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Controversial roles of oxygen in organismal responses to climate warming

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Abstract:	Despite the global ecological importance of climate change, controversy surrounds how oxygen affects the fate of aquatic ectotherms under warming. Disagreements extend to the nature of oxygen bioavailability, and whether oxygen usually limits growth under warming, explaining smaller adult size. These controversies affect two influential hypotheses: Gill Oxygen Limitation, and Oxygen- and Capacity-Limited Thermal Tolerance. Here, we promote deeper integration of physiological and evolutionary mechanisms. We first clarify the nature of oxygen bioavailability in water, developing a new mass-transfer model that can be adapted to compare warming impacts on organisms with different respiratory systems and flow regimes. By distinguishing aerobic energy costs of moving oxygen from environment to tissues from costs of all other functions, we predict a decline in energy-dependent fitness during hypoxia despite approximately constant total metabolic rate before reaching critically low environmental oxygen. A new measure of oxygen bioavailability that keeps costs of generating water convection constant, predicts a higher thermal sensitivity of oxygen uptake (Q10 ~1.24) in an amphipod model, than do previous oxygen supply indices. More importantly, by incorporating size- and temperature-dependent costs of generating water flow, we propose that oxygen limitation at different body sizes and temperatures can be modelled mechanistically. We then report little evidence for oxygen limitation of growth and adult size under benign warming. Yet occasional oxygen limitation, we argue, may, along with other selective pressures, help maintain adaptive plastic responses to warming. Finally, we discuss how to overcome flaws in a commonly-used growth model that undermine predictions of warming impacts.

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Controversial roles of oxygen in organismal responses to climate warming

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Abbreviations used in text: OSI, Oxygen Supply Index; TSR, Temperature-Size Rule.

Key words: oxygen limitation, mass-transfer, temperature-size rule, thermal adaptation.

Abstract

Despite the global ecological importance of climate change, controversy surrounds how oxygen affects the fate of aquatic ectotherms under warming. Disagreements extend to the nature of oxygen bioavailability, and whether oxygen usually limits growth under warming, explaining smaller adult size. These controversies affect two influential hypotheses: Gill Oxygen Limitation, and Oxygen- and Capacity-Limited Thermal Tolerance. Here, we promote deeper integration of physiological and evolutionary mechanisms. We first clarify the nature of oxygen bioavailability in water, developing a new mass-transfer model that can be adapted to compare warming impacts on organisms with different respiratory systems and flow regimes. By distinguishing aerobic energy costs of moving oxygen from environment to tissues from costs of all other functions, we predict a decline in energy-dependent fitness during hypoxia despite approximately constant total metabolic rate before reaching critically low environmental oxygen. A new measure of oxygen bioavailability that keeps costs of generating water convection constant, predicts a higher thermal sensitivity of oxygen uptake (Q_{10} ~1.24) in an amphipod model, than do previous oxygen supply indices. More importantly, by incorporating size- and temperature-dependent costs of generating water flow, we propose that oxygen limitation at different body sizes and temperatures can be modelled mechanistically. We then report little evidence for oxygen limitation of growth and adult size under benign warming. Yet occasional oxygen limitation, we argue, may, along with other selective pressures, help maintain adaptive plastic responses to warming. Finally, we discuss how to overcome flaws in a commonly-used growth model that undermine predictions of warming impacts.

Introduction

One of the main global challenges facing humanity is how to mitigate and adapt to ongoing climate change (IPCC, 2021). Here, we focus on the role of oxygen in understanding and predicting impacts of warming, including heat waves, on water-breathing ectotherms. For decades, warming, combined with nutrients discharged into aquatic systems, have led to strongly decreased oxygen availability in oceans and lakes by accelerated consumption of oxygen by microbial respiration, reduced oxygen solubility and a reduction in oxygen replenishment from the atmosphere to water beneath the surface (Breitburg *et al*., 2018). Impacts of low oxygen (i.e. hypoxia) are pervasive, affecting all level of biological organization (Woods *et al*., synthetic manuscript). However, whether and how oxygen limitation is responsible for warming-induced changes in growth, adult size and survival outside such oxygen-depleted zones (i.e. under normoxia) is controversial (e.g. Schulte, 2015; Verberk *et al*., 2016b; Lefevre *et al*., 2017; Pauly and Cheung, 2018; Pörtner *et al*., 2017; Jütfelt *et al*., 2018; Audzijonyte *et al*., 2019; Pauly, 2021; Verberk *et al*., 2021), and is a major focus of this paper.

Oxygen limitation arises when the oxygen supplied to tissues is insufficient to meet metabolic demands. But oxygen limitation may be manifest not just as direct physiological impacts on the organisms (e.g. anaerobiosis, reduced growth and reproduction), but also as changes in allocation of metabolic energy among different functions (e.g. locomotion or anti-predator defense; e.g. Roman *et al*., 2019). Adaptive responses should therefore allocate energy or oxygen in ways that minimize (oxygen-dependent) reductions in fitness.

Warming-induced oxygen limitation is considered a particular risk for water-breathers, which rely strongly on the ability to move water - a dense and viscous medium - quickly over respiratory exchange surfaces (Forster *et al*., 2012; Verberk *et al*., 2011). Addressing the many controversies in this field is beyond the scope of this paper, and we do not expect a consensus about the importance of warming-induced oxygen limitation until we have sufficient relevant data that includes not just measurements of organism performance across temperatures and oxygen bioavailabilities, preferably in field experiments, but also include other conditions found in nature that impose high levels of demand (e.g. large body sizes, high levels of locomotion and other metabolic activity) (Verberk *et al*., 2021). However, we will discuss two aspects of oxygen limitation where we both clarify concepts and propose ways to improve investigations: (i) how warming affects oxygen bioavailability to the tissues of animals, and (ii) whether warming causes oxygen limitation of animal growth and adult size under conditions typically experienced in the field.

For the first aspect, related to the bioavailability of oxygen, we will account for the crucial but typically omitted role of water flow, by presenting a new mass-transfer model. Flowing water, whether imposed by the environment or by active ventilation, helps organisms to cope with warm and low-oxygen conditions, as demonstrated for anurans and salamanders (Pinder and Feder, 1990; Rollinson and Rowe, 2018), fishes (Rubalcaba *et al*., 2020; Dahlke *et al*., 2020, 2022) and aquatic insects (Verberk *et al*., 2016c; Jones *et al*. 2018; Frake *et al*., 2021). But moving oxygen from the surrounding water to tissues can be costly, amounting to about 10% of resting metabolism in rainbow trout (Jones and Schwarzfeld, 1974), which

is likely to increase under challenges of reduced oxygen availability or increased demand, thus likely constituting an important component of oxygen limitation.

The second aspect, considering whether, under normoxia, oxygen generally limits aspects of organism performance at increased temperatures, has produced disagreements which we expect will be reduced when an evolutionary approach is incorporated into the physiological analysis. We specifically focus on controversies that arise from, firstly, not fully distinguishing proximate physiological from ultimate evolutionary factors and, secondly, from using a classic growth model that contains inappropriate assumptions, which lead to unrealistic predictions.

1. Climate controversies: Oxygen bioavailability

(i) Contrasting viewpoints, and an alternative approach to estimating oxygen bioavailability

Ecologists and physiologists have traditionally emphasized the importance of different metrics of oxygen bioavailability and thus its environmental sensitivity. Ecologists have focused on correlating the concentration of oxygen in water with organismal properties such as their size, abundance and diversity. Chapelle and Peck (1999), for instance, demonstrated a positive linear relationship between maximum amphipod body length and oxygen concentration across a wide range of habitat salinities and temperatures. In contrast, physiologists have focused on the physical process of oxygen movement within water and across a diffusion barrier, and the importance of the oxygen partial pressure difference (Δp_{O2}) in driving the rate of oxygen diffusion ($\dot{M}_{O2}$) through media with different

oxygen-carrying properties, such as water and blood, and across a diffusion barrier (Piiper *et al*., 1971; Dejours, 1975; Spicer and Gaston, 1999). The partial pressure (p_{O2}) and concentration of oxygen in water (c_{O2}) are linked through the solubility coefficient of oxygen in water (α_{wO2}) in Henry's Law ($c_{O2} = \alpha_{wO2}.p_{O2}$).

Verberk *et al*. (2011), attempted to reconcile these two viewpoints, applying Fick's first law of diffusion to a static diffusion barrier (Piiper *et al*., 1971) to produce a metric of oxygen bioavailability (the Oxygen Supply Index, or OSI), which is proportional to the product of partial pressure of oxygen in water, and the solubility and diffusion (D_{wO2}) coefficients of oxygen in water ($OSI \propto p_{O2}.\alpha_{wO2}.D_{wO2}$). Verberk *et al*., (2011) estimated temperature effects on oxygen bioavailability by combining the thermal sensitivities of solubility and diffusivity, as was done previously (Woods, 1999; Atkinson *et al*., 2006). In this Oxygen Supply Index, reductions in oxygen solubility (here measured as the factorial change with 10°C warming, $Q_{10,} \sim 0.81$ in freshwater and 0.83 in seawater of salinity 35 p. s. u.) are slightly outweighed by increases in diffusivity (thermal sensitivity, $Q_{10,} \sim 1.3–1.4$; see Table S1, Supplement S1, available online), thereby slightly increasing oxygen bioavailability according to the OSI ($Q_{10} = 1.05–1.16$). Consequently, they concluded that reductions in aerobic performance in warmer waters do not arise from lower oxygen concentrations, but instead through organismal oxygen demand exceeding supply.

Although our theoretical analyses agree with this qualitative conclusion, our predictions are quantitatively different. Moreover, we will show that the OSI does not account for the complexities of oxygen delivery from water to blood, and how environmental sensitivity of

oxygen bioavailability crucially depends on water flow (Pinder and Feder, 1990; Frakes *et al*., 2021) including the diverse flow regimes involved in oxygen uptake by aquatic ectotherms. To illustrate, we provide an alternative, mass-transfer, perspective that accounts for different respiratory flow regimes and their energetic costs, and which can produce estimates of the thermal sensitivity of oxygen bioavailability different from that of the OSI.

Mass-transfer formulae can incorporate combinations of convection and diffusion to describe the physical factors influencing the movement of solutes in flowing liquids, or through static layers (Cussler, 2009). Although well established in industrial processes, such models have been applied only infrequently to gas transfer at respiratory surfaces despite the development of formulae that describe gas transfer scenarios applicable to at least some aquatic respiratory gas exchange systems (Cussler, 2009). Hills and Hughes (1970) applied the concept of mass transfer to the body mass-scaling of oxygen uptake at fish gills. Rubalcaba *et al*., (2020) used mass-transfer modelling to predict thermal sensitivity to oxygen delivery through an external boundary layer, balanced against metabolic oxygen demand. Deutsch *et al*. (2022) also used mass-transfer equations to model oxygen bioavailability across a size range from unicells to large fish.

A benefit of applying mass-transfer models to oxygen transfer, from high to low partial pressure across a respiratory diffusion barrier, is a simplified quantitative estimation of the thermal sensitivity of multiple physical factors (including kinematic viscosity, diffusivity, solubility) affecting uptake. Empirically-derived and biologically-realistic mass-transfer

154 correlations (Cussler, 2009) enable the effects of environmental change, including
155 temperature, on oxygen uptake to be quantified for organisms with different respiratory
156 water flow regimes (e.g. laminar *versus* turbulent flow through open *versus* closed channels
157 or along differently shaped surfaces).
158
159 Our illustrative example of mass-transfer modeling (Supplement S1, available online) uses
160 amphipods, a group of aquatic crustaceans that, at rest, generate a turbulent water current
161 through a ventral groove *via* the rhythmic beating of their pleopods (Sutcliffe, 1984).
162 Hemolymph flows through the lacunar space within gill plates, situated at the inward base
163 of their thoracic limbs (Steele and Steele, 1991). These gill plates, suspended within the
164 ventral groove, are assumed here to be the principal site of respiratory gas exchange in our
165 idealized adult amphipod model, even though other, extrabranchial sites of respiratory gas
166 exchange, may occur (Steele and Steele, 1991; Spicer & Gaston, 1999).
167
168 The full derivation of the model is presented in Supplement S1, available online. Here, we
169 focus on those salient features that help improve exploration and understanding of
170 mechanism and the quantification of oxygen bioavailability in response to warming.
171
172 ***(ii) Potential declines in partial pressure differences with warming***
173 The fundamental equation that underlies the movement of oxygen across a respiratory gas
174 exchange surface is:
175

$\dot{M}_{O2}/A = \Delta p_{O2w\text{-}h}/R_{O2}$

[Equation 1]

(Hills and Hughes, 1970), where $\dot{M}_{O2}/A$ is rate of movement of oxygen (uptake, mol. s^{-1}) per unit respiratory gas exchange surface area (A, cm^2). $\Delta p_{O2w\text{-}h}$ is the oxygen partial pressure difference (atm) between water and blood, or hemolymph, on either side of the respiratory exchange surface, and R_{O2} is the total resistance (s. atm. cm^2. mol^{-1}) to oxygen movement in the direction of the partial pressure gradient.

