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COMMENTARY

Is hypoxia vulnerability in fishes a by-product of maximum metabolic rate?

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ABSTRACT

The metabolic index concept combines metabolic data and known thermal sensitivities to estimate the factorial aerobic scope of animals in different habitats, which is valuable for understanding the metabolic demands that constrain species' geographical distributions. An important assumption of this concept is that the O_2 supply capacity (which is equivalent to the rate of oxygen consumption divided by the environmental partial pressure of oxygen: $\dot{M}_{O_2}/P_{O_2}$) is constant at O_2 tensions above the critical O_2 threshold (i.e. the P_{O_2} where O_2 uptake can no longer meet metabolic demand). This has led to the notion that hypoxia vulnerability is not a selected trait, but a by-product of selection on maximum metabolic rate. In this Commentary, we explore whether this fundamental assumption is supported among fishes. We provide evidence that O_2 supply capacity is not constant in all fishes, with some species exhibiting an elevated O_2 supply capacity in hypoxic environments. We further discuss the divergent selective pressures on hypoxia- and exercise-based cardiorespiratory adaptations in fishes, while also considering the implications of a hypoxia-optimized O_2 supply capacity for the metabolic index concept.

KEY WORDS: Metabolic index, Aerobic scope, Exercise, Critical oxygen threshold, Climate change, Ocean warming

Introduction

Global oceans and other aquatic habitats are changing at unprecedented rates as a result of natural and anthropogenic forces driving climate change. These changes include ocean warming, deoxygenation and acidification, all of which have been the subject of intense study by marine biologists in recent years (e.g. Keeling et al., 2010; Bozinovic and Portner, 2015; Esbaugh, 2018). Much of this work has focused on identifying and understanding the physiological traits that confer tolerance and sensitivity to environmental changes in different species. From a physiological perspective, there has been particular focus on respiratory performance, as all three climate change stressors pose specific challenges to respiratory gas transport. Respiratory performance is often quantified through the determination of aerobic scope (AS; see Glossary), which is the difference between the baseline cost of living (i.e. standard metabolic rate, SMR; see Glossary) and the maximum capacity to transport oxygen from the environment to mitochondria (maximum metabolic rate, MMR; see Glossary) (Clark et al., 2013). AS has become an important ecophysiological metric because it quantifies all the energy available for non-vital functions such as activity, reproduction and growth. Environmental stressors can act as either 'limiting' or 'loading' stressors that constrain oxygen (O_2) supply or raise metabolic costs, respectively.

The global scope of climate change has pushed researchers to seek broad-scale unifying approaches to assess the effects on species' performance and biogeography (i.e. latitudinal shifts in species distribution), the most recent of which is the metabolic index concept (see Glossary; Deutsch et al., 2015; Deutsch et al., 2020). This concept estimates the energy available to aquatic ectotherms (i.e. the metabolic index, ϕ) under varying environmental conditions using the thermal sensitivities of O_2 supply and demand, as well as the effects of O_2 partial pressure (P_{O_2}) on O_2 supply. This concept has been used to estimate the energy available to aquatic ectotherms at equatorial and depth range limits, providing important information on the possible energetic limitations at these geographic boundaries (Deutsch et al., 2015; Deutsch et al., 2020). Notably, energetic limitations are not based on baseline costs of living (i.e. SMR), but instead are the cumulative result of baseline and routine activity costs across a wide variety of aquatic ectotherms. Approaches such as the metabolic index concept are critical to allow us to understand and predict the effects of climate change on ecosystems. However, it is equally important to critically evaluate whether these concepts are truly universal, and to identify species that may require alternative approaches. This seems particularly important for fishes, which are the most diverse vertebrate group on the planet and which occupy a plethora of different environments and ecological niches (Ravi and Venkatesh, 2008; Nelson et al., 2016). In this Commentary, we ask whether the metabolic index concept, in its current iteration, can be effectively applied to all fish species. Specifically, we seek to examine whether O_2 supply capacity (see Glossary) of fishes is constant across O_2 partial pressures, and whether it may be optimized to offset hypoxia vulnerability in some species.

The metabolic index

Obtaining O_2 is a fundamental physiological challenge for fish because of its naturally low solubility in water. Pioneering work by Fry and Hart (1948) demonstrated that warming exacerbates this challenge by simultaneously raising SMR and constraining MMR when temperatures surpass a species' thermal optimum (Fig. 1A). These findings were used as the mechanistic basis for the O_2- and capacity-limited thermal tolerance (OCLTT) hypothesis (see Glossary), which is a unifying conceptual framework used to predict the impacts of climate change on aquatic ectotherms (Portner and Knust, 2007; Pörtner and Farrell, 2008; Portner, 2010). Although the OCLTT remains a source of debate (e.g. Jutfelt et al., 2018), in part because not all aquatic ectotherms exhibit O_2-dependent thermal limitations (Ern et al., 2016; Ern et al., 2017), the underlying mechanism of observed thermal constraints on MMR are supported in many species (e.g. Fry and Hart, 1948; McBryan et al., 2013; Lefevre, 2016; Slesinger et al., 2019). The metabolic

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List of symbols and abbreviations

AS	aerobic scope
$(a-v)_{O_2}$	the difference in arterial and venous blood oxygen content
CT_{max}	the upper temperature limit for a species
E_D	the thermal sensitivity of oxygen demand
E_O	the thermal sensitivity of hypoxia vulnerability (i.e. P_{crit})
E_S	the thermal sensitivity of oxygen supply (i.e. MMR)
FAS	factorial aerobic scope
MMR	maximum metabolic rate
$\dot{M}_{O_2}$	oxygen consumption rate
P_{crit}	critical oxygen threshold
$P_{crit,max}$	the critical oxygen threshold for maximum metabolic rate
SMR	standard metabolic rate
V_b	cardiac output
α_D	oxygen demand (i.e. standard metabolic rate)
α_S	oxygen supply capacity
ϕ	the metabolic index
ϕ_{crit}	the metabolic index found at the equatorial or depth limit of a species distribution

