Oxygen limitation may affect the temperature and size dependence of metabolism in aquatic ectotherms

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Both oxygen and temperature are fundamental factors determining metabolic performance, fitness, ecological niches, and responses of many aquatic organisms to climate change. Despite the importance of physical and physiological constraints on oxygen supply affecting aerobic metabolism of aquatic ectotherms, ecological theories such as the metabolic theory of ecology have focused on the effects of temperature rather than oxygen. This gap currently impedes mechanistic models from accurately predicting metabolic rates (i.e., oxygen consumption rates) of aquatic organisms and restricts predictions to resting metabolism, which is less affected by oxygen limitation. Here, we expand on models of metabolic scaling by accounting for the role of oxygen availability and temperature on both resting and active metabolic rates. Our model predicts that oxygen limitation is more likely to constrain metabolism in larger, warmer, and active fish. Consequently, active metabolic rates are less responsive to temperature than are resting metabolic rates, and metabolism scales to body size with a smaller exponent whenever temperatures or activity levels are higher. Results from a metaanalysis of fish metabolic rates are consistent with our model predictions. The observed interactive effects of temperature, oxygen availability, and body size predict that global warming will limit the aerobic scope of aquatic ectotherms and may place a greater metabolic burden on larger individuals, impairing their physiological performance in the future. Our model reconciles the metabolic theory with empirical observations of oxygen limitation and provides a formal, quantitative framework for predicting both resting and active metabolic rate and hence aerobic scope of aquatic ectotherms.

biophysical modeling | aerobic scope | fish | teleost | metabolic scaling

Aerobic metabolism fueling the physiological performance of ectotherms depends strongly on environmental temperature and can be limited by insufficient supply of oxygen (1, 2). Both temperature and oxygen availability are thus key environmental variables shaping the fitness of ectotherms and hence their geographical distributions (3–5). The limiting effect of oxygen may be especially important for aquatic ectotherms, for which obtaining oxygen is more challenging (6–10). However, mechanistic theories such as the metabolic theory of ecology (MTE), which have been widely adopted by ecologists to investigate organismal responses to climate change (11), have placed the thermal dependence of metabolic rates at the center of their synthesis (12, 13). While MTE successfully models the factors determining resting metabolism, predictions for active metabolic rates are far less accurate, likely because higher metabolic rates are progressively more constrained by oxygen supply. Here we extend models of the temperature dependence of metabolic scaling to include mechanistic descriptions of how oxygen uptake may constrain metabolic rates (i.e., measured as the rate of oxygen consumption) of active, warm, large-bodied aquatic ectotherms. We propose that such models will be crucial for making accurate predictions under future climate scenarios.

The temperature dependence of metabolism in many ecological theories relies on the Boltzmann–Arrhenius kinetics, $b_0 M^b e^{-\frac{E}{kT}}$ (12), where b_0 is the normalization constant, k is the Boltzmann constant, T is the absolute temperature, E is activation energy, M is body mass, and b is the scaling exponent. Within the normal operating temperature range of a species, this formula expresses how 1) metabolic rate increases with temperature with a constant factor E, given by the average activation energy of the enzymatic reactions comprising the individual's metabolism (i.e., $E \sim 0.6$ eV) (12) and 2) that metabolic rate scales with body size with a constant exponent b, determined by the internal architecture of the transport network that distributes resources (i.e., $b = 3/4$) (14, 15). The MTE therefore assumes that oxygen supply does not alter how metabolic rate increases with rising temperature or physical activity (Fig. 1).

It is well known that metabolic rates can be limited by insufficient oxygen, especially during intense activity or in hypoxia (1, 16), and that oxygen is linked to both how temperature affects metabolism (2, 17) and how metabolic rate scales with body size (18–22). At high temperatures or under intense physical activity, the oxygen supply can no longer meet the increase in oxygen demand, at which point the metabolic rate approaches a limit (Fig. 1 and refs. 2, 5, 17, 23, 24). The difference between this upper limit, or maximum metabolic rate, and the resting

Significance

Organismal responses to climate change are mediated through its effects on physiology and metabolism. In aquatic environments, both water temperature and oxygen availability may modulate these responses by altering the aerobic metabolism fueling physiological performance. However, ecological models aimed at predicting how environmental factors shape aerobic metabolism disregard the role of oxygen supply. Here, we expand on these models by explicitly incorporating oxygen supply. Our results show that warmer water increases oxygen demand relative to supply, and the resulting reduction in aerobic scope appears to be stronger in larger individuals. Smaller aerobic scopes in warming water imply that climate change will reduce energy budgets needed to support the activities of aquatic animals and their physiological performance in the future.