Indices of oxygen bioavailability such as the OSI assume that the oxygen partial pressure difference is temperature independent because the effect of temperature on the total sum of all atmospheric gases via changes in water vapour pressure is negligible at sea level and across the realistic range of water temperatures (Verberk *et al*., 2011). However, when applied along a path of water flow where oxygen is being extracted, as in the amphipod ventral groove (Fig. 1) this assumption may need to be revisited. Although the system is open anatomically, it is treated physiologically as a closed gas exchange space, with no oxygen diffusion or admixture of water from outside, and a fully formed turbulent boundary layer within the ventral groove.

As oxygen is extracted at the gill plates, the partial pressure of oxygen in inflowing water (p_{O2i}) pumped through the ventral groove falls in line with the decline in the mass of dissolved oxygen. At lower solubilities (such as at higher temperature), p_{O2} will fall more rapidly for a fixed $\dot{M}_{O2}$ and rate of water volume flow $\dot{V}_w$. This effect cannot occur in

isolation, however, because the partial pressure difference between water and hemolymph across the gill diffusion barrier ($\Delta p_{O2w\text{-}h}$) will also decline, which decreases the potential driving oxygen movement from water to hemolymph, which reduces $\dot{M}_{O2}$ (Eqn. 1). The model (Supplement S1, available online) therefore utilizes iteration to estimate how variation in environmental p_{O2}, $\dot{M}_{O2}$, water volume flow ($\dot{V}_w$) and oxygen solubility coefficient (α_{wO2}) influences $\Delta p_{O2w\text{-}h}$, and consequently feeds back to affect $\dot{M}_{O2}$, .

(iii) The mass-transfer coefficient accounts for flow regime

The product of the mass-transfer coefficient for oxygen in water (k_{wO2}) and solubility coefficient of oxygen in water (α_{wO2}) is inversely proportional to the resistance in the fundamental model of oxygen movement (R_{O2} in Eqn. 1; also Eqn. S3). In mass-transfer scenarios involving both forced convective flow and diffusion, convective flow is expressed using the Reynolds number (ratio of inertial to viscous forces, which increases with increasing linear water velocity and turbulence) (Cussler, 2009). Diffusion is expressed using the Schmidt number (ratio of momentum diffusivity, or kinematic viscosity, to molecular diffusivity) (Cussler 2009). These dimensionless numbers are combined to determine the mass-transfer coefficient k which is expressed within the dimensionless Sherwood number (ratio of convective mass transfer to diffusive mass transport) (Cussler, 2009). Thus:

$$\text{Sherwood number} = k_{wO2}.h/D_{wO2} = \text{Constant}.(\text{Reynolds number})^{x}.(\text{Schmidt number})^{y} \quad \text{[Equation 2]}$$

where h is the width of the channel and D_{wO2} is the diffusion coefficient of oxygen in water. All mass-transfer coefficients are determined empirically using forms of the above equation that apply to different flow regimes and yield the exponents x and y for the Reynolds and Schmidt numbers, respectively (Cussler, 2009; e.g., Eqn. S10, Supplement S1, available online).

The Sherwood number for turbulent water flow through a horizontal channel, which is applicable to the amphipod system (Figure 2), can be rearranged to derive a mass-transfer coefficient that is incorporated into the fundamental model of oxygen movement (Eqn. 1) for a constant environmental partial pressure of oxygen, thus:

$$\dot{M}_{O2} \propto \Delta p_{O2w\text{-}h}.A.\alpha_{wO2}.D_{wO2}^{2/3}.v_w^{4/5}/(\upsilon_w^{7/15}.h^{1/5}) \qquad \text{[Equation 3]}$$

Where α_{wO2} is the solubility coefficient of oxygen in water, v_w is linear water velocity through the channel and υ_w is the kinematic viscosity of water (Supplement S1, available online).

In conclusion, incorporating mass-transfer coefficients into oxygen uptake models, as in Eqn. 3, alters the temperature-sensitive contributions to oxygen uptake rate, relative to those predicted by the OSI. Here, for example, the contribution of the diffusivity coefficient to the temperature sensitivity of $\dot{M}_{O2}$ is now raised to the power of 2/3 rather than 1, and a dependency on warming-induced changes in the kinematic viscosity of water to the power of -7/15 is introduced. A further benefit is that the effects of phenotypic adjustments of organism-specific, structural features of gas exchange systems, such as the width h of the

244 respiratory water channel, on warming-induced changes in oxygen uptake rate can be
245 assessed.
246
247 ***(iv) Temperature-dependence of oxygen bioavailability – controlling for oxygen***
248 ***movement costs***
249 Incorporating the energy cost involved in generating the water flow is essential because
250 the actual variation in oxygen available to the organism will be the whole-organism $\dot{M}_{O2}$
251 minus the aerobic cost of generating that $\dot{M}_{O2,}$ (residual $\dot{M}_{O2} = {}_{r}\dot{M}_{O2}$). In our idealized
252 amphipod example, we consider aerobic metabolism associated with ventilation of water
253 (${}_{v}\dot{M}_{O2}$), as an index of the aerobic costs of oxygen movement from environment to tissues
254 (including ventilation and circulation). The cost of ventilation is generally considered a
255 potential limiting factor in hypoxia in fishes (e.g. Wood, 2018), and ventilatory costs are
256 predicted to be considerably higher than circulatory costs in fishes (Farrell & Steffensen,
257 1987). Moreover, information is currently insufficient to allow modeling of circulatory
258 costs in amphipods; but future modeling of circulatory costs may be incorporated into
259 costs of oxygen movement for species with more information on circulation, such as fishes.
260
261 To compare oxygen bioavailability across temperatures for particular respiratory flow
262 regimes, $\dot{M}_{O2}$ can be predicted where ${}_{v}\dot{M}_{O2}$ does not change: this is a measure of the
263 temperature-dependence of oxygen bioavailability from the perspective of all metabolic
264 functions other than those directly associated with the physical movement of oxygen.
265

The rate of aerobic energy expenditure or power used to pump water can be expressed in the same fundamental units as $\dot{M}_{O2}$. Therefore, changes in power spent pumping water through the central groove (P_w) and ${}_v\dot{M}_{O2}$ are directly interchangeable, assuming constant aerobic muscle efficiency. Vogel (1994) presented formulae expressing power spent in ventilation as proportional to the product of resistance to water flow and the square of the rate of flow of water volume; and in these equations the resistance to water flow through a narrow horizontal channel was presented as being proportional to the dynamic viscosity of water (μ_w). Therefore, when P_w is kept constant and body dimensions are unchanged, the rate of flow of water volume is proportion to $\mu_w^{-1/2}$ (Supplement S1, available online). When ratios of body dimensions are invariant, the rate of flow of water volume is also proportional to the linear water flow rate.

By fixing P_w at a constant value and assuming body dimensions are invariant, Eqn. 3 can be modified (Eqn. S18, Supplement S1, available online) to indicate how $\dot{M}_{O2}$ and its contributory components vary with temperature when the aerobic cost of ventilating the ventral groove remains constant. These contributions of components of $\dot{M}_{O2}$ responses to temperature are shown in Fig. 3.

(v) Thermal sensitivity of components of oxygen bioavailability with constant ${}_v\dot{M}_{O2}$

Figure 4 contrasts the thermal sensitivities of uptake that relies on solubility alone; the combination of solubility, diffusivity and p_{O2} (the latter being constant) in the OSI (Verberk *et al.*, 2011); and the variation in ${}_r\dot{M}_{O2}$ predicted in the amphipod system, with constant ${}_v\dot{M}_{O2}$.

289

290 Thus, when the amphipod makes no change in the ventilatory effort of physically moving
291 oxygen from water towards tissues, warming from 0 to 30°C increases oxygen
292 bioavailability for other aerobic metabolism (${}_{r}\dot{M}_{O2}$) by about 90% ($Q_{10} \sim 1.24$) as a result of
293 the interaction of the physical variables described in Fig. 3 and Eqn. 3. The greater increase
294 in oxygen bioavailability with temperature in amphipods, in comparison with the OSI, is
295 largely due to the inclusion of decreasing water viscosity at higher temperatures, which not
296 only directly increases $\dot{M}_{O2}$ in turbulent flow regimes, but also makes ventilation less costly
297 and thus allows greater ventilation under a constant effort (see also Verberk and Atkinson,
298 2013 for discussion of viscosity effects). Moreover, warming-induced increases in $\dot{M}_{O2}$ (and
299 hence ${}_{r}\dot{M}_{O2}$) at constant ${}_{v}\dot{M}_{O2}$ may be greater still if the aerobic efficiency of muscle function
300 increases with temperature.
301

302 This mass-transfer modeling framework highlights how physiologically-realistic estimates
303 of oxygen movement and associated energetics can provide a more realistic view of oxygen
304 supply capacity and hence the (residual) energy available to an organism when faced with
305 warmer water. The framework is flexible and can also include effects of increasing rates of
306 oxygen movement in response to diverse and interacting factors (e.g. hypoxia, activity,
307 increasing temperature or body size), and be used to partition oxygen bioavailability for all
308 metabolic processes not associated with oxygen movement (e.g. maintenance,
309 reproduction and growth). Consequently, it provides more general, mechanism-based
310 predictions of the consequences of life history evolution.
311

(vi) The importance of oxygen supply capacity

When an increase in ventilation is unable to satisfy the requirements of residual metabolism, the oxygen supply capacity is reached, and oxygen becomes limiting. Therefore, mass-transfer models that incorporate the cost of increasing ventilation will determine not just oxygen bioavailability or supply that balances instantaneous demand, but also the oxygen supply *capacity*, hence the point at which oxygen becomes limiting.

To prevent oxygen limitation, organisms must therefore be able to boost oxygen delivery to meet tissue oxygen demand. Aside from finding locations with greater oxygen availability (Kramer, 1987), organisms can boost oxygen bioavailability by: increasing ventilation that generates water convection over exchange surfaces; increasing the respiratory exchange surface area or conductance (Nilsson et al., 2012; Funk *et al*., 2021); and various adjustments in the internal oxygen exchange and delivery systems (Woods and Moran, 2020). Therefore, temperature effects on oxygen limitation should compare thermal sensitivity of metabolic rate (oxygen demand) with the thermal sensitivity of oxygen supply *capacity,* which accounts for organism's behavioral and plastic responses to improve oxygen uptake with warming (Deutsch *et al*., 2015; Kielland *et al*., 2019; Seibel and Deutsch, 2020), rather than with simple measures of oxygen bioavailability (*DO, pO_2, OSI*).

Uptake capacity may be estimated in conditions at or below critical oxygen partial pressures, $p_{crit O2}$ from the change in oxygen uptake by the organism per unit increase in environmental oxygen partial pressure (Kielland *et al*., 2019; Seibel and Deutsch, 2020). The thermal sensitivity of oxygen supply capacity compares how temperature increases

this measure, However, although this empirical approach may be simpler than mechanistic mass-transfer modeling, it does not isolate the costs of moving oxygen, which our model predicts will increase as pO_2 is reduced towards $p_{crit O2}$. Consequently, rather than being constant, ${}_r\dot{M}_{O2}$ is predicted to decline as pO_2 is reduced towards $p_{crit O2}$.

2. Climate controversies: Oxygen limitation

(i) Limitation – proximate or ultimate?