Glossary

Aerobic scope
The energy available for all non-vital functions.
Bradycardia
A reduced heart rate.
Cardiac output
The volume of blood pumped by the heart per unit time.
Cardiac power
The ability of the heart to generate output against pressure.
Critical oxygen threshold
A measure of hypoxia vulnerability. The partial pressure of oxygen where oxygen uptake can no longer meet the requirements of standard metabolic rate.
Critical oxygen threshold maximum
A measure of the effects of reduced oxygen on maximum metabolic rate. The partial pressure of oxygen that causes a decline in maximum metabolic rate.
Factorial aerobic scope
The energy available for all non-vital functions expressed as the ratio of maximum metabolic rate to standard metabolic rate.
Maximum metabolic rate
The maximum oxygen consumption rate available to an individual during exhaustive exercise.
Metabolic index
The ratio of oxygen supply to demand after accounting for thermal sensitivities and environmental P_{O_2}.
Oxygen- and capacity-limited thermal tolerance
A theory that suggests critical thermal limitations of aquatic ectotherms are due to a collapse of aerobic scope.
Oxygen supply capacity
The ratio of maximum oxygen consumption rate to ambient P_{O_2}.
Root effect
The tendency of fish hemoglobin to exhibit reduced carrying capacity of O_2 at low pH.
Standard metabolic rate
The oxygen consumption rate required to meet the energetic costs of vital physiological functions.
Tachycardia
An elevated heart rate.
Thermal sensitivity
Describes the degree of change in a metabolic trait (e.g. SMR, MMR, P_{crit}) as a consequence of warming, expressed by an exponential Arrhenius function.

index concept can be considered a refinement of the OCLTT that focuses on AS the determinative endpoint, as opposed to the critical thermal maximums (i.e. CT_{max}). This concept attempts to explain biogeographical patterns based on respiratory traits and the thermal sensitivity of those traits (Deutsch et al., 2015; Deutsch et al., 2020). The formula for a species' metabolic index (ϕ) is:

$$\Phi = \frac{\alpha_S}{\alpha_D}\beta^{\varepsilon}P_{O_2}\exp\left\{\frac{E_O}{k_B}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right\}, \quad (1)$$

where α_S is O_2 supply capacity, α_D is O_2 demand (i.e. SMR), β^{ε} is body mass and the associated metabolic scaling coefficient, P_{O_2} is the environmental O_2 partial pressure, k_B is the Boltzmann constant, E_O is the temperature sensitivity of hypoxia vulnerability (i.e. the critical oxygen threshold, P_{crit}; see Glossary), and T and T_{ref} are temperature and reference temperature (i.e. the temperature where metabolic traits were measured), respectively (Deutsch et al., 2015; Deutsch et al., 2020). Although it is complex, this formula can be thought of as a variation on factorial AS (FAS; see Glossary) that incorporates thermal sensitivity and O_2 availability. More simply, ϕ=FAS under normoxia at the reference temperature. Importantly, ϕ only addresses hypoxia vulnerability using P_{crit}, which is an aerobic trait that defines the P_{O_2} at which an animal can no longer maintain SMR and where AS falls to zero. One important assumption behind the ϕ concept is that α_S is constant across P_{O_2}. From a physiological perspective, this would mean that the O_2 supply constraints that limit O_2 uptake in exercising fish at high P_{O_2} are similar to those that limit O_2 uptake in resting fish at P_{crit}. A second assumption is that the thermal sensitivity of P_{crit} (E_O) represents the cumulative effects of temperature on O_2 supply (E_S) and demand (E_D) (i.e. $E_O = E_D - E_S$). In other words, the P_{crit} of a fish at a given temperature is the product of the cumulative thermal constraints on α_D and α_S, the latter of which can be defined using MMR (i.e. MMR=$\alpha_S P_{O_2}$ when P_{O_2}=21 kPa). Furthermore, it is assumed that the natural log of thermal sensitivities is linear across the entire thermal range of a species. Below, we consider the validity of these assumptions for fishes in more detail.

Is oxygen supply capacity constant in fishes?

The metabolic index concept defines α_S as the O_2 consumption rate divided by P_{O_2} at which the O_2 consumption was measured. This can be measured as SMR/P_{crit} (Seibel and Deutsch, 2020). The effect of P_{O_2} on O_2 consumption, and thus α_S, is generally described in one of two ways (Fig. 1B). Firstly, through a classical description of the line of O_2 limitation, which is often hyperbolic (e.g. Fry, 1947; McBryan et al., 2013; Claireaux and Chabot, 2016). Secondly, and more recently, models have described a linear relationship followed by a plateau (e.g. Wood, 2018; Seibel and Deutsch, 2020). The breakpoint is defined as the $P_{crit,max}$ (see Glossary), the P_{O_2} below which MMR is constrained. A comprehensive assessment of $P_{crit,max}$ across a variety of animal phyla, including mollusks, arthropods and chordates, was recently performed by Seibel and Deutsch (2020), which used available SMR, MMR and P_{crit} values to predict $P_{crit,max}$ ($P_{crit,max}$=MMR×P_{crit}/SMR) in a variety of species from different phyla. Of the 52 species tested – 28 of which were fish – 73% demonstrated a $P_{crit,max}$ of approximately 21 kPa O_2. This led to the suggestion that hypoxia tolerance, as defined by P_{crit}, is simply a by-product of selective pressures on MMR (Seibel and Deutsch, 2020). This conclusion was based on the premise that maximizing MMR and AS in normoxia is a prominent evolutionary driver of respiratory performance and ecological success in aquatic ectotherms. If α_S is similar at P_{crit} and $P_{crit,max}$, then it is logical to suggest that hypoxia