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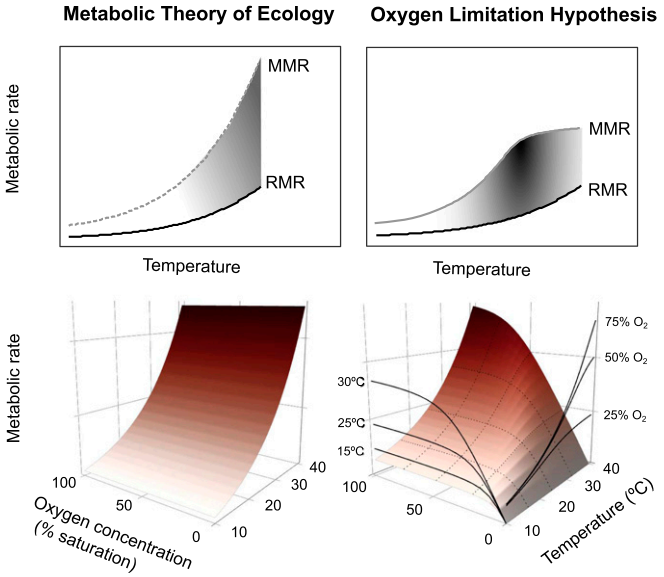

Fig. 1. General frameworks of the MTE and the oxygen limitation hypothesis. While MTE focuses on the exponential relationship between RMRs and temperature, the oxygen limitation hypothesis emphasizes that metabolism is constrained by oxygen availability, especially at high temperatures or during intense activity (MMR). Therefore, MTE cannot be used to predict MMR (dashed line) and the aerobic scope (i.e., the difference between MMR and RMR, shaded areas), which according to the oxygen limitation hypothesis, represents the energy available for fitness-enhancing activities such as reproduction, feeding, or fighting.

metabolic rate is the aerobic scope, which represents the rate of energy available for fitness-enhancing activities like movement, feeding, digestion, growth, and reproduction. According to the oxygen limitation hypothesis, the aerobic scope is functionally related to physiological performance and fitness of aquatic ectotherms (2).

Although recent models have investigated the effects of hypoxia on physiological performance (25–27), we still lack a mechanistic, quantitative framework that derives oxygen supply and demand from first principles. Here, we build upon the MTE equation of temperature and mass dependence (12) to show that oxygen limitation emerges from fundamental biophysical principles. We model the effect of oxygen limitation by introducing Michaelis–Menten kinetics for oxygen consumption. Although originally developed to describe saturation effects in individual chemical reactions, the Michaelis–Menten equation also captures how more complex, multicomponent systems, like metabolism, respond to limitation in supply (10, 28, 29). We use our model to investigate how oxygen limitation influences the temperature dependence and mass scaling of resting and maximum metabolic rates, and we show that the model successfully predicts patterns of resting and active metabolic rate across 286 species of fishes. More broadly, this analysis shows that predictions from the MTE represent a special case of a more general model where the affinity for oxygen is infinitely high.

Methods

Model Description. The metabolic reactions that comprise whole-organism metabolic rate depend on temperature and the concentration of substrates such as carbohydrates and oxygen. In enzyme-catalyzed chemical reactions, the dependence on substrate concentration is given by the Hill kinetic equation, $v = V_{max}\frac{\rho^H}{\rho^H + (K_M)^H}$, a generalization of the Michaelis–Menten equation, where V_{max} represents the maximum reaction rate, ρ (mg m^{-3}) is the concentration of the substrate, K_M (mg m^{-3}) is the Michaelis constant representing the concentration at which the reaction velocity is half of V_{max}, and H is the Hill coefficient representing the role of cooperative binding of oxygen to enzymes (29), e.g., through respiratory pigments. Because metabolic processes take place via enzyme-catalyzed reactions, the metabolic rate under oxygen limitation, $\dot{M}_{O_2}^{met}$ (mg$_{O2}$ s^{-1}), can be approximated using the kinetic equation:

$$\dot{M}_{O_2}^{met} = \delta b_0 M^b e^{-\frac{E}{kT}} \frac{{\rho_{O_2,gill}}^H}{{\rho_{O_2,gill}}^H + {K_M}^H}, \quad [1]$$

where $\rho_{O2,gill}$ is the concentration of oxygen at the gill surface (mg m^{-3}). Eq. **1** incorporates an additional constant δ representing the increase in metabolic demand above the minimum (e.g., due to activities such as locomotion, feeding, or fighting). $\delta = 1$ thus represents the minimum metabolic requirements (resting metabolic rate, RMR), whereas $\delta > 1$ simulates increasing metabolic demand (up to maximum metabolic rate, MMR). Thus, the parameter δ is equivalent to the factorial aerobic scope (FAS = MMR/RMR), which provides a means to investigate the effect of oxygen limitation at different activity levels.

