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

One sentence summary: Metabolism is not oxygen limited

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Abstract: Physiological oxygen supply capacity is associated with athletic performance and cardiovascular health and is thought to cause hypometabolic scaling in diverse species. Environmental oxygen is widely believed to be limiting of metabolic rate and aerobic scope, setting thermal tolerance and body size limits with implications for species diversity and biogeography. Here we derive a quantifiable linkage between maximum and basal metabolic rate and their temperature, size and oxygen dependencies. We show that, regardless of size or temperature, the capacity for oxygen supply precisely matches the maximum evolved demand at the highest persistently available oxygen pressure which, for most species assessed, is the current atmospheric pressure. Any reduction in oxygen partial pressure from current values will result in a decrement in maximum metabolic performance. However, oxygen supply capacity does not constrain thermal tolerance and does not cause hypometabolic scaling. The critical oxygen pressure, typically viewed as an indicator of hypoxia tolerance, instead reflects adaptations for aerobic scope. This simple new relationship redefines many important physiological concepts and alters their ecological interpretation.

Main: The maximum rate of aerobic metabolism (MMR) is an important measure of physiological performance and fitness that integrates neural, cardiovascular, and metabolic systems. Hill and Lupton(1) believed that MMR is limited by oxygen supply, a view still widely accepted today (2, 3) (4) (5). The difference between MMR and the standard or basal metabolic rate (BMR), measured under resting, fasted condition, is thought to represent the absolute aerobic scope (AAS) available for organisms to supply oxygen in support of all aerobic function (6, 7). The AAS typically increases with temperature to a peak and then declines at a higher “pejus” temperature. The peak in aerobic scope is believed to represent an optimal temperature while its decline at higher temperatures is thought to result from a failure of oxygen supply.

Similarly, geometric constraints on oxygen uptake and delivery are widely believed to cause hypometric scaling, in which the metabolic rate increases at less than direct proportionality to body mass, and may restrict body size at high temperatures (8, 9). Selection for maximum aerobic activity is further believed to be an important driver of the evolution of endothermy in vertebrates(10). Oxygen supply capacity is strongly linked to athletic performance, cardiovascular health, temperature and hypoxia tolerance in diverse species including humans with implications for species diversity, abundance, distribution, life history and response to climate change.(8, 11, 12), (13), (7, 14), (15, 16)

Despite its ecological and medical importance, oxygen supply capacity is rarely directly measured and the selective pressures acting on it are poorly understood. Here we estimate the

physiological oxygen supply capacity (α , $\mu\text{mol O}_2/\text{hr}/\text{gram}/\text{kPa}$) for 47 species from across the tree of life, including mollusks, arthropods and vertebrates, from marine, freshwater and terrestrial environments (Table S1). We compare α derived under two commonly measured, yet distinct, oxygen supply challenges; environmental hypoxia at rest and maximum aerobic exercise. We hypothesize that the physiological oxygen transport system has evolved to supply sufficient oxygen to meet maximum demand at the prevailing environmental oxygen partial pressure (PO_2). The prevailing PO_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 (S) must meet or exceed its O_2 demand as described by the *Metabolic Index*(15). At any particular temperature and workload, $S = \text{PO}_2 * \alpha$. Oxygen demand is simply the metabolic rate (MR), here estimated by the rate of oxygen consumption at the same workload and temperature. As ambient oxygen declines, a critical PO_2 (P_{crit}) is reached at which a species' oxygen supply capacity is fully exploited and below which the basal (resting and fasted) metabolic rate (BMR) can no longer be maintained (Fig. 1). When the environmental PO_2 reaches P_{crit} , α can be estimated ($\alpha = \text{BMR}/P_{\text{crit}}$). In the present dataset, α ranges over 2 orders of magnitude, from 0.17 in the cephalopod, *Nautilus pompilius*, to 15.28 in the grasshopper, *Schistocerca americana* (Table S1).

At MMR, which is typically elicited during intense exercise, α is similarly fully utilized and can be independently estimated as $\alpha = \text{MMR}/P_{\text{crit-max}}$ (the critical oxygen pressure at MMR; Fig. 1). However, $P_{\text{crit-max}}$ is rarely measured. We hypothesize that $P_{\text{crit-max}}$ is the prevailing PO_2 in a species' environment under which their capacity for activity has evolved. To assess the equivalence of α under both environmental and physiological oxygen supply challenge, we first

estimated $P_{crit-max} = MMR/\alpha$ using the α derived from BMR and P_{crit} .

Despite more than two orders of magnitude variation in MMR and in α , the $P_{crit-max}$ for diverse species is tightly constrained near atmospheric PO_2 . The median value is 19.45 kPa, while the mean is 17.98 ± 5.04 kPa. However, of the 47 species examined, 13 fell below 21 kPa by more than one standard deviation (Fig. 2A). All of those 13 species 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. While many marine species experience diel or tidal variability in PO_2 (e.g. intertidal and diel migrating animals), their estimated $P_{crit-max}$ values are within one standard deviation of atmospheric PO_2 and they are known to employ anaerobic metabolic pathways and metabolic suppression to survive short-term oxygen limitation(17, 18). For 9 of the 13 “hypoxic” species, $P_{crit-max}$ has been experimentally determined and it closely matches the predicted values (Table S2). For the additional 4 hypoxic species, the estimated $P_{crit-max}$ values are similar to published environmental PO_2 values from each species habitat (Table S2). The remaining 34 species (hereafter referred to as “normoxic”) have a mean estimated $P_{crit-max}$ value of 20.62 ± 2.09 and are, thus, expected to achieve maximum activity only at or near atmospheric PO_2 . With few exceptions(19), hyperoxia ($PO_2 > 21$ kPa) does not result in increases in MMR suggesting that species do not typically evolve the excess supply capacity required to transport the additional oxygen, nor the oxidative capacity to consume any excess oxygen that is delivered to the tissues (Fig. 2B; (20)).

For the 34 normoxic species, the oxygen supply capacity, estimated as $\alpha = BMR/P_{crit}$, is strongly correlated with that derived as $\alpha = MMR/21$, while for hypoxic species, it is correlated with the measured $P_{crit-max}$ or the prevailing environmental PO_2 (Table S1; Fig. 2C; $y = -0.03 + 1.01x$; R^2

= 0.998; $p < 0.0001$). Thus α is equivalent at the limiting oxygen level for any metabolic level (e.g. MMR, BMR or any routine MR between), allowing us to derive a simple relationship between MMR and BMR and their respective critical oxygen pressures (equation 1).

Eq. 1

$$\frac{MMR}{BMR} = \frac{P_{crit-max}}{P_{crit}}$$

The maximum metabolic rate can be estimated as $MMR = BMR * P_{crit-max} / P_{crit}$. Assuming that $P_{crit-max}$ is air-saturation for normoxic species, and using the estimated environmental PO_2 and measured $P_{crit-max}$ values for hypoxic species (Table S2), the predicted MMR is precisely correlated with the measured MMR (Fig. 2D; $y = 0.26 + 1x$; $R^2 = 0.998$; $p < 0.0001$).

The decrement in MMR due to environmental hypoxia can be directly calculated and is proportional to $1/P_{crit-max}$, or $4.7\% \text{ kPa}^{-1}$ for normoxic species. For hypoxic species, $P_{crit-max}$ is lower, which drives a larger relative change in MMR for an equivalent absolute change in PO_2 (up to $36\% \text{ kPa}^{-1}$ in *Gnathophausia ingens* living in the oxygen minimum zone in the California Current). Because of the sigmoidal shape of the oxyhemoglobin dissociation curve, the linear relationship between MO_2 and PO_2 revealed by our analysis was unexpected. However, we hypothesize that the combined effect of reduced arterial PO_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 PO_2 (Fig. 2C). This is supported experimental work in humans, which demonstrates that arterial PO_2 decreases linearly with inspired PO_2 under maximum exercise (21). The maximum metabolic rates measured under hypoxia for several fish species(22-25) and for the subterranean mole rat(26) are all much lower than measurements made at or above P_{crit-}

$\alpha_{\max}$ and are within 10-20% of the values predicted as α^*PO_2 (Fig. 2B; Table S1). In humans, the measured decrement in MMR with increasing altitude (decreasing PO_2 ; (11, 27)) also closely matches the predicted reduction (Fig. 2B(21)). Human populations adapted to high altitude have a similar MMR but lower $P_{\text{crit-max}}$ and higher α compared to those at sea level(28).