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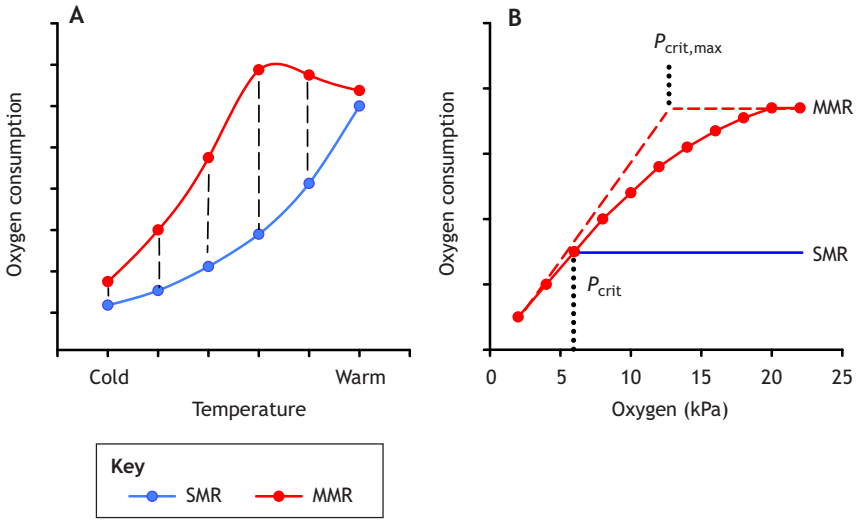

Fig. 1. Illustration of the theoretical effects of temperature and hypoxia on oxygen consumption. (A) Standard metabolic rate (SMR) increases with temperature at an exponential rate defined by the Arrhenius thermal sensitivity coefficient. Maximum metabolic rate (MMR) also increases with temperature at an exponential rate, but in some fish this reaches a breakpoint after which the effects of temperature plateau or reverse. The dashed black lines denote aerobic scope. (B) As ambient P_{O_2} decreases, MMR declines along the line of oxygen limitation (oxygen supply capacity), which is defined by α_S ($\dot{M}_{O_2}/P_{O_2}$). The diagonal dashed red line denotes a constant α_S between the critical oxygen threshold (P_{crit}) and the critical oxygen threshold on MMR ($P_{crit,max}$). The horizontal dashed red line denotes the point at which excess oxygen provides no benefit to MMR. The solid red line denotes a hyperbolic relationship between P_{O_2} and MMR, which results from a declining α_S as P_{O_2} increases. The blue line denotes SMR.

tolerance as defined by P_{crit} is a by-product of the evolutionary pressures on MMR. This is probably true for those species with a $P_{crit,max}$ of 21 kPa; however, it is less certain for the other 27% of species.

Seibel and Deutsch (2020) suggested that species with a $P_{crit,max}$ below approximately 21 kPa are adapted to hypoxic environments, but argue that adaptations to these environments are based on maximizing α_S at $P_{crit,max}$ (i.e. maximizing MMR). However, this conclusion was reached by extrapolating only a single measurement of α_S (i.e. SMR/P_{crit}) per species to the measured MMR of that species. Here, we compiled datasets from seven different fish species that allow the calculation of α_S across a P_{O_2} range (MMR/P_{O_2}; Fig. 2; Table S1). These seven species have $P_{crit,max}$ values that range from 8.35 to 17.2 kPa, and P_{crit} values that range from 2.3 to 6.2 kPa. Of these seven, the red drum (*Sciaenops ocellatus*) (Ackerly and Esbaugh, 2020) and pumpkinseed (*Lepomis gibbosus*) (Crans et al., 2015) generally follow the pattern of a constant α_S until $P_{crit,max}$. In contrast, four of the species – the largemouth bass (*Micropterus salmoides*), rock bass (*Ambloplites rupestris*), blue gill (*Lepomis macrochirus*) (Crans et al., 2015) and common sole (*Solea solea*) (Lefrancois and Claireaux, 2003) – deviate from the described pattern with a gradual decline in α_S as P_{O_2} increases from P_{crit}. The pattern of the last species, the black-axil chromis (*Chromis atripectoralis*) (Ern et al., 2017), is ambiguous but it is included for completeness. All four species without a constant α_S have at least one data point between P_{crit} and $P_{crit,max}$ (Fig. 2). The physiological explanation for the patterns described above is uncertain. It is possible that fish species with a calculated $P_{crit,max}$ below 21 kPa have an α_S that is selected for specifically on the basis of hypoxia tolerance (i.e. α_S at P_{crit}), not based on MMR at $P_{crit,max}$. Regardless of the mechanism, it is clear that α_S should not be assumed to be constant across P_{O_2} values in all fishes.

Are the physiological responses to hypoxia and exhaustive exercise similar in fish?

As discussed above, the available data suggest that α_S is not constant across P_{O_2} levels in all fish, which suggests that species exhibit an α_S that is optimized for performance under either hypoxia or exercise. Here, we will consider whether this dichotomy is consistent with our current understanding of respiratory physiology in fishes. For fish, exercise has often been grouped with other biotic factors such as anemia, acidosis and changes in gill structure that cause 'functional hypoxia' (Farrell and Richards, 2009). Although this observation is true in a general sense, the question we will address here is whether there is a mechanistic basis to assume that exercise-induced hypoxia and acute environmentally derived hypoxia are similar from a physiological perspective, and whether there is reason to believe that separate selective pressures can shape exercise performance and hypoxia tolerance (see Table 1). We will use the Fick equation as a functional framework:

$$\dot{M}_{O_2} = V_b \times (a - v)_{O_2}, \quad (2)$$

where $\dot{M}_{O_2}$ refers to metabolic O_2 consumption, V_b is cardiac output (see Glossary) and $(a-v)_{O_2}$ is the difference between arterial and venous O_2 content. At rest, fish in hypoxia maintain $\dot{M}_{O_2}$ (Fry, 1947; Holeton and Randall, 1967; Negrete and Esbaugh, 2019), whereas exercising fish increase $\dot{M}_{O_2}$ (e.g. Kiceniuk and Jones, 1977; Norin and Clark, 2016). These different challenges necessitate that fish make distinct changes to their cardiorespiratory system by manipulating V_b and $(a-v)_{O_2}$ to meet O_2 demand. Note that this is not intended as a comprehensive review of hypoxia, exercise or cardiorespiratory physiology, as such reviews can be found elsewhere (e.g. Farrell et al., 2009; Gamperl and Driedzic, 2009; Richards, 2011; Eliason and Farrell, 2016).

