seibel, 2011; Wishner et al., 2018; Mandic et al., 2009; Richards, 2011; Regan et al., 2019). Whether variation in P_{crit} and α reflects adaptation for increased metabolic capacity or hypoxia adaptation can only be known if MMR (and thus $P_{crit-max}$) is also known (Fig. 8A). In the absence of such data, one can tentatively infer whether a high α is an adaptation for hypoxia or for metabolic capacity if it is assumed that FAS for the species of interest falls within the typical range ($< \sim 7$; Seibel and Deutsch, 2020; Killen et al., 2016; Peterson et al., 1989). Thus α multiplied by 21 kPa ($P_{crit-max}$ for normoxic species) should result in an estimated MMR less than $\sim 7X$ SMR. A greater value suggests that $P_{crit-max}$ is less than 21 kPa by some degree and that oxygen supply mechanisms are adapted for hypoxia. Regardless, selection acts on the oxygen supply capacity in support of MMR and its $P_{crit-max}$, not SMR and its P_{crit} .

The maximum achievable metabolic rate (MMR) is linearly related to PO_2 below $P_{crit-max}$ and MMR reaches an oxygen limit at α , just as SMR does (Figs. 1, 7). For example, in the freshwater pumpkinseed fish, *Lepomis gibbosus* (Crans et al., 2015), measurements of P_{crit} at SMR and measurements of MMR at different oxygen levels show that the maximum achievable metabolic rate always becomes oxygen limited when $PO_2 = MR/\alpha$. The MMR measured in normoxia falls off the line, suggesting that *L. gibbosus* is hypoxia tolerant with a $P_{crit-max}$ near 11 kPa (Fig. 7A). Similarly, *Gnathophausia ingens*, a deep-sea lophogastrid crustacean, maintained MMR down to ~ 5 kPa while intermediate activity levels reached P_{crit} at proportionally lower PO_2 equal to MR/α (Childress and Seibel, 1998). Distinct activity levels or metabolic states produce equivalent estimates of α for a variety of other animals including fishes (Ern et al., 2016; Ultsch et al., 1981), prawn (Dall, 1986) and octopus (Seibel and Childress, 2000; Fig. 7).

Both P_{crit} and SMR typically increase with temperature (Rogers et al., 2016 for review), although the temperature sensitivities (E , eV) for each metric are quite different. The typically lower temperature sensitivity of P_{crit} , relative to SMR, has led some to suggest that oxygen supply

does not keep pace with oxygen demand (Pörtner et al., 2017; Keilland et al., 2019; Deutsch et al., 2020). However, this makes little sense given that oxygen supply is sufficient to meet MMR, which is typically many times higher. In contrast, oxygen supply matches MMR with increasing temperature. If MMR increased with temperature as fast as SMR, P_{crit} would not change (Fig. 8B). Mathematically, the E value (exponent) for α must equal the difference between the E values for SMR and P_{crit} . If SMR increases faster with temperature than P_{crit} , E_{α} must be less than E_{SMR} , leading to the assumption that oxygen supply does not match demand at high temperatures. However, since P_{crit} for MMR ($P_{crit-max}$) is effectively the maximum environmental PO_2 , it does not change with temperature for most species ($E_{P_{crit-max}} = 0$). Thus E_{α} exactly matches E_{MMR} and oxygen supply increases with temperature to match maximum demand (Fig. 6A). For example, in the lumpfish, the measured E_{α} (0.29 eV), derived from SMR and P_{crit} , is very similar to the measured E_{MMR} (0.27 eV). The fact that P_{crit} and α do not increase with temperature in proportion to SMR *in no way* indicates oxygen limitation at high temperature. To the contrary, oxygen supply evolved to meet maximum demand regardless of temperature.