Across both normoxic and hypoxic species, α is correlated with both basal and maximum oxygen demand, but the correlation is much stronger with MMR (Fig. 3A). Furthermore, hypoxic species have a higher α than normoxic species at a given metabolic rate (ANCOVA; $p < 0.001$; Fig. 3A). Thus, natural selection acts on the oxygen supply pathways primarily in support of maximum metabolic demand at the prevailing environmental PO_2 , which is $P_{\text{crit-max}}$. Athletic performance is achieved by concomitant adjustments in both oxygen demand (i.e. muscle oxidative capacity) and oxygen supply at a given environmental PO_2 .

While cardiac output is thought by many to limit MMR, limitation of aerobic performance in hypoxia is typically attributed to gas exchange surface area (lungs or gills) or the oxygen affinity of respiratory proteins(17, 18, 29). Despite the many differences between the physiological responses to changing PO_2 and workload(30), the present findings argue strongly that environmental and physiological oxygen supply challenges are met by an equivalent oxygen supply capacity. We suggest that the $P_{\text{crit-max}}$ and, thus hypoxia tolerance (see below), is set by the efficacy of oxygen exchange between the environment and the blood (respiratory protein oxygen affinity and gas exchange surface area), while the metabolic rate is primarily determined by the oxygen carrying capacity of the blood (respiratory protein concentration) and cardiac output.

The fact that the oxygen supply capacity is equivalent for species across a range of body sizes and temperatures further argues that α evolves and acclimates to meet changing demands with body size and temperature. As a result, not only are MMR, BMR, and their respective critical oxygen partial pressures linked (Eq. 1), but their temperature and body mass scaling coefficients are also mechanistically and quantifiably linked. Temperature (T) and body mass (M) effects on physiological processes or rates can be estimated as $aM^b \exp(-E/k_B T)$ (9), where a is the species-specific metabolic rate or P_{crit} , b is the mass-dependence, E is the temperature dependence and k_B is the Boltzmann constant. Inserting these coefficients into eq. 1, we see that $E_{BMR} = E_{MMR} + E_{P_{crit}} - E_{P_{crit-max}}$ and, similarly, $b_{BMR} = b_{MMR} + b_{P_{crit}} - b_{P_{crit-max}}$.

The PO_2 available in air or in air-saturated water is consistently near 21 kPa regardless of temperature. Thus, $P_{crit-max}$ for normoxic species must be constant across a body size and temperature range (i.e. the temperature and scaling coefficients, E and b , for $P_{crit-max}$ 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 $P_{crit-max}$ may change with size or temperature (see below). However, if $P_{crit-max}$ is constant, MMR and α have equivalent temperature and mass coefficients at any temperature within the natural range. This suggests that the physiological oxygen supply capacity matches maximum demand at the prevailing PO_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 the current relationships is sparse. Furthermore, factorial aerobic scope (MMR/BMR) and

P_{crit} must scale with opposite slopes in response to both body mass and temperature (e.g. $b_{MMR} - b_{BMR} = -b_{P_{crit}}$).

Temperature Effects

Only for a handful of species (Table S3) have MMR, BMR and P_{crit} all been measured at more than one temperature. For those species, the temperature coefficients for BMR (E_{BMR}), predicted as $E_{MMR} + E_{P_{crit}} + E_{P_{crit-max}}$, are strongly correlated with the measured coefficients (Fig. 4A) and the measured MMR correlates with the predicted MMR across the temperature range for each species (Fig. 4B). This suggests that oxygen supply capacity increases with temperature to match increasing metabolic oxygen demand across a temperature range.

Aerobic scope (AS), defined as the absolute (AAS = MMR-BMR) or factorial (FAS = MMR/BMR) difference between maximum and resting metabolic rates, is believed to represent the capacity to perform all aerobic activities beyond maintenance metabolism. The pioneering studies of Fry and Hart(6), 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 (Fig. 1C; 4C). 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(7). Oxygen supply limitation is believed by many (though not all(31, 32)) to be an important determinant of temperature and biogeographic limits for ectothermic animals. As such, it has played a prominent role in efforts to predict species' responses to climate change(5).

This "oxygen- and capacity-limited thermal tolerance hypothesis"(7) is testable using the new relationship (eq. 1) revealed here. We find that, at temperatures that cause a decrement in the measured MMR, the MMR (and AAS) predicted from BMR and P_{crit} continues to increase in the

species for which data are available (Fig. 4C). 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. 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. 4C, D).

Absolute aerobic scope is driven almost entirely by changes in MMR(33) and, like MMR, it increases with temperature up to a critical temperature and declines at higher temperatures (Figure 1B, C). 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}), defined as the temperature at which BMR equals MMR, P_{crit} equals $P_{crit-max}$, and aerobic scope is nil, is reached only at very high temperatures beyond those experienced by most species. 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 a CT_{max} near 65°C (Fig. 5). Thus, the measured peak temperature for AAS (near 24-27°C for Black Sea Bass) is not optimum, but rather is a peak in the metabolic cost to the organism within their evolved temperature range, usually at or just below a critical temperature that elicits complete physiological failure (27-30°C for Black Sea Bass).

FAS and the Metabolic Index(15) are quantitatively equivalent within each species native habitat and, in contrast to AAS, both decline with increasing temperature toward minimal values that are required to support populations. FAS is similar (~3-6) across species due to evolved adjustments

in the oxygen supply capacity and in maintenance costs. These adjustments effectively reduce the temperature sensitivity of BMR across species relative to within species. The mean intraspecific E_{BMR} is 0.63 eV while that across species follows E_{MMR} (0.3 eV). The temperature coefficient for FAS is opposite in sign but equal to that for P_{crit} (e.g. $E_{\text{MMR}} - E_{\text{BMR}} = -E_{\text{Pcrit}}$).

Body mass effects

For most species, $P_{\text{crit-max}}$ is constant through ontogeny and will be invariant with body size. For such species, the oxygen supply capacity, α , and MMR must scale with identical exponents (eq. 1; $b_{\alpha} = b_{\text{MMR}} + b_{P_{\text{crit-max}}}$). 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 α . This undermines a central assumption of the gill-oxygen limitation hypothesis(8), which suggests that as ocean warming elevates metabolic rate, 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.

The limited data available suggests that P_{crit} is largely size-invariant in fishes(34) and insects(35). In tropical damselfish, P_{crit} declines slightly with body mass ($b = -0.1$) over several orders of magnitude size range and Pan et al.(36) found that P_{crit} *increased* slightly with body mass in the Red Drum, *Sciaenops ocellatus*. Interestingly, Red Drum spend early life in hypoxic estuaries and migrates out to air-saturated coastal waters at larger sizes. Thus, $P_{\text{crit-max}}$ likely increases with size for this species. If both P_{crit} and $P_{\text{crit-max}}$ are constant, or scale with similar exponents, then α , MMR and BMR must scale with similar exponents. FAS scales with a coefficient that is opposite in sign but equal to that for P_{crit} and will thus typically be size invariant ($b_{\text{FAS}} = b_{\text{MMR}} -$

$b_{BMR} = -b_{P_{crit}}$). 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(37, 38). 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), $P_{crit-max}$ would increase with size and hyperoxia would be required to exploit the maximum 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 PO_2 gradients, P_{crit} is roughly correlated with habitat PO_2 (17, 39, 40). However, P_{crit} must also respond to selection for an increase in aerobic scope, via increased MMR or reduced BMR (eq. 1). In fact, P_{crit} is strongly, inversely correlated with FAS (Fig. 3B). 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} reflects selective pressure to enhance aerobic scope at the prevailing PO_2 . Without measuring MMR, it cannot be determined whether a low P_{crit} reflects specific adaptation for hypoxia tolerance or elevated aerobic scope. Thus the “incipient limiting oxygen level” ($P_{crit-max}$; (6, 41)), which we view as the environmental PO_2 under which a species is most active and to which the oxygen transport capacity has evolved, is a more useful hypoxia-tolerance metric than P_{crit} . $P_{crit-max}$ is the PO_2 below which a decrement in MMR is certain.