Oxygen limitation of aerobic scope (difference between maximum and standard metabolic rates) has been implicated in heat-induced reduction in organismal performance (Pörtner 2010, 2017). Oxygen limitation has also been proposed as the proximate mechanism causing growth rates to decline in water-breathing ectotherms as size increases, especially at increased temperatures (Pauly,1981, 2010, 2021). The widespread phenotypically plastic decline in late-ontogeny growth rate and a reduced mature or final body size under benign environmental warming, when stressfully high temperatures, food shortage and reduced environmental oxygen bioavailability are all avoided, has been called the Temperature-Size Rule (TSR; Atkinson, 1994). Under these benign conditions the idea of oxygen limitation as a proximate mechanism becomes contentious (Audzijonyte *et al*., 2019; Seibel and Deutsch, 2020; Pauly 2021; Verberk *et al*., 2021; Wootton *et al*., 2022).

Experimental tests of direct oxygen limitation should employ increased oxygen bioavailability above levels normally experienced by the organisms (e.g. hyperoxia for species adapted to normoxic conditions) at different temperatures. If oxygen generally limits growth and mature size, such tests would reveal that hyperoxia enhances late growth

and causes animals to mature at a larger size, especially in the warm. But such tests using various air- and water-breathers do not provide strong support for oxygen limitation, as they have either produced no or very small increases in mature body sizes, in contrast to the much greater size response (reductions) under hypoxia (Verberk *et al*., 2021; Funk *et al*., 2021). Experimental reduction in oxygen availability (growth responses reviewed in Verberk *et al*., 2021) are not appropriate tests of oxygen-limitation, as they only show that oxygen can become limiting if made less available, but not that oxygen becomes limiting under the warmer, normoxic conditions where declines in growth rate late in ontogeny or other measures of performance are still observed. This general principle of employing hyperoxia to test for oxygen limitation has also been adopted by Seibel and Deutsch (2020), who collated data on Maximum Metabolic Rate at different oxygen conditions, including hyperoxic conditions, to derive the critical oxygen partial pressures, $p_{crit02,}$ of Maximum Metabolic Rate. Their findings, for terrestrial and shallow-living aquatic species (38 species, mainly arthropods, mollusks and chordates) supported the idea that oxygen starts to limit Maximum Metabolic Rate below 100% air-saturation (i.e. normoxia), but again that 100% air-saturation is not limiting within the normal temperature range of the species.

Other evidence presented to support the idea that oxygen normally limits growth of water-breathing ectotherms requires further scrutiny. One example is the assertion by Pauly (2021) that the change in enzymes in tissues from mainly oxidative to mainly glycolytic as water-breathers grow supports his hypothesis of Gill Oxygen Limitation. But a fuller evaluation of all relevant evidence would include identifying which tissues, hence which functions, are affected. If changes are mainly to white muscle, an alternative explanation to

gill oxygen limitation is maintaining length-specific burst speeds (Childress and Somero, 1990). Also, in the context of climate warming, the role of temperature on any shift towards glycolysis with increased size should be included in the analysis, since the problem to be solved constitutes a three-way interaction between size, temperature and oxygen (Woods & Moran, 2020; Verberk *et al*., 2021). Moreover, alternative hypotheses should be evaluated, including that the lower mass-specific metabolic rate of large animals is not determined by oxygen limitation (Glazier, 2014); instead, these animals may have an advantage over small animals when having to rely on glycolysis because small animals will reach lethal levels of anaerobic end-products faster (Nilsson and Ostlund-Nilsson, 2008).

Despite the poor support for oxygen limitation persistently slowing growth and reducing body size with warming within the physiological range and under oxygen partial pressures typical for a species, further evidence still suggests a role for oxygen. Warming-induced reductions in mass-scaling exponents of maximum but not resting metabolic rates in European Perch (*Perca fluviatilils*) (Christensen *et al*., 2020) and in a quantitative analysis of 286 fish species (Rubalcaba *et al*., 2020) did suggest that larger individuals may be more susceptible to oxygen limitation, especially if they are in warmer water and if they are active.

To reconcile the apparently conflicting evidence, we propose that oxygen limitation may play mainly a selective (ultimate) role, rather than proximately limiting growth. When oxygen limitation is considered an ultimate factor, a reduction in growth rate during late ontogeny especially at warmer temperatures, evolves as a phenotypically plastic response

to temperature that helps maintain sufficient capacity for oxygen uptake (e.g. a safety margin, such as aerobic scope, see Atkinson *et al*., 2006) under a range of conditions. Most frequently these conditions will not tax an animal to its capacity limits and the animal is able to avoid oxygen limitation. But more extreme events could act as a selection pressure: these include episodes of hypoxia reducing oxygen bioavailability, or of events that increase oxygen demand such as extreme warming, disease, predator attack, or digestion of large meals (Jütfelt *et al*., 2021) or their combinations (i.e. warm water and high activity; Rubalcaba *et al*., 2020). During these selective events, actual, episodic oxygen limitation can occur. If large individuals with lower ratio of respiratory surface area to oxygen-consuming body mass are more likely disadvantaged, they will have been selected against during such events. This selection explains the evolution of phenotypes that reduce their growth rate during late ontogeny especially at warmer temperatures, hence maintaining safety margins for oxygen uptake - an idea termed 'The Ghost of Oxygen Limitation Past' (Verberk *et al*., 2021).

The finding that gill surface area does not generally decrease relative to standard, and maximum metabolic rate (MMR) as teleost fish grow (Scheuffele *et al*., 2021) provides more evidence (especially from MMR) against the Gill Oxygen Limitation hypothesis of Pauly (1981, 2010, 2021), which is based on a progressively increasing surface area limitation. The solitary empirical test of temperature effects (Li et al., 2018), shows the need for further such investigations. Li *et al*. (2018) appear to find a suggestion that gill surface area, when expressed as a ratio of standard metabolic rate, is reduced during the growth of goldfish (*Carassius auratus*) at higher (25°C) but not at lower (15°C) acclimation

temperature; but neither ontogenetic trend was significantly different from zero (Scheuffele *et al*., 2021). Consequently, the available gill surface evidence does not support direct Gill Oxygen Limitation; yet it may not contradict the Ghost of Oxygen Limitation Past, which invokes selection producing phenotypes that have avoided oxygen limitation at all sizes. Thus, the selected fish could have gill surfaces that can normally satisfy oxygen uptake requirements throughout ontogeny *via* a combination of: (i) matching gill surface area and other contributions to oxygen uptake with maximum oxygen demand and (ii) body size reduction in warm water that avoids gill surface limitation of oxygen uptake at the largest sizes, where a geometric challenge of matching gill surface area to demand would become more difficult.

Testing ultimate explanations or "seeing ghosts" can be challenging. The "Ghost of Oxygen Limitation Past" could be tested using experimental evolution (Atkinson *et al*., 2006; Walczynska and Sobczyk, this volume). Such experiments should decouple the proximate cause or environmental cue (temperature) from the ultimate cause (protecting aerobic scope from oxygen limitation). Thus, each selective regime would simulate fluctuations in both temperature and oxygen partial pressure, with regimes differing by having oxygen partial pressures either predictably positively, negatively or uncorrelated with temperature. Oxygen limitation in the warm would be reduced by a positive temperature-partial pressure correlation and increased by a negative correlation, which is predicted to select for traits that would protect aerobic scope (e.g. a strong reduction in adult size with warming). As such experiments will be tightly controlled, and likely done in the laboratory, they can only demonstrate that the evolution of body size responses to temperature *can* be

generated by selection to protect aerobic scope, but would not demonstrate that this *is* the cause of the warming-induced reductions in late growth and adult size in the field.

A remaining argument is that the "Ghost of Oxygen Limitation Past" may be deemed superfluous if there is little or no evidence for oxygen limitation across a wide range of body sizes and temperatures at field oxygen partial pressures, as described by Seibel and Deutsch (2020). A counter-argument to this is that, within each species, selection from extreme events on the oxygen budget, which may be infrequent, can help to maintain the adaptive match of oxygen supply to demand that is observed most of the time for all sizes, temperatures and activity levels.

In conclusion, evidence does not favour oxygen usually limiting growth directly (proximately) under warming within the physiological range and under typical oxygen partial pressure, as proposed in the Gill Oxygen Limitation hypothesis (Pauly, 2021). But occasional oxygen limitation that produces evolutionary effects on growth rates at large sizes ('the Ghost of Oxygen Limitation Past') cannot be ruled out as an explanation for widespread warming-induced deceleration of growth and reduced adult size in aquatic ectotherms. Moreover, away from the benign conditions of a species' normal oxygen bioavailability and physiological temperatures, environmental warming that places new and increased demands on an animal's oxygen budget, may then lead to oxygen limitation.

(ii) Avoiding critical flaws in growth models

Several models that explore how oxygen limits growth and which quantitatively predict the Temperature-size rule (TSR) have greatly overestimated warming-induced reductions in body size. For instance, the magnitude of warming-induced body size reduction in an aquatic crustacean, predicted from a model that assumed oxygen limitation, overestimated body size reductions by about an order of magnitude (Einum *et al*., 2021). Similar overestimation of body size reduction occurs in fish (Cheung *et al*., 2013; Lefevre *et al*., 2018). The quantitative mismatch between observed and predicted size reduction recorded by Einum *et al*., (2021) occurred even when they avoided problems of comparing demand with passive oxygen uptake by instead using their measure of oxygen supply capacity that accounts for improvements in uptake with warming (Kielland *et al*., 2019).

One source of error comes from the choice of growth models used. For over a century, classic models of organism growth and metabolism have been used to predict reduced adult or final body size at increased temperatures (Pütter, 1920, von Bertalanffy, 1960; Cheung *et al*. 2013, Kearney 2021). These models express growth rate as the difference between the rate of surface-related acquisition of resources available to supply growth and other metabolic activities (variously considered as 'anabolism' (Pütter, 1920, von Bertalanffy, 1960) or 'assimilation' (first term on right side of Eqn. 4) and the rate of metabolic breakdown of organic resources (second term on right side of Eqn.4) - characterized as 'catabolism' (Pütter, 1920; von Bertalanffy, 1960) or 'maintenance metabolism' (Cheung *et al*., 2013; Kearney, 2021).

$$dm/dt = Hm^a - Km^c \qquad \text{[Equation 4]}$$

where m is mass, t is time, H and K are coefficients of anabolism (or assimilation) and catabolism (or maintenance metabolism) respectively, and a and c are their respective exponents. In these models, relative to the rates of anabolism (assimilation), the rate of catabolism (maintenance metabolism) scales more steeply with increasing body mass ($c > a$) and the coefficient for catabolism is more sensitive to temperature ($dK/dT > dH/dT$, where T is temperature) (Atkinson and Sibly, 1997; Kearney, 2021; Pauly, 2021). These models can predict the observed increase in initial growth rate with warming (Atkinson and Sibly, 1997) as well as the decrease in growth rate with warming later in ontogeny.

However, it is important that any modeled decline in growth should allow for individuals to have sufficient capacity for oxygen uptake and energy acquisition in order to complete reproduction (Kozłowski *et al*., 2004; Kearney, 2019; Pauly, 2021). Moreover, growth models should incorporate the amount of resources diverted from late growth towards reproduction (Day and Taylor, 1997; Kozłowski *et al*., 2004; Kearney, 2019; Marshall and White, 2019), which is predicted by life-history optimization to arise from selection by external mortality (Kozłowski *et al*., 2004). We suggest that part of the ongoing controversy about what determines the shape of growth curves when resources are abundant (Pauly, 2019; Marshall and White, 2019; Kearney, 2019; White and Marshall, 2019) may be reduced if: (i) explanations incorporating the ultimate (selective) effect of oxygen or other resource limitation (Section 2i, above) are prioritized over proximate constraints of oxygen or energy shortage on growth, which we have shown is unlikely to apply widely in favorable conditions, and (ii) the debate is not polarized as selection either on increased

517 reproduction or on avoiding oxygen (or other resource) limitation; both may need to be
518 considered.
519
520 Additionally, a particularly crucial flaw in these classic models, including the variant by
521 Einum *et al*. (2021), is that they predict a reduction in growth efficiency with warming; this
522 runs counter to the typically increased efficiency observed under benign conditions in the
523 physiological temperature range (Angilletta and Dunham, 2003). However, it would be
524 informative to investigate whether warming enhances efficiency at small but not at large
525 sizes during ontogeny - which would mirror the observed effects of temperature on growth
526 rate during ontogeny.
527
528 It remains to be determined whether this failure to avoid unrealistic reductions in
529 warming-induced growth efficiency applies also to a recent model that quantitatively
530 predicts the TSR across a range of sizes from microbes to large fish (Deutsch *et al*., 2022).
531 Observations used to parameterize this model indicate that hypoxia tolerance typically
532 declines with size during growth - slightly in fish and more strongly in smaller species - and
533 also with increased temperature. This model did not examine growth trajectories explicitly,
534 though it is worth exploring whether its predicted warming-induced body size reduction
535 arises from assumptions that match those used in the classic growth models. Specifically,
536 does the greater temperature dependence and shallower mass-scaling of metabolic or
537 oxygen demand than of oxygen supply efficacy also predict a warming-induced reduction in
538 growth efficiency at all sizes? Such a finding would run counter to the observed warming-
539 enhanced growth efficiency under conditions relevant to the TSR (Angilletta and Dunham,

2003). Unlike the previous models of the TSR, that of Deutsch *et al.* (2022) predicts well the interspecific mean and variability of body size reduction with warming. It is therefore important to know whether unrealistic warming-induced reductions in growth efficiency are mostly avoided in this model, or whether the improved predictions of TSR emerge despite an unrealistic formulation.

