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RESEARCH ARTICLE

Oxygen supply capacity in animals evolves to meet maximum demand at the current oxygen partial pressure regardless of size or temperature

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ABSTRACT

The capacity to extract oxygen from the environment and transport it to respiring tissues in support of metabolic demand reportedly has implications for species' thermal tolerance, body size, diversity and biogeography. Here, we derived a quantifiable linkage between maximum and basal metabolic rate and their oxygen, temperature and size dependencies. We show that, regardless of size or temperature, the physiological capacity for oxygen supply precisely matches the maximum evolved demand at the highest persistently available oxygen pressure and this is the critical P_{O_2} for the maximum metabolic rate, $P_{crit\text{-}max}$. For most terrestrial and shallow-living marine species, $P_{crit\text{-}max}$ is the current atmospheric pressure, 21 kPa. Any reduction in oxygen partial pressure from current values will result in a calculable decrement in maximum metabolic performance. However, oxygen supply capacity has evolved to match demand across temperatures and body sizes and so does not constrain thermal tolerance or cause the well-known reduction in mass-specific metabolic rate with increasing body mass. The critical oxygen pressure for resting metabolic rate, typically viewed as an indicator of hypoxia tolerance, is, instead, simply a rate-specific reflection of the oxygen supply capacity. A compensatory reduction in maintenance metabolic costs in warm-adapted species constrains factorial aerobic scope and the critical P_{O_2} to a similar range, between ∼2 and 6, across each species' natural temperature range. The simple new relationship described here redefines many important physiological concepts and alters their ecological interpretation.

KEY WORDS: Maximum metabolic rate, Hypoxia tolerance, Critical thermal maximum, Oxygen supply capacity, Metabolic theory, Aerobic scope

INTRODUCTION

The maximum rate of aerobic metabolism (MMR) is an important measure of physiological performance and fitness that integrates neural, cardiovascular, respiratory and metabolic systems. Hill and Lupton (1923) believed that MMR is limited by the physiological capacity to supply oxygen, including adaptations for gill oxygen diffusion, blood–oxygen binding and cardiac function, a view still widely accepted today (Bassett and Howley, 2000; Spurway et al., 2012; Wagner, 2015; Farrell, 2016). Physiological oxygen supply limitation has also been implicated in temperature- and size-related reductions in metabolism and in aerobic scope for growth and reproduction with consequences for species diversity, abundance, distribution, life history and response to climate change (Brown et al., 2004; Hochachka, 1985; Falkowski et al., 2005; Lefevre, 2016; Pörtner et al., 2017; Deutsch et al., 2015; Roman et al., 2019; Cheung et al., 2013; Gillooly et al., 2001).

Despite its ecological and medical importance, the oxygen supply capacity (α, μmol O_2 h^{-1} g^{-1} kPa^{-1}) is rarely directly measured and the selective pressures acting on it are poorly understood. Here, we estimated α for 52 species from across the tree of life, including mollusks, arthropods and vertebrates, from marine, freshwater and terrestrial environments (Table S1). We compared α derived under two commonly measured, yet distinct, oxygen supply challenges: environmental hypoxia at rest and maximum aerobic exercise. We hypothesized that the physiological oxygen transport system has evolved to supply sufficient oxygen to meet maximum demand at the prevailing environmental oxygen partial pressure (P_{O_2}). The prevailing P_{O_2} is that under which a species' capacity for activity has evolved, regardless of metabolic rate, body size or temperature.

To obtain sufficient energy for survival, the O_2 supplied to an aerobic organism must meet or exceed its O_2 demand (as described by the 'metabolic index'; Deutsch et al., 2015). At any particular temperature and at maximum workload, the total oxygen supply is the product of the environmental P_{O_2} and α. Oxygen demand is simply the metabolic rate (MR), here estimated by the rate of oxygen consumption. As ambient oxygen declines, physiological supply (i.e. inclusive of ventilation and cardiac output) increases until a critical P_{O_2} (P_{crit}) is reached at which a species' physiological oxygen supply capacity is fully exploited and below which oxygen demand, typically measured as the standard or basal metabolic rate (BMR), can no longer be maintained (Fig. 1). When the environmental P_{O_2} reaches P_{crit}, α can be estimated (α=BMR/P_{crit}). At MMR, which is typically elicited during intense activity, α is similarly fully utilized and can be independently estimated as α=MMR/$P_{crit\text{-}max}$ (where $P_{crit\text{-}max}$ is the critical oxygen pressure at MMR; Fig. 1). $P_{crit\text{-}max}$ is rarely measured, but we hypothesize that it is the prevailing P_{O_2} in a species' environment under which their capacity for activity has evolved. If α is equivalent at P_{crit} and MMR, then $P_{crit\text{-}max}$ can be calculated.

MATERIALS AND METHODS

The physiological parameters MMR, BMR and P_{crit} were gathered from published laboratory measurements for 52 species of animals. The original studies are referenced in Tables S1–S3. The measurements range over 5 orders of magnitude in body mass, span nearly the entire range of habitable temperatures, and are from 3 phyla (Arthropoda, Chordata and Mollusca). The data compiled here are derived from studies using diverse methodologies as

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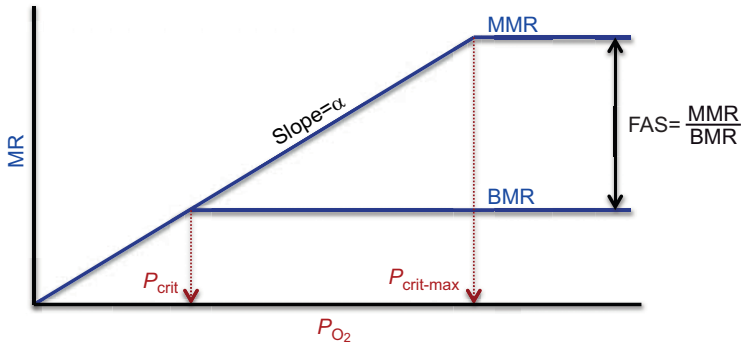

Fig. 1. Schematic illustration of the relationship between metabolic parameters, oxygen and temperature. Maximum metabolic rate (MMR) and basal metabolic rate (BMR) and the corresponding critical oxygen partial pressures ($P_{crit\text{-}max}$ and P_{crit}) are related via the oxygen supply capacity, α, which is the slope of the metabolic rate–oxygen partial pressure (MR–P_{O_2}) curve. The factorial aerobic scope (MMR/BMR)=$P_{crit\text{-}max}/P_{crit}$.

appropriate for the diversity of taxa represented. We included all available taxa for which all three physiological metrics have been measured. We did not discriminate on the basis of methodological differences (e.g. closed, flow-through or intermittent flow respirometry; chase or swim flume measurement) or the acclimation period allowed, as long as a distinct critical P_{O_2} could be identified at a specified metabolic rate. Where multiple datasets exist for a single species, preference was given to those for which all three variables were reported in the same study, or by the same authors in multiple studies, or in reviews that summarized all available data.