Discussion

The maximum metabolic rate 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(15, 20, 22, 25, 42-44), but the precise equivalency of the oxygen supply cascade 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 PO₂ and temperature. This simple relationship calls for a conceptual reassessment of aerobic scope, hypoxia tolerance, metabolic scaling and related ecological implications.

For example, in contrast to current thinking(7), 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. Although, given the evolved match between supply capacity and demand, many physiological systems likely fail at similar temperatures beyond the evolved tolerance range. The exact limitation cannot be known from aerobic scope alone. What becomes clear from the present analysis is that the temperature peak for absolute aerobic scope does not represent an optimum temperature. Similarly, 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(12) and the Gill-Oxygen Limitation Theory(8) because the oxygen supply capacity evolves to meet increasing demand at large size.

Also in contrast to current thinking, the critical PO₂ for basal metabolic rate does not specifically reflect hypoxia tolerance. Persistent hypoxia selects for enhanced oxygen supply capacity, which results in a relatively low incipient limiting oxygen level (P_{crit-max}). P_{crit} reflects selection

for enhanced aerobic scope at a particular environmental PO_2 . Reductions in oceanic PO_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(45) (46). It is important to note, however, that most shallow marine environments will remain in equilibrium with the atmosphere. While reduced oxygen concentration at constant PO_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 ($1/P_{crit-max}$; $\sim 5\% \text{ kPa}^{-1}$ for most shallow-living and terrestrial species), with consequences for vertically mobile and mesopelagic species(39).

The ecological and fitness implications of small hypoxia-induced decrements in MMR or aerobic scope ($\sim 5\% \text{ kPa}^{-1}$) are difficult to know, but may impair predator-prey interactions or restrict growth and reproduction(5). A recent analysis for marine animals demonstrates that population limits occur below a mean critical value of the Metabolic Index (equivalent to factorial aerobic scope) of ~ 3 (15). In other words, a factorial aerobic scope of ~ 3 is required to support a population and an environmental PO_2 ~ 3 times higher than the critical PO_2 is required to support that aerobic scope. Evolutionary adjustments in oxygen supply capacity and in maintenance costs result in similar FAS ($\sim 3-6$) in species across the temperature range occupied by animals.

Some reduction in the capacity for aerobic activity will occur for any species exposed to an O_2 partial pressure below their $P_{crit-max}$. 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 PO_2 remains at or above $P_{crit-max}$, the evolved maximum metabolic capacity at the higher temperature can be fully realized. Providing excess oxygen (beyond $P_{crit-max}$) will not elevate aerobic scope toward values achieved at lower temperatures. Temperature and hypoxia stress are linked, but their consequences for metabolism and aerobic scope are not equivalent. Acute changes in temperature result in a change to the oxygen supply capacity whereas changes to oxygen partial pressure do not.

In summary, we find 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(47)(48, 49). Proponents of this view suggest that organisms possess little or no excess capacity for oxygen supply nor for its use.

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 PO_2 , but with a reduced incipient limiting oxygen level(6) ($P_{crit-max}$). However, enhanced oxygen supply capacity at a given environmental PO_2 serves only to elevate MMR. Because BMR and MMR are linked, enhanced aerobic scope must be achieved via efficiency adaptations to reduce maintenance costs thereby reducing BMR relative to MMR. The P_{crit} for BMR is a simple consequence of those balancing selective pressures and is not under direct selection for hypoxia tolerance. Absolute aerobic scope primarily mirrors MMR and does not provide an obvious additional fitness benefit.

328 Species do not evolve excess capacity to supply oxygen nor excess capacity for its use. The
329 traditional views of aerobic scope and hypoxia tolerance are refined in this light. The clear and
330 quantifiable connection between MMR, BMR and P_{crit} provides new power to test and predict
331 the response of animals to changing environmental conditions.
332

Figures

Figure 1. Schematic illustrations of the relationship between metabolic parameters, oxygen and temperature. A) Maximum (MMR) and basal metabolic rates (BMR) and the corresponding critical oxygen partial pressures (P_{crit}) are related via the oxygen supply capacity, α , which is the slope of the MR- PO_2 curve.

Figure 2. A. Dot plot of $P_{crit-max}$ estimated for the 47 species in this study (Table S1; $P_{crit-max} = MMR \cdot P_{crit} / BMR$). The mean for all species combined is 17.98 ± 5.04 kPa ($n = 47$). The black symbols are species for which the estimated $P_{crit-max}$ is below atmospheric PO_2 (21 kPa) by more than one standard deviation. Those species are all known to inhabit environments with sustained (longer than diel or tidal cycles) hypoxia. Normoxic species (blue symbols) have a mean of 20.62 ± 2.09 (blue line, $n = 34$). B. The oxygen-dependence of MMR as a percentage of the MMR at $P_{crit-max}$. C. Oxygen supply capacity (α) estimated as BMR / P_{crit} as a function of that predicted as $MMR / P_{crit-max}$ ($y = -0.01 + 1X$; $R^2 = 1.0$; Table S1; Table S2). D. The MMR, predicted as $(BMR \cdot PO_{2env}) / P_{crit}$, as a function of the measured MMR ($MMR_{pred} = 0.43 + 1MMR_{meas}$; $R^2 = 0.998$; $p < 0.0001$) across diverse species of mollusks, arthropods and chordates.

Figure 3. Maximum metabolic rate and critical oxygen levels are related to the oxygen supply capacity (α) in normoxic (blue symbols) and hypoxic (black symbols) species. A. MMR as a function of α (defined as BMR/P_{crit} ; $MMR = 21 \alpha^{0.98}$; $R^2 = 0.998$). For hypoxic species, $MMR = 13 \alpha^{1.0}$ ($R^2 = 0.99$). B. Factorial Aerobic Scope (FAS = MMR/BMR) as a function of critical PO_2 (P_{crit} kPa). For normoxic species, $FAS = 18 P_{crit}^{-0.89}$ ($R^2 = 0.86$). For hypoxic species, $FAS = 13 P_{crit}^{-1.0}$ ($R^2 = 0.92$).

Figure 4. 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, estimated as $E_{BMR} = E_{MMR} + E_{P_{crit}}$, as a function of that directly measured ($y = 0.01 + 0.98x$; $R^2 = 0.94$). B) Measured MMR values (solid symbols, dashed lines) and values estimated as $BMR * P_{crit-max} / P_{crit}$ (open symbols, solid lines). C, D. *Centropomus striatus* (Black Seabass) and *Carassius auratus* (goldfish). The measured MMR (C) and aerobic scope (D) closely match predicted values until high temperatures. The decline in MMR at high temperature is not due to a failure of oxygen supply, which would similarly impact P_{crit} and the predicted values. See supplementary tables for data and references.

Figure 5. A. Maximum (MMR) and basal metabolic rates (BMR) and the corresponding critical oxygen partial pressures (P_{crit}) for Black Sea Bass are shown across temperature range from 12-30°C. The measured temperature coefficients are used to extrapolate to higher temperatures showing that MMR meets BMR, and P_{crit} meets $P_{crit-max}$ (air-saturation), at the traditionally defined critical thermal maximum ($CT_{max} \sim 65^\circ C$), which is well beyond the tolerated temperature range of the species (Slesinger et al., 2019). Note that the measured MMR declines beyond 27°C despite a functional oxygen supply capacity. B. Factorial ($FAS=MMR/BMR$) and Absolute ($AAS=MMR-BMR$) aerobic scope are depicted across a temperature range using the temperature coefficients from Fig. 5A. The CT_{max} is reached where AAS declines to zero, FAS declines to 1, and the P_{crit} equals $P_{crit-max}$. The temperature resulting in a peak in aerobic scope is often considered optimal (T_{opt}).