This problem with how the growth models are applied to predicting the TSR (Angilletta and Dunham, 2003) is not new, but the continued use of such models requires measures to overcome the problem. More generally, the models poorly capture effects of size and temperature on metabolism. Thus, instead of a simple difference in temperature-dependence of assimilation and maintenance metabolism in the classic growth model, a more realistic growth model would capture changes in multiple costs, efficiencies and resource allocations with: (i) temperature (e.g. enzyme titers and efficiencies; viscosity effects on movement in water; Verberk and Atkinson, 2013); (ii) body size (e.g. size-scaling of costs of locomotion and ventilation); and (iii) combined size and temperature (e.g. viscosity effects on scaling of ventilation; Section 1; Verberk and Atkinson, 2013; Verberk *et al*., 2021). At different sizes and temperatures, adjustments may be made to the capacity for uptake of limiting resources (e.g. Sollid *et al*., 2005; Sollid and Nilsson, 2006) (the upper metabolic limit, considered the upper boundary of the oxygen supply safety margin above routine expenditure), to the size of the safety margin, and to the amount of other routine investment, which is not just tissue maintenance but may also include routine costs of locomotion and resource capture (Verberk *et al*., 2021). Not accounting for these adjustments and how they vary between species is likely to produce erroneous predictions

563 of warming-induced shrinkage in adult body size. However, modeling all these adjustments
564 individually will likely be prohibitive, and therefore alternative ways of capturing size-
565 dependent responses to temperature will be required. One step towards resolving the
566 problem could include allowing not just the coefficients to be temperature-dependent, but
567 also the exponents, *a* and *c* as individuals grow larger (Kozłowski et al., 2004), which is
568 more likely to reflect observed metabolic responses (e.g. Rubalcaba *et al*., 2020). It is also
569 important to correctly characterize empirically the two terms on the right side of Equation
570 4, so that their difference equates to growth and nothing else. For instance, if standard
571 metabolic rate is used as a measure of maintenance metabolism or catabolism, its growth
572 overheads will need to be accounted for (Rosenfeld *et al*., 2015). Moreover, an assumption
573 of isometric scaling is not often supported for metabolism (hence catabolism) at various
574 levels of activity including standard, resting and routine (Glazier, 2005.
575
576 One potential solution to prevent overestimating warming-induced reductions of body size
577 may be to incorporate mass-transfer dynamics. Einum *et al*. (2021) did not use such
578 dynamics in their model that overestimated size reduction, and assumed that uptake
579 capacity scaled with surface area (exponent around 0.67). However, Deutsch *et al*. (2022),
580 used mass-transfer modeling to compare responses of species across the size range from
581 aerobic microbes to large metazoans, examining the relative roles of convection and
582 diffusion on oxygen movement through the stagnant boundary layer surrounding
583 respiratory exchange surfaces. They predicted a much lower scaling exponent of around
584 0.3 for microbes, leading to a lesser warming-induced body size reduction compared with
585 that of larger metazoans, as is generally observed (Forster *et al*., 2012).

Conclusion

To improve estimates of oxygen bioavailability and to provide mechanistic models of oxygen limitation, we have applied mass-transfer modeling to quantify how water flow regime at external gas exchange surfaces affects oxygen bioavailability. Applying this modeling framework to amphipods, we found a greater increase in oxygen bioavailability with warming ($Q_{10} \sim 1.24$) when costs of moving oxygen are kept constant, compared with using the Oxygen Supply Index (Verberk *et al*., 2011) ($Q_{10} \sim 1.05$-1.16). Unlike the OSI, our measure of oxygen bioavailability is not a single generic index, but can be customized to particular respiratory systems (e.g. laminar *versus* turbulent flow through open *versus* closed channels or along differently shaped surfaces) and levels of respiratory activity. The framework therefore provides a more complete mechanistic approach to understanding variation in oxygen bioavailability among diverse water-breathers. However, a full exploration of impacts of different flow regimes and respiratory structures will be the subject of future work. A major further benefit from such modelling arises from its potential to generate realistic estimates of both the rate of oxygen uptake, and the aerobic cost of achieving such uptake. Thus, maximizing the oxygen available for all metabolic processes not associated with oxygen movement (e.g. maintenance, reproduction and growth) ($_{r}\dot{M}_{O2}$) depends on how much oxygen is devoted to moving oxygen from the environment to tissues. A consequence of this distinction between types of metabolic cost is that we predict an increase in costs of moving water and oxygen while $_{r}\dot{M}_{O2}$ decreases as pO_2 is reduced towards $p_{crit O2}$. This prediction challenges the idea that oxygen limitation of metabolic rate at $p_{crit O2}$ reflects the start of oxygen limitation on fitness. Instead, we predict

609 that reductions in metabolic activities contributing to fitness occur before p_{crit02} is reached.
610 The appearance of an approximately invariant total metabolic rate as oxygen partial
611 pressure is reduced below normoxia results from a simultaneous increase in investment in
612 generating water and oxygen movement. The modeling framework can be developed
613 further to provide mechanistic predictions of oxygen limitation and life history (e.g. body
614 size). Thus, by incorporating into the models the costs of moving water and oxygen, and
615 how these costs vary with size and temperature, oxygen limitation at different body sizes
616 and temperatures can help predict combinations of body size and temperature that avoid
617 oxygen limitation.
618
619 We argue that a physiological understanding should be combined with evolutionary
620 principles to clearly distinguish between oxygen as a proximate and as an ultimate factor.
621 We describe how experimental treatments that increase rather than reduce oxygen tension
622 should be used to test for proximate oxygen limitation. Under benign growth conditions
623 within the normal range of oxygen tensions and physiological temperatures used to
624 describe the Temperature-Size Rule, experimental hyperoxia provided little evidence that
625 oxygen normally limited growth and adult size. Overall, we found little support for
626 warming-induced oxygen limitation usually decelerating growth and producing small
627 adults. However, evidence was consistent with oxygen as an ultimate or evolutionary factor
628 leading to slowed growth and smaller size at increased temperatures that avoided oxygen
629 limitation – termed the Ghost of Oxygen Limitation Past. We proposed that experiments to
630 test this idea should decouple the proximate cause or environmental cue (temperature)
631 from the ultimate cause (protecting aerobic scope from oxygen limitation). We suggest that

part of the ongoing controversy about what determines the shape of growth curves when resources are abundant may be reduced if: (i) explanations incorporating the ultimate (selective) effect of oxygen or other resource limitation are prioritized over proximate constraints of oxygen or energy shortage on growth, which we showed was unlikely to apply widely in favorable conditions, and (ii) the debate is not polarized by arguing for selection just on increased reproduction or just on avoiding oxygen (or other resource) limitation; both may need to be considered. Finally, we highlight a crucial flaw in classic growth models that led to a predicted reduction in growth efficiency with warming, which runs counter to the typically increased efficiency observed under benign conditions in the physiological temperature range. Overall, this paper shows the importance of a multi-disciplinary approach, as advocated by Verberk *et al*. (2016a) and Audzijonyte *et al*., (2019), which here combines perspectives from evolutionary ecology and physiology.

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Figure legends

Figure 1. A schematic of the change in oxygen partial pressure through the amphipod ventral groove (arrow indicates direction of water flow), assuming the partial pressure of oxygen in hemolymph is constant. As oxygen is extracted at the gill plates, the partial pressure of oxygen at the inflow (p_{O2i}) falls in line with the decline in the mass of dissolved oxygen (solid line). At lower solubilities (such as at higher temperature, which also causes a much stronger increase metabolic oxygen demand), p_{O2} (dashed line) will fall more rapidly for a fixed water volume flow rate ($\dot{V}_w$) and rate of movement of oxygen from water to blood ($\dot{M}_{O2}$). This effect cannot occur in isolation however, because the decline in the average partial pressure in water along the gill plates from inflow to outflow $p_{O2w} = (p_{O2i} + p_{O2e})/2$ and hence the partial pressure difference Δp_{O2w-h} between water and hemolymph across the gill diffusion barrier also reduces $\dot{M}_{O2}$, which will have a corresponding effect on variation in Δp_{O2i-e}.

Figure 2. A schematic of the idealised amphipod respiratory gas exchange system. Turbulent water flow is generated by beating abdominal limbs (pleopods, not shown) and passes posteriorly (arrow denotes direction of flow) through a narrow channel of width *h*, within the lateral walls formed by 6 pairs of gill plates. In our idealized amphipod gas exchange channel, and in order to simplify the mass transfer modelling, we treat the 6 gill plates flanking one side of the channel as a single liquid-solid interface where gas exchange occurs over the surface of area *l.d*. Although the system is open anatomically, it is treated physiologically as a closed gas exchange space, with no oxygen diffusion or admixture of

866 water assumed to occur from outside the groove. Although there is evidence of a role for
867 extra-branchial gas exchange in some amphipod species (Spicer and Gaston, 1999) and life
868 stages (Spicer and McMahon, 1992), this is not incorporated into our modelling, which
869 assumes the gills are the principal site of respiratory gas exchange.
870
871 **Figure 3**. A semi-$\log_{10}$ plot of variation in the rate of movement of oxygen ($\dot{M}_{O2}$; dotted)
872 from water to hemolymph in our amphipod gas transfer model, where the aerobic cost to
873 the animal of pumping water through the channel remains constant and the initial fraction
874 of oxygen extraction from the water current is 25%; all values are normalised to 0°C.
875 Physical variables are predicted to drive variation in $\dot{M}_{O2}$ with temperature, according to
876 the equation $\dot{M}_{O2} \propto \Delta p_{O2w\text{-}h}.A.\alpha_{wO2}.D_{wO2}^{2/3}.\nu_w^{4/5}/(\upsilon_w^{7/15}.h^{1/5})$ (Eqn. 3). These variables are
877 diffusivity of oxygen in water ($\dot{M}_{O2} \propto D_{wO2}^{2/3}$; fine dash), kinematic viscosity of water ($\dot{M}_{O2} \propto$
878 $\upsilon_w^{-7/15}$; coarse dash), the partial pressure gradient of oxygen ($\dot{M}_{O2} \propto \Delta p_{O2w\text{-}h}$; dash dot dash),
879 solubility of oxygen in water ($\dot{M}_{O2} \propto \alpha_{wO2}$; dash dot dot dash), and linear water velocity
880 through the channel ($\dot{M}_{O2} \propto \nu_w^{4/5}$; medium dash). Linear water velocity varies proportional
881 to dynamic viscosity of water (μ_w) to the power -1/2 when assuming a constant power
882 spent in ventilation. These physical variables combine to produce an increase in $\dot{M}_{O2}$ with
883 increasing temperature. Residual $\dot{M}_{O2}$ ($_r\dot{M}_{O2}$; solid) represents the oxygen bioavailability for
884 functions not involved in the physical process of oxygen movement (i.e. those pumping
885 external water and hemolymph) and shows an approximate Q_{10} of 1.24.
886
887 **Figure 4**. Variation in three different proposed metrics of oxygen bioavailability with
888 changing temperature, normalised to 0°C and displayed on a semi-$\log_{10}$ plot. All models

assume constant environmental partial pressure of oxygen (p_{O2i} in the analysis presented here). Water oxygen content will decline in line with the solubility coefficient of oxygen in water (α_{wO2}; dash dot dot dash); $Q_{10} \simeq 0.83$. The Oxygen Supply Index, or OSI (product of solubility and diffusivity coefficient variation; dash), increases slightly with temperature; $Q_{10} \simeq 1.11$. Our definition of oxygen bioavailability, as mass-transfer-derived variation in the rate of oxygen movement ($\dot{M}_{O2}$), when costs of oxygen movement are constant and subtracted from whole organism $\dot{M}_{O2}$ ($_r\dot{M}_{O2}$; solid) and initial fraction of oxygen extraction from the water current is 25%, also increases with temperature in amphipods, but to a greater extent than the OSI; $Q_{10} \simeq 1.24$.