The first component of the Fick equation is cardiac output, which is a combination of heart rate and stroke volume and refers to the total amount of blood pumped by the heart per unit time. When fish are exposed to hypoxia, they respond with a series of physiological adjustments intended to maintain O_2 delivery to the heart and brain. In most fishes, hypoxia exposure causes significant bradycardia (see Glossary) accompanied by increased stroke volume (Farrell, 2007; Gamperl and Driedzic, 2009) stimulated by elevated venous pressure (Sandblom and Axelsson, 2005) – although, in some species, this response does not occur until the fish approaches P_{crit} (e.g. Atlantic cod, *Gadhus morhua*) (McKenzie et al., 2009; Petersen and Gamperl, 2011). The observed change in cardiac output under hypoxia depends on whether increased stroke volume fully compensates for the bradycardia (Farrell, 2007; Gamperl and Driedzic, 2009). In some species, such as Adriatic sturgeon (*Acipenser naccarii*; Agnisola et al., 1999) and rainbow trout (*Oncorhynchus mykiss*; Sandblom and Axelsson, 2005), cardiac output is fully maintained. In other species, such as sea bass (*Dicentrarchus labrax*; Axelsson et al., 2002) and tilapia

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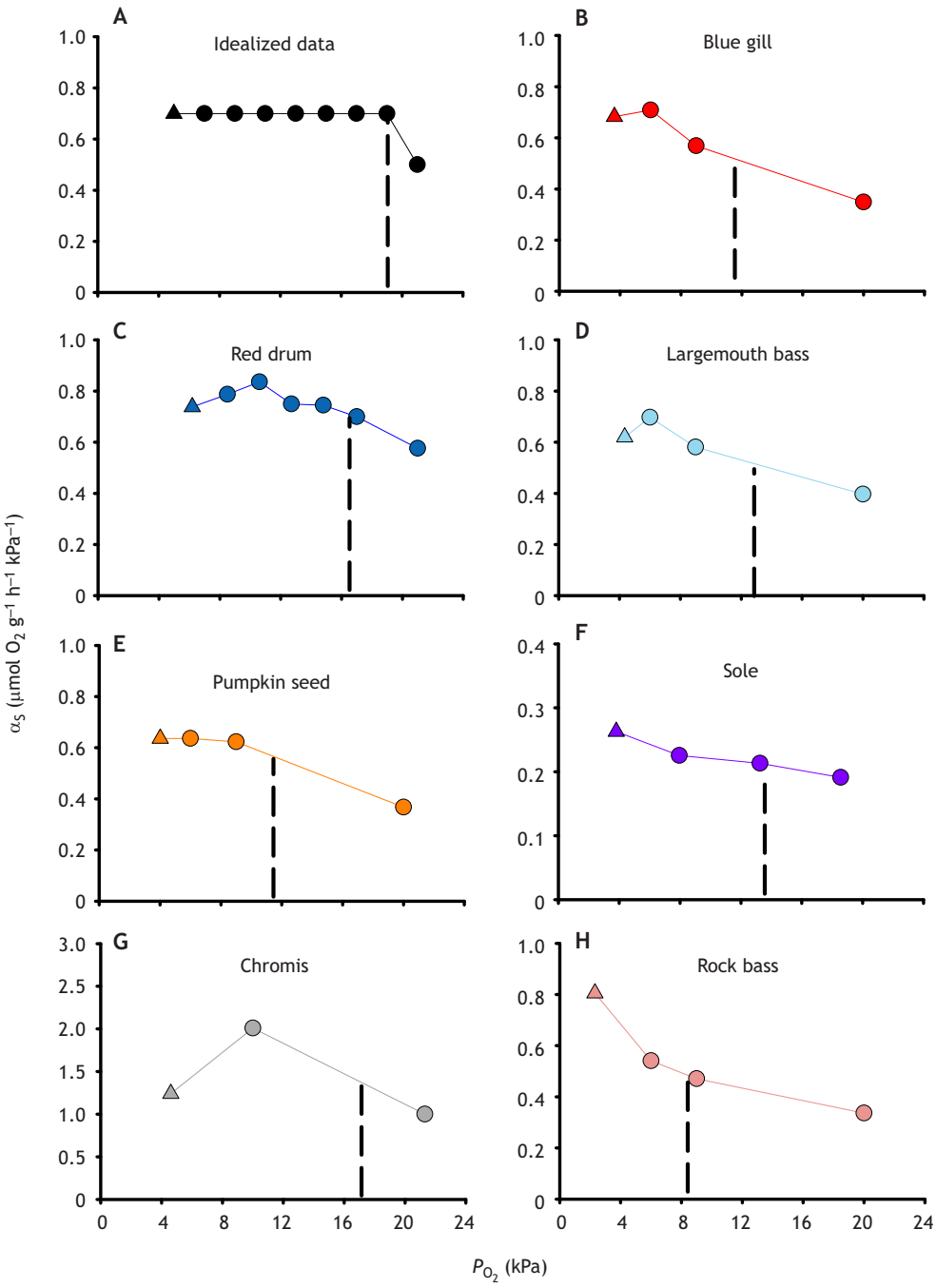

Fig. 2. Oxygen supply capacity at various oxygen partial pressures in seven teleost species. (A) The expected data trace of a species that exhibits a constant α_S between P_{crit} and $P_{crit,max}$. Note the decline in α_S after $P_{crit,max}$ (indicated by the dashed vertical line). (B–H) Species-specific traces from data compiled from the literature. Several species (B,D,F,H) exhibit a decline in α_S between P_{crit} and $P_{crit,max}$, which is indicative of an elevated α_S at lower oxygen tensions. Note that P_{crit} is the left-most point on all traces (denoted by a triangle). Raw data are shown in Table S1.

(*Oreochromis* hybrid sp.; Speers-Roesch et al., 2010), stroke volume does not compensate for the bradycardia, reducing cardiac output under hypoxia. Because these responses occur in conjunction with reduced arterial blood pressure (Sandblom and Axelsson, 2005; Speers-Roesch et al., 2010) it is hypothesized that their purpose is to reduce the cardiac power (see Glossary), and thus reduce O_2 demand. Put simply, the acute hypoxia strategy of fish prioritizes reduced cardiac O_2 demand as opposed to increased work to support uptake and delivery via perfusion.