Literature Cited

1. A. V. Hill, H. Lupton, Muscular Exercise, Lactic Acid, and the Supply and Utilization of Oxygen. *QJM: An International Journal of Medicine* **16**, 135-171 (1923).
2. P. D. Wagner, CrossTalk proposal: Diffusion limitation of O₂ from microvessels into muscle does contribute to the limitation of $\dot{V}O_{2\max}$. *The Journal of Physiology* **593**, 3757-3758 (2015).
3. N. C. Spurway, B. Ekblom, T. D. Noakes, P. D. Wagner, What limits $\dot{V}O_{2\max}$? *Sports Medicine* **30**, 517-531 (2012).
4. D. R. J. Bassett, E. T. Howley, Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc* **32**, 70-84 (2000).
5. A. P. Farrell, Pragmatic perspective on aerobic scope: peaking, plummeting, pejus and apportioning. *Journal of Fish Biology* **88**, 322-343 (2016).
6. F. E. J. Fry, J. S. Hart, The relation of temperature to oxygen consumption in the goldfish. *Biological Bulletin* **94**, 66-77 (1948).
7. H.-O. Pörtner, C. Bock, F. C. Mark, Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. *Journal of Experimental Biology* **220**, 2685-2696 (2017).
8. W. W. L. Cheung *et al.*, Shrinking of fishes exacerbates impacts of global ocean changes on marine ecosystems. *Nature Climate Change* **3**, 254-258 (2013).
9. J. F. Gillooly, J. H. Brown, G. B. West, V. M. Savage, E. L. Charnov, Effects of size and temperature on metabolic rate. *Science* **293**, 2248-2251 (2001).
10. A. F. Bennett, J. A. Ruben, Endothermy and activity in vertebrates. *Science* **206**, 649-654 (1979).
11. P. W. Hochachka, in *Circulation, Respiration, and Metabolism. Proceedings in Life Sciences.*, R. Gilles, Ed. (Springer, Berlin, Heidelberg, 1985).
12. J. H. Brown, J. F. Gillooly, A. P. Allen, V. M. Savage, G. B. West, Toward a Metabolic Theory of Ecology. *Ecology* **85**, 1771-1789 (2004).
13. P. G. Falkowski *et al.*, The Rise of Oxygen over the Past 205 Million Years and the Evolution of Large Placental Mammals. *Science* **309**, 2202-2204 (2005).
14. S. Lefevre, Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO₂ and their interaction. *Conservation Physiology* **4**, (2016).
15. C. Deutsch, A. Ferrel, B. Seibel, H.-O. Pörtner, R. B. Huey, Climate change tightens a metabolic constraint on marine habitats. *Science* **348**, 1132-1135 (2015).
16. M. R. Roman, S. B. Brandt, E. D. Houde, J. J. Pierson, Interactive Effects of Hypoxia and Temperature on Coastal Pelagic Zooplankton and Fish. *Frontiers in Marine Science* **6**, 139 (2019).
17. B. A. Seibel, Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones. *Journal of Experimental Biology* **214**, 326-336 (2011).
18. M. Mandic, A. E. Todgham, J. G. Richards, Mechanisms and evolution of hypoxia tolerance in fish. *Proceedings of the Royal Society B* **276**, 735-744 (2009).
19. J. Brijs *et al.*, Experimental manipulations of tissue oxygen supply do not affect warming tolerance of European perch. *Journal of Experimental Biology* **218**, 2448-2454 (2015).

20. G. Claireaux, D. Chabot, Responses by fishes to environmental hypoxia: integration through Fry's concept of aerobic metabolic scope. *J Fish Biol* **88**, 232-251 (2016).
21. J. P. Wehrlin, J. Hallen, Linear decrease in VO₂max and performance with increasing altitude in endurance athletes. *Eur. J. Appl. Physiol.* **96**, 404-412 (2006).
22. R. Ern, T. Norin, A. K. Gamperl, A. J. Esbaugh, Oxygen dependence of upper thermal limits in fishes. *J Exp Biol* **219**, 3376-3383 (2016).
23. R. Ern, J. L. Johansen, J. L. Rummer, A. J. Esbaugh, Effects of hypoxia and ocean acidification on the upper thermal niche boundaries of coral reef fishes. *Biol Lett* **13**, (2017).
24. C. Lefrançois, G. Claireaux, Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole *Solea solea*. *Marine Ecology Progress Series* **259**, 273-284 (2003).
25. K. D. Crans, N. A. Prankevicus, G. R. Scott, Physiological tradeoffs may underlie the evolution of hypoxia tolerance and exercise performance in sunfish (Centrarchidae). *Journal of Experimental Biology* **218**, 3264-3275 (2015).
26. R. Arieli, A. Ar, A. Shkolnik, Metabolic Responses of a Fossorial Rodent (*Spalax ehrenbergi*) to Simulated Burrow Conditions. *Physiological Zoology* **50**, 61-75 (1977).
27. C. S. Fulco, P. B. Rock, A. Cymerman, Maximal and submaximal exercise performance at altitude. *Aviat Space Environ Med* **69**, 793-801 (1998).
28. G. Ferretti, C. Moia, J.-M. Thomet, B. Kayser, The decrease of maximal oxygen consumption during hypoxia in man: a mirror image of the oxygen equilibrium curve. *Journal of Physiology* **489**, 231-237 (1997).
29. J. A. L. Calbet, C. Lundby, Air to muscle O₂ delivery during exercise at altitude. *High Altitude Medicine and Biology* **10**, 123-134 (2009).
30. A. P. Farrell, E. J. Eliason, E. Sandblom, T. D. Clark, Fish cardiorespiratory physiology in an era of climate change. *Canadian Journal of Zoology* **87**, 835-851 (2009).
31. T. D. Clark, E. Sandblom, F. Jutfelt, Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *Journal of Experimental Biology* **216**, 2771-2782 (2013).
32. F. Jutfelt *et al.*, Oxygen- and capacity-limited thermal tolerance: blurring ecology and physiology. *Journal of Experimental Biology* **221**, (2018).
33. S. S. Killen *et al.*, Ecological Influences and Morphological Correlates of Resting and Maximal Metabolic Rates across Teleost Fish Species. *The American Naturalist* **187**, 592-606 (2016).
34. G. E. Nilsson, S. Östlund-Nilsson, Does size matter for hypoxia tolerance in fish? *Biological Reviews* **83**, 173-189 (2008).
35. J. F. Harrison, C. J. Klok, J. S. Waters, Critical PO₂ is size-independent in insects: implications for the metabolic theory of ecology. *Current Opinion in Insect Science* **4**, 54-59 (2014).
36. Y. K. Pan, R. Ern, P. R. Morrison, C. J. Brauner, A. J. Esbaugh, Acclimation to prolonged hypoxia alters hemoglobin isoform expression and increases hemoglobin oxygen affinity and aerobic performance in a marine fish. *Sci Rep* **7**, 7834 (2017).