Legend for Supplement S1, available online.

Oxygen bioavailability and its response to warming is modelled using a mass-transfer approach. We illustrate the modeling using an amphipod model of respiration. The model is described step-by-step under the following headings: Fundamentals of oxygen transfer across respiratory gas exchange surfaces; Mass-transfer treatment; Energetics of water flow; Normalising temperature effects on total and residual $\dot{M}_{O2}$ to a 0°C reference; Physical variables contributing to thermal sensitivity of total and residual $\dot{M}_{O2}$ in the amphipod gill model; Table S1. Q_{10} values for a range of physical variables relevant to the modelling of respiratory gas transfer presented in this study; Table S2. Abbreviations for terms used in modeling in this study. Supplementary references.

Fig 1

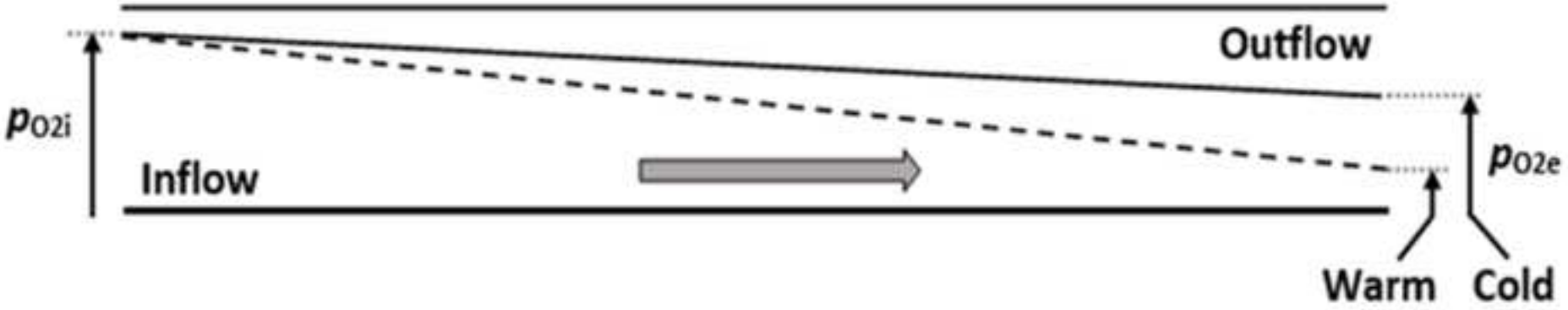


Fig 2

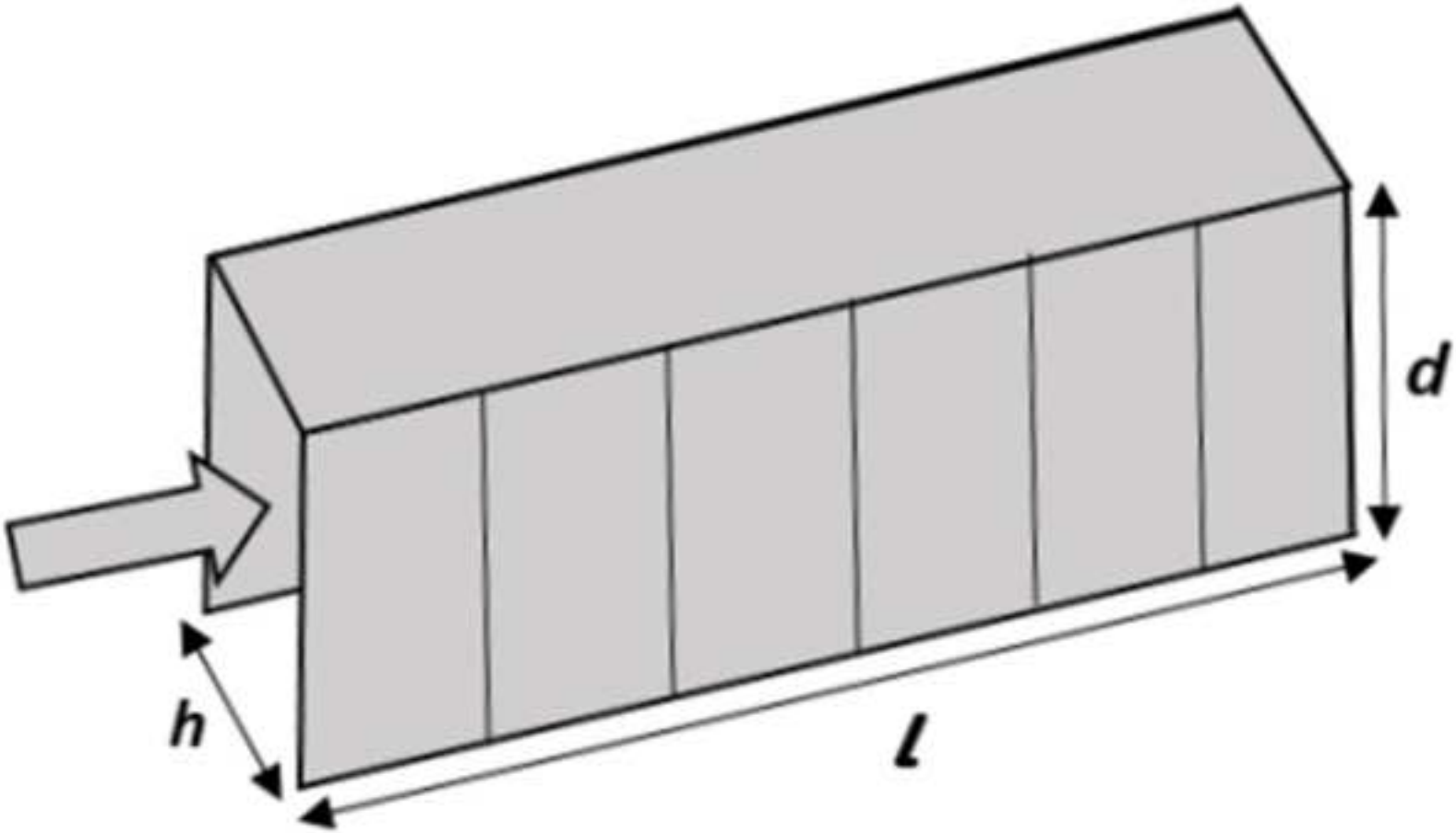


Figure 3

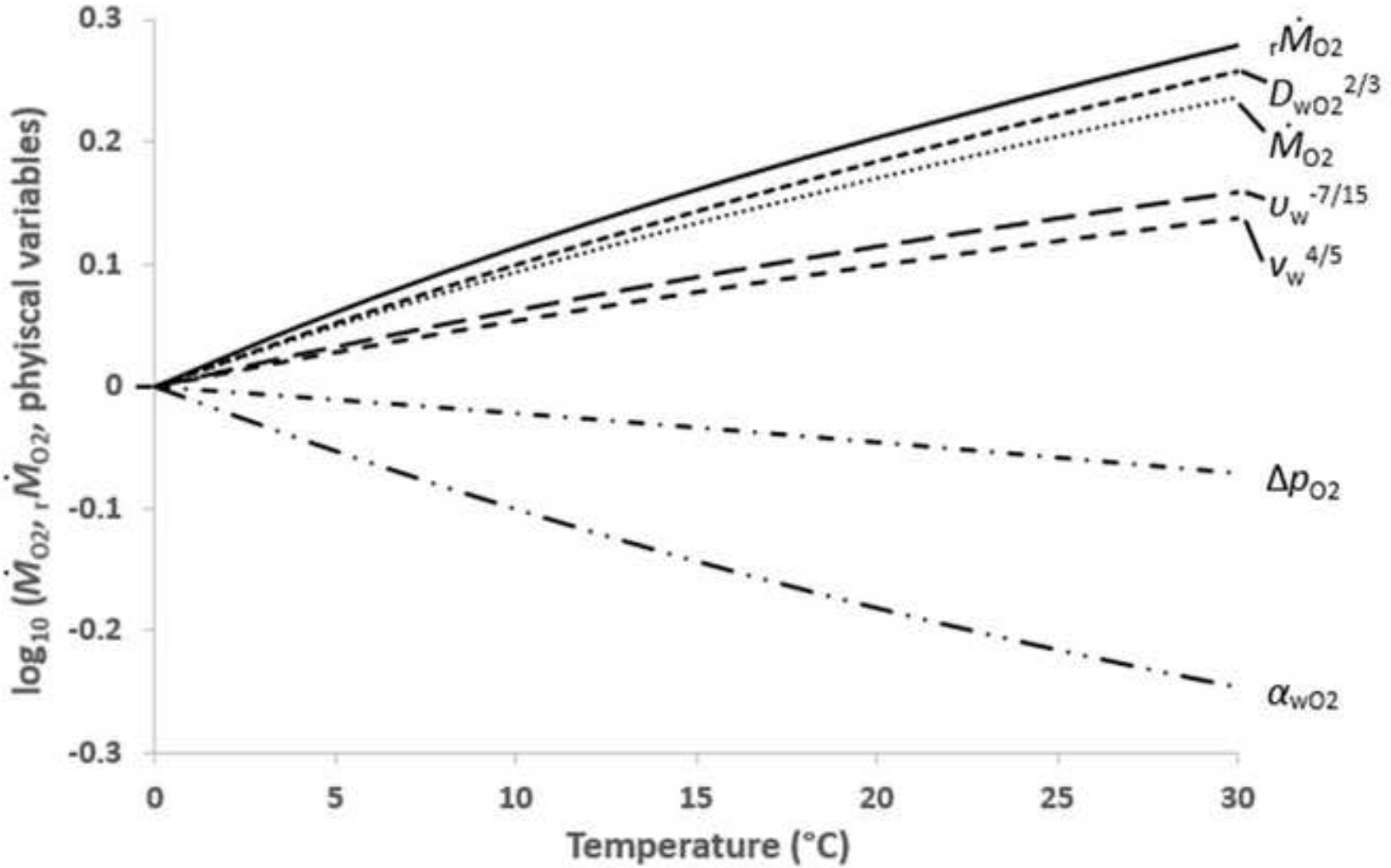
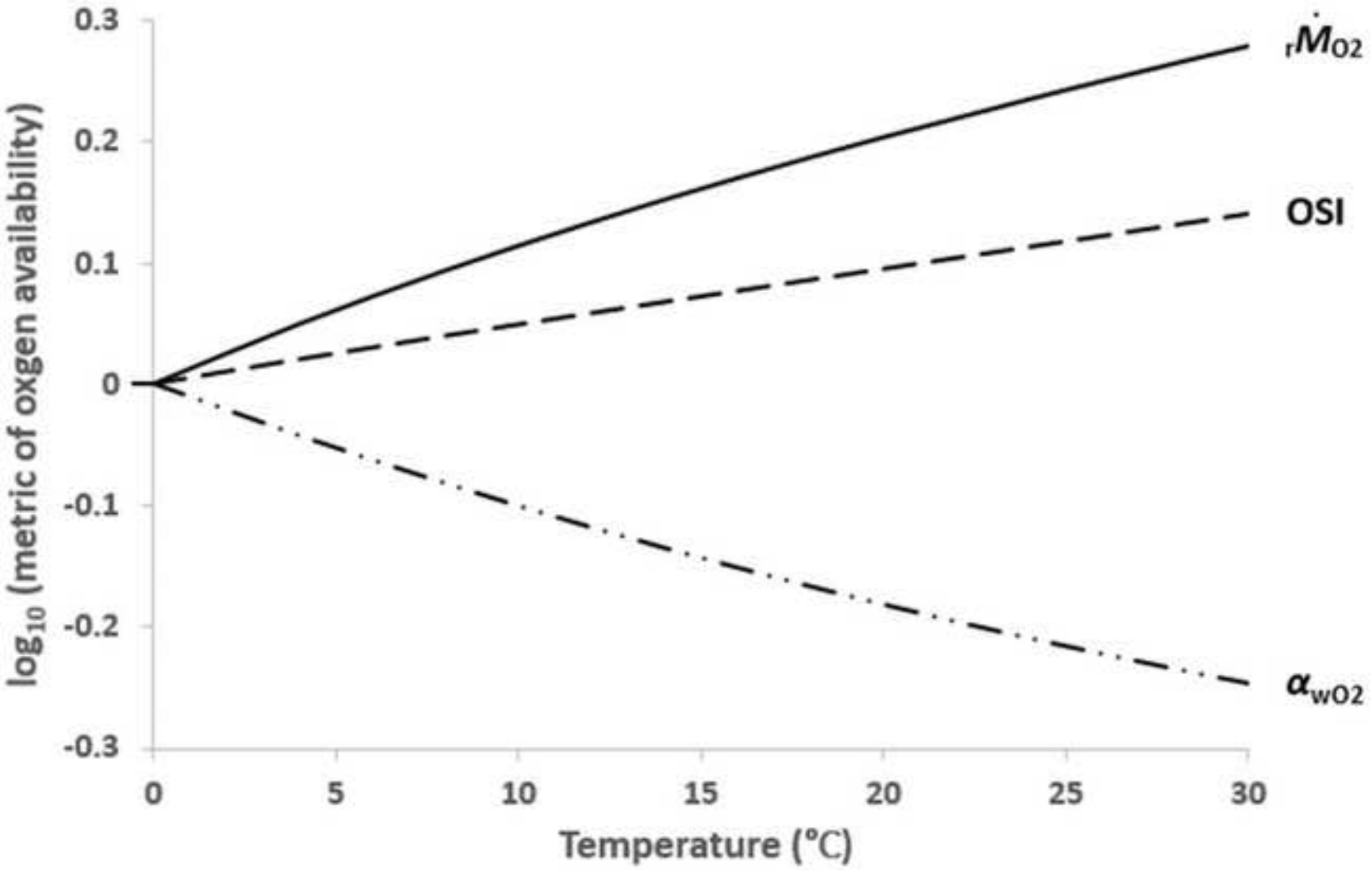