The key challenge is to model the oxygen concentration at the gill surface, $\rho_{O2,gill}$, as a function of environmental variables together with the animal's morphology and physiology. In water-breathing ectotherms, oxygen intake depends on the rate of convective oxygen transfer, which involves both simple diffusion governed by gradients in partial pressure of oxygen, and the motion of water powered by branchial ventilation (6, 7, 30–32). To model this convective process, we need to apply an analog of Fick's law that also incorporates the advective motion of water at the gill surface:

$$\dot{M}_{O_2}^{supply} = -A \cdot h_m\left(\rho_{O_2,gill} - \rho_{O_2,water}\right), \quad [2]$$

where $\dot{M}_{O_2}^{supply}$ is the rate of oxygen supply (mg$_{O2}$ s^{-1}), A is the respiratory surface area (m^2), h_m is the mass transfer coefficient (m s^{-1}), and $\rho_{O2,water}$ is the concentration of oxygen in the environment (mg m^{-3}), i.e., the product of partial pressure and solubility calculated as a function of water temperature (33). The oxygen-transfer coefficient captures the effects of water motion on oxygen transfer at the gill surface, which depends on water viscosity, μ (Pa s), and density, ρ (kg m^{-3}), the diffusion coefficient of oxygen, D (m^2 s^{-1}), and the geometry of the gill, characterized by its length L (m), and the velocity of the flow of water at the gill, v (6, 30, 32). Here, we use an expression for the oxygen-transfer coefficient that involves the Sherwood (Sh), Reynolds (Re), and Schmidt numbers (Sc) given by $h_m = \frac{D}{L}\left[2 + 0.6\left(\frac{L\rho v}{\mu}\right)^{\frac{1}{2}}\left(\frac{\mu}{\rho D}\right)^{\frac{1}{3}}\right]$. This approach allows us to incorporate the dynamics of water at the gill surface by explicitly including velocity of flow at the gill, v, which is influenced by branchial ventilation (6, 30, 34).

Now we have the ingredients to model oxygen consumption by metabolism, $\dot{M}_{O_2}^{met}$ (Eq. **1**), as a function of oxygen supply $\dot{M}_{O_2}^{supply}$ (Eq. **2**). In the steady state, oxygen consumption must equal the rate of oxygen supply, and we can exploit this linkage to derive the oxygen concentration at the respiratory surface. For instance, perturbations of the steady state, such as a transient increase in metabolic rate, will deplete oxygen at the respiratory surface (reducing $\rho_{O2,gill}$), and the resulting larger gradient in oxygen concentration will increase rates of diffusive transport. Lower oxygen concentrations at the respiratory surface will also prevent increases in oxygen consumption rates as K_M is approached. This negative feedback will result in a new dynamic equilibrium. Ultimately, we are interested in the steady-state condition, where the rate of oxygen consumption (Eq. **1**) equals the rate of oxygen intake by diffusion (Eq. **2**):

$$\delta b_0 M^b e^{-\frac{E}{kT}} \frac{\left(\rho_{O_2,gill}^*\right)^H}{\left(\rho_{O_2,gill}^*\right)^H + {K_M}^H} = -Ah_m\left(\rho_{O_2,gill}^* - \rho_{O_2,water}\right),$$

with

$$h_m = \frac{D}{L}\left[2 + 0.6\left(\frac{L\rho v}{\mu}\right)^{\frac{1}{2}}\left(\frac{\mu}{\rho D}\right)^{\frac{1}{3}}\right] \quad [3]$$

where $\rho_{O2,gill}^*$ is the steady-state concentration of oxygen at the respiratory surface. The left side of Eq. **3** contains the relationship between metabolic rate and both temperature (i.e., the Boltzmann–Arrhenius equation) and oxygen availability (i.e., the Michalis–Menten kinetic equation). The right side describes the rate of oxygen intake from the environment, modeled here via boundary-layer effects on external oxygen transport. A K_M of 0 thus indicates that the affinity for oxygen is infinitely high and that there is no

oxygen limitation. Under this condition, metabolic rate increases exponentially with temperature following the Arrhenius equation, consistent with how this relationship is expressed in the MTE. Higher K_M values make metabolism increasingly dependent on the rate of oxygen intake given by the right side of the equation. This equation also contains several variables that the animal can potentially alter to increase oxygen intake rates, for example, increasing the velocity of the current of water at the gill surface, v (increasing ventilation rates) or the surface area of the respiratory organs, A (increasing exposure of respiratory organs). We analyzed the effect of each parameter by exploring Eq. **3** numerically (*SI Appendix, Numerical analyses*).

Model Predictions. We first calibrated the model by comparing predicted and observed metabolic rates of eight species of teleost fishes to obtain realistic values of model parameters b_0, E, K_M, and δ (*SI Appendix, Model calibration*). We then used the model to analyze how incorporating oxygen limitation changes both 1) the thermal dependence of metabolism and 2) the mass scaling of metabolism. Following MTE, we used Arrhenius plots, which present metabolic rates as a function of inverse temperature, $(kT)^{-1}$, where k is the Boltzmann constant (eV K^{-1}) and T is absolute temperature (K). The slope of this relationship describes the sensitivity of metabolism to changes in temperature (more negative slope means greater dependence on temperature) and, according to MTE, this slope is determined by the average activation energy of the enzymatic reactions of metabolism. In our model, this slope also depends on the availability of and affinity for oxygen and thereby on temperature and activity level (*SI Appendix, Numerical analyses*). Specifically, at higher temperatures, more intense activity, or both, steady-state oxygen uptake rates are more and more constrained by oxygen availability, reducing the slope of the relationship of metabolism vs. inverse temperature (Fig. 2*A*). This predicted effect of oxygen limitation on the slope indicates that we should expect a significant interaction between temperature and activity level on metabolic rate.