- 479 37. S. S. Killen, I. Costa, J. A. Brown, A. K. Gamperl, Little left in the tank: metabolic scaling in
480 marine teleosts and its implications for aerobic scope. *Proceedings of the Royal Society B*
481 **274**, 431-438 (2007).
- 482 38. J. F. Gillooly, J. P. Gomez, E. V. Mavrodiev, A broad-scale comparison of aerobic activity
483 levels in vertebrates: endotherms versus ectotherms. *Proceedings of the Royal Society B*
484 **284**, (2017).
- 485 39. K. F. Wishner *et al.*, Ocean deoxygenation and zooplankton: Very small oxygen
486 differences matter. *Science Advances* **4**, eea5180 (2018).
- 487 40. J. G. Richards, Physiological, behavioral and biochemical adaptations of intertidal fishes
488 to hypoxia. *Journal of Experimental Biology* **214**, 191-199 (2011).
- 489 41. G. Claireaux, D. Chabot, Responses by fishes to environmental hypoxia: integration
490 through Fry's concept of aerobic metabolic scope. *Journal of Fish Biology* **88**, 232-251
491 (2016).
- 492 42. K. Anttila *et al.*, Variation in temperature tolerance among families of Atlantic salmon
493 (*Salmo salar*) is associated with hypoxia tolerance, ventricle size and myoglobin level.
494 *Journal of Experimental Biology* **216**, 1183-1190 (2013).
- 495 43. E. J. Gangloff, R. S. Telemeco, High Temperature, Oxygen, and Performance: Insights
496 from Reptiles and Amphibians. *Integrative and Comparative Biology* **58**, 9-24 (2018).
- 497 44. Y. Zhang, T. M. Healy, W. Vandersteen, P. M. Schulte, A. P. Farrell, A rainbow trout
498 *Oncorhynchus mykiss* strain with higher aerobic scope in normoxia also has superior
499 tolerance of hypoxia. *Journal of Fish Biology* **92**, 487-503 (2018).
- 500 45. D. Breitburg *et al.*, Declining oxygen in the global ocean and coastal waters. *Science* **359**,
501 (2018).
- 502 46. R. J. Matear, A. C. Hirst, Long-term changes in dissolved oxygen concentrations in the
503 ocean caused by protracted global warming. *Global Biogeochemical Cycles* **17**, 1125
504 (2003).
- 505 47. E. R. Weibel, C. R. Taylor, H. Hoppeler, The concept of symmorphosis: a testable
506 hypothesis of structure-function relationship. *Proceedings of the National Academy of*
507 *Sciences* **88**, 10357-10361.
- 508 48. S. L. Lindstedt, K. E. Conley, Human aerobic performance: too much ado about limits to
509 $\dot{V}O_2$. *Journal of Experimental Biology* **204**, 3195-3199 (2001).
- 510 49. R. K. Suarez, Oxygen and the upper limits to animal design and performance. *Journal of*
511 *Experimental Biology* **201**, 1065-1072 (1998).
- 512 50. M. A. Birk, E. L. McLean, B. A. Seibel, Ocean acidification does not limit squid
513 metabolism via blood oxygen supply. *J Exp Biol* **221**, (2018).
- 514 51. M. E. Demont, R. K. O'Dor, The effects of activity, temperature and mass on the
515 respiratory metabolism of the squid, *Illex illecebrosus*. *J. Mar. Biol. Ass. U. K.* **64**, 535-543
516 (1984).
- 517 52. R. Rosa, B. A. Seibel, Metabolic physiology of the Humboldt Squid, *Dosidicus gigas*:
518 implications for vertical migration in a pronounced oxygen minimum zone. *Prog.*
519 *Oceanography* **86**, 72-80 (2010).
- 520 53. R. K. O'Dor, D. M. Webber, Invertebrate athletes: Trade-off between transport efficiency
521 and power density in cephalopod evolution. *J Exp Biol* **160**, 93-112 (1991).

54. R. K. O'Dor, Respiratory metabolism and swimming performance of the squid, *Loligo opalescens*. *Canadian Journal of Fisheries and Aquatic Science* **39**, 580-587 (1982).
55. M. J. Wells, R. T. Hanlon, P. G. Lee, F. P. DiMarco, Respiratory and cardiac performance in *Lolliguncula brevis* (Cephalopoda, Myopsida): The effects of activity, temperature and hypoxia. *J Exp Biol* **138**, 17-36 (1988).
56. E. Finke, H.-O. Pörtner, P. O. Lee, D. M. Webber, Squid (*Lolliguncula brevis*) life in shallow waters: Oxygen limitation of metabolism and swimming performance. *J Exp Biol* **199**, 911-921 (1996).
57. I. K. Bartol, R. Mann, M. R. Patterson, Aerobic respiratory costs of swimming in the negatively buoyant brief squid, *Lolliguncula brevis*. *J Exp Biol* **204**, 3639-3653 (2001).
58. J. C. Valverde, B. G. Garcia, Suitable dissolved oxygen levels for common octopus (*Octopus vulgaris* cuvier, 1797) at different weights and temperatures: analysis of respiratory behavior. *Aquaculture* **224**, 303-314 (2005).
59. M. J. Wells, R. K. O'Dor, K. Mangold, J. Wells, Feeding and metabolic rate in Octopus. *Marine Behaviour and Physiology* **9**, 305-317 (1983).
60. M. J. Wells, R. K. O'Dor, K. Mangold, J. Wells, Diurnal changes in activity and metabolic rate in *Octopus vulgaris*. *Marine Behaviour and Physiology* **9**, 275-287 (1983).
61. R. K. O'Dor, J. Wells, M. J. Wells, Speed, jet pressure and oxygen consumption relationships in free-swimming *Nautilus*. *J Exp Biol* **154**, 383-396 (1990).
62. J. F. Staples, J. J. Hershkowitz, R. G. Boutilier, Effects of ambient PO₂ and temperature on oxygen uptake in *Nautilus pompilius*. *J. Comp. Physiol. B.* **170**, 231-236 (2000).
63. S. Lefevre, S.-A. Watson, P. L. Munday, G. E. Nilsson, Will jumping snails prevail? Influence of near-future CO₂, temperature and hypoxia on respiratory performance in the tropical conch *Gibberulus gibberulus gibbosus*. *J Exp Biol* **218**, 2991-3001 (2015).
64. D. V. Le, A. C. Alfaro, N. L. C. Ragg, Z. Hilton, N. King, Aerobic scope and oxygen regulation of New Zealand geoduck (*Panopea zelandica*) in response to progressive hypoxia. *Aquaculture* **463**, 28-36 (2016).
65. J. J. Childress, B. A. Seibel, Life at stable low oxygen: Adaptations of animals to oceanic oxygen minimum layers. *J Exp Biol* **201**, 1223-1232 (1998).
66. D. L. Cowles, J. J. Childress, Swimming Speed and Oxygen Consumption in the Bathypelagic Mysid *Gnathopausia ingens*. *Biological Bulletin* **175**, 111-121 (1988).
67. R. W. Brill, P. G. Bushnell, T. A. Elton, H. J. Small, The ability of blue crab (*Callinectes sapidus*, Rathbun 1886) to sustain aerobic metabolism during hypoxia. *Journal of Experimental Marine Biology and Ecology* **471**, 126-136 (2015).
68. C. E. Booth, B. McMahon, Aerobic capacity of the blue crab, *Callinectes sapidus*. *Physiological Zoology* **65**, 1074-1091 (1992).
69. R. Ern *et al.*, Some like it hot: Thermal tolerance and oxygen supply capacity in two eurythermal crustaceans. *Sci Rep* **5:10743**, (2015).
70. B. Salvato, V. Cuomo, P. D. Muro, M. Beltramini, Effects of environmental parameters on the oxygen consumption of four marine invertebrates: a comparative factorial study. *Marine Biology* **138**, 659-668 (2001).
71. J. J. Childress, The respiratory rate of midwater crustaceans as a function of depth of occurrence and relation to the oxygen minimum layer off southern California.