Three species were excluded from analysis despite availability of data, the Antarctic icefish, *Pagothenia borchgrevinki*, the Nile tilapia, *Oreochromis niloticus*, and the European perch, *Perca fluviatilis*. Measurements from multiple studies of the icefish and tilapia were inconsistent and highly variable (Davison et al., 1990; Lowe and Davison, 2006; Mamun et al., 2013; Lapointe et al., 2018; Burggren et al., 2019; Forster et al., 1987). The inconsistency in the icefish may result from the lack of a respiratory protein in this species or the high sensitivity of this species to experimental handling. In tilapia, the variability likely results from the use of differently acclimatized populations and a highly adaptable physiology (Burggren et al., 2019). The European perch was excluded because it appears not to have reached a true MMR under normoxic conditions, perhaps because of the chase protocol used to elicit MMR, which resulted in an apparent increase in MMR under hyperoxia (Brijs et al., 2015; Thuy et al., 2010). Several studies note that the chase protocol does not elicit a maximum metabolic rate in some species (e.g. Slesinger et al., 2019; Clark et al., 2013).

In all cases (except humans), BMR and P_{crit} were taken from the same study. For humans, P_{crit} was estimated from the maximum decrement in MMR at altitude (Table S1). MMR for all species is achieved during or just after activity protocols induced via either treadmills or swim flumes or following an exhaustive chase. BMR, as usually defined, is equivalent to a standard metabolic rate (SMR) and is the lowest rate achieved by a particular species at a specified temperature. The BMR in the present dataset was typically measured in a resting, fasted state, although this was not a requirement for inclusion. Many of the BMR values were measured under unspecified activity levels and are thus more appropriately termed a 'routine' rate. All reported resting or routine rates were accepted as BMR, providing a corresponding P_{crit} was available. Any elevation in metabolic rate above a true BMR will lead to an underestimated aerobic scope. However, MR and P_{crit} are linearly related. Thus, an accurate MMR can be calculated, as described below, from any paired MR and P_{crit} measurement. Temperature (T) and body mass (M) effects on physiological processes or rates were estimated as $aM^b \exp(-E/k_BT)$ (Deutsch et al., 2015), where a is the normalization constant for species-specific metabolic rate or P_{crit}, b is the mass dependence, E is the temperature dependence and k_B is the Boltzmann constant (eV K^{-1}). The temperature dependence (E) of each metabolic metric was, thus, determined from the slope of the linear regression of $1/k_BT$ versus the natural log of the metric.

RESULTS

In the present dataset, the oxygen supply capacity (α=BMR/P_{crit}) varied by 2 orders of magnitude, from 0.17 in the cephalopod *Nautilus pompilius* to 15.28 in the grasshopper *Schistocerca americana* (Table S1). To assess the equivalence of environmental and physiological oxygen supply challenges, we first estimated $P_{crit\text{-}max}$=MMR/α using the α value derived from BMR and P_{crit}. Despite >2 orders of magnitude variation in MMR and in α, the $P_{crit\text{-}max}$ for diverse species was tightly constrained near atmospheric P_{O_2} (21 kPa). The median value for all species was 19.45 kPa, while the mean (±s.d.) was 18.06±4.95 kPa. However, of the 52 species examined, 14 are known to inhabit persistently hypoxic environments (longer than diel or tidal cycles), including deep-sea oxygen minimum zones, estuaries, poorly ventilated ponds, subterranean burrows and high altitude. In all of these species, hereafter referred to as 'hypoxic species', the calculated $P_{crit\text{-}max}$ fell below 21 kPa by more than 1 s.d. (4.95 kPa; Fig. 2A). While many otherwise normoxic marine species experience diel or tidal variability in P_{O_2}, they are routinely exposed to air-saturated water and their estimated $P_{crit\text{-}max}$ values were within 1 s.d. of atmospheric P_{O_2}. Many species are known to employ anaerobic metabolic pathways and metabolic suppression to survive short-term oxygen limitation (Mandic et al., 2009; Seibel, 2011). For 9 of the 14 hypoxic species, $P_{crit\text{-}max}$ had been experimentally determined and it closely matched the predicted values (Table S2). For the remaining 5 hypoxic species, the estimated $P_{crit\text{-}max}$ values were similar to the highest published environmental P_{O_2} values from each species' habitat (Table S2). The other 38 species (hereafter referred to as 'normoxic') had a mean (±s.d.) estimated $P_{crit\text{-}max}$ value of 21.12±2.05 kPa (median 20.74 kPa) and were, thus, expected to achieve maximum activity only at or near atmospheric P_{O_2}. With few exceptions (Brijs et al., 2015), hyperoxia (P_{O_2}>21 kPa) did not result in an increase in MMR, suggesting that species do not typically evolve the excess supply capacity required to transport the additional oxygen, nor presumably the oxidative capacity to consume any excess oxygen that would be delivered to the tissues (Fig. 2B; Claireaux and Chabot, 2016).

For the 38 normoxic species, the oxygen supply capacity, estimated as α=BMR/P_{crit}, was strongly correlated with that derived as α=MMR/21, while for hypoxic species, it was correlated with that derived from MMR divided by the measured $P_{crit\text{-}max}$ or the prevailing environmental P_{O_2} (Tables S1 and S2; Fig. 2C; y=−0.03+1.01x; R^2=0.998; P<0.0001). Thus, α was equivalent at the limiting oxygen level for any metabolic level (e.g. MMR, BMR or any routine MR