Figure 4



Dear Wilco (Symposium Guest Editor),

Thank you very much for your very helpful, thoughtful and constructive comments.

Below, we address the request of the editorial staff in bold blue font.

Ref.: Ms. No. 22040R1ocx
Controversial roles of oxygen in organismal responses to climate warming The Biological Bulletin

Dear David,

I have now read the revised manuscript and am happy to accept the paper pending minor revisions. I found the paper to be much improved in terms of organization and scope and the coherence of the different ideas. I have annotated the word file with some further suggestions for improvement, which mostly pertain to wording and which I hope will prove useful and not too much work to process. (You can find this as an attachment in your main menu.)

Our editorial staff has requested the following changes, to conform with Biological Bulletin style:
- Please compose figure legends with enough information to make the figure intelligible without reference to the text, or other tables or figures.
We have now amended the figure legends so that they are intelligible without reference to other material.
- It's Biological Bulletin policy to not thank assigned editors or staff in the Acknowledgments. It's our job!
Now removed from Acknowledgments
When you submit the revision, please include a letter describing the suggested revisions that were made and outlining any points with which you disagree. To submit a revision, go to https://www.editorialmanager.com/biolbull/ and log in as an Author. You will see a menu item called Submission Needing Revision. You will find your submission record there.

Please resubmit your revision within two weeks. If you need more time to complete an assignment or revision please contact the office.

We look forward to receiving your revised manuscript.

Best regards,
Wilco

Wilco Verberk
Guest Symposium Editor
The Biological Bulletin
As the Guest Editor comments were added directly to the submitted ms, we considered that an efficient response would be directly to your marked-up version, which we submitted as Atkinson Biol Bull revised ms - responses to eds comments. Please note that we accepted most of your suggestions verbatim and simply incorporated those corrections and removed the comments, leaving just your comments and responses where we needed to explain our response. Thank you again for your help in considerably improving the ms.

Running Head: Warming-oxygen controversies

Controversial roles of oxygen in organismal responses to climate warming

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Abbreviations used in text: OSI, Oxygen Supply Index; TSR, Temperature-Size Rule.

Key words: oxygen limitation, mass-transfer, temperature-size rule, thermal adaptation.

Abstract

Despite the global ecological importance of climate change, controversy surrounds how oxygen affects the fate of aquatic ectotherms under warming. Disagreements extend to the nature of oxygen bioavailability, and whether oxygen usually limits growth under warming, explaining smaller adult size. These controversies affect two influential hypotheses: Gill Oxygen Limitation, and Oxygen- and Capacity-Limited Thermal Tolerance. Here, we promote deeper integration of physiological and evolutionary mechanisms. We first clarify the nature of oxygen bioavailability in water, developing a new mass-transfer model that can be adapted to compare warming impacts on organisms with different respiratory systems and flow regimes. By distinguishing aerobic energy costs of moving oxygen from environment to tissues from costs of all other functions, we predict a decline in energy-dependent fitness during hypoxia despite approximately constant total metabolic rate before reaching critically low environmental oxygen. A new measure of oxygen bioavailability that keeps costs of generating water convection constant, predicts a higher thermal sensitivity of oxygen uptake (Q_{10} ~1.24) in an amphipod model, than do previous oxygen supply indices. More importantly, by incorporating size- and temperature-dependent costs of generating water flow, we propose that oxygen limitation at different body sizes and temperatures can be modelled mechanistically. We then report little evidence for oxygen limitation of growth and adult size under benign warming. Yet occasional oxygen limitation, we argue, may, along with other selective pressures, help maintain adaptive plastic responses to warming. Finally, we discuss how to overcome flaws in a commonly-used growth model that undermine predictions of warming impacts.

Commented [AD1]: Would have inserted 'a' between these words, as suggested by the guest editor if it had not exceeded the word count.

Commented [AD2]: Wilco said: "Or do these costs also pertain to cardiac output?"
We agree that in principle, the costs do include cardiac output, and are not just ventilatory. We decided to improve clarity by using a more explicit description of the costs. Then we had to reduce number of words elsewhere to hit the target word count.

Introduction

One of the main global challenges facing humanity is how to mitigate and adapt to ongoing climate change (IPCC, 2021). Here, we focus on the role of oxygen in understanding and predicting impacts of warming, including heat waves, on water-breathing ectotherms. For decades, warming, combined with nutrients discharged into aquatic systems, have led to strongly decreased oxygen availability in oceans and lakes by accelerated consumption of oxygen by microbial respiration, reduced oxygen solubility and a reduction in oxygen replenishment from the atmosphere to water beneath the surface (Breitburg *et al*., 2018). Impacts of low oxygen (i.e. hypoxia) are pervasive, affecting all level of biological organization (Woods *et al*., synthetic manuscript). However, whether and how oxygen limitation is responsible for warming-induced changes in growth, adult size and survival outside such oxygen-depleted zones (i.e. under normoxia) is controversial (e.g. Schulte, 2015; Verberk *et al*., 2016b; Lefevre *et al*., 2017; Pauly and Cheung, 2018; Pörtner *et al*., 2017; Jütfelt *et al*., 2018; Audzijonyte *et al*., 2019; Pauly, 2021; Verberk *et al*., 2021), and is a major focus of this paper.

Oxygen limitation arises when the oxygen supplied to tissues is insufficient to meet metabolic demands. But oxygen limitation may be manifest not just as direct physiological impacts on the organisms (e.g. anaerobiosis, reduced growth and reproduction), but also as changes in allocation of metabolic energy among different functions (e.g. locomotion or anti-predator defense; e.g. Roman *et al*., 2019). Adaptive responses should therefore allocate energy or oxygen in ways that minimize (oxygen-dependent) reductions in fitness.

Commented [WV3]: A suggestion would be to first say here that: "Effects of low oxygen (i.e. hypoxia) on performance are pervasive affecting all levels of biological organization (Woods et al., synthetic manuscript).".

Commented [AD4R3]: Now incorporated. A minor change to the suggested text was to remove 'performance', as some interpret that to apply to organisms and to lower but not higher levels of biological organization. I also changed "Effects" to "Impacts".

Commented [AD5]: I have incorporated the editor's suggested improvement, except I have used the phrase 'oxygen-dependent' rather than 'hypoxia-induced', as the latter implies deviation from environmental normoxia (at least for ecologists), yet oxygen effects on fitness may also occur under environmental normoxia.

Warming-induced oxygen limitation is considered a particular risk for water-breathers, which rely strongly on the ability to move water - a dense and viscous medium - quickly over respiratory exchange surfaces (Forster *et al*., 2012; Verberk *et al*., 2011). Addressing the many controversies in this field is beyond the scope of this paper, and we do not expect a consensus about the importance of warming-induced oxygen limitation until we have sufficient relevant data that includes not just measurements of organism performance across temperatures and oxygen bioavailabilities, preferably in field experiments, but also include other conditions found in nature that impose high levels of demand (e.g. large body sizes, high levels of locomotion and other metabolic activity) (Verberk *et al*., 2021). However, we will discuss two aspects of oxygen limitation where we both clarify concepts and propose ways to improve investigations: (i) how warming affects oxygen bioavailability to the tissues of animals, and (ii) whether warming causes oxygen limitation of animal growth and adult size under conditions typically experienced in the field.

For the first aspect, related to the bioavailability of oxygen, we will account for the crucial but typically omitted role of water flow, by presenting a new mass-transfer model. Flowing water, whether imposed by the environment or by active ventilation, helps organisms to cope with warm and low-oxygen conditions, as demonstrated for anurans and salamanders (Pinder and Feder, 1990; Rollinson and Rowe, 2018), fishes (Rubalcaba *et al*., 2020; Dahlke *et al*., 2020, 2022) and aquatic insects (Verberk *et al*., 2016c; Jones *et al*. 2018; Frake *et al*., 2021). But moving oxygen from the surrounding water to tissues can be costly, amounting to about 10% of resting metabolism in rainbow trout (Jones and Schwarzfeld, 1974), which

is likely to increase under challenges of reduced oxygen availability or increased demand, thus likely constituting an important component of oxygen limitation.

The second aspect, considering whether, under normoxia, oxygen generally limits aspects of organism performance at increased temperatures, has produced disagreements which we expect will be reduced when an evolutionary approach is incorporated into the physiological analysis. We specifically focus on controversies that arise from, firstly, not fully distinguishing proximate physiological from ultimate evolutionary factors and, secondly, from using a classic growth model that contains inappropriate assumptions, which lead to unrealistic predictions.

Commented [WV6]: i.e. under normoxia?

Commented [AD7R6]: Yes. Now inserted.

1. Climate controversies: Oxygen bioavailability

(i) Contrasting viewpoints, and an alternative approach to estimating oxygen bioavailability

Ecologists and physiologists have traditionally emphasized the importance of different metrics of oxygen bioavailability and thus its environmental sensitivity. Ecologists have focused on correlating the concentration of oxygen in water with organismal properties such as their size, abundance and diversity. Chapelle and Peck (1999), for instance, demonstrated a positive linear relationship between maximum amphipod body length and oxygen concentration across a wide range of habitat salinities and temperatures. In contrast, physiologists have focused on the physical process of oxygen movement within water and across a diffusion barrier, and the importance of the oxygen partial pressure difference (Δp_{O2}) in driving the rate of oxygen diffusion ($\dot{M}_{O2}$) through media with different

oxygen-carrying properties, such as water and blood, and across a diffusion barrier (Piiper *et al*., 1971; Dejours, 1975; Spicer and Gaston, 1999). The partial pressure (p_{O2}) and concentration of oxygen in water (c_{O2}) are linked through the solubility coefficient of oxygen in water (α_{wO2}) in Henry's Law ($c_{O2} = \alpha_{wO2}.p_{O2}$).