- 565 *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **50**,
566 (1975).
- 567 72. D. L. Cowles, Swimming speed and metabolic rate during routine swimming and
568 simulated diel vertical migration of *Sergestes similis* in the laboratory. *Pac. Sci.* **55**, 215-
569 226 (2001).
- 570 73. G. N. Lyons, L. G. Halsey, E. C. Pope, J. D. Eddington, J. D. R. Houghton, Energy
571 expenditure during activity in the American lobster *Homarus americanus*: Correlations
572 with body acceleration. *Comparative Biochemistry and Physiology Part A: Molecular &*
573 *Integrative Physiology* **166**, 278-284 (2013).
- 574 74. B. McMahon, J. L. Wilkens, Respiratory and circulatory responses to hypoxia in the
575 lobster *Homarus americanus*. *J Exp Biol* **62**, 637-655 (1975).
- 576 75. B. J. Crear, G. N. R. Forteach, The effect of extrinsic and intrinsic factors on the oxygen
577 consumption by the southern rock lobster, *Janus edwardsii*. *J. Exp. Mar. Biol. Ecol.* **252**,
578 129-147 (2000).
- 579 76. S. D. Kirkton, J. A. Niska, J. F. Harrison, Ontogenetic effects on aerobic and anaerobic
580 metabolism during jumping in the American locust, *Schistocerca Americana*. *J Exp Biol*
581 **208**, 3003-3012 (2005).
- 582 77. D. Lapointe, W. K. Vogelbein, M. C. Fabrizio, D. T. Gauthier, R. W. Brill, Temperature,
583 hypoxia, and mycobacteriosis: effects on adult striped bass *Morone saxatilis* metabolic
584 performance. *Dis Aquat Organ* **108**, 113-127 (2014).
- 585 78. E. Slesinger *et al.*, The effect of ocean warming on black sea bass (*Centropristis striata*)
586 aerobic scope and hypoxia tolerance. *PLOS One*, (in press).
- 587 79. D. Chabot, G. Claireaux, Environmental hypoxia as a metabolic constraint on fish: The
588 case of Atlantic cod, *Gadus morhua*. *Marine Pollution Bulletin* **57**, 6-12 (2008).
- 589 80. T. Norin, H. Malte, T. D. Clark, Aerobic scope does not predict the performance of a
590 tropical eurythermal fish at elevated temperatures. *J Exp Biol* **217**, 244-251 (2014).
- 591 81. G. M. Collins, T. D. Clark, J. L. Rummer, A. G. Carton, Hypoxia tolerance is conserved
592 across genetically distinct sub-populations of an iconic, tropical Australian teleost (*Lates*
593 *calcarifer*). *Conserv Physiol* **1**, cot029 (2013).
- 594 82. S. M. Mamun, U. Focken, K. Becker, A respirometer system to measure critical and
595 recovery oxygen tensions of fish under simulated diurnal fluctuations in dissolved
596 oxygen. *Aquacult.Int.* **21**, 31-44 (2013).
- 597 83. D. Lapointe *et al.*, Predicted impacts of climate warming on aerobic performance and
598 upper thermal tolerance of six tropical freshwater fishes spanning three continents.
599 *Conserv Physiol* **6**, (2018).
- 600 84. G. Thillart, J. D. Via, G. Vitali, P. Cortesi, Influence of long-term hypoxia exposure on the
601 energy metabolism of *Solea solea*. I. Critical O₂ levels for aerobic and anaerobic
602 metabolism. *Mar. Ecol. Prog. Ser.* **104**, 109-117 (1994).
- 603 85. V. K. Maxime, K. Pichavant, G. Boeuf, G. Nonnotte, Effects of hypoxia on respiratory
604 physiology of turbot, *Scophthalmus maximus*. *Fish Physiol. Biochem.* **22**, 51-59 (2000).
- 605 86. R. Mallekh, J. P. La-gardere, Effect of temperature and dissolved oxygen concentration
606 on the metabolic rate of the turbot and the relationship between metabolic scope and
607 feeding demand. *J. Fish Biology*, (2002).

87. T. M. Healy, P. M. Schulte, Thermal acclimation is not necessary to maintain a wide thermal breadth o aerobic scope in the common killifish (*Fundulus heteroclitus*). *Physiological Biochemical Zoology* **85**, 107-119 (2012).
88. T. L. McBryan, T. M. Healy, K. L. Haakons, P. M. Schulte, Warm acclimation improves hypoxia tolerance in *Fundulus heteroclitus*. *J Exp Biol* **219**, 474-484 (2016).
89. H. Seth *et al.*, Metabolic scope and interspecific competition in sculpins of Greenland are influenced by increased temperatures due to climate change. *PLOS One* **8**, e62859 (2013).
90. T. J. McArley, A. J. R. Hickey, L. Wallace, A. Kunzmann, N. A. Herbert, Intertidal triplefin fishes have a lower critical oxygen tension (Pcrit), higher maximal aerobic capacity, and higher tissue glycogen stores than their subtidal counterparts. *J. Comp. Physiol. B.*, (2019).
91. G. G. Duthie, The respiratory metabolism of temperature-adapted flatfish at rest and during swimming activity and the use of anaerobic metabolism at moderate swimming speeds. *J Exp Biol* **97**, 359-373 (1982).
92. G. Claireaux, J.-P. Lagardere, Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. *J. Sea Res.* **42**, 157-168 (1999).
93. G. Claireaux, C. Couturier, A. L. Groison, Effect of temperature on maximum swimming speed and cost of transport in juvenile European sea bass (*Dicentrarchus labrax*). *Journal of Experimental Biology* **209**, 3420-2428 (2006).
94. Y. Zhang, T. M. Healy, V. Andersteen, P. M. Schulte, A. P. Farrell, A rainbow trout *Oncorhynchus mykiss* strain with higher aerobic scope in normoxia also has superior tolerance of hypoxia. *J. Fish Biology* **92**, 487-503 (2018).
95. D. D. Tuong *et al.*, Clown knifefish (*Chitala ornata*) oxygen uptake and its partitioning in present and future temperature environments. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **216**, 52-59 (2018).
96. L. Y. Lewis, R. E. J. Gatten, Aerobic metabolism of American alligators, *Alligator mississippiensis*, under standard conditions and during voluntary activity. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **80**, 441-447 (1985).
97. M. M. Berger, M. P. W. Grocott, *Facing acute hypoxia: from the mountains to critical care medicine* **118**, 283-328 (2017).
98. J. R. Day, H. B. Rossiter, E. M. Coats, A. Skasick, B. J. Whipp, The maximally attainable VO₂ during exercise in humans: the peak vs. maximum issue. *J. Appl. Physiol.* **95**, 1901-1907 (2003).
99. J. Lechner, Metabolic performance during hypoxia in native and acclimated pocket gophers. *J. Appl. Physiol.* **43**, 965-970 (1977).
100. I. K. Bartol, R. Mann, M. Vecchione, Distribution of the euryhaline squid, *Lolliguncula brevis*, in Chesapeake Bay: effects of selected abiotic factors. *Mar. Ecol. Prog. Ser.* **226**, 235-247 (2002).
101. G. W. Stunz, T. J. Minello, P. S. Levin, Growth of newly settled red drum *Sciaenops ocellatus* in different estuarine habitat types. *Mar. Ecol. Prog. Ser.* **238**, 227-236 (2002).
102. R. E. Cochran, L. E. Burnett, Respiratory responses of the salt marsh animals, *Fundulus heteroclitus*, *Leiostomus xanthurus*, and *Palaemoetes pugio* to environmental hypoxia

- and hypercapnia and the organophosphate pesticide, azinphosmethyl. *J. Exp. Mar. Biol. Ecol.* **195**, 125-144 (1996).
103. W. Burggren *et al.*, Developmental changes in oxygen consumption and hypoxia tolerance in the heat and hypoxia-adapted tabasco line of the Nile tilapia *Oreochromis niloticus*, with a survey of the metabolic literature for the genus *Oreochromis*. *J. Fish Biology*, 1-13 (2019).
104. H. Schurmann, J. F. Steffenson, Effects of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. *J. Fish Biology* **50**, 1166-1180 (1997).
105. R. Barnes, H. King, C. G. Carter, Hypoxia tolerance and oxygen regulation in Atlantic salmon, *Salmo salar* from a Tasmanian population. *Aquaculture* **318**, 397-401 (2011).
106. M. Hvas, O. Fokedal, A. Imsland, F. Oppedal, The effect of thermal acclimation on aerobic scope and critical swimming speed in Atlantic salmon, *Salmo salar*. *J Exp Biol* **220**, 2757-2764 (2017).

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List of Supplementary Materials:

Methods
Table S1-3
References (50-106)

Supplementary Materials for

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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This PDF file includes:

Materials and Methods
Tables S1 to S3

Methods

The physiological parameters, MMR, BMR and P_{crit} , are laboratory measurements taken from published literature. The original studies are given 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 appropriate for the diversity of taxa represented. We included all available taxa for which all three physiological metrics have been measured, regardless of the methods employed (e.g. closed, flow-through or intermittent flow respirometry) or the acclimation period allowed. Maximum metabolic rate (MMR) for all species is achieved during or just after exercise protocols induced either via treadmills or swim flumes or following an exhaustive chase. Basal metabolic rate (BMR) is the lowest rate achieved by a particular species at a specified temperature, typically in a resting, fasted state. The critical oxygen partial pressure (P_{crit}) is defined as the oxygen partial pressure below which the basal rate of metabolism can no longer be maintained.