During exercise, fishes prioritize increased O_2 delivery to skeletal muscle through changes to blood flow (e.g. Kolok et al., 1993). To facilitate the increased skeletal muscle O_2 demand, the heart rate will increase (tachycardia; see Glossary), as will stroke volume and cardiac output (reviewed by Farrell, 1991). In most fishes, an increase in stroke volume is more important than tachycardia – but this is species dependent. For example, yellowfin tuna (*Thunnus albacares*) exhibit a reduced stroke volume with exercise, yet cardiac output is still elevated because of substantial tachycardia (Korsmeyer et al., 1997). Exercise also has been shown to elevate arterial blood pressure (Stevens and Randall, 1967; Kiceniuk and Jones, 1977). Combined with elevated cardiac output, these changes work to substantially increase cardiac power, a critical limiting factor for maximal swim performance in fishes (e.g. Cox et al., 2017). O_2 delivery is also a crucial determinant of cardiac power, which is most evident in fishes with coronary circulation (e.g. Ekström et al., 2018). Yet, coronary circulation is generally considered as an adaptation to an athletic lifestyle, and we are unaware of any suggestion of hypoxia-induced evolution of this trait in fishes. As such, it appears that the

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Table 1. Generalized summary of select cardiorespiratory responses that accompany acute hypoxia exposure as fish approach their critical oxygen threshold (P_{crit}) compared with those as fish approach maximum sustained aerobic exercise capacity (i.e. maximum metabolic rate)

	Hypoxia (approaching P_{crit})	Exercise (approaching MMR)
Cardiovascular		
Heart rate	Bradycardia	Tachycardia
Cardiac output	Normal[1]	Elevated
Arterial blood pressure	Normal	High
Power output	Low	High
Respiratory		
Hematocrit	Elevated	Elevated
Red blood cell [NTP]	Reduced	Reduced
Arterial blood oxygen content	Reduced	Normal
Venous blood oxygen content	Exhausted	Reserved
Selection on high Hb affinity	Yes	No evidence
Selection on Root effect	Against	Yes
B-NHE response	Beneficial	Beneficial
Red blood cell intracellular pH	Normal	Acidified
Metabolic		
Respiratory acidosis	Yes	Yes
Metabolic acidosis	No[2]	Yes

See text for references. [1]This response shows substantial interspecies variability (see text).
[2]This refers to hypoxia exposure $\geq P_{crit}$.

selective pressures of aerobic exercise and acute hypoxia on cardiac performance are fundamentally different in fishes, with exercise-adapted fish prioritizing increased O_2 delivery and hypoxia-adapted fish prioritizing decreased O_2 demand.

The second component of the Fick equation is the arteriovenous difference in total O_2 content, which is a combination of P_{O_2} and hemoglobin (Hb)–O_2 (i.e. O_2 bound to Hb). Hypoxia exposure and exercise both induce a number of similar physiological responses in the blood that protect total blood O_2 content, including elevated hematocrit (reviewed by Gallaugher and Farrell, 1998) and the initiation of a catecholamine response to protect red blood cell intracellular pH (Primmett et al., 1986; Tetens and Christensen, 1987). The former raises the total blood O_2-carrying capacity, whereas the latter protects Hb–O_2 binding affinity during periods of systemic acidosis. But these similarities between the responses to hypoxia and exercise belie a number of important differences that can be the focus of selective pressure. The first notable difference is that although both hypoxia and exercise result in decreased blood P_{O_2}, only hypoxia significantly reduces the total blood O_2 content, at least with respect to the species studied to date (e.g. Holeton and Randall, 1967; Kiceniuk and Jones, 1977; Bushnell and Brill, 1992; Furimsky et al., 2003; McKenzie et al., 2004). The best datasets for illustrative purposes come from rainbow trout, where fish exercised at maximal swimming speed do not exhibit significantly reduced arterial total oxygen content (Brauner et al., 2000; McKenzie et al., 2004). Conversely, in response to hypoxia (4 kPa, 30 mmHg), the arterial blood of rainbow trout is significantly reduced to 37% saturation, as compared with ≥95% saturation under normoxia (Holeton and Randall, 1967). Differences between the responses to exercise and hypoxia also extend to venous blood, where evidence suggests that maximal exercise does not fully exhaust the available blood oxygen content, whereas hypoxia-exposed fish generally deplete venous oxygen reserves to maintain cardiac function. For example, the venous blood total O_2 content of maximally exercised rainbow trout is ∼23% that of the arterial blood (Brauner et al., 2000). Conversely, exposure to a P_{O_2} of 4 kPa – a value approximately 1.5 kPa above the reported P_{crit} for rainbow trout (Svendsen et al., 2011) – lowers venous P_{O_2} to 6 mmHg with only 3% Hb–O_2 saturation (Holeton and Randall, 1967).

The data described above suggest that the O_2-supply limitations that govern O_2 uptake during exhaustive exercise (i.e. MMR) and O_2 uptake at low ambient O_2 levels (i.e. P_{crit}) are fundamentally different in fishes. During exercise, increased cardiac output and hyperventilation (Stevens and Randall, 1967) maintain arterial blood O_2 saturation and augment delivery to muscles, suggesting that aerobic constraints relate to O_2-extraction efficiency, particularly in the heart. Conversely, hypoxia limitations are clearly governed by O_2 uptake, which is benefited by hyperventilation (e.g. Ern and Esbaugh, 2016) in combination with high Hb–O_2 binding affinity. This dichotomy is further supported by our understanding of the contribution of different Hb characteristics to O_2 uptake and delivery. Several studies have highlighted the mechanistic link between high Hb–O_2 affinity, whereby a low or reduced P_{50} (i.e. the O_2 tension when Hb is 50% saturated with O_2) improves aerobic performance in hypoxic environments (e.g. Mandic et al., 2009; Speers-Roesch et al., 2012; Pan et al., 2017). Conversely, recent data have highlighted an important role for the Root effect (see Glossary) in driving O_2 extraction at the tissues when combined with plasma-accessible carbonic anhydrase activity (Rummer et al., 2013; Alderman et al., 2016; Harter et al., 2019), red blood cell Na^+/H^+ exchange (Rummer and Brauner, 2011) and cytoplasmic carbonic anhydrase activity (Dichiera and Esbaugh, 2020; Dichiera et al., 2020). This system has been specifically highlighted in the eye (Damsgaard et al., 2020), heart (Alderman et al., 2016) and red muscle (Rummer et al., 2013) of fishes, but has also been suggested to act to benefit systemic O_2 delivery more broadly (Randall et al., 2014; Rummer and Brauner, 2015; Shu et al., 2018; Harter et al., 2019). Interestingly, an 8 day hypoxia acclimation significantly reduced the magnitude of the Root effect in red drum, while simultaneously improving Hb–O_2 affinity. This further supports the notion of divergent evolutionary pressures exerted by hypoxia and exercise on fishes.