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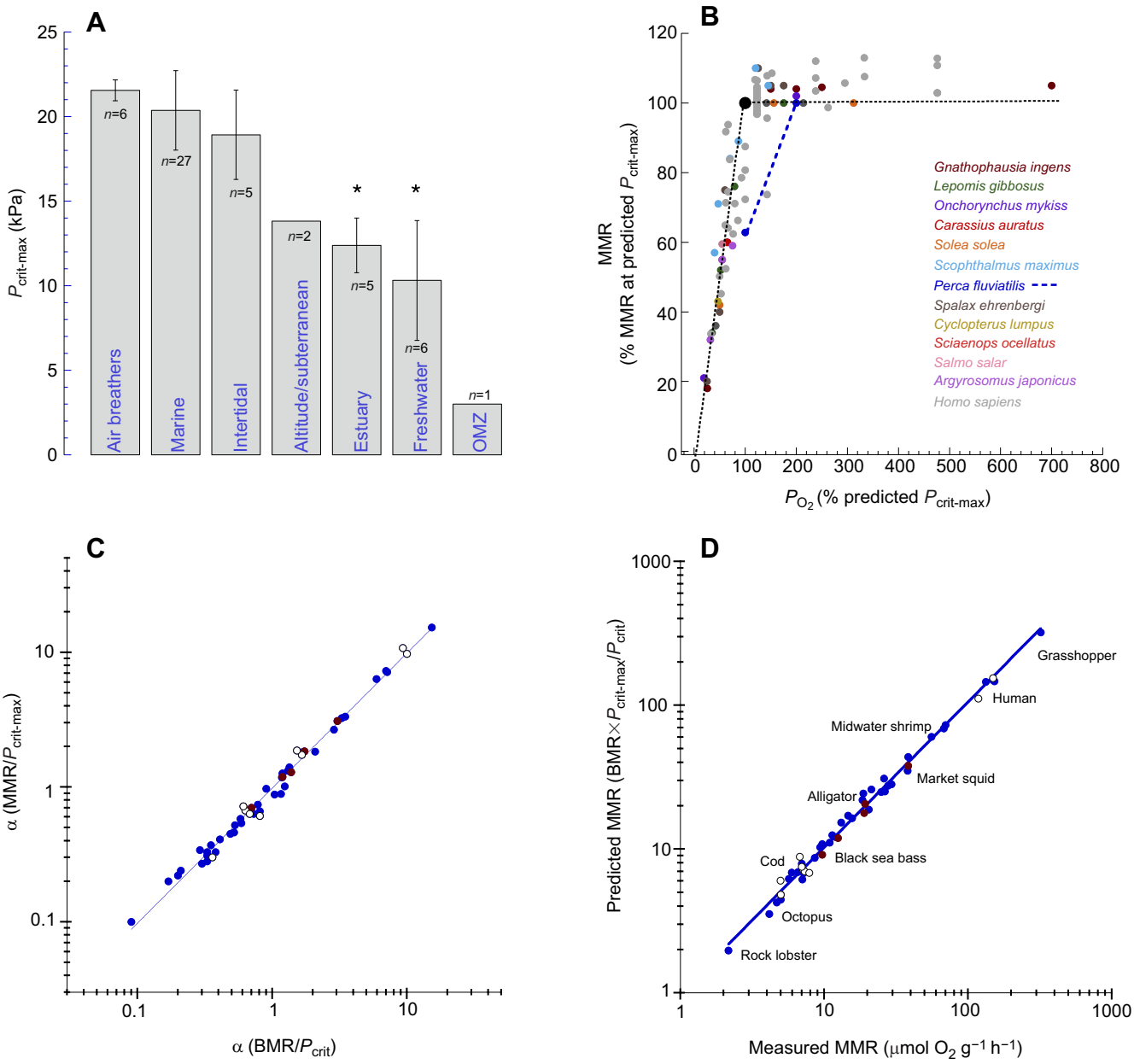

Fig. 2. Oxygen supply capacity. (A) $P_{crit-max}$ estimated for the diverse species in this study (Table S1; $P_{crit-max}$=MMR×P_{crit}/BMR). The mean (±s.d.) for all species combined is 18.06±4.95 kPa (n=52). $P_{crit-max}$ is lower for species specifically known to inhabit environments with persistent (longer than diel or tidal cycles) hypoxia, such as estuaries, high altitude, subterranean burrows and oxygen minimum zones (OMZ). Normoxic species ('air breathers' and shallow marine, including intertidal, species) have a mean of 21.12±2.05 kPa (n=38) and are not known to experience persistent hypoxia. $P_{crit-max}$ for estuarine and freshwater species is significantly lower than that of normoxic species (*P>0.05). (B) MMR, shown as a percentage of the MMR at predicted $P_{crit-max}$, plateaus at P_{O_2} levels above $P_{crit-max}$. The European perch, *Perca fluviatilis* (dashed blue line), achieved higher MMR under hyperoxia. (C) Oxygen supply capacity (α) estimated as MMR/$P_{crit-max}$ as a function of that predicted as BMR/P_{crit} (y=−0.01+1x; R^2=1.0; Tables S1 and S2). (D) MMR, predicted as (BMR×$P_{crit-max}$)/P_{crit}, as a function of the measured MMR (MMR_{pred}=MMR_{meas}; R^2=0.99; P<0.0001) across diverse species of mollusks, arthropods and chordates. The $P_{crit-max}$ used to estimate α and MMR in C and D was 21 kPa for normoxic species (blue circles). For the other species, $P_{crit-max}$ was either directly measured (open circles) or estimated as the highest environmental P_{O_2} to which a species is exposed (red circles). $P_{crit-max}$ values used in C and D are reported in Tables S1 and S2.

between), allowing us to derive a simple relationship between MMR and BMR and their respective critical oxygen pressures:

$$\frac{\text{MMR}}{\text{BMR}} = \frac{P_{\text{crit}-\text{max}}}{P_{\text{crit}}}. \quad (1)$$

The MMR can be estimated as BMR×$P_{crit-max}$/P_{crit}. Assuming that $P_{crit-max}$ is 21 kPa (air saturation) for normoxic species, and for hypoxic species, using the measured $P_{crit-max}$ values or highest estimated environmental P_{O_2} (Table S2), the predicted MMR is precisely correlated with the measured MMR (Fig. 2D; y=0.26+1x; R^2=0.998; P<0.0001; n=52). The decrement in MMR due to environmental hypoxia can be directly calculated and is proportional to 1/$P_{crit-max}$, or 4.7% kPa^{-1} for normoxic species (for accurate measurement of MMR, it is therefore very important to maintain experimental P_{O_2} at or above $P_{crit-max}$). For hypoxic species, $P_{crit-max}$ is lower, which drives a larger relative change in MMR for an equivalent absolute change in P_{O_2} (up to 36% kPa^{-1} in

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Gnathophausia ingens living in the oxygen minimum zone in the California Current).

Because of the typical sigmoidal shape of oxygen dissociation curves, the linear relationship between MR and P_{O_2} revealed by our analysis was unexpected. However, we hypothesize that the combined effect of reduced arterial P_{O_2} and a right-shift of the oxyhemoglobin curve due to reduced arterial pH during exercise results in a linear decline in MMR with decreasing P_{O_2} (Fig. 2C). This is supported by experimental work in humans (Wehrlin and Hallén, 2006) and fish (Speers-Roesch et al., 2012), which demonstrates that blood-oxygen saturation decreases linearly with inspired P_{O_2}. Similarly, the MMR for several fish species (Crans et al., 2015; Ern et al., 2017, 2016; Lefrançois and Claireaux, 2003; Oldham et al., 2019) and for the subterranean mole rat (Arieli et al., 1977) is directly proportional to P_{O_2} when measured below $P_{crit\text{-}max}$ (Fig. 2B). In humans, the measured decrement in MMR with increasing altitude (decreasing P_{O_2}; Fulco et al., 1998; Hochachka, 1985) also closely matches the predicted reduction (Fig. 2B; Wehrlin and Hallén, 2006). Human populations adapted to high altitude have a similar MMR but lower $P_{crit\text{-}max}$ and, thus, higher α compared with those at sea level (Ferretti et al., 1997). Across both normoxic and hypoxic species, α is correlated with both basal (Deutsch et al., 2020) and maximum oxygen demand (Fig. 3A). Furthermore, hypoxic species have a higher α than normoxic species despite similar metabolic rates (ANCOVA; $P<0.001$; Fig. 3A).