Despite the variable methods used, the relationship from equation 1 is extremely robust as indicated by the precision with which MMR is estimated (Fig. 2D). Deviation from the relationship described here may result from 1) a failure to experimentally achieve a truly maximum metabolic rate, 2) a decrement in oxygen demand relative to supply capacity, for example, at temperatures beyond the tolerated range, 3) inaccurate estimate of $P_{crit-max}$, or 4) error in the P_{crit} determination. Several studies note, for example, discrepancies between the MMR achieved by fishes during excess post-exercise oxygen consumption (EPOC; i.e. the “chase” method) and that obtained during maximum sustained swimming using a flume(52)(31).

Similarly, most of the variation in P_{crit} determination (e.g. between studies of the same species) results from variation in measured BMR, rather than in the determination of P_{crit} itself. Such error most often results from organismal stress or spontaneous activity leading to an elevated rate that is better termed a “routine rate” (RMR). Any elevation in BMR will lead to an underestimated aerobic scope. However, BMR and P_{crit} covary along the MO_2 - PO_2 curve (the slope of which is α , the oxygen supply capacity). Thus, an accurate MMR can be calculated from any paired MR and P_{crit} measurement. Assuming a value for $P_{crit-max}$ that exceeds the true $P_{crit-max}$ (the environmental PO_2 under which maximum capacity has evolved) will lead to an overestimate of MMR.

The temperature dependence (E) of each metabolic metric was determined from the slope of the linear regression of $1/k_B T$ versus the natural log of the metric (T in °Kelvin and k_B is the Boltzmann constant; Table S3). The temperature and body mass dependencies for each metric are related according to equation 2.

Eq. 2

$$\frac{MMR e^{(-E_{MMR}/k_B T)} M^{b_{MMR}}}{BMR e^{(-E_{BMR}/k_B T)} M^{b_{BMR}}} = \frac{P_{crit-max} e^{(-E_{P_{crit-max}}/k_B T)} M^{b_{P_{crit-max}}}}{P_{crit} e^{(-E_{P_{crit}}/k_B T)} M^{b_{P_{crit}}}}$$

Table S1.

Table S1: Metabolic measurements for diverse species.										
Taxa	Common Name	P_{crit-max} = MMR * Pcrit/BMR	P_{crit} (kPa)	BMR ($\mu\text{mol O}_2$ $\text{g}^{-1}\text{h}^{-1}$)	MMR ($\mu\text{mol O}_2$ $\text{g}^{-1}\text{h}^{-1}$)	MMR = BMR * Pcrit-max/Pcrit	α MMR/ Pcrit-max	α BMR/ Pcrit	T °C	References
Mollusca										
Cephalopoda										
<i>Illex illecebrosus</i>	Short-fin Squid	19.45	4.87	13.97	55.80	60.24	2.66	2.87	13	(53) (54)
<i>Dosidicus gigas</i>	Jumbo Squid	20.13	4.6	16	70.00	73.04	3.33	3.48	25	(53, 55)
<i>Doryteuthis pealei</i>	Long-fin Squid	22.80	3.9	6.5	38.00	35.00	1.81	1.67	15	(53, 56)
<i>Doryteuthis opalescens</i>	Market Squid	18.53	4.96	10.30	38.48	43.61	1.83	2.08	15	(57) (57)
<i>Lolliguncula brevis</i>	Brief Squid	12.63	7.7	23.47	38.50	37.92	3.09	3.05	24	(58) (59) (60)
<i>Octopus vulgaris</i>	Octopus	19.25	8	2.36	5.68	6.20	0.27	0.30	25	(61) (62, 63)
<i>Nautilus pompilius</i>	Nautilus	24.69	6.47	1.09	4.16	3.54	0.20	0.17	21	(64, 65)
Gastropoda										
<i>G. gibberulus gibbosus</i>	Jumping Conch	16.17	3.5	4.06	18.76	24.36	0.89	1.16	33	(66)
Bivalvia										
<i>Panopea zelandica</i>	Geoduck Clam	20.01	8	6.25	15.63	16.41	0.74	0.78	15	(67)
Arthropoda										
Crustacea										
<i>Gnathophausia ingens</i>	Lophogastrid	3.01	0.8	1.33	5.00	4.80	1.73	1.66	5.5	(68, 69)
<i>Calinectes sapidus</i>	Blue crab	20.73	7.98	4.2	10.91	11.05	0.52	0.53	10	(70) (71)
<i>Penaeus monodon</i>	Tiger prawn	22.80	5.37	4.81	20.42	18.81	0.97	0.90	30	(72) (73)
<i>Sergestes similis</i>	Deep shrimp	20.74	3.05	10	68.00	68.85	3.24	3.28	13	(74) (75)
<i>Homarus americanus</i>	American Lobster	23.15	4.6	0.93	4.68	4.25	0.22	0.20	15	(76, 77)
<i>Janus edwardsii</i>	Rock Lobster	23.11	7.66	0.72	2.16	1.97	0.10	0.09	13	(78)
Insecta										
<i>Schistocerca americana</i>	Grasshopper	21.01	5	76.4	321.00	320.88	15.3	15.3	30	(79)
Chordata - Vertebrata										
Teleostei										
<i>Morone saxatilis</i>	Striped Bass	20.81	5.5	2.27	8.59	8.67	0.41	0.41	20	(80)
<i>Centropomus striata</i>	Black Sea Bass	21.01	4.73	2.72	12.08	12.08	0.58	0.58	22	(52)
<i>Gadus morhua</i>	Atlantic Cod	18.16	6.42	2.1	5.94	6.87	0.28	0.33	15	(50)
<i>Sciaenops ocellatus</i>	Red Drum	14.02	4.2	5.78	19.30	20.64	1.29	1.38	24	(22)
<i>Cyclopterus lumpus</i>	Lumpfish	20.53	7.13	2.5	7.20	6.80	0.37	0.35	10	(22)
<i>Lates calcarifer</i>	Barramundi	21.11	4.42	5.81	27.75	27.60	1.32	1.31	29	(81) (82)
<i>Oreochromis niloticus</i>	Nile Tilapia	14.62	4.2	4.31	15.00	15.39		1.03	27	(83) (84)
<i>Solea solea</i>	Atlantic Sole	13.89	3.57	1.28	4.98	6.02	0.30	0.36	20	(24, 85)
<i>Scophthalmus maximus</i>	Turbot	20.00	4	1.32	6.60	6.93	0.31	0.33	17	(86, 87)
<i>Fundulus heteroclitus</i>	killifish	10.98	5.2	9	19.00	17.83	1.84	1.73	20	(88, 89)
<i>Chromis atripectoralis</i>	Black-axil	22.12	4.31	5.15	26.43	25.09	1.26	1.19	31	(23)
<i>Myoxocephalus scorpius</i>	Sculpin	23.61	7.13	1.51	5.00	4.45	0.24	0.21	10	(90) (40)
<i>Bellapiscis medius</i>	Intertidal triplefin	17.21	2.00	2.47	21.25	25.93	1.01	1.24	18	(91)
<i>Forsterygion lapillum</i>	Intertidal triplefin	17.69	3.00	3.13	18.43	21.91	0.88	1.04	18	(91)
<i>Forsterygion varium</i>	Subtidal triplefin	18.11	3.70	3.00	14.66	17.03	0.66	0.81	18	(91)
<i>Forsterygion malcolmi</i>	Subtidal triplefin	17.98	4.70	3.43	13.13	15.32	0.63	0.73	18	(91)
<i>Limanda limanda</i>	Common Dab	24.09	5.46	1.6	7.06	6.15	0.34	0.29	15	(92)
<i>Dicentrarchus labrax</i>	European Bass	18.58	4.46	1.68	7.00	7.91	0.33	0.38	11	(93) (94)
<i>Onchorynchus mykiss</i>	Trout	19.17	3.15	1.54	9.37	10.27	0.45	0.49	7.4	(95)
<i>Carassius auratus</i>	Goldfish	4.69	2.49	3.79	7.14	7.15	1.87	1.52	20	(6)
<i>Chitala ornata</i>	Clown knifefish	21.02	8.7	2.91	7.03	7.02	0.33	0.33	33	(96)