The definition of $P_{crit-\alpha}$, provided here, requires reassessment of the concept of oxygen consumption regulation (oxyregulation). Regulation identifies the ability of a species to increase oxygen provision as oxygen availability declines. However, a variable or declining metabolic rate during a respirometry experiment may merely reflect behavioral changes or changes in metabolic state unrelated to oxygen and is not necessarily diagnostic of a physiological inability to regulate. Oxyconformation (a continuous decline in MO_2 throughout a respirometry trial), in contrast, implies that an individual is operating at its maximum oxygen supply capacity throughout a trial regardless of PO_2 . Accordingly, it suggests that either the individual is operating at the maximum achievable metabolic rate for a given PO_2 or that it completely lacks aerobic scope (i.e. MMR equals SMR regardless of oxygen availability). For conformation to occur, physiological oxygen supply mechanisms must be either absent, always operating at their maximum capacity, or incapable of regulating their output. Thus, true conformation can be diagnosed by a constant oxygen supply (MR/ PO_2) as PO_2 declines (Gnaiger, 1993). For example, a reanalysis of a representative respirometry trial for *Sipunculus nudus*, a worm that is widely regarded as an oxyconformer (Pörtner and Grieshaber, 1993), shows that α increases as PO_2 declines (Fig. 5), suggesting some level of active regulation, or what Gnaiger (1993) referred to as microoxic regulation. Oxyconformation is seemingly rare (Ultsch and Regan,

2019), even among animals that lack complex circulatory systems (Rutherford and Thuesen, 2005).

The determination of α , rather than P_{crit} alone, not only alters our perception of the ecological and evolutionary significance of P_{crit} , it is also a refinement of the respirometry methodology required to determine P_{crit} . Typically, P_{crit} and SMR are determined during the same trials, requiring a lengthy acclimation period to ensure a resting, fasted state. Not only are such experiments extremely time-consuming, but an acclimatory response in the oxygen supply system may influence P_{crit} , especially in fishes with highly elastic physiologies. For example, goldfish are known to remodel gills during hypoxic exposure, which reduces P_{crit} (Sollid et al., 2005; Regan and Richards, 2017). The present method eliminates the requirements for long experiments and for slow hypoxic induction to determine P_{crit} . For example, trials using a conventional broken stick method must extend to oxygen levels well below P_{crit} solely to produce sufficient data points for a reliable regression of the oxygen dependent portion of the curve. In contrast, $P_{crit-\alpha}$ trials can be truncated by starting at relatively low PO_2 and/or by measuring relatively high (active) metabolic rates where oxygen supply is maximized above potentially lethal oxygen levels. $P_{crit-\alpha}$ trials can be terminated as soon as α has plateaued or reached a summit, thus avoiding potential distress in experimental animals in hypoxic conditions just to satisfy statistical requirements for data below P_{crit} . An additional benefit of this method is that once α has been determined for a given species and temperature, $P_{crit-\alpha}$ can be calculated for any physiological state or metabolic rate of interest (e.g. following feeding or exercise) simply by measuring their respective metabolic rates in normoxia. This facilitates modeling of habitable environments based on the metabolic responses to a variety of environmental and physiological conditions (Deutsch et al., 2020; 2015).

Conclusions

The critical oxygen pressure is redefined here ($P_{crit-\alpha}$) as the PO_2 at which the oxygen transport machinery reaches maximum capacity. The advantages of this definition include 1) $P_{crit-\alpha}$ is directly measured without the need for complex statistics that hinder measurement and interpretation; 2) it makes clear that P_{crit} is a measure of oxygen supply, which does not necessarily reflect hypoxia tolerance; 3) it alleviates many of the methodological constraints inherent in existing methods; 4) it provides a means of predicting the maximum metabolic rate

436 achievable at any PO_2 , 5) $P_{crit-\alpha}$ sheds light on the temperature- and size-dependence of oxygen
 437 supply and metabolic rate and 6) $P_{crit-\alpha}$ can be determined with greater precision than traditional
 438 P_{crit} . We argue that $P_{crit-\alpha}$ provides important physiological information that is lost in non-linear
 439 analyses such as the *Regulation Index*. Moreover, we argue that the concept of oxyregulation
 440 should be redefined with reference to the physiological adjustments that increase oxygen
 441 delivery as environmental PO_2 declines, rather than the maintenance of an arbitrary metabolic
 442 rate. $P_{crit-\alpha}$ provides greater predictive power and enables testing of previously obscured
 443 hypotheses regarding metabolic scope.