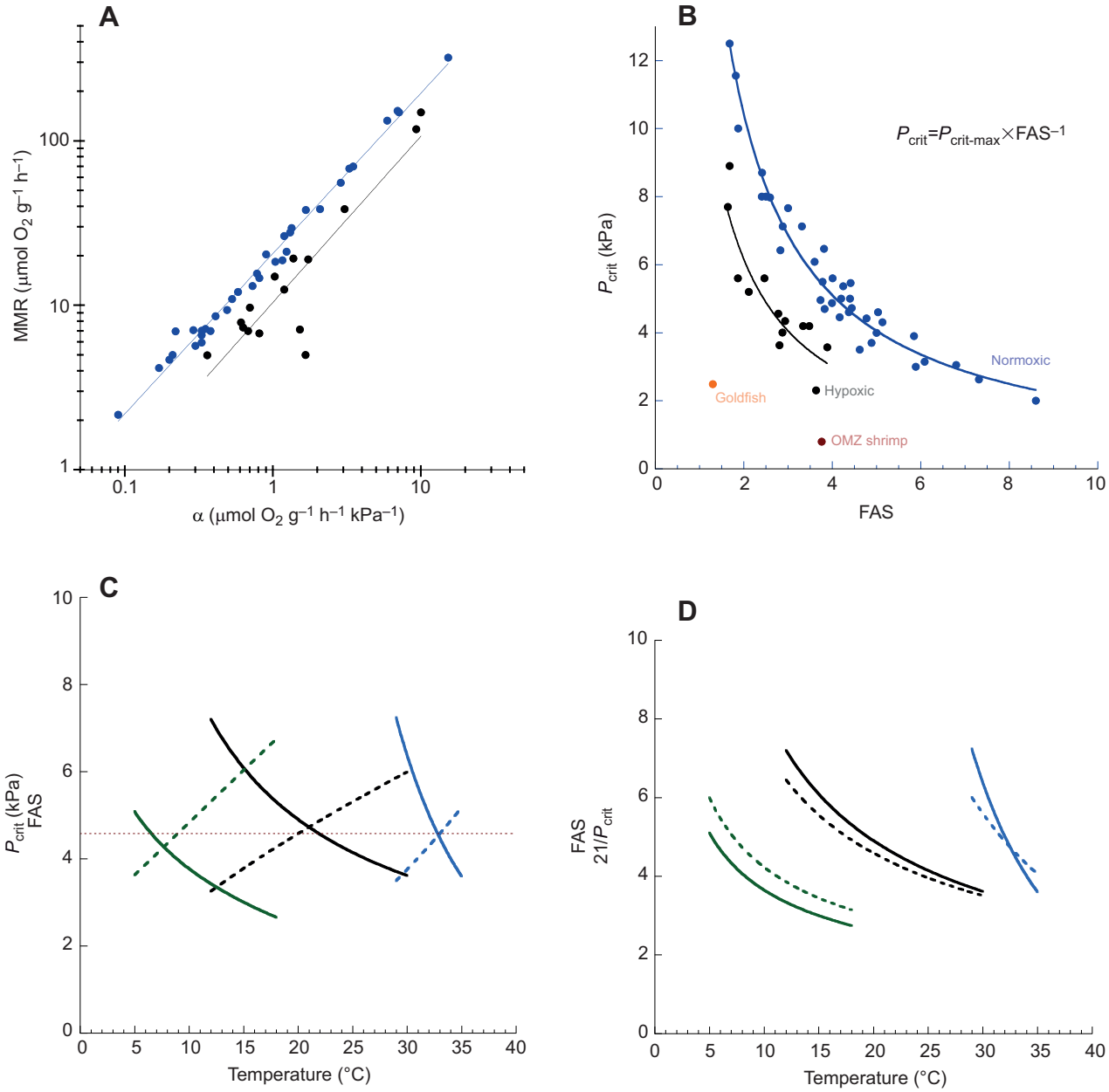

Fig. 3. MMR and P_{crit} are related to α in normoxic (n=38) and hypoxic (n=14) species. (A) MMR as a function of α (defined as BMR/P_{crit}). For normoxic species (blue): $MMR=21\alpha^{0.98}$; $R^2=0.998$. For hypoxic species (black): $MMR=13\alpha^{1.0}$; $R^2=0.99$. (B) P_{crit} as a function of FAS (MMR/BMR). For normoxic species, $P_{crit}=21.4FAS^{-1.03}$; $R^2=0.95$. For hypoxic species, $P_{crit}=12.1FAS^{-1.17}$; $R^2=0.53$. Goldfish and the OMZ shrimp *Gnathophausia ingens* have among the lowest $P_{crit\text{-}max}$ values reported here. (C) FAS (solid lines) and P_{crit} (dashed lines) have equal temperature sensitivity but opposite in sign. Three example normoxic species are shown; Atlantic cod (green), black sea bass (black) and black-axil damselfish (blue). The red dashed line is placed at 4.58, which is the square root of 21 (P_{O_2} at air saturation in kPa) and is the value at which FAS should equal P_{crit} according to Eqn 1 ($FAS\times P_{crit}=21$). (D) FAS (solid lines) and metabolic index ($21/P_{crit}$; Deutsch et al., 2015; dashed lines) shown across a temperature range for those same three species.

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DISCUSSION

Natural selection acts on the oxygen supply pathways primarily in support of maximum metabolic demand at the prevailing environmental P_{O_2}, which is $P_{crit\text{-}max}$. Athletic performance is achieved by concomitant adjustments in both oxygen demand (i.e. muscle oxidative capacity) and oxygen supply at a given environmental P_{O_2}. While limitation of maximum activity is typically attributed to cardiac output, aerobic performance in hypoxia is thought to be limited by gas exchange surface area (lungs or gills) or the oxygen affinity of respiratory proteins (Calbet and Lundby, 2009; Mandic et al., 2009; Seibel, 2011). Despite the many differences between the physiological responses to changing P_{O_2} and workload (Farrell et al., 2009), the present findings argue strongly that environmental and physiological oxygen supply challenges are equivalent and met by a single oxygen supply capacity. We suggest that the $P_{crit\text{-}max}$ is set by the physiological ability to saturate the respiratory protein at maximum exercise under the prevailing P_{O_2}, while the MMR is primarily supported by the rate of blood oxygen loading and unloading, the total oxygen carrying capacity of the blood (respiratory protein concentration, hematocrit) and cardiac output. Thus, hypoxia-tolerant species typically have a respiratory protein with a high affinity for oxygen (Childress and Seibel, 1998; Seibel, 2011) that facilitates oxygen binding in the gills under maximum activity despite a reduced oxygen gradient. While $P_{crit\text{-}max}$ and P_{crit} are linked, we argue that selection is acting to supply oxygen in support of MMR in hypoxic conditions. The result is a low $P_{crit\text{-}max}$.

The fact that the $P_{crit\text{-}max}$ is the atmospheric P_{O_2} for diverse normoxic species across a range of body sizes and temperatures argues that oxygen supply capacity evolves and acclimates to meet changing demands with body size and temperature at a stable prevailing P_{O_2}. As a result, not only are MMR, BMR (Fig. 4) and their respective critical oxygen partial pressures linked (Eqn 1) but also their temperature and body mass scaling coefficients are mechanistically and quantifiably linked. Inserting the temperature (E) and body mass (b) coefficients for species-specific physiological processes or rates into Eqn 1, we determined the relationships between the mass and temperature sensitivity of MMR, BMR, P_{crit} and $P_{crit\text{-}max}$ ($E_{BMR}=E_{MMR}+E_{P_{crit}}-E_{P_{crit\text{-}max}}$ and $b_{BMR}=b_{MMR}+b_{P_{crit}}-b_{P_{crit\text{-}max}}$).