We would like to close this section by clarifying that we are not suggesting that exercise-based adaptations cannot benefit hypoxia tolerance, and vice versa. Elevated hematocrit, cardiac morphology and hyperventilatory responses are obvious examples that would benefit O_2 supply capacity under any circumstance, and are known to respond positively to exercise and hypoxia acclimation in fishes (e.g. Yamamoto, 1987; Anttila et al., 2015). We fully support the notion that fish not specifically adapted to hypoxia will likely have a hypoxia tolerance that is a by-product of the selective pressures on MMR (Seibel and Deutsch, 2020). However, we suggest that hypoxia adaption can also include a suite of unique respiratory traits subject to selection, which may optimize α_S at P_{crit} to avoid an over-reliance on unsustainable anaerobic metabolism.

Implications of hypoxia-optimized α_S for predicting the effects of hypoxia on ϕ

The effects of hypoxia on ϕ are specific to the supply (S) side of Eqn 1, which can be simplified for a reference body mass and temperature as $S=\alpha_S P_{O_2}$. In those species where α_S is not constant, there is a trend of gradually declining α_S as the environment approaches normoxia (Fig. 2). The rate of change in α_S is the determining factor in the accuracy of the ϕ estimate. To illustrate this, we have evaluated three different datasets as examples (Fig. 3). Red drum generally conform to the bisecting line model and have a $P_{crit,max}$ relatively close to normoxia (16.6 kPa; 79% air saturation). In this case, the estimated ϕ deviates from the measured values by <5%, and this only occurs near

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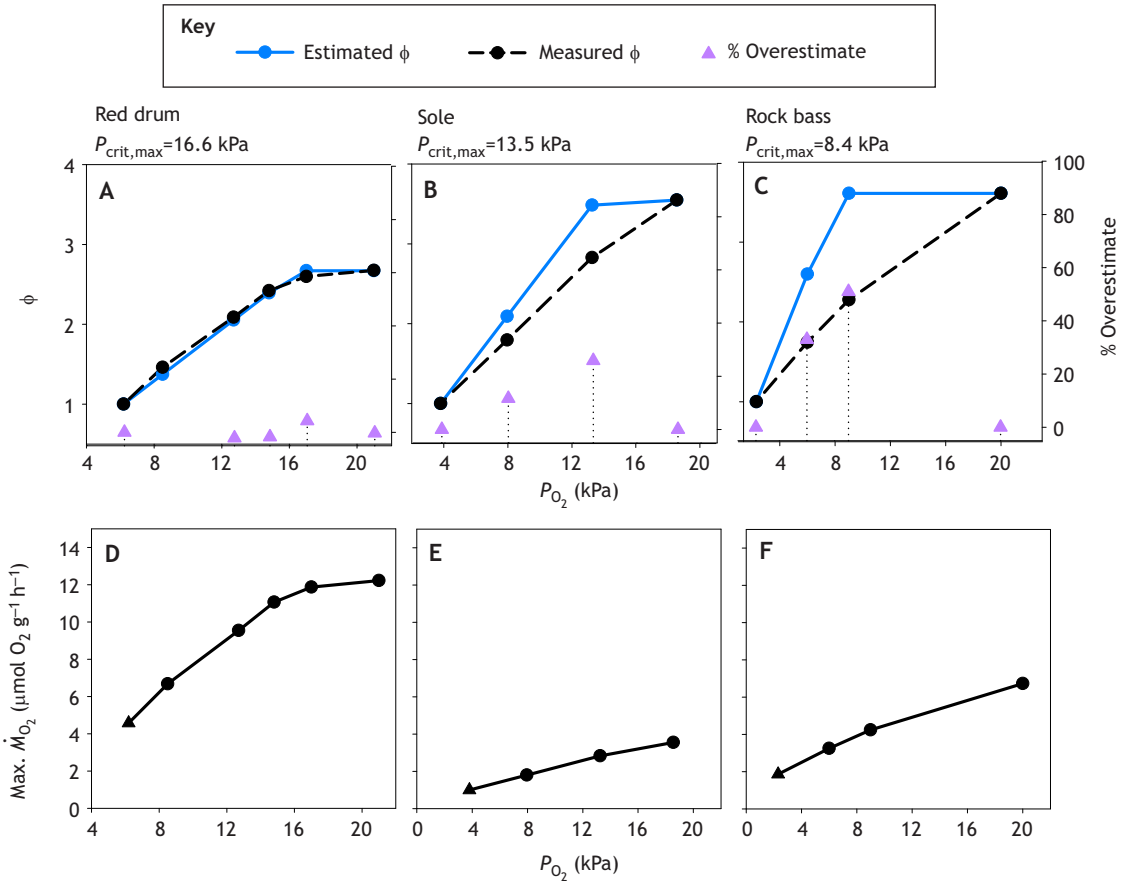