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Table 1. $P_{crit-\alpha}$ compared to published P_{crit} measurements for diverse aquatic animals. The methods used for published P_{crit} estimates include those described by YU (Yeager and Ultsch, 1989), LLO (Reemeyer and Rees, 2019), and SMR (Chabot and Claireaux, 2008). See Reemeyer and Rees (2019), Ultsch and Regan (2019) and Wood (2018) for recent reviews.

Species	Common Name	T°C	MO ₂ ($\mu\text{mol g}^{-1}\text{h}^{-1}$)	Reported P_{crit} (kPa)	Method Reported	α ($\mu\text{mol g}^{-1}\text{h}^{-1}\text{kPa}^{-1}$)	$P_{crit-\alpha}$ (kPa)	Reference
Cnidaria								
<i>Phacellophora camtschatica</i>	Jellyfish	10	0.18	0.91	Broken-stick	0.18	1.0	Rutherford and Thuesen, 2005
Echinodermata								
<i>Hemipholis elongata</i>	Brittlestar	24	1.89	4.86	Broken-stick	0.74	2.57	Christiansen and Colasino 2000
Mollusca								
<i>Doryteuthis pealeii</i>	Long-fin squid	15	6.5	3.9	SMR	1.65	3.61	Birk et al., 2018
<i>Octopus bimaculoides</i>	Two-spot octopus	10	0.55	2.11	SMR	0.26	2.11	Seibel and Childress, 2000
<i>Japetella diaphana</i>	Bathypelagic octopod	5	0.5	2.53	SMR	0.75	0.66	Birk et al., 2019
<i>Gibberulus gibbosus</i>	Jumping snail	28	2.81	2.5	Broken-stick	1.65	1.7	Lefevre et al., 2015
<i>Panopea zelandica</i>	Geoduck clam	15	16 (g^{-1}DW)	3.57	Michaelis Menten	0.93 (units)	2	Le et al., 2016
<i>Argopecten purpuratus</i>	Peruvian scallop	25	6.06/ind	4.49	Broken-stick	1.68	3.72	Aguirre-Velarde et al., 2016
Arthropoda								
<i>Lithodes santolla</i>	King crab	12	3.88	9.0	Eye-ball	0.69	5.66	Paschke et al., 2010
<i>Callinectes sapidus</i>	Blue Crab	23	4.08	7.58	SegReg	0.66	6.21	Brill et al., 2015
<i>Janus edwardsii</i>	Rock Lobster	13	0.63	7.66	Broken-stick	0.13	4.75	Crear and Forteach, 2000
<i>Euphausia pacifica</i>	Pacific Krill	10	3.80	2.38	Broken-stick	2.20	1.73	Childress, 1975
<i>Gnathophausia ingens</i>	Bathypelagic lophogastrid	5.5	0.56	0.46	Broken-stick	2.03	0.28	Childress and Seibel, 1998
<i>Lucicutia hulsemannae</i>	Bathypelagic copepod	8	0.8	0.38	SMR	2.20	0.36	Wishner et al., 2018
<i>Penaeus esculentus</i>	Prawn	25	2.81	5.28	Eye-ball	0.71	3.96	Dall 1986
Vertebrata								
<i>Hemiscyllium ocellatum</i>	Epaulette shark	28	2.4	5.1	YU	0.5	4.80	Speers-Roesch et al., 2012
<i>Aptychotrema rostrata</i>	Shovelnose ray	28	2.6	7.23	YU	0.38	6.87	Speers-Roesch et al., 2012
<i>Chitala ornata</i>	Clown Knifefish	27	1.47	6.1	SMR	0.25	5.98	Tuong et al., 2018
<i>Oreochromis niloticus</i>	Nile Tilapia	30	0.8	2.11	YU	0.39	2.07	Burggren et al., 2019a
<i>Mayaheros urophthalmus</i>	Mayan Cichlid	23	2.9	2.6	YU	1.30	2.23	Burggren et al., 2019b
<i>Dicentrarchus labrax</i>	European Bass	15	2.1	5.65	LLO	0.37	5.65	Claireaux and Chabot 2016
<i>Reinhardtius hippoglossoides</i>	Greenland Halibut	5	1.00	2.67	calcO2crit	0.40	2.5	Claireaux and Chabot 2016
<i>Sciaenops ocellatus</i>	Red Drum	26	6.25	4.0	SMR	1.63	3.84	Negrete and Esbaugh, 2019
<i>Fundulus grandis</i>	Killifish	27	7.8	4.1	SMR	1.74	4.48	Reemeyer and Rees, 2019
<i>Cyclopterus lumpus</i>	Lumpfish	10	2.5	7.18	SMR	0.43	5.87	Ern et al., 2016
<i>Opsanus tau</i>	Toadfish	22	1.41	4.33	SMR	0.37	3.78	Ultsch et al., 1981
<i>Perca fluviatilis</i>	European Perch		1.88	3.5	SMR	0.53	3.53	Thuy et al., 2010
<i>Lates calcarifer</i>	Barramundi	26	5.81	4.42	SMR	1.62	3.58	Collins et al., 2013
<i>Mogurnda adspersa</i>	Gudgeon	25	1.88	2.5	SMR	0.94	2.0	Stoffels 2015
<i>Paralichthys dentatus</i>	Flounder	22	0.78	5.67	Two segment	0.14	5.46	Caposella 2012
<i>Carrasius auratus</i>	Goldfish	13	2.98	1.85	YU	3.54	0.84	Fu et al., 2011