Verberk *et al*. (2011), attempted to reconcile these two viewpoints, applying Fick's first law of diffusion to a static diffusion barrier (Piiper *et al*., 1971) to produce a metric of oxygen bioavailability (the Oxygen Supply Index, or OSI), which is proportional to the product of partial pressure of oxygen in water, and the solubility and diffusion (D_{wO2}) coefficients of oxygen in water (OSI $\propto p_{O2}.\alpha_{wO2}.D_{wO2}$). Verberk *et al*., (2011) estimated temperature effects on oxygen bioavailability by combining the thermal sensitivities of solubility and diffusivity, as was done previously (Woods, 1999; Atkinson *et al*., 2006). In this Oxygen Supply Index, reductions in oxygen solubility (here measured as the factorial change with 10°C warming, $Q_{10,}$ ~0.81 in freshwater and 0.83 in seawater of salinity 35 p. s. u.) are slightly outweighed by increases in diffusivity (thermal sensitivity, $Q_{10,}$ ~1.3–1.4; see Table S1, Supplement S1, available online), thereby slightly increasing oxygen bioavailability according to the OSI (Q_{10} = 1.05–1.16). Consequently, they concluded that reductions in aerobic performance in warmer waters do not arise from lower oxygen concentrations, but instead through organismal oxygen demand exceeding supply.

Although our theoretical analyses agree with this qualitative conclusion, our predictions are quantitatively different. Moreover, we will show that the OSI does not account for the complexities of oxygen delivery from water to blood, and how environmental sensitivity of

oxygen bioavailability crucially depends on water flow (Pinder and Feder, 1990; Frakes *et al*., 2021) including the diverse flow regimes involved in oxygen uptake by aquatic ectotherms. To illustrate, we provide an alternative, mass-transfer, perspective that accounts for different respiratory flow regimes and their energetic costs, and which can produce estimates of the thermal sensitivity of oxygen bioavailability different from that of the OSI.

Mass-transfer formulae can incorporate combinations of convection and diffusion to describe the physical factors influencing the movement of solutes in flowing liquids, or through static layers (Cussler, 2009). Although well established in industrial processes, such models have been applied only infrequently to gas transfer at respiratory surfaces despite the development of formulae that describe gas transfer scenarios applicable to at least some aquatic respiratory gas exchange systems (Cussler, 2009). Hills and Hughes (1970) applied the concept of mass transfer to the body mass-scaling of oxygen uptake at fish gills. Rubalcaba *et al*., (2020) used mass-transfer modelling to predict thermal sensitivity to oxygen delivery through an external boundary layer, balanced against metabolic oxygen demand. Deutsch *et al*. (2022) also used mass-transfer equations to model oxygen bioavailability across a size range from unicells to large fish.

A benefit of applying mass-transfer models to oxygen transfer, from high to low partial pressure across a respiratory diffusion barrier, is a simplified quantitative estimation of the thermal sensitivity of multiple physical factors (including kinematic viscosity, diffusivity, solubility) affecting uptake. Empirically-derived and biologically-realistic mass-transfer

154 correlations (Cussler, 2009) enable the effects of environmental change, including
155 temperature, on oxygen uptake to be quantified for organisms with different respiratory
156 water flow regimes (e.g. laminar *versus* turbulent flow through open *versus* closed channels
157 or along differently shaped surfaces).
158
159 Our illustrative example of mass-transfer modeling (Supplement S1, available online) uses
160 amphipods, a group of aquatic crustaceans that, at rest, generate a turbulent water current
161 through a ventral groove *via* the rhythmic beating of their pleopods (Sutcliffe, 1984).
162 Hemolymph flows through the lacunar space within gill plates, situated at the inward base
163 of their thoracic limbs (Steele and Steele, 1991). These gill plates, suspended within the
164 ventral groove, are assumed here to be the principal site of respiratory gas exchange in our
165 idealized adult amphipod model, even though other, extrabranchial sites of respiratory gas
166 exchange, may occur (Steele and Steele, 1991; Spicer & Gaston, 1999).
167
168 The full derivation of the model is presented in Supplement S1, available online. Here, we
169 focus on those salient features that help improve exploration and understanding of
170 mechanism and the quantification of oxygen bioavailability in response to warming.
171
172 ***(ii) Potential declines in partial pressure differences with warming***
173 The fundamental equation that underlies the movement of oxygen across a respiratory gas
174 exchange surface is:
175

176 $\dot{M}_{O2}/A = \Delta p_{O2w\text{-}h}/R_{O2}$

177 [Equation 1]

178

179 (Hills and Hughes, 1970), where $\dot{M}_{O2}/A$ is rate of movement of oxygen (uptake, mol. s^{-1})

180 per unit respiratory gas exchange surface area (A, cm^2). $\Delta p_{O2w\text{-}h}$ is the oxygen partial

181 pressure difference (atm) between water and blood, or hemolymph, on either side of the

182 respiratory exchange surface, and R_{O2} is the total resistance (s. atm. cm^2. mol^{-1}) to oxygen

183 movement in the direction of the partial pressure gradient.

184

185 Indices of oxygen bioavailability such as the OSI assume that the oxygen partial pressure

186 difference is temperature independent because the effect of temperature on the total sum

187 of all atmospheric gases via changes in water vapour pressure is negligible at sea level and

188 across the realistic range of water temperatures (Verberk *et al.*, 2011). However, when

189 applied along a path of water flow where oxygen is being extracted, as in the amphipod

190 ventral groove (Fig. 1) this assumption may need to be revisited. Although the system is

191 open anatomically, it is treated physiologically as a closed gas exchange space, with no

192 oxygen diffusion or admixture of water from outside, and a fully formed turbulent

193 boundary layer within the ventral groove.

194

195 As oxygen is extracted at the gill plates, the partial pressure of oxygen in inflowing water

196 (p_{O2i}) pumped through the ventral groove falls in line with the decline in the mass of

197 dissolved oxygen. At lower solubilities (such as at higher temperature), p_{O2} will fall more

198 rapidly for a fixed $\dot{M}_{O2}$ and rate of water volume flow $\dot{V}_w$. This effect cannot occur in

Commented [WV8]: Moles of Oxygen?

Commented [AD9R8]: Units now added

Commented [WV10]: Maybe add the units so the reader can see that the units match up with the left hand side of the equation?

Commented [LG11R10]: Units of 1/k are sec/cm
Units of 1/αwO2 are atm.cm3/mol
Therefore R units are sec.atm.cm2/mol
The units therefore balance in Eqn. 1

Commented [AD12R10]: Units now added.

isolation, however, because the partial pressure difference between water and hemolymph across the gill diffusion barrier ($\Delta p_{O2w\text{-}h}$) will also decline, which decreases the potential driving oxygen movement from water to hemolymph, which reduces $\dot{M}_{O2}$ (Eqn. 1). The model (Supplement S1, available online) therefore utilizes iteration to estimate how variation in environmental p_{O2}, $\dot{M}_{O2}$, water volume flow ($\dot{V}_w$) and oxygen solubility coefficient (α_{wO2}) influences $\Delta p_{O2w\text{-}h}$, and consequently feeds back to affect $\dot{M}_{O2}$, .

(iii) The mass-transfer coefficient accounts for flow regime

The product of the mass-transfer coefficient for oxygen in water (k_{wO2}) and solubility coefficient of oxygen in water (α_{wO2}) is inversely proportional to the resistance in the fundamental model of oxygen movement (R_{O2} in Eqn. 1; also Eqn. S3). In mass-transfer scenarios involving both forced convective flow and diffusion, convective flow is expressed using the Reynolds number (ratio of inertial to viscous forces, which increases with increasing linear water velocity and turbulence) (Cussler, 2009). Diffusion is expressed using the Schmidt number (ratio of momentum diffusivity, or kinematic viscosity, to molecular diffusivity) (Cussler 2009). These dimensionless numbers are combined to determine the mass-transfer coefficient k which is expressed within the dimensionless Sherwood number (ratio of convective mass transfer to diffusive mass transport) (Cussler, 2009). Thus:

$$\text{Sherwood number} = k_{wO2}.h/D_{wO2} = \text{Constant}.(\text{Reynolds number})^{x}.(\text{Schmidt number})^{y} \quad \text{[Equation 2]}$$

where h is the width of the channel and D_{wO2} is the diffusion coefficient of oxygen in water. All mass-transfer coefficients are determined empirically using forms of the above equation that apply to different flow regimes and yield the exponents x and y for the Reynolds and Schmidt numbers, respectively (Cussler, 2009; e.g., Eqn. S10, Supplement S1, available online).

The Sherwood number for turbulent water flow through a horizontal channel, which is applicable to the amphipod system (Figure 2), can be rearranged to derive a mass-transfer coefficient that is incorporated into the fundamental model of oxygen movement (Eqn. 1) for a constant environmental partial pressure of oxygen, thus:

$$\dot{M}_{O2} \propto \Delta p_{O2w\text{-}h}.A.\alpha_{wO2}.D_{wO2}^{2/3}.v_w^{4/5}/(\upsilon_w^{7/15}.h^{1/5}) \qquad \text{[Equation 3]}$$

Where α_{wO2} is the solubility coefficient of oxygen in water, v_w is linear water velocity through the channel and υ_w is the kinematic viscosity of water (Supplement S1, available online).

In conclusion, incorporating mass-transfer coefficients into oxygen uptake models, as in Eqn. 3, alters the temperature-sensitive contributions to oxygen uptake rate, relative to those predicted by the OSI. Here, for example, the contribution of the diffusivity coefficient to the temperature sensitivity of $\dot{M}_{O2}$ is now raised to the power of $2/3$ rather than 1, and a dependency on warming-induced changes in the kinematic viscosity of water to the power of -7/15 is introduced. A further benefit is that the effects of phenotypic adjustments of organism-specific, structural features of gas exchange systems, such as the width h of the

Commented [WV13]: Maybe give a short rationale for why the water flow is best considered to be turbulent? I associate turbulent flow mainly with high flow velocity, but I reckon the rhythmic beating of the pleopods results in flow turbulence?

Commented [LG14R13]: Stated in line 194

244 respiratory water channel, on warming-induced changes in oxygen uptake rate can be
245 assessed.
246
247 ***(iv) Temperature-dependence of oxygen bioavailability – controlling for oxygen***
248 ***movement costs***
249 Incorporating the energy cost involved in generating the water flow is essential because
250 the actual variation in oxygen available to the organism will be the whole-organism $\dot{M}_{O2}$
251 minus the aerobic cost of generating that $\dot{M}_{O2,}$ (residual $\dot{M}_{O2} = {}_{r}\dot{M}_{O2}$). In our idealized
252 amphipod example, we consider aerobic metabolism associated with ventilation of water
253 (${}_{v}\dot{M}_{O2}$), as an index of the aerobic costs of oxygen movement from environment to tissues
254 (including ventilation and circulation). The cost of ventilation is generally considered a
255 potential limiting factor in hypoxia in fishes (e.g. Wood, 2018), and ventilatory costs are
256 predicted to be considerably higher than circulatory costs in fishes (Farrell & Steffensen,
257 1987). Moreover, information is currently insufficient to allow modeling of circulatory
258 costs in amphipods; but future modeling of circulatory costs may be incorporated into
259 costs of oxygen movement for species with more information on circulation, such as fishes.
260
261 To compare oxygen bioavailability across temperatures for particular respiratory flow
262 regimes, $\dot{M}_{O2}$ can be predicted where ${}_{v}\dot{M}_{O2}$ does not change: this is a measure of the
263 temperature-dependence of oxygen bioavailability from the perspective of all metabolic
264 functions other than those directly associated with the physical movement of oxygen.
265

The rate of aerobic energy expenditure or power used to pump water can be expressed in the same fundamental units as $\dot{M}_{O2}$. Therefore, changes in power spent pumping water through the central groove (P_w) and ${}_v\dot{M}_{O2}$ are directly interchangeable, assuming constant aerobic muscle efficiency. Vogel (1994) presented formulae expressing power spent in ventilation as proportional to the product of resistance to water flow and the square of the rate of flow of water volume; and in these equations the resistance to water flow through a narrow horizontal channel was presented as being proportional to the dynamic viscosity of water (μ_w). Therefore, when P_w is kept constant and body dimensions are unchanged, the rate of flow of water volume is proportion to $\mu_w^{-1/2}$ (Supplement S1, available online). When ratios of body dimensions are invariant, the rate of flow of water volume is also proportional to the linear water flow rate.

By fixing P_w at a constant value and assuming body dimensions are invariant, Eqn. 3 can be modified (Eqn. S18, Supplement S1, available online) to indicate how $\dot{M}_{O2}$ and its contributory components vary with temperature when the aerobic cost of ventilating the ventral groove remains constant. These contributions of components of $\dot{M}_{O2}$ responses to temperature are shown in Fig. 3.