The predicted scaling of metabolic rates also depends on temperature and oxygen availability. Warmer water increases metabolism and thereby the intercept of the scaling relationship. However, the model predicts that the limits to oxygen supply are more readily experienced in larger fish, as indicated in a declining slope of the scaling relationship. Such a shallower scaling relationship is especially pronounced under intense activity and in warm waters (Fig. 2*B*). Specifically, the mass-scaling slope falls from its initial value, set here to $b = 0.75$, and approaches ~0.60 with increasing demand for oxygen. These specific values of the mass-scaling slope in relation to temperature also depend on how other traits involved in oxygen transport scale with body size (30). We performed an additional sensitivity analysis to examine how the mass scaling of gill surface (A), velocity of the gill water flow (v), and activity level (δ) influence the predicted relationship between metabolism and body mass (*SI Appendix, Sensitivity analysis*). This predicted effect of temperature on the slope of the allometric scaling means that we should expect an interaction between temperature and mass on metabolic rate, and that this interaction should be more evident on maximum than on resting metabolic rates.

Data Analyses. We tested model predictions for temperature dependence and mass scaling of RMR and MMR using empirical data from 286 species of fishes. These data were taken from White et al. (35) and supplemented with data from FishBase (36). The full database includes both intra- and interspecific data comprising 3,291 RMR measurements (241 species) and 1,212 MMR measurements (95 species).

We analyzed the effects of temperature, body mass, and activity level (either RMR or MMR) on oxygen consumption rate. We first included the three independent variables and analyzed their interactions to test the first prediction of the model (metabolic rates are determined by an interaction between activity level and temperature). We then split the data and performed separate analyses for RMR and MMR to test the second prediction of the model (the interaction between temperature and mass is more important for maximum than resting metabolic rates). We used mixed-effect models including the species identity as a random factor, allowing for both random intercepts and random slopes for each species. We also controlled for the phylogenetic relatedness between species using phylogenetic information in a subset of 103 fish species (*SI Appendix, Phylogenetic analysis*). We used the R package MCMCglmm (37) to perform both phylogenetically controlled and ordinary mixed-effect models in R v3.5.3 (38).

Results

Temperature Dependence. Fish metabolic rates (both resting and maximum) were negatively and linearly related to inverse temperature ($1/kT$), which means that increasing water temperature increases oxygen demand (Table 1). The slope of this relationship, however, varied between resting and maximum metabolic rates; resting metabolic increased with temperature more strongly than maximum metabolic rates (Fig. 3*A*). This interaction term suggests that oxygen is more likely to be limiting for maximum metabolic rates, reducing their sensitivity to temperature. This result supports the first prediction of our model (Fig. 3*A*).

Mass Scaling. Although both RMR and MMR scaled with body mass, the scaling exponent varied with temperature, as indicated by the significant interaction between mass and temperature on MMR (Table 1). Specifically, increasing water temperature reduced the slope of the mass-scaling relationship and this effect was more evident for MMRs (Table 1). This result supports the second prediction of our model that oxygen limitation has a greater impact on large animals at higher water temperature or under intense activity (Fig. 3*B*). The results of both temperature dependence and the mass scaling were consistent with those obtained from phylogenetic models (*SI Appendix, Phylogenetic analysis*).

Discussion

The model provides quantitative predictions to evaluate how oxygen availability may shape the temperature dependence and mass scaling of metabolism in aquatic ectotherms. The model's key predictions are that MMR is less sensitive to temperature variation than is RMR, and that metabolism scales to body size with a smaller exponent whenever temperatures are warmer or

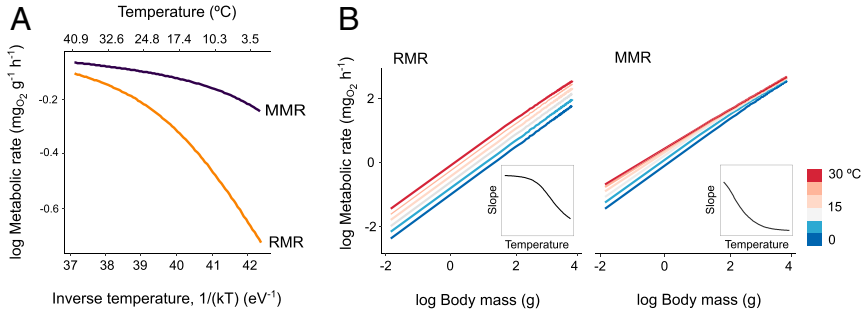

Fig. 2. Biophysical model predictions for temperature dependence (*A*) and mass scaling (*B*) of resting, RMR, and MMR. RMRs are predicted to exhibit greater temperature dependence than MMRs, which are more constrained by oxygen limitation. The metabolic scaling exponent (the slope of the log-MR vs. log-mass relationship) is predicted to vary with temperature, especially for MMR, where greater oxygen limitation with increasing temperature and larger body size gives rise to a shallower scaling relationship.