<i>Ammodytes tobianus</i>	Sand Eel	10	2.16	4.1	SMR	0.60	3.62	Behrens and Steffensen, 2007
<i>Gadus morhua</i>	Atlantic Cod	5	0.97	3.57	SMR	0.30	3.19	Schurmann and Steffensen, 1997
<i>Cymatogaster aggregata</i>	Shiner Perch	14	7.0	5.90	SMR	1.48	4.74	Snyder et al., 2016
<i>Melanocetes johnsoni</i>	Bathypelagic Anglerfish	5	0.35	3.43	Broken-stick	0.09	4.02	Cowles and Childress, 1995
<i>Danio rerio</i>	Zebrafish larvae (10 dpf)	28	80	4.35	Broken-stick	16.65	4.80	Mandic et al., 2020
<i>Pseudophryne bibronii</i>	Toad	12	90	4.0-6.0	Multiple	units	3.46	Mueller and Seymour, 2011
<i>Crinia georgiani</i>	Tadpole	12	55	4.1	Broken-stick	units	2.9	Marshall et al., 2013
<i>Centropristis striata</i>	Black Sea Bass	12	1.45	4.31	SMR	0.44	3.26	Slesinger et al., 2019
<i>Scolopsis bilineata</i>	Bream (juv)	30	11.71	5.88	Broken-stick	0.41	4.75	Nilsson and Östland- Nilsson, 2004

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Table 2. $P_{crit-\alpha}$ compared to P_{crit} derived using LLO (Reemeyer and Rees, 2019) or broken-stick analyses (Yeager and Ultsch, 1989) for complete datasets to assess intraspecific variability.

Species	taxa	T°C	n	MO ₂ ($\mu\text{mol g}^{-1}\text{h}^{-1}$)	P _{crit} (kPa)	α ($\mu\text{mol g}^{-1}\text{h}^{-1}\text{kPa}^{-1}$)	P _{crit-α} (kPa)	Reference
<i>Euphausia mucronata</i>	Krill	10	22	5.68 ± 2.63	2.27 ± 1.11	4.08 ± 2.69	1.95 ± 1.44	Welsh unpubl.
<i>Farfantepenaeus duorarum</i>	Pink Shrimp	23	5	4.59 ± 0.86	n.a.	1.67 ± 0.17	2.71 ± 0.27	Burns, unpubl.
<i>Squalus acanthias</i>	Dogfish shark	13	7	1.25 ± 0.41	4.48 ± 0.89	0.41 ± 0.13	4.04 ± 0.93	Andres et al., submitted.
		21	8	2.03 ± 0.27	5.57 ± 0.89	0.67 ± 0.10	3.02 ± 0.10	
<i>Centropristis striata</i>	Black Seabass	12	5	1.47	3.36	0.42	3.50	Slesinger et al., 2019
		17	4	2.07	3.99	0.58	3.60	
		22	4	3.07	4.73	0.67	4.59	
		28	4	4.55	5.07	0.75	6.04	
		30	4	5.42	5.62	0.80	6.81	
<i>Illex illecebrosus</i>	Short-fin Squid	20	3	13.95 ± 2.24	5.28 ± 0.56	2.69 ± 0.37	5.18 ± 0.18	Birk, unpubl.
<i>Sthenoteuthis oualaniensis</i>	Purple-back Squid	23	3	12.35 ± 1.47	6.38 ± 1.99	3.16 ± 0.87	4.04 ± 0.67	Birk, unpubl.