Fig. 3. Implications of variable oxygen supply capacity on estimates of factorial aerobic scope using the metabolic index calculation. (A) Red drum exemplify a species where the observed and estimated factorial aerobic scope (FAS) are in agreement. (B) Common sole and (C) rock bass demonstrate patterns where α_S optimized for low oxygen results in deviation between estimated and measured MMR at intermediate P_{O_2} levels. The maximum error will occur at $P_{crit,max}$. (D–F) The maximum $\dot{M}_{O_2}$ across oxygen tensions for (D) red drum, (E) common sole and (F) rock bass. Raw data are available in Table S1. The measured metabolic index (ϕ) is calculated as the maximum oxygen consumption divided by the SMR, the latter of which is denoted by the oxygen consumption at P_{crit} (i.e. the left-most point in D–F; black triangles). The estimated ϕ is derived from the α_S calculated at P_{crit} and extrapolated to higher oxygen tensions up to $P_{crit,max}$. Note that temperature is not incorporated into these estimates of ϕ because all measurements were performed at the same temperature. See text for additional details on ϕ estimation.

the $P_{crit,max}$ point. Common sole have a lower $P_{crit,max}$ and a more pronounced 26% deviation between measured and estimated ϕ values at 13.3 kPa. A more extreme example is shown for rock bass, which reveals a 51% overestimate of ϕ when compared with the measured value at 9 kPa. We would stress that the existence of a $P_{crit,max}$ below normoxia is not necessarily indicative of a species with an α_S optimized for P_{crit}, as evident in pumpkinseed (Fig. 2). However, $P_{crit,max}$ will represent the point of greatest deviation between estimated and measured ϕ in such fish species.

With these illustrative examples in mind, we have two recommendations for those applying the ϕ concept at an organismal level. The first is that a calculated $P_{crit,max}$ approximating normoxia (i.e. >80% air saturation) is suitable evidence that a species has a constant α_S. The second recommendation is that when a $P_{crit,max}$ below 80% air saturation is observed (e.g. pumpkinseed), researchers should experimentally validate α_S at the equivalent $P_{crit,max}$ oxygen tension. This will allow easy characterization of the consistency of α_S, and quantify the maximum estimate error as a result of a non-linear α_S.

Implications of hypoxia-optimized α_S for predicting the effects of warming on ϕ

A hyperbolic relationship between O_2 supply capacity and P_{O_2} is unlikely to directly impact the thermal sensitivity coefficients (E; see Glossary) of aerobic parameters, and the data of Lefrancois and Claireaux (2003) support this assumption. Their dataset measured MMR at multiple O_2 tensions at three different temperatures, as well as SMR and P_{crit} for those temperatures. Using these data, we calculated relatively similar values for thermal sensitivity of oxygen supply (E_S) across the experimental P_{O_2} range. Specifically, the E_S values were 0.23 at P_{crit}, 0.22 at 8 kPa, 0.23 at 13.3 kPa and 0.27 at 18.6 kPa (all P_{O_2} values are equal to the mean of the reported measurement intervals).

A more important consideration with respect to warming was observed by Seibel and Deutsch (2020), who noted that the relative relationship between E_S and the thermal sensitivity of oxygen demand (E_D) may result in SMR exceeding MMR at very high temperatures, and more specifically that E_S could not effectively predict MMR beyond a breakpoint that the authors designated as CT_{max}. This breakpoint is defined by the occurrence of a thermal plateau, or decline, in MMR that deviates from the exponential increase observed at lower temperatures. The authors used this relationship to suggest that impaired α_S is not related to the decline in MMR because such an impairment would also be observed in P_{crit}. This conclusion assumes that α_S is constant at normoxic MMR and P_{crit}, which as described above may not be true for a number of fish species. In fact, the available evidence suggests that α_S

limitations with warming in fishes are related to the ability to maintain cardiac output (Steinhausen et al., 2008; Eliason et al., 2011; Eliason et al., 2013), which, as described above, is not a major driver of P_{crit}. It is important to note the practical implications of these findings with respect to predicting ϕ under warming conditions. The numerator of the ϕ formula is equivalent to MMR (e.g. $MMR=\alpha_S \times P_{O_2,normoxia}$). This suggests that care should be taken when estimating ϕ at any temperature beyond which a thermally induced plateau in MMR is observed, as such extrapolation would result in an overestimate of ϕ.

As with the situation described above for hypoxia, the potential to overestimate ϕ is likely to affect only a subset of fishes. For example, species such as goldfish (*Carassius auratus*) (Fry and Hart, 1948), killifish (McBryan et al., 2013) and black sea bass (*Centropristis striata*) (Slesinger et al., 2019) all have documented thermal plateaus in MMR. Conversely, many species, such as Atlantic halibut (*Hippoglossus hippoglossus*) (Grans et al., 2014) and barramundi (*Lates calcarifer*) (Norin et al., 2014), show a consistent increase in MMR with warming. A thorough examination of the effects of warming on metabolic rate across a wide range of species can be found in Lefevre (2016). In the context of ϕ it is important that focus is placed on defining whether MMR collapses with warming, and identifying the temperature at which such breakpoints occur in species. To date, reduced cardiac performance has been highlighted as a likely cause for warming-induced collapses in MMR (e.g. Eliason et al., 2013); however, we should continue to explore additional causative factors that may contribute to this phenomenon. Intraspecies variation in such traits will ultimately be the focus of the selective processes that define the trajectory of species with climate change.

Why does an α_S optimized for low O_2 matter?

Thus far we have demonstrated that respiratory performance in fishes is not consistent with the assumption that α_S is singularly evolved to meet the demands of exercise in all fishes. Instead, we hypothesize that the evolutionary pressures that have shaped respiratory performance in fishes can be divided into two categories. The first category contains those species adapted for exercise in their environment, which would be represented by a constant α_S between P_{crit} and $P_{crit,max}$ (e.g. red drum; Fig. 2C) (see Seibel and Deutsch, 2020). The second category contains those fishes whose α_S is adapted to meet O_2 demands at P_{crit}, which is identifiable by declining α_S as P_{O_2} increases from P_{crit} to a theoretical $P_{crit,max}$ (e.g. rock bass; Fig. 2H). It is important to recognize these patterns when studying the physiological mechanisms related to hypoxia tolerance and vulnerability in fishes. When α_S is adapted for performance under normoxia, hypoxia vulnerability is likely to be a by-product of the selective pressures on MMR. Conversely, fishes with an α_S that increases as O_2 declines are likely to have specific adaptations to reduce hypoxia vulnerability.