Table 1. Results of the comparative mixed-effects models relating metabolic rates with inverse temperature (1/*k*T), log-body mass (mass), and activity level (resting, RMR, and MMR)

RMR and MMR, n = 286 sp	Coefficient	−95% CI	+95% CI	pMCMC
Intercept	8.051	7.063	8.874	<0.001
Mass	0.758	0.225	1.214	0.002
1/*k*T	−0.220	−0.242	−0.197	<0.001
Activity	4.603	2.432	6.614	<0.001
Mass × 1/*k*T	6.60×10^{-4}	−0.011	0.013	0.938
Mass × activity	−3.535	−4.699	−2.419	<0.001
1/*k*T × activity	−0.104	−0.154	−0.049	<0.001
Mass × 1/*k*T × activity	0.088	0.060	0.117	<0.001
Species - intercept	8.00×10^{-3}	3.05×10^{-7}	0.040	
Species - 1/*k*T	3.81×10^{-5}	1.50×10^{-5}	5.31×10^{-5}	
Species - mass	0.026	0.020	0.035	
RMR, n = 241 sp				
Intercept	9.111	8.295	10.135	<0.001
Mass	0.148	−0.347	0.619	0.560
1/*k*T	−0.247	−0.269	−0.223	<0.001
Mass × 1/*k*T	0.015	3.33×10^{-3}	0.027	0.018
Species - intercept	0.050	8.83×10^{-4}	0.081	
Species - 1/*k*T	1.48×10^{-5}	4.31×10^{-9}	4.22×10^{-5}	
Species - mass	0.022	0.016	0.029	
MMR, n = 95 sp				
Intercept	12.082	9.517	14.585	<0.001
Mass	−1.991	−3.313	−0.607	0.006
1/*k*T	−0.310	−0.370	−0.242	<0.001
Mass × 1/*k*T	0.071	0.037	0.105	<0.001
Species - intercept	2.12×10^{-7}	1.83×10^{-16}	8.10×10^{-8}	
Species - 1/*k*T	3.90×10^{-5}	2.35×10^{-5}	5.66×10^{-5}	
Species - mass	1.31×10^{-2}	6.29×10^{-3}	2.02×10^{-2}	

Mixed models include random intercepts and slopes for each species.

activity levels are higher (Fig. 2). Predictions on both temperature and size dependence were supported by a metaanalysis of metabolic rates in 286 species of fishes (Fig. 3). We propose that a parsimonious explanation for the observed interactions between body size, water temperature, and activity is the influence of physical processes shaping oxygen uptake dynamics in water. By integrating oxygen limitation (Michaelis–Menten kinetics), temperature dependence (Arrhenius kinetics), and oxygen transport dynamics, our model provides a mechanistic framework that integrates effects of temperature dependence, mass scaling, and activity on the aerobic metabolism of aquatic ectotherms.

Including both temperature and substrate dependence in metabolic models provides a more general view and better predictions than theories focused on temperature dependence alone (28). The first attempt to include oxygen limitation in the MTE was the metabolic index by Deutsch et al. (3), which focused on the net effects of oxygen supply and demand rather than on modeling each explicitly. Their metabolic index highlighted the need to incorporate both water temperature and oxygenation as determinants of species geographical ranges, as animals can lose aerobic scope due to warming and deoxygenation (3, 27). Dynamic energy budget theory (DEB) offers another framework to model the effects of metabolic fluxes of mass and energy on self-maintenance, growth, and reproduction (39, 40). Recent models have integrated the effect of hypoxia on metabolic fluxes (26) within the context of DEB theory (25), relying on indices and empirical formulas to describe the relationship between oxygen consumption and availability, rather than deriving them from underlying biophysical and chemical processes. Here we take a first-principles approach to modeling

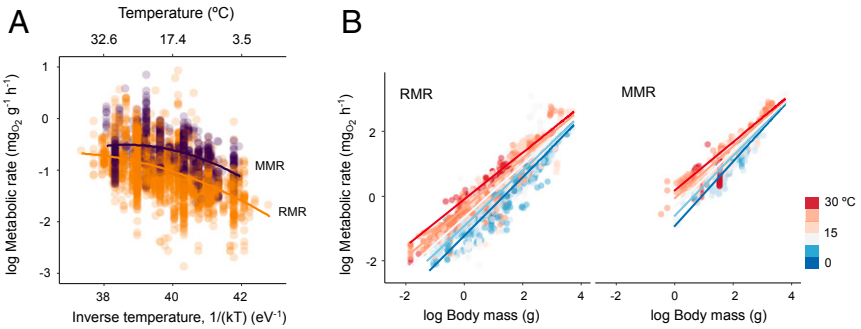

Fig. 3. Empirical patterns for temperature dependence (*A*) and mass scaling (*B*) of resting, RMR, and MMR of fishes. Lines represent fits to empirical data (note that analyses were performed using linear mixed models that control for species identity).

rates of oxygen supply and consumption. By linking metabolism to environmental factors and the physical properties of water that determine oxygen supply, such an approach offers the possibility of integrating temperature and oxygen dependence more mechanistically into theoretical frameworks such as MTE or DEB.