Figures

Figure 1. Schematic illustrating a new method for P_{crit} determination, termed $P_{crit-\alpha}$. A) The relationship between standard (SMR, blue), routine (RMR, green) and maximum (MMR, red) metabolic rate and oxygen partial pressure (PO_2). The oxygen supply capacity (α , dashed line, derived in panel B) is the slope of the relationship between $P_{crit-\alpha}$ and PO_2 . B) For each metabolic level (e.g. SMR), the physiological oxygen provision (α_0) increases toward the maximum capacity, α (dashed line). While α_0 must be greater to meet a higher metabolic rate at a given PO_2 , α is the same for all three rates. All three metabolic levels reach a critical PO_2 where oxygen provision is at maximum capacity (where $\alpha_0 = \alpha$). As a result, α is the slope of the MO_2 vs PO_2 curve, allowing P_{crit} to be calculated at any metabolic rate and the maximum achievable metabolic rate to be calculated for any PO_2 .

Figure 2. A new method for P_{crit} determination using a representative respirometry trial for *Fundulus grandis* (Reemeyer and Rees, 2019). A) Oxygen consumption rate (MO_2 , blue dots) as a function of PO_2 . $P_{crit-\alpha}$ (red line; $P_{crit-\alpha} = MO_2/\alpha$) is the MO_2 value divided by the oxygen supply capacity, α (derived in panel B). Thus, $P_{crit-\alpha}$ can be determined for any MR. B) Each MO_2 bin (panel A) divided by its corresponding PO_2 is the physiological oxygen provision (α_0 ; $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1} \text{ kPa}^{-1}$). As PO_2 declines, oxygen provision increases toward the oxygen supply capacity ($\alpha = 0.027$, dashed line). Using several common methods for P_{crit} determination, including the traditional broken-stick method, non-linear statistics, and a segmented approach with independent SMR determination, P_{crit} for this trial varied from 2.9 to 9.9 (Reemeyer and Rees, 2019).

Figure 3. Critical oxygen partial pressure ($P_{\text{crit-}\alpha}$, kPa), determined using the present method, as a function of that determined using other published methods (P_{crit} reported) at an equivalent metabolic rate. P_{crit} measured using the LLO method (Reemeyer and Rees, 2018; closed symbols, solid line) is most strongly correlated ($P_{\text{crit-}\alpha} = 0.046 + 0.92P_{\text{crit-reported}}$; $R^2 = 0.90$). Open symbols (dashed line) were measured with a variety of other methods (Table 1; $P_{\text{crit-}\alpha} = 0.27 + 0.72 P_{\text{crit-reported}}$; $R^2 = 0.81$). The black line is a unity line indicating a 1:1 relationship.

Figure 4. Three common response types of physiological oxygen provision (α_0) vs oxygen partial pressure. In each pair (A, B and C) the left-hand panel shows the oxygen consumption rate (blue dots) as a function of PO_2 , with $P_{crit-\alpha}$ for each rate indicated by the red line ($P_{crit-\alpha} = MO_2/\alpha$). The oxygen supply capacity (α) is derived in the right panel. The physiological oxygen provision ($\alpha_0 = MO_2/PO_2$) increases toward capacity (α) as PO_2 declines. A) Epaulette shark, *Hemiscyllium ocellatum* (Speers-Roesch et al., 2012). The oxygen provision plateaus at P_{crit} , meaning that oxygen supply is maintained at full capacity until oxygen is exhausted. B) *Pseudophryne bibronii*, post-hatch (Mueller and Seymour, 2011). Oxygen provision peaks at P_{crit} and declines at lower PO_2 , meaning that the animal is failing at PO_2 less than P_{crit} . C) Sand eel, *Ammodytes tobianus* (Behrens and Steffensen, 2007). Oxygen provision peaks at the lowest PO_2 , which suggests that MO_2 begins to decline just before P_{crit} is reached. Note that all MO_2 and α values in this figure are expressed in the units as originally published.