Our second take home message is that a variable α_S violates a central assumption of the metabolic index concept, and results in overestimated ϕ. The significance of such an error depends on the species, the scope of the error and how ϕ is used by researchers. For example, the recent work by Deutsch et al. (2020) used biogeographical and physiological data to predict the energetic limitations that constrain animal species distributions. Note that this work included a wide variety of marine animal phyla, and reported an average ϕ_{crit} (i.e. the value of ϕ found at the equatorial or depth limit of a species distribution) of 3.3±0.3 (mean±s.e.m.; N=22) for marine fish species. For context, a subset of those species with both ϕ_{crit} estimates and FAS data across a thermal range are shown in Fig. 4 (Table S2; data from Seibel and Deutsch, 2020; Ackerly and Esbaugh, 2021). Note that ϕ_{crit} is an estimate of FAS, and that ϕ_{crit} is quite high when compared with the FAS at the upper thermal range of laboratory studies. If we assume that the thermal ranges employed for the studies on FAS (Fig. 4A) are environmentally relevant, then the estimates of ϕ_{crit} (Fig. 4B) suggest that the energetic limitations for many of these fishes equal the entirety of the theoretical AS. However, in cases where fish exhibit flexible α_S across O_2 tensions, or exhibit a thermally induced MMR collapse, the estimated ϕ_{crit} is likely to be lower than reported.

An overestimated ϕ_{crit} in a subset of fishes will have little bearing on the overall conclusions of Deutsch et al. (2020); namely, that the

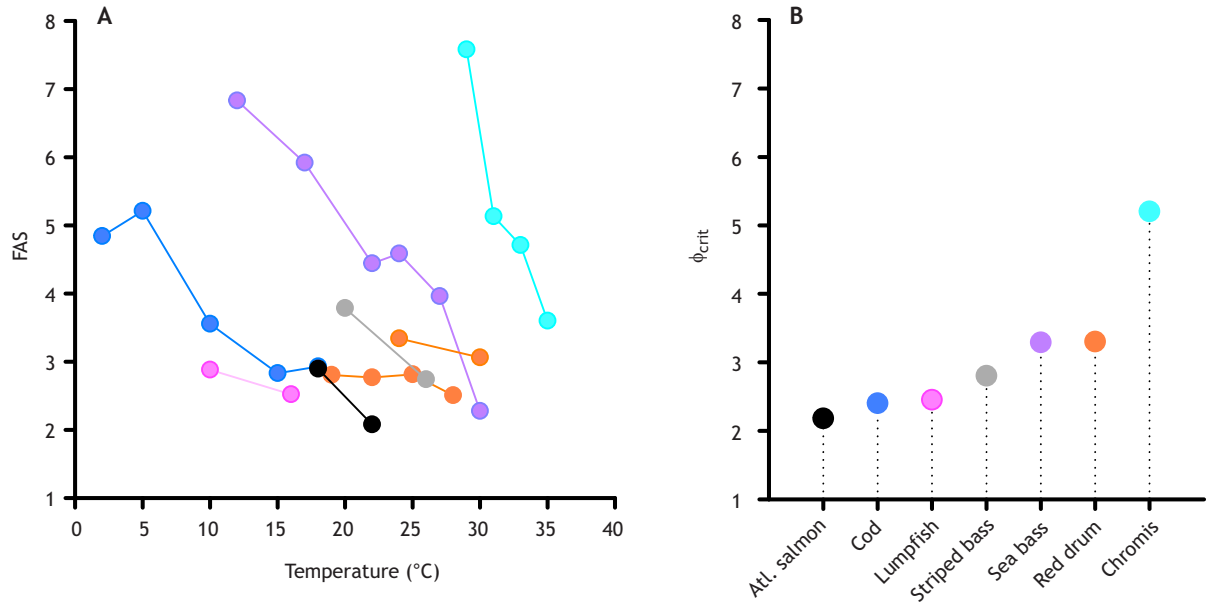

Fig. 4. FAS and metabolic habitat index of seven teleost species. (A) FAS declines with elevated temperature (see Table S2 for raw data). (B) The calculated metabolic habitat limit (ϕ_{crit}) ranges between a FAS of 2.2 and 5.2 (data from Deutsch et al., 2020).

energetic limitations that define aquatic ectotherm species ranges are due to the combined effects of exercise, temperature and hypoxia. However, overestimates can change our understanding of the absolute energetic minimums that species experience, and how ϕ is used to predict the effects of climate change. In some ways, ϕ_{crit} is an estimate of the buffer a species has against further warming and deoxygenation before they are forced to rely on anaerobic metabolism to meet their energetic demands. Species such as the black-axil chromis (ϕ_{crit}=5.2) have a large buffer against respiratory stress, whereas Atlantic salmon (ϕ_{crit}=2.2) have relatively little capacity for further O_2 supply constraints. It is also important to recognize that species' responses to climate change are complex, and survival may depend on poleward migration, phenotypic plasticity or other adaptations that allow species to maintain their existing ϕ_{crit}. If we are to properly understand the role that ϕ_{crit} may have in shaping biogeography and performance in fishes, it is important that we work with the best available estimates.

Conclusion

The metabolic index concept has placed a new and important significance on α_S and the relationship between O_2 consumption and P_{O_2}, particularly at intermediate levels of hypoxia. In this Commentary, we reinforced the premise that this relationship can vary among species (Fry, 1947), and that many species show hyperbolic curves representative of an elevated α_S as the animal approaches P_{crit}. Although these findings have clear implications for the calculation of ϕ and ϕ_{crit}, we would like to stress that this critique should not be viewed as a broad criticism of the metabolic index concept overall. We view any framework that can effectively predict the metabolic scope of a majority of aquatic ectotherms in response to environmental change as an incredibly powerful tool. But unifying theories are challenging, and inevitably there will be species that defy expectation. We argue that it is important to acknowledge such species and to develop alternative approaches that ensure a complete understanding of the effects of environmental change on animal performance. In particular, we hope this work places renewed focus on those species that may be adapted to hypoxic environments. This should include continued efforts to understand the physiological traits that define α_S in these species, while also exploring the compounding effects of warming and deoxygenation on hypoxia vulnerability.

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Competing interests

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