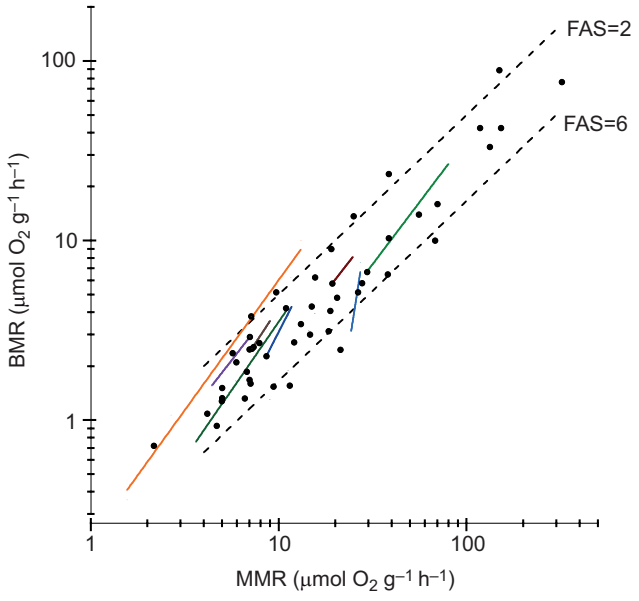

Fig. 4. Relationship between MMR and BMR for the diverse species from Table S1 (*n*=52). Species measured at more than one temperature are indicated by colored lines (Table S3). Note that, intraspecifically, BMR generally increases faster than MMR, while across species, BMR and MMR increase proportionally. Across each species' temperature range, the FAS ranges between ~2 and 6 (dashed lines).

The P_{O_2} available in air or in air-saturated water is consistently near 21 kPa regardless of temperature. Thus, $P_{crit\text{-}max}$ within a species must be constant across a body size and temperature range (i.e. the temperature and scaling coefficients, E and b, for $P_{crit\text{-}max}$ are zero). Some species may migrate, ontogenetically or on a diel or seasonal basis, across an oxygen and/or temperature gradient. In that case, the evolved $P_{crit\text{-}max}$ may change with size or temperature (see below). However, if $P_{crit\text{-}max}$ is constant, MMR and α have equivalent temperature and mass coefficients across the natural temperature range. Furthermore, FAS (MMR/BMR) and P_{crit} must scale with opposite slopes (but equal in magnitude) in response to both body mass and temperature (e.g. $b_{MMR}-b_{BMR}=-b_{P_{crit}}$). This suggests that the physiological oxygen supply capacity matches maximum demand at the prevailing P_{O_2} regardless of temperature (within the natural range) or body size. Despite nearly a century of research into the effects of both body mass and temperature on metabolism, the data required to test these relationships are sparse.

Temperature effects

Only for a handful of species have MMR, BMR and P_{crit} all been measured at more than one temperature (Table S3). For those species, the measured temperature coefficients for BMR (E_{BMR}) are strongly correlated with those predicted as $E_{MMR}+E_{P_{crit}}-E_{P_{crit\text{-}max}}$ (Fig. 5A) and the measured MMR correlates with the predicted MMR across the temperature range for each species (Fig. 5B). This suggests that oxygen supply capacity changes with temperature to match maximum metabolic oxygen demand across a temperature range.

A minimum aerobic scope, defined as the absolute (AAS=MMR−BMR) or factorial (FAS=MMR/BMR) difference between maximum and resting metabolic rates, must be maintained to perform all aerobic activities beyond maintenance metabolism (Deutsch et al., 2015, 2020). The pioneering studies of Fry and Hart (1948), as well as many studies since, show that MMR and AAS increase with temperature to a peak and then plateau or decline in many species. The temperature at which AAS peaks is widely held as an optimum temperature (T_{opt}), while loss of aerobic scope at higher temperatures is often interpreted as an oxygen supply limitation (Pörtner et al., 2017). Oxygen supply limitation is believed by many (though not all; Clark et al., 2013; Jutfelt et al., 2018) to be an important determinant of existing temperature and biogeographic range limits for ectothermic animals. As such, it has played a prominent role in efforts to predict species' responses to climate change (Farrell, 2016; Deutsch et al., 2020).

This 'oxygen- and capacity-limited thermal tolerance hypothesis' (Pörtner et al., 2017) is testable using the new relationship (Eqn 1) revealed here. We found that, at temperatures that cause a decrement in the measured MMR, the predicted MMR (and AAS) continues to increase in the few species for which data are available (Fig. 5C). In other words, there is no decrement in the oxygen supply capacity, α, within the measured temperature range. This suggests that the limitation on MMR at high temperature results from something other than oxygen supply, at least for these species. A failure of oxygen supply capacity would affect both P_{crit} and MMR, resulting in a decline in both measured and predicted MMR and AAS (Fig. 5C,D).

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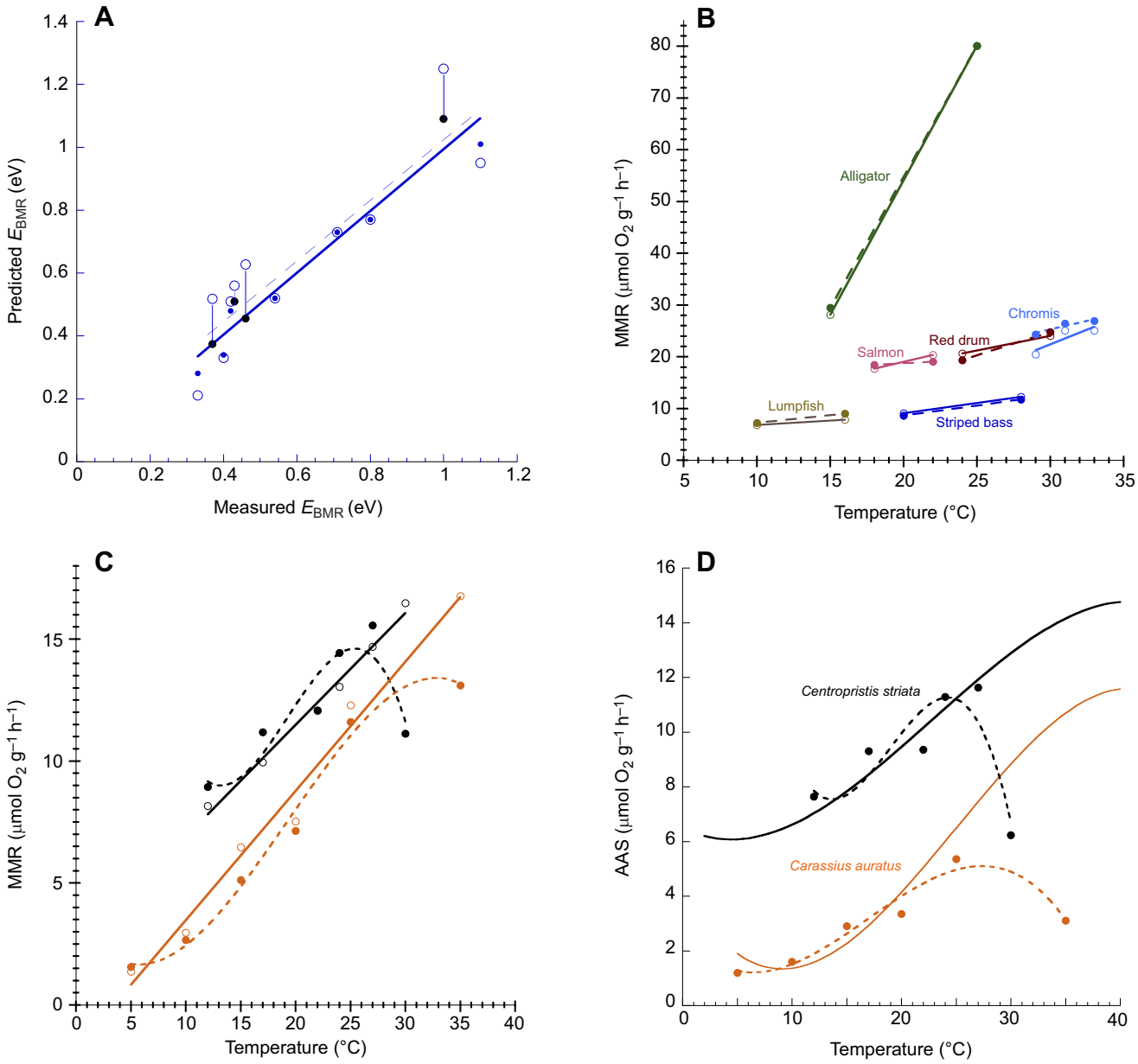