A.

B.

C.

Figure 5. Apparent oxyconformation in A) the clown knifefish, *Chitala ornata*, and B) in the worm, *Sipunculus nudus*. In the knifefish (*C. ornata*, 33°C), oxygen supply (right, red dots) is always near capacity and does not change appreciably as PO₂ declines, indicating that this individual was operating near the maximum achievable metabolic rate throughout the trial. B) The worm, *S. nudus*, oxygen supply increases as PO₂ and MO₂ decline, suggesting some active regulation despite apparent oxyconformation. The *S. nudus* data were corrected for PO₂ error (Timpe et al., in prep; gray points in B), which led to a reduction in α from 0.052 to 0.017 (gray and red lines). As a result, the ratio of maximum to minimum α_0 (a measure of the factorial aerobic scope) was reduced from 11.3 to 3.8, which is within the range of most species. Units are as in the original publications.

Figure 6. Temperature, methodological, and inter-individual variation in $P_{crit-\alpha}$ for existing datasets. A-C) Shrimp, *Farfantepenaeus duorarum* (Burns, unpubl.): Oxygen consumption rate as a function of PO₂ for an individual animal was calculated over time bins varying from 1 to 20 minutes. The 5- (A) and 20-min (B) time bins are shown. Oxygen supply capacity (α , slope of the red $P_{crit-\alpha}$ line) was strongly influenced by increasing MO₂ outliers at small bin sizes. C) High α (orange) at small bin size lead to unrealistically low $P_{crit-\alpha}$ (blue). No significant differences were found among values obtained using 10 to 20 min bin sizes. Trials were ~12 h duration and oxygen was recorded every 30 s. D) Black Seabass (*Centropristis striata*, Slesinger et al., 2019): Oxygen supply capacity (α) increases with temperature in proportion to maximum (MMR), rather than standard (SMR) metabolic rate. B)

Figure 7. $P_{crit-\alpha}$ determined for the maximum achievable metabolic rate (MMR) at varying oxygen levels for seven aquatic species. A) The freshwater fish, *Lepomis gibbosus* (Crans et al., 2015): The data suggest that P_{crit} for MMR is less than air-saturation, meaning that the maximum rate recorded at 20 kPa could be maintained until ~ 11.4 kPa PO_2 . B) *Gnathophausia ingens*, a deep-sea lophogastrid crustacean (Childress and Seibel, 1998). Three individuals measured at differing activity levels between maximum and rest. C) Lumpfish (*Cyclopterus lumpus*; Ern et al. 2016). D) Toadfish (*Opsanus tau*; Ultsch, 1989). Routine and standard metabolic rates. E) The prawn, *Penaeus esculentus* (Dall, 1986). F) *Octopus bimaculoides* (Seibel and Childress, 2000): Three individuals measured after varying acclimation periods resulting in variation in the metabolic rate. Units are as originally published.

Figure 8. Schematics showing the relationships between SMR, MMR, P_{crit} , oxygen supply capacity (α , slopes) and oxygen partial pressure. A) Two hypothetical species with identical SMR (1, blue; 2, black). Species 2 has a lower P_{crit} and, thus, a higher α (SMR/ P_{crit} ; higher slope). Two possible MMR values (open circles) are shown for species 2. If both species have the same MMR, then $P_{crit-max}$ (P_{crit} for MMR) must be lower for species 2, indicating hypoxia tolerance. Alternatively, the higher oxygen supply capacity may support a higher MMR and greater athleticism in normoxia for species 2. $P_{crit-max}$ must be known to distinguish between these two alternatives. B) A single species depicted at two temperatures (warm, red; cold, blue). Black dashed arrows represent the increase in each metric (SMR, MMR and P_{crit}) with temperature. The oxygen supply capacity increases with temperature to meet the higher MMR. SMR commonly increases with temperature faster than MMR (as shown here), causing P_{crit} to increase.