<i>Ambloplites rupestris</i>	Rock Bass	8.10	2.31	1.86	6.76	8.86	0.61	0.81	15	(25)
<i>Lepomis gibbosus</i>	Pumpkinseed sunfish	11.54	4.01	2.55	7.33	7.00	0.67	0.63	15	(25)
<i>Lepomis macrochirus</i>	Bluegill	10.20	3.63	2.48	6.97	7.52	0.63	0.68	15	(25)
<i>Micropterus salmoides</i>	Largemouth Bass	12.73	4.34	2.69	7.89	6.82	0.72	0.61	15	(25)
Reptilia										
<i>Alligator mississippiensis</i>	Alligator	22.00	5	6.7	29.48	28.14	1.40	1.34	15	(43, 97)
Mammalia										
<i>Spalax ehrenbergi</i>	Mole Rat	12.69	4.56	42.4	118	111.58	10.73	9.30	30	(26)
<i>Rattus norvegicus</i>	Lab rat	21.88	6.08	42.4	152.6	146.45	7.27	6.97	37	(26)
<i>Homo sapiens</i>	Human	22.40	5.6	33.2	133	145.60	6.33	5.93	37	(98) (99)
<i>Thomomys bottae</i>	Pocket gopher	21.00	12.5	89	149.50	149.52	7.12	7.12	37	(100)
<i>T. umbrinus melanotis</i>	Altitude gopher	14.95	8.9	89	149.50	154.00	9.71	10.0	37	(100)

* $P_{\text{crit-max}}$ is assumed to be 21 kPa for MMR and α estimates except for the highlighted species, which are known to inhabit persistently hypoxic environments (for longer than diel or tidal cycles). $P_{\text{crit-max}}$ and environmental PO_2 values for those hypoxic species are listed in Table S2.

Table S2.

Table S2. $P_{crit-max}$ and environmental PO_2 (PO_{2env}) in diverse species.				
Species	Predicted $P_{rit-max}$ (kPa) $= \frac{MMR * P_{crit}}{BMR}$	PO_{2env} or Measured $P_{crit-max}$	Description of PO_{2env}	Ref
<i>Lolliguncula brevis</i>	12.63	12.44	Summer PO_2 in Chesapeake Bay when squids are present (4 mg/l at 26°C).	(101)
<i>Gnathophausia ingens</i>	3.01	2.89	$P_{crit-max}$ is 2.89 kPa (0.95 ml/l at 5.5°C), approximate PO_2 maximum in California Current between 400-800 m depth.	(68)
<i>Sciaenops ocellatus</i>	14-15.48	15	PO_2 high of 15 kPa (5 mg at 20°C) in estuarine habitat.	(102)
<i>Solea solea</i>	13.89	16.8	$P_{crit-max}$ is 16.8 kPa. The bottom waters of the Adriatic Sea are known to be hypoxic but a precise figure could not be determined.	(85)
<i>Fundulus heteroclitus</i>	10.98	10.3	PO_2 range day to night is ~6.7 to 10.3 kPa	(103)
<i>Carassius auratus</i>	4.69	3.96	$P_{crit-max}$ (20°C) is 3.96 kPa (Incipient lethal oxygen level). Goldfish ponds are also known to be hypoxic but highly variable.	(6)
<i>Oreochromis niloticus</i>	14.62		Highly variable hypoxic environments.	(51)
<i>Ambloplites rupestri</i>	8.40	11	$P_{crit-max}$ estimates consistent with MMR measurements at 6 and 9 kPa.	(25)
<i>Lepomis gibbosus</i>	11.54	11	$P_{crit-max}$ estimates consistent with MMR measurements at 6 and 9 kPa.	(25)
<i>Lepomis macrochirus</i>	10.20	11	$P_{crit-max}$ estimates consistent with MMR measurements at 6 and 9 kPa.	(25)
<i>Micropterus salmoides</i>	12.73	11	$P_{crit-max}$ estimates consistent with MMR measurements at 6 and 9 kPa.	(25)
<i>Spalax ehrenbergi</i>	12.69	11	11 kPa is approximate P_{crit} for MMR. Mole rats are known to inhabit hypoxic underground tunnels.	(26)
<i>T. umbrinus melanotis</i>	14.95	15.4	Collected at 3200 m = 15.4 kPa. Measured $P_{crit-max}$ is 14.95.	(100)

Table S3.

Table S3. Temperature effects on metabolic rate and critical oxygen pressures.										
Species	T°C	P _{crit-max}	BMR	P _{crit}	MMR	E (eV)				References
						BMR	MMR	P _{crit}	P _{c-max}	
<i>Morone saxatilis</i>	20	19.9	2.27	5.5	8.59	0.80	= 0.38	+ 0.39	- 0.0	(80)
	26	20.1	4.28	7.43	11.72		= 0.77			
<i>Centropristis striata</i>	12	23.0	1.31	3.36	8.95	0.54	= 0.27	+ 0.25	- 0.0	(52)
	17	23.6	1.89	3.99	11.19		= 0.52			
	22	21.0	2.72	4.73	12.08					
	24	23.2	3.15	5.07	14.44					
	27	22.3	3.93	5.62	15.56					
	30	14.2	4.89	6.23	11.12					
<i>Gadus morhua</i>	18	18.8	3.84	6.42	11.24	0.71	= 0.39	+ 0.34	- 0.0	(104)
	5	18.2	0.9	3.49	4.69		= 0.73			
	10	17.5	1.66	4.91	5.9					(50)
	15	18.2	2.1	6.42	5.94					
	2	15.5	0.75	3.2	3.63					
<i>Sciaenops ocellatus</i>	24	14.0	5.78	4.2	19.30	0.43	= 0.32	+ 0.24	- 0.05	(22)
	30	15.4	8.09	5.05	24.80		= 0.51			
<i>Cyclopterus lumpus</i>	10	20.5	2.5	7.13	7.20	0.42	= 0.25	+ 0.26	- 0.03	(22)
	16	22.2	3.57	8.83	9.00		= 0.48			
<i>Chromis atriplectus</i>	35	17.6	7.56	4.88	27.21	1.10	= 0.20	+ 0.75	+ 0.06	(23)
	33	22.6	5.72	4.8	26.94		= 1.01			
	31	22.1	5.15	4.31	26.43					
	29	24.9	3.21	3.29	24.34					
<i>Carassius auratus</i>	5	2.25	0.36	0.52	1.56	1.00	= 0.71	+ 0.54	- 0.16	(6)
	10	2.62	1.07	1.05	2.67		= 1.09			
	15	3.03	2.23	1.32	5.13					
	20	4.69	3.79	2.49	7.14					
	25	4.16	6.25	2.24	11.60					
	35	4.32	10	3.3	13.10					
<i>Salmo salar</i>	18	21.9	6.37	7.56	18.44	0.33	= 0.021	+ 0.19	+ 0.07	(105, 106)
	22	19.6	9.17	9.45	19.06		= 0.28			
<i>Alligator mississippiensis</i>	15	22	6.70	5.0	29.48	0.40	= 0.19	+ 0.14	+ 0.01	(43) (97)
	25	21	26.70	7.0	80.10		= 0.34			
Basal (BMR) and maximum (MMR) metabolic rates in $\mu\text{mol O}_2 \text{ g}^{-1}\text{h}^{-1}$ and the critical PO_2 (P _{crit}) in kPa. Temperature coefficients (E) for metrics (Y = BMR, P _{crit} or MMR). The temperature coefficient is the slope of the relationship between lnY and 1/k _B T; Y = exp(-E/1k _B T), T in °Kelvin and k _B is the Boltzmann constant), calculated within the tolerated temperature range for each species (excluded temperatures in red). E _{Pcrit-max} is near zero for all species in this table except the goldfish, <i>Carassius auratus</i> , which sees hypoxic water during winter. Estimated P _{crit-max} values for <i>C. auratus</i> are very similar to those directly measured (Fry and Hart, 1948). As described above, E _{BMR} = E _{MMR} + E _{Pcrit} - E _{Pcrit-max} .										