Fig. 5. Temperature dependence of measured and predicted MMR and absolute aerobic scope (MMR−BMR) in species for which MMR, BMR and P_{crit} have all been measured at more than one temperature. (A) The temperature coefficient for BMR, E_{BMR}, estimated as E_{MMR}+$E_{P_{crit}}$ (open symbols; y=0.96x; R^2=0.81) and, for those same species, as E_{MMR}+$E_{P_{crit}}$−$E_{P_{crit\text{-}max}}$ (blue, normoxic species; black, hypoxic species; y=0.98x; R^2=0.96) as a function of measured E_{BMR} (n=11). For all normoxic species, calculated $E_{P_{crit\text{-}max}}$ is near zero and does not significantly influence the correlation. (B–D) Measured MMR values (solid symbols, dashed lines) and values estimated as BMR×21/P_{crit} (open symbols, solid lines; data in Table S3). For red drum, a $P_{crit\text{-}max}$ value of 15 kPa was used (Table S2). (C,D) Measured MMR (C) and AAS (D) for *Centropristis striata* (black sea bass; black) and *Carassius auratus* (goldfish; orange) closely match predicted values up to a critical temperature. The decline in MMR beyond that temperature is not due to a failure of oxygen supply, which would similarly impact P_{crit} and the predicted values. See Tables S1–S3 for data and references.

AAS is driven largely by changes in MMR (Killen et al., 2016; Fig. 6). As such, the increase in AAS with temperature represents increasing costs associated primarily with increasing demands for aerobic activity. The critical thermal maximum (CT_{max}) is operationally defined by a failure in some performance metric, often AAS. However, the aerobic thermal limit (AT_{max}), the temperature at which aerobic scope is projected to reach zero, is, for many species, beyond those experienced in their natural range (Deutsch et al., 2020; Ern et al., 2016). For example, in black sea bass, extrapolation of the temperature curves for BMR, MMR and P_{crit} suggests a peak in AAS above 45°C and AAS declines to zero with a projected AT_{max} near 65°C (Fig. 6B). Thus, the measured peak temperature for AAS (near 24–27°C for black sea bass) is not an optimum temperature, but rather is a peak in the metabolic cost to the organism within their evolved temperature range, usually at or just below the measured (operational) CT_{max}, which elicits some physiological failure (27–30°C for black sea bass).

FAS, in contrast to AAS, typically declines with increasing temperature toward a minimal value that is required to support a population (Deutsch et al., 2015, 2020). FAS usually decreases from ∼6 to 2 across each species' natural temperature range as a result of evolved adjustments in maintenance costs and in the oxygen supply

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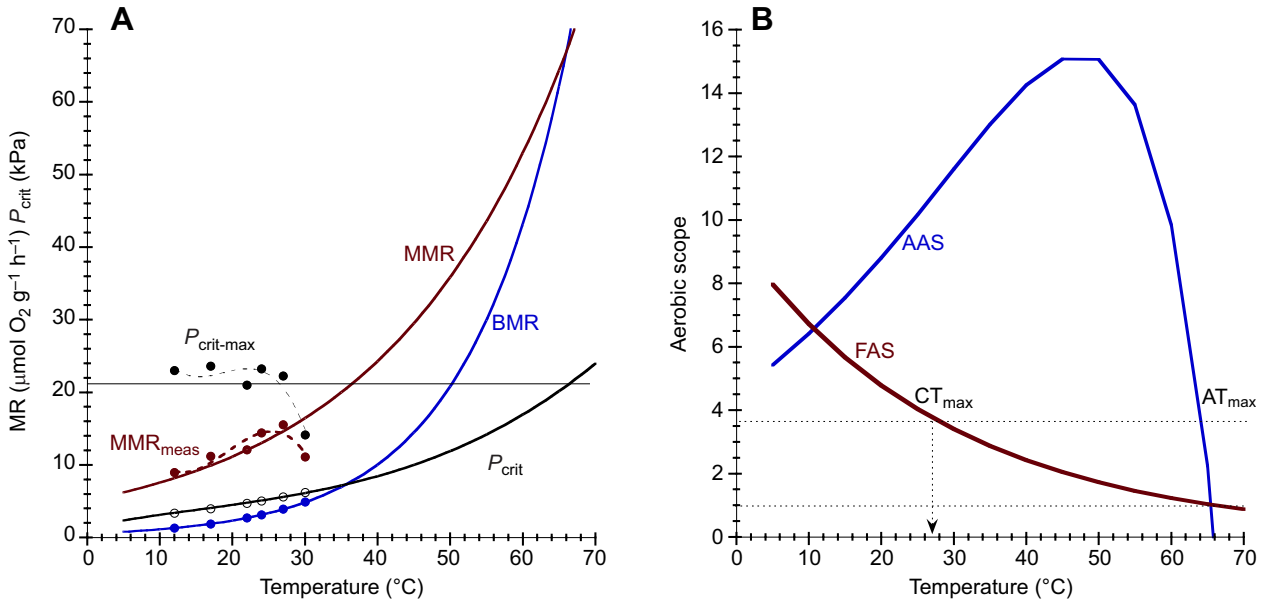