Oxygen moves from environment to mitochondria via a cascade that includes external transport, absorption across respiratory surfaces, distribution by internal transport systems, and diffusion within tissues and cells. Constraints on oxygen supply can arise at any or all of these levels (20, 22). While the MTE focuses on the internal transport network to predict the scaling exponent (14), the role of external factors such as temperature and notably oxygen availability have received less attention (40). Here we show that modeling external transport explicitly has two major implications for understanding metabolic scaling under oxygen limitation. First, this approach takes into consideration key compensatory mechanisms used by aquatic ectotherms to mitigate constraints on oxygen supply, such as increasing gill surface area (A), and the velocity of water at the gill surface via active branchial ventilation (v), or reducing the activity level by adopting a lifestyle with lower oxygen demands (δ). It has been argued that oxygen supply capacity can evolve to meet demand at the current oxygen partial pressure (41), and the increase in gill surface area with mass may be critical to reducing the impacts of oxygen limitation (42, 43). Here, we find that, when considering ranges of variation of gill surface, velocity of gill water flow, and activity level that are representative for fish, size constraints on oxygen supply remain evident at higher temperatures and activity levels (*SI Appendix*, *Sensitivity analysis*), and the empirical results are consistent with the model prediction of increased susceptibility to oxygen limitation in larger, active, hot fish (Fig. 3). Although fish exhibit a variety of adaptations to enhance oxygen supply or reduce oxygen demand, they are apparently not able to fully mitigate such oxygen constraints. A likely reason is that these adaptations themselves carry costs and involve trade-offs with other life-history traits that ultimately limit the effectiveness of selection. Although our observational results are consistent with predictions based on well-established physical principles on mass transfer, further experimental research is necessary to provide definitive support for oxygen limitation as the primary mechanism driving these patterns.

The second key implication of modeling external transport results from the fact that the oxygen-transfer coefficient depends on variables such as water temperature, current velocity, flow regime, and body size, making metabolic scaling exponents dependent on environmental variables and the activity of the animal. Fry et al. (1) first noticed that metabolic scaling exponents depend on environmental factors such as salinity and the activity of the animal. The metabolic-level boundaries hypothesis by Glazier (18) later postulated that both intrinsic and extrinsic factors can affect metabolic scaling and predicted a negative relationship between the intercept and the slope of the scaling relationship (19, 44). Killen et al. (19), found support for this prediction for resting metabolic rates: as the intercept of the scaling relationship increased with temperature, the mass-scaling exponent decreased. Here, we propose that stronger constraints on oxygen supply at warmer temperatures and larger body sizes are the main mechanism underpinning this negative relationship, and further show that the effect is more evident in maximum than in resting metabolic rates (Fig. 3*B*). Because our model incorporates both internal (parameter b from MTE) and external constraints on oxygen supply, it provides a comprehensive framework for quantitatively predicting scaling exponents under oxygen limitation. The Arrhenius exponential equation adopted by the MTE describes the temperature dependence of metabolic rates when oxygen is not limiting. In contrast, our model better captures responses to higher temperatures, higher activity levels, and environments with low oxygen availability, representing an advance in the field of metabolic scaling over previous studies which investigated differences in scaling between RMR and MMR but did not consider temperature effects on oxygen limitation (18, 19, 44).

Because the diffusivity of oxygen is much lower in water than in air, the effects of oxygen limitation on both temperature dependence and scaling exponents of metabolic rates should be more apparent in aquatic than in terrestrial taxa. Thus, for example, Gillooly et al. (12) showed that the Arrhenius slopes for fishes and amphibians are less negative than in other taxa but did not provide an explanation. According to our model, these shallower slopes result from greater risks of oxygen limitation in aquatic and semiaquatic than in terrestrial organisms. A second-order extrapolation of our model is that, in aquatic ectotherms, larger individuals likely experience greater oxygen limitation whenever temperatures are warmer, or activity levels are higher. Therefore, large animals may have some aerobic scope left to fuel activity, growth, and reproduction in cold waters but not in warmer waters—a prediction that is consistent with the temperature-size rule, i.e., the tendency of many ectotherms to grow to smaller final sizes when reared in warmer temperatures (45). Thus, Forster et al. (46) found that the temperature-size rule is more pronounced in aquatic than terrestrial species. Furthermore, Horne et al. (47) found that body sizes of aquatic species generally decrease more than those of terrestrial organisms with both warming and decreasing latitude. Finally, the evidence supporting the role of oxygen limitation of thermal tolerance limits is stronger for water- than for air-breathing arthropods (8, 9). Overall, these observations support the conclusion that oxygen limitation is a major factor driving patterns of body-size variations and physiological traits in aquatic but not in terrestrial organisms. This result does not contradict the idea that oxygen supply capacity in animals has evolved to meet demand at the current oxygen partial pressure (41), but it suggests that many aquatic ectotherms can readily reach their maximum supply capacity during bursts of activity. Warmer water temperatures will make demand for oxygen increasingly outpace supply (7, 31), which will curtail performance, especially at larger body sizes.