Fig. 6. MMR, BMR and the corresponding P_{crit} for black sea bass across a temperature range from 12 to 30°C. (A) The measured temperature coefficients were used to extrapolate to higher temperatures, showing that MMR meets BMR, and P_{crit} meets $P_{crit\text{-}max}$ (air saturation) at the projected critical aerobic thermal maximum (AT_{max}, ~65°C), which is well beyond the tolerated temperature range of the species (Slesinger et al., 2019). Note that the measured MMR declines beyond 27°C (the operationally defined critical thermal maximum, CT_{max}) despite a functional oxygen supply capacity as calculated from BMR and P_{crit}. (B) FAS (MMR/BMR) and AAS (MMR−BMR) depicted across a temperature range using the temperature coefficients from Fig. 5A. The AT_{max} is reached where AAS declines to zero, FAS declines to 1, and P_{crit} equals $P_{crit\text{-}max}$.

capacity required to support them. These adjustments effectively reduce the temperature sensitivity of BMR across species (0.3 eV) relative to that within species (0.69 eV; Deutsch et al., 2020) and result in a similar P_{crit} range in all normoxic species. Thus, E_{BMR} across species is equivalent to E_{α} and E_{MMR} while P_{crit} and $P_{crit\text{-}max}$ are usually constant (E=0) across species regardless of temperature (Figs 3C, 6). Thus 'metabolic cold adaptation', apparent as an elevation of BMR in cold-adapted species above the value predicted using a typical intraspecific temperature coefficient (Clarke, 1991), may instead be thought of as 'metabolic heat adaptation' by which BMR in low-latitude species is reduced to maintain aerobic scope in warm water. Fig. 4 shows clearly that, within a species, BMR increases faster than does MMR with temperature while, interspecifically, they increase with similar coefficients.

Body mass effects

If, as suggested above, $P_{crit\text{-}max}$ is the prevailing P_{O_2} to which species' oxygen supply capacity has evolved, then it should, in the absence of ontogenetic migrations across correlated temperature and oxygen gradients, be constant through ontogeny and invariant with body size ($b_{P_{crit\text{-}max}}$=0). For such species, the oxygen supply capacity, α, and MMR must scale with identical exponents ($b_{\alpha}=b_{MMR}-b_{P_{crit\text{-}max}}$). In other words, the oxygen supply capacity increases to meet increasing demand at larger sizes. If P_{crit} is also size invariant, then BMR must scale with an identical slope to MMR and α.

The limited data available suggest that P_{crit} is largely size invariant in fishes (Nilsson and Östlund-Nilsson, 2008) and insects (Harrison et al., 2014). In tropical damselfish, P_{crit} declines slightly with body mass (b=−0.1) over a size range of several orders of magnitude and Pan et al. (2017) found that P_{crit} increased slightly with body mass in the red drum, *Sciaenops ocellatus*. Interestingly, red drum spend their early life in hypoxic estuaries and migrate out to air-saturated coastal waters at larger sizes. Thus, $P_{crit\text{-}max}$ likely increases with size for this species. If both P_{crit} and $P_{crit\text{-}max}$ are constant, or scale with similar exponents, then α, MMR and BMR must scale with similar exponents.

This undermines a central assumption of the gill-oxygen limitation hypothesis (Cheung et al., 2013), which suggests that as ocean warming increases metabolic demand, fish size will become limited by oxygen supply because the two-dimensional surface area of respiratory organs (i.e. gills or lungs) cannot keep pace with the increasing three-dimensional volume of respiring tissues as fish grow. Our analysis thus supports previous criticism of the gill-oxygen limitation hypothesis (Lefevre et al., 2017). Oldham et al. (2019) recently published maximum and basal metabolic rates, as well as maximum rates under reduced oxygen (11.5 kPa), in three different size classes of the Atlantic salmon, *Salmo salar*. Neither P_{crit} nor $P_{crit\text{-}max}$ was measured, but the MMR under low oxygen effectively provides a P_{crit} for that submaximal rate, which can be used to estimate P_{crit} for BMR in each size class. The scaling coefficient for the estimated P_{crit} (b_{Pcrit}=0.15) precisely matches that calculated from b_{MMR} (−0.20) and b_{BMR} (−0.35).

Although several studies have found small but significant differences in the scaling coefficients for MMR and BMR, recent work suggests that they scale with similar slopes in fishes and mammals (Gillooly et al., 2017; Killen et al., 2007). Thus, as whole-animal metabolic rate increases with size, the oxygen supply capacity increases to match it. If that were not true (i.e. if α did not increase with size in proportion to metabolic demand), $P_{crit\text{-}max}$ would increase with size and hyperoxia would be required to exploit the available oxidative and muscle capacity. The present findings refute that possibility (Fig. 2B).

Hypoxia tolerance

A low P_{crit} is usually interpreted as an indication of hypoxia tolerance and, across large P_{O_2} gradients, P_{crit} is correlated with habitat P_{O_2} (Richards, 2011; Childress and Seibel, 1998; Wishner

et al., 2018). However, according to Eqn 1, P_{crit} must also respond to changes in aerobic scope, via increased MMR or reduced BMR. In fact, P_{crit} is strongly, inversely correlated with FAS (Fig. 3), whereas $P_{crit\text{-}max}$ is not. Hypoxic species have a similar range in FAS but a lower P_{crit} at a given FAS (Fig. 3B), suggesting that, rather than hypoxia tolerance per se, P_{crit} is a reflection of the oxygen supply capacity which evolves to meet MMR and the prevailing P_{O_2}. Thus, interspecific variation in $P_{crit\text{-}max}$ reflects specific adaptations in the oxygen supply machinery for hypoxia tolerance while variation in P_{crit} reflects changing FAS with temperature. Thus the 'incipient limiting oxygen level' ($P_{crit\text{-}max}$; Claireaux and Chabot, 2016; Fry and Hart, 1948), which we view as the environmental P_{O_2} under which a species is most active and to which the oxygen transport capacity has evolved, provides direct information regarding the response of species to hypoxia. P_{crit} does not. $P_{crit\text{-}max}$ is the P_{O_2} below which a decrement in MMR is certain. Species are equipped with enhanced capacity for anaerobic metabolism and metabolic suppression to survive short-term exposure to sub-critical oxygen pressure (Seibel, 2011).

Implications

MMR and aerobic scope are quantifiably linked to resting metabolic rate and critical oxygen pressures. Several recent studies have noted a relationship between metabolic performance and hypoxia tolerance (Anttila et al., 2013; Claireaux and Chabot, 2016; Crans et al., 2015; Deutsch et al., 2015, 2020; Ern et al., 2016; Gangloff and Telemeco, 2018; Zhang et al., 2018), but the precise equivalency of the oxygen supply challenges during both physical exertion and environmental hypoxia has gone unrecognized. The equivalency revealed here provides the ability to precisely predict the changes in species' aerobic metabolism with changing P_{O_2} and temperature. This simple relationship demands a conceptual reassessment of aerobic scope, hypoxia tolerance, metabolic scaling and their ecological implications.