The biological impacts of climate warming are mediated primarily through its effects on organismal physiology (11, 48), and our model has general implications for predicting these impacts. First, rising water temperatures are predicted to raise resting metabolic rates more than MMRs, which may reduce the overall aerobic scopes of aquatic ectotherms. Smaller aerobic scopes imply that less energy remains for supporting physiological performance and fitness-related activities (17, 23, 49). Second, large individuals are likely more susceptible to oxygen limitation and therefore they could experience a greater reduction in aerobic scope with warming. This prediction supports the idea that oxygen limitation is at least partly responsible for driving the reduction of body size in response to global warming (32, 45, 49). Finally, risks of oxygen limitation will be greater in aquatic ecosystems. Consequently, oxygen limitation of aerobic metabolism may be a fundamental mechanism driving the response of aquatic ectotherms to climate warming.

Data Availability. All study data are included in the article and *SI Appendix*.

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1. F. E. J. Fry, "The effect of environmental factors on the physiology of fish" in *Fish Physiology. Volume VI Environmental Relations and Behaviour*, W. S. Hoar, D. J. Randall, Eds. (Academic Press Inc, London, 1947), pp. 1–98.
2. H. O. Pörtner, Climate change and temperature-dependent biogeography: Oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* **88**, 137–146 (2001).
3. C. Deutsch, A. Ferrel, B. Seibel, H. O. Pörtner, R. B. Huey, Ecophysiology. Climate change tightens a metabolic constraint on marine habitats. *Science* **348**, 1132–1135 (2015).
4. W. C. E. P. Verberk, I. Durance, I. P. Vaughan, S. J. Ormerod, Field and laboratory studies reveal interacting effects of stream oxygenation and warming on aquatic ectotherms. *Glob. Change Biol.* **22**, 1769–1778 (2016).
5. R. Ern, A mechanistic oxygen- and temperature-limited metabolic niche framework. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **374**, 20180540 (2019).
6. P. Dejours, *Principles of Comparative Respiratory Physiology* (Elsevier, Amsterdam, 1981).
7. W. C. E. P. Verberk, D. T. Bilton, P. Calosi, J. I. Spicer, Oxygen supply in aquatic ectotherms: Partial pressure and solubility together explain biodiversity and size patterns. *Ecology* **92**, 1565–1572 (2011).
8. W. C. E. P. Verberk, D. T. Bilton, Respiratory control in aquatic insects dictates their vulnerability to global warming. *Biol. Lett.* **9**, 20130473 (2013).
9. F. P. Leiva, P. Calosi, W. C. E. P. Verberk, Scaling of thermal tolerance with body mass and genome size in ectotherms: A comparison between water- and air-breathers. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **374**, 20190035 (2019).
10. N. Galic, T. Hawkins, V. E. Forbes, Adverse impacts of hypoxia on aquatic invertebrates: A meta-analysis. *Sci. Total Environ.* **652**, 736–743 (2019).
11. M. E. Dillon, G. Wang, R. B. Huey, Global metabolic impacts of recent climate warming. *Nature* **467**, 704–706 (2010).
12. 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).
13. 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).
14. G. B. West, J. H. Brown, B. J. Enquist, A general model for the origin of allometric scaling laws in biology. *Science* **276**, 122–126 (1997).
15. J. R. Banavar *et al*., A general basis for quarter-power scaling in animals. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 15816–15820 (2010).
16. H. O. Pörtner, Climate variations and the physiological basis of temperature dependent biogeography: Systemic to molecular hierarchy of thermal tolerance in animals. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **132**, 739–761 (2002).
17. H. O. Pörtner, R. Knust, Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* **315**, 95–97 (2007).
18. D. S. Glazier, A unifying explanation for diverse metabolic scaling in animals and plants. *Biol. Rev. Camb. Philos. Soc.* **85**, 111–138 (2010).
19. S. S. Killen, D. Atkinson, D. S. Glazier, The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. *Ecol. Lett.* **13**, 184–193 (2010).
20. J. F. Gillooly, J. P. Gomez, E. V. Mavrodiev, Y. Rong, E. S. McLamore, Body mass scaling of passive oxygen diffusion in endotherms and ectotherms. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 5340–5345 (2016).
21. F. P. Leiva *et al*., Differences in the respiratory response to temperature and hypoxia across four life-stages of the intertidal porcelain crab *Petrolisthes laevigatus*. *Mar. Biol.* **165**, 146 (2018).