For example, in contrast to the oxygen- and capacity-limited thermal tolerance hypothesis (Pörtner et al., 2017), we suggest that, in many species, the measured decrement in performance at high temperatures results from a failure of the metabolic machinery to use oxygen (e.g. muscle oxidative performance) or an inability of the muscles to produce equivalent work rather than an inability to provide sufficient oxygen or a reduction in aerobic scope. This conclusion stems from three lines of evidence. (1) The oxygen supply capacity, determined from BMR and P_{crit}, is unaffected at temperatures that cause a large decrement in MMR in the species analyzed here. If oxygen supply was restricting MMR at those temperatures, P_{crit} would also be affected. (2) Extrapolation of the relationships between MMR, BMR and P_{crit} with temperature shows that AAS would be enhanced at temperatures well beyond the critical temperature for MMR (Figs 5D, 6; Deutsch et al., 2020). (3) FAS decreases continuously to a minimum of ∼2–3 near the upper temperature in each species' range (Fig. 3). In other words, a FAS of ∼2–3 may be required to support a population, and an environmental P_{O_2} ∼2–3 times higher than the P_{crit} is required to support that aerobic scope. Evolutionary adjustments in oxygen supply capacity and in maintenance costs result in similar FAS (∼2–6) in species across the temperature range occupied by animals. Beyond the upper temperature range of each species (beyond the temperature that results in a FAS of ∼2–3), no selective pressure exists to maintain any function, including oxidative capacity or oxygen supply. Thus, FAS and the metabolic index (Deutsch et al., 2015, 2020) are reasonable indicators of future habitat restrictions due to climate-related changes in temperature and oxygen. However, while this minimum FAS could be construed as a metabolic constraint resulting in current biogeography, we believe that, instead, biogeography has driven the evolution of oxygen supply to meet this minimum FAS requirement. Biogeography determines physiology, not the other way around.

Given the evolved match between supply capacity and maximum demand, many physiological systems likely fail at similar temperatures beyond the evolved tolerance range. The exact physiological limitation cannot be known from aerobic scope alone. What becomes clear from the present analysis is that the temperature peak for AAS does not represent an optimum temperature. Moreover, metabolic scaling and temperature-induced reductions in body size cannot result from a size-related oxygen supply limitation as posited by the metabolic theory of ecology (Brown et al., 2004) and the gill-oxygen limitation theory (Cheung et al., 2013) because the oxygen supply capacity evolves to meet increasing demand at large size (Lefevre et al., 2017).

Also, in contrast to current thinking, the P_{crit} (at BMR) does not reflect hypoxia tolerance. Persistent hypoxia does select for enhanced oxygen supply capacity, which, for any given metabolic rate, results in a relatively low P_{crit}. However, the enhanced supply capacity is selected to meet maximum, not resting, demand. Thus, α matches MMR. The only reason P_{crit} varies between normoxic species is because resting and maximum metabolism have differing temperature sensitivities. The increase in P_{crit} that is typically observed with increasing temperature reflects this higher temperature sensitivity of BMR relative to α and MMR. The P_{crit} among normoxic species ranges from 2 to 12 kPa, yet 95% of that variability is explained by FAS (Fig. 3B) and none can be linked directly to environmental oxygen. Species with very high P_{crit} (>8) and, thus, low FAS include animals whose MMR was measured in an active state, such as the ram-ventilating sandbar shark (Crear et al., 2019), and endotherms that have very high BMR. Lower P_{crit} in hypoxic species follows from selection on MMR in reduced oxygen and, thus, low $P_{crit\text{-}max}$.

Reductions in oceanic P_{O_2} have been observed in many subsurface and coastal regions due to warming-induced ocean deoxygenation, upwelling of low-oxygen waters and eutrophication. This ocean deoxygenation is projected to accelerate into the future (Breitburg et al., 2018; Matear and Hirst, 2003). It is important to note, however, that most shallow marine environments will remain in equilibrium with the atmosphere regardless of temperature. While reduced oxygen concentration at constant P_{O_2} will not result in reduced metabolic rate (e.g. in most shallow and terrestrial habitats), reduced oxygen partial pressure at depth or due to eutrophication will result in a precise decrement in MMR ($1/P_{crit\text{-}max}$=4.7% kPa^{-1} for most shallow-living and terrestrial species), with consequences for vertically mobile and mesopelagic species (Wishner et al., 2018). The ecological and fitness implications of small hypoxia-induced decrements in MMR or aerobic scope are difficult to know, but may impair predator–prey interactions or restrict growth and reproduction (Farrell, 2016).

Some reduction in the capacity for aerobic activity will occur for any species exposed to a P_{O_2} below their $P_{crit\text{-}max}$. Increasing temperature within a species' natural range also elevates metabolic demand, with BMR increasing faster than MMR, leading to reduced FAS. However, as long as the P_{O_2} remains at or above $P_{crit\text{-}max}$, the evolved maximum metabolic capacity at the higher temperature can be fully realized. Providing excess oxygen (beyond $P_{crit\text{-}max}$) will not elevate FAS toward values achieved at lower temperatures. In other words, each species is capable of meeting its evolved MMR at the upper end of their natural temperature range, whereas the same is

not true in hypoxia at an equivalent FAS. Temperature and hypoxia stress are linked, but their consequences for metabolism and aerobic scope are not equivalent. Acute changes in temperature usually result in a change to the oxygen supply capacity whereas changes in P_{O_2} do not.

In summary, we found that strong selective pressure acts on the oxygen supply system to meet the maximum oxygen demand, despite wide interspecific, temperature- and size-related variation. This finding is consistent with 'symmorphosis', a concept in which each step in the oxygen supply cascade has evolved in concert, without a single rate-limiting step (Lindstedt and Conley, 2001; Suarez, 1998; Weibel et al., 1991). Proponents of this view suggest that organisms possess little or no excess capacity for oxygen supply nor for its use. While our analysis does not preclude the possibility that organisms possess excess oxidative and muscle capacity, it strongly suggests that organisms have a particular ecological need for energy and that the oxygen supply capacity evolves to meet it.

The oxygen supply capacity matches MMR across a size and temperature range (Fig. 3A). The selective pressure on α is enhanced for species living in persistent hypoxia, allowing them to achieve metabolic rates similar to those of species living at atmospheric P_{O_2}, but with a reduced incipient limiting oxygen level ($P_{crit\text{-}max}$; Fry and Hart, 1948). However, enhanced oxygen supply capacity at a given environmental P_{O_2} evolves in support of an elevated MMR. Because BMR and MMR are linked, enhanced aerobic scope in warm-water species must be achieved via efficiency adaptations to reduce maintenance costs, thereby reducing BMR relative to MMR. Improved efficiency reduces the temperature sensitivity across species relative to that within species, as has been observed in fishes (Clarke and Johnston, 1999). As a result, warm-water species achieve a similar FAS to those living in colder environments despite the intraspecific tendency of FAS to decline with temperature. Our findings thus suggest partial metabolic compensation for warm, rather than cold, temperature. Selection acts on warm-water species to increase FAS, rather than in cold-water species to enhance metabolic rate as has long been postulated (see Clarke, 1991, for review).

The P_{crit} for BMR is a simple consequence of the balancing selective pressures described above and is not under direct selection for hypoxia tolerance. AAS primarily mirrors MMR and does not provide an obvious additional fitness benefit. Species do not evolve excess capacity to supply oxygen nor excess capacity for its use. The traditional views of aerobic scope and hypoxia tolerance are refined in this light. The clear and quantifiable connection between MMR, BMR and P_{crit} provides new power to test and predict the response of animals to changing environmental conditions.

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The authors declare no competing or financial interests.

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