22. J. Kozłowski, M. Konarzewski, M. Czarnoleski, Coevolution of body size and metabolic rate in vertebrates: A life-history perspective. *Biol. Rev. Camb. Philos. Soc.* **95**, 1393–1417 (2020).
23. G. Claireaux, C. Lefrançois, Linking environmental variability and fish performance: Integration through the concept of scope for activity. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **362**, 2031–2041 (2007).
24. P. M. Schulte, The effects of temperature on aerobic metabolism: Towards a mechanistic understanding of the responses of ectotherms to a changing environment. *J. Exp. Biol.* **218**, 1856–1866 (2015).
25. Y. Thomas *et al*., Effects of hypoxia on metabolic functions in marine organisms: Observed patterns and modelling assumptions within the context of Dynamic Energy Budget (DEB) theory. *J. Sea Res.* **143**, 231–242 (2019).
26. P. Neubauer, K. H. Andersen, Thermal performance of fish is explained by an interplay between physiology, behaviour and ecology. *Conserv. Physiol.* **7**, coz025 (2019).
27. C. Deutsch, J. L. Penn, B. A. Seibel, Metabolic trait diversity shapes marine biogeography. *Nature* **585**, 557–562 (2020).
28. B. Aguiar-González, T. T. Packard, E. Berdalet, S. Roy, M. Gómez, Respiration predicted from an enzyme kinetic model and the metabolic theory of ecology in two species of marine bacteria. *J. Exp. Mar. Biol. Ecol.* **412**, 1–12 (2012).
29. C. M. Wood, The fallacy of the P_{crit} -are there more useful alternatives? *J. Exp. Biol.* **221**, jeb163717 (2018).
30. B. A. Hills, G. M. Hughes, A dimensional analysis of oxygen transfer in the fish gill. *Respir. Physiol.* **9**, 126–140 (1970).
31. H. A. Woods, Egg-mass size and cell size: Effects of temperature on oxygen distribution. *Am. Zool.* **39**, 244–252 (1999).
32. W. C. E. P. Verberk, D. Atkinson, Why polar gigantism and palaeozoic gigantism are not equivalent: Effects of oxygen and temperature on the body size of ectotherms. *Funct. Ecol.* **27**, 1275–1285 (2013).
33. H. E. García, L. I. Gordon, Oxygen solubility in seawater: Better fitting equations. *Limnol. Oceanogr.* **37**, 1307–1312 (1992).
34. D. Randall, H. Lin, P. A. Wright, Gill water flow and the chemistry of the boundary layer. *Physiol. Zool.* **64**, 26–38 (1991).
35. C. R. White *et al*., The origin and maintenance of metabolic allometry in animals. *Nat. Ecol. Evol.* **3**, 598–603 (2019).
36. R. Froese, D. Pauly, FishBase Version 12/2019. https://www.fishbase.in/search.php. Accessed December 2019.
37. J. D. Hadfield, MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. *J. Stat. Softw.* **33**, 1–22 (2010).
38. R Core Team, R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria). https://www.R-project.org/ (2019).
39. S. A. L. M. Kooijman, *Dynamic Energy Budget Theory for Metabolic Organisation* (Cambridge University Press, Cambridge, UK, 2010).
40. M. R. Kearney, C. R. White, Testing metabolic theories. *Am. Nat.* **180**, 546–565 (2012).
41. B. A. Seibel, C. Deutsch, Oxygen supply capacity in animals evolves to meet maximum demand at the current oxygen partial pressure regardless of size or temperature. *J. Exp. Biol.* **223**, jeb210492 (2020).
42. S. Lefevre, D. J. McKenzie, G. E. Nilsson, Models projecting the fate of fish populations under climate change need to be based on valid physiological mechanisms. *Glob. Change Biol.* **23**, 3449–3459 (2017).
43. D. Pauly, W. W. L. Cheung, Sound physiological knowledge and principles in modeling shrinking of fishes under climate change. *Glob. Change Biol.* **24**, e15–e26 (2018).
44. D. Glazier, Scaling of metabolic scaling within physical limits. *Systems* **2**, 425–450 (2014).
45. W. C. E. P. Verberk *et al*., Shrinking body sizes in response to warming: Explanations for the temperature-size rule with special emphasis on the role of oxygen. *Biol. Rev. Camb. Philos. Soc.*, 10.1111/brv.12653 (2020).
46. J. Forster, A. G. Hirst, D. Atkinson, Warming-induced reductions in body size are greater in aquatic than terrestrial species. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 19310–19314 (2012).
47. C. R. Horne, A. G. Hirst, D. Atkinson, Temperature-size responses match latitudinal-size clines in arthropods, revealing critical differences between aquatic and terrestrial species. *Ecol. Lett.* **18**, 327–335 (2015).
48. C. A. Deutsch *et al*., Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 6668–6672 (2008).
49. H. O. Pörtner, A. P. Farrell, Ecology. Physiology and climate change. *Science* **322**, 690–692 (2008).