(v) Thermal sensitivity of components of oxygen bioavailability with constant ${}_v\dot{M}_{O2}$

Figure 4 contrasts the thermal sensitivities of uptake that relies on solubility alone; the combination of solubility, diffusivity and p_{O2} (the latter being constant) in the OSI (Verberk *et al*., 2011); and the variation in ${}_r\dot{M}_{O2}$ predicted in the amphipod system, with constant ${}_v\dot{M}_{O2}$.

Thus, when the amphipod makes no change in the ventilatory effort of physically moving oxygen from water towards tissues, warming from 0 to 30°C increases oxygen bioavailability for other aerobic metabolism (${}_{r}\dot{M}_{O2}$) by about 90% ($Q_{10} \sim 1.24$) as a result of the interaction of the physical variables described in Fig. 3 and Eqn. 3. The greater increase in oxygen bioavailability with temperature in amphipods, in comparison with the OSI, is largely due to the inclusion of decreasing water viscosity at higher temperatures, which not only directly increases $\dot{M}_{O2}$ in turbulent flow regimes, but also makes ventilation less costly and thus allows greater ventilation under a constant effort (see also Verberk and Atkinson, 2013 for discussion of viscosity effects). Moreover, warming-induced increases in $\dot{M}_{O2}$ (and hence ${}_{r}\dot{M}_{O2}$) at constant ${}_{v}\dot{M}_{O2}$ may be greater still if the aerobic efficiency of muscle function increases with temperature.

This mass-transfer modeling framework highlights how physiologically-realistic estimates of oxygen movement and associated energetics can provide a more realistic view of oxygen supply capacity and hence the (residual) energy available to an organism when faced with warmer water. The framework is flexible and can also include effects of increasing rates of oxygen movement in response to diverse and interacting factors (e.g. hypoxia, activity, increasing temperature or body size), and be used to partition oxygen bioavailability for all metabolic processes not associated with oxygen movement (e.g. maintenance, reproduction and growth). Consequently, it provides more general, mechanism-based predictions of the consequences of life history evolution.

312 ***(vi)*** **The importance of oxygen supply capacity**

313 When an increase in ventilation is unable to satisfy the requirements of residual
314 metabolism, the oxygen supply capacity is reached, and oxygen becomes limiting.
315 Therefore, mass-transfer models that incorporate the cost of increasing ventilation will
316 determine not just oxygen bioavailability or supply that balances instantaneous demand,
317 but also the oxygen supply *capacity*, hence the point at which oxygen becomes limiting.
318
319 To prevent oxygen limitation, organisms must therefore be able to boost oxygen delivery to
320 meet tissue oxygen demand. Aside from finding locations with greater oxygen availability
321 (Kramer, 1987), organisms can boost oxygen bioavailability by: increasing ventilation that
322 generates water convection over exchange surfaces; increasing the respiratory exchange
323 surface area or conductance (Nilsson et al., 2012; Funk *et al.*, 2021); and various
324 adjustments in the internal oxygen exchange and delivery systems (Woods and Moran,
325 2020). Therefore, temperature effects on oxygen limitation should compare thermal
326 sensitivity of metabolic rate (oxygen demand) with the thermal sensitivity of oxygen
327 supply *capacity,* which accounts for organism's behavioral and plastic responses to
328 improve oxygen uptake with warming (Deutsch *et al.*, 2015; Kielland *et al.*, 2019; Seibel and
329 Deutsch, 2020), rather than with simple measures of oxygen bioavailability (*DO, pO_2, OSI*).
330
331 Uptake capacity may be estimated in conditions at or below critical oxygen partial
332 pressures, $p_{crit O2}$ from the change in oxygen uptake by the organism per unit increase in
333 environmental oxygen partial pressure (Kielland *et al.*, 2019; Seibel and Deutsch, 2020).
334 The thermal sensitivity of oxygen supply capacity compares how temperature increases

Commented [WV15]: There appears to be some ambiguity of wat the costs of oxygen movement (vMO2) include. Is it only ventilation (as indicated here and alluded to by the v-prefix) or does it also include circulation. My suggestion would be to restrict it to ventilation which is more in the domain of the mass transfer and how size and temperature affect availability via viscosity and boundary layers, both of which are less explored for circulation.

Commented [LG16R15]:

Commented [AD17R15]: Yes. In lines 251-261 (Section iv), we now clarify the distinction between the theoretical ideal (ventilation and circulation costs) and what is practicable, and hence used in the amphipod model (ventilation costs only). We also justify our focus on ventilation.

Commented [WV18]: Or simply by dividing smr by pcrit?

Commented [AD19R18]: This is the same only if the line goes through the origin (zero SMR at zero pO2), which is debatable, as organisms may die (zero SMR) before complete anoxia is reached). We therefore wish to retain our original wording.

this measure, However, although this empirical approach may be simpler than mechanistic mass-transfer modeling, it does not isolate the costs of moving oxygen, which our model predicts will increase as pO_2 is reduced towards $p_{crit O2}$. Consequently, rather than being constant, ${}_r\dot{M}_{O2}$ is predicted to decline as pO_2 is reduced towards $p_{crit O2}$.

2. Climate controversies: Oxygen limitation

(i) Limitation – proximate or ultimate?

Oxygen limitation of aerobic scope (difference between maximum and standard metabolic rates) has been implicated in heat-induced reduction in organismal performance (Pörtner 2010, 2017). Oxygen limitation has also been proposed as the proximate mechanism causing growth rates to decline in water-breathing ectotherms as size increases, especially at increased temperatures (Pauly,1981, 2010, 2021). The widespread phenotypically plastic decline in late-ontogeny growth rate and a reduced mature or final body size under benign environmental warming, when stressfully high temperatures, food shortage and reduced environmental oxygen bioavailability are all avoided, has been called the Temperature-Size Rule (TSR; Atkinson, 1994). Under these benign conditions the idea of oxygen limitation as a proximate mechanism becomes contentious (Audzijonyte *et al*., 2019; Seibel and Deutsch, 2020; Pauly 2021; Verberk *et al*., 2021; Wootton *et al*., 2022).

Experimental tests of direct oxygen limitation should employ increased oxygen bioavailability above levels normally experienced by the organisms (e.g. hyperoxia for species adapted to normoxic conditions) at different temperatures. If oxygen generally limits growth and mature size, such tests would reveal that hyperoxia enhances late growth

and causes animals to mature at a larger size, especially in the warm. But such tests using various air- and water-breathers do not provide strong support for oxygen limitation, as they have either produced no or very small increases in mature body sizes, in contrast to the much greater size response (reductions) under hypoxia (Verberk *et al.*, 2021; Funk *et al.*, 2021). Experimental reduction in oxygen availability (growth responses reviewed in Verberk *et al.*, 2021) are not appropriate tests of oxygen-limitation, as they only show that oxygen can become limiting if made less available, but not that oxygen becomes limiting under the warmer, normoxic conditions where declines in growth rate late in ontogeny or other measures of performance are still observed. This general principle of employing hyperoxia to test for oxygen limitation has also been adopted by Seibel and Deutsch (2020), who collated data on Maximum Metabolic Rate at different oxygen conditions, including hyperoxic conditions, to derive the critical oxygen partial pressures, $p_{crit02,}$ of Maximum Metabolic Rate. Their findings, for terrestrial and shallow-living aquatic species (38 species, mainly arthropods, mollusks and chordates) supported the idea that oxygen starts to limit Maximum Metabolic Rate below 100% air-saturation (i.e. normoxia), but again that 100% air-saturation is not limiting within the normal temperature range of the species.

Other evidence presented to support the idea that oxygen normally limits growth of water-breathing ectotherms requires further scrutiny. One example is the assertion by Pauly (2021) that the change in enzymes in tissues from mainly oxidative to mainly glycolytic as water-breathers grow supports his hypothesis of Gill Oxygen Limitation. But a fuller evaluation of all relevant evidence would include identifying which tissues, hence which functions, are affected. If changes are mainly to white muscle, an alternative explanation to

Commented [WV20]: This needs to be contextualized as initially, growth rates are enhanced in warmer water.

Commented [AD21R20]: Agreed. Now inserted 'late in ontogeny'

Commented [WV22]: Does this number exclude the species living in hypoci habitats and for which Pcrit max was found to be limiting by oxygen levels below normoxia?

Commented [AD23R22]: Yes. I filtered out the hypoxia specialists to enable me to make the simple point about normoxia vs hyperoxia, without a potentially more complicated wording that also included increased environmental oxygen availability relative to hypoxic oxygen norms.

gill oxygen limitation is maintaining length-specific burst speeds (Childress and Somero, 1990). Also, in the context of climate warming, the role of temperature on any shift towards glycolysis with increased size should be included in the analysis, since the problem to be solved constitutes a three-way interaction between size, temperature and oxygen (Woods & Moran, 2020; Verberk *et al*., 2021). Moreover, alternative hypotheses should be evaluated, including that the lower mass-specific metabolic rate of large animals is not determined by oxygen limitation (Glazier, 2014); instead, these animals may have an advantage over small animals when having to rely on glycolysis because small animals will reach lethal levels of anaerobic end-products faster (Nilsson and Ostlund-Nilsson, 2008).

Despite the poor support for oxygen limitation persistently slowing growth and reducing body size with warming within the physiological range and under oxygen partial pressures typical for a species, further evidence still suggests a role for oxygen. Warming-induced reductions in mass-scaling exponents of maximum but not resting metabolic rates in European Perch (*Perca fluviatilils*) (Christensen *et al*., 2020) and in a quantitative analysis of 286 fish species (Rubalcaba *et al*., 2020) did suggest that larger individuals may be more susceptible to oxygen limitation, especially if they are in warmer water and if they are active.

To reconcile the apparently conflicting evidence, we propose that oxygen limitation may play mainly a selective (ultimate) role, rather than proximately limiting growth. When oxygen limitation is considered an ultimate factor, a reduction in growth rate during late ontogeny especially at warmer temperatures, evolves as a phenotypically plastic response

Commented [WV24]: Since I am an author on this paper, my perception on how relevant the paper is may be biased. As such, please feel free to dismiss this suggestion.

Commented [AD25R24]: First, congratulations on a very nice, valuable paper. After considering this suggestion carefully, I decided that its interspecific, rather than intraspecific focus, would make it less relevant to our particular discussion. Given that Pauly/Cheung and Lefevre et al have muddied the arguments by supporting intraspecific arguments with interspecific evidence, I was keen not to do the same. We retained Rubalcaba et al (2020) because we think there is an intraspecific size component within its findings.

to temperature that helps maintain sufficient capacity for oxygen uptake (e.g. a safety margin, such as aerobic scope, see Atkinson *et al.*, 2006) under a range of conditions. Most frequently these conditions will not tax an animal to its capacity limits and the animal is able to avoid oxygen limitation. But more extreme events could act as a selection pressure: these include episodes of hypoxia reducing oxygen bioavailability, or of events that increase oxygen demand such as extreme warming, disease, predator attack, or digestion of large meals (Jütfelt *et al.*, 2021) or their combinations (i.e. warm water and high activity; Rubalcaba *et al.*, 2020). During these selective events, actual, episodic oxygen limitation can occur. If large individuals with lower ratio of respiratory surface area to oxygen-consuming body mass are more likely disadvantaged, they will have been selected against during such events. This selection explains the evolution of phenotypes that reduce their growth rate during late ontogeny especially at warmer temperatures, hence maintaining safety margins for oxygen uptake - an idea termed 'The Ghost of Oxygen Limitation Past' (Verberk *et al.*, 2021).

The finding that gill surface area does not generally decrease relative to standard, and maximum metabolic rate (MMR) as teleost fish grow (Scheuffele *et al.*, 2021) provides more evidence (especially from MMR) against the Gill Oxygen Limitation hypothesis of Pauly (1981, 2010, 2021), which is based on a progressively increasing surface area limitation. The solitary empirical test of temperature effects (Li et al., 2018), shows the need for further such investigations. Li *et al.* (2018) appear to find a suggestion that gill surface area, when expressed as a ratio of standard metabolic rate, is reduced during the growth of goldfish (*Carassius auratus*) at higher (25°C) but not at lower (15°C) acclimation

Commented [WV26]: I added warming and the combination with warming as this forms an important component of the thermal reaction norms that you want to explain.

Commented [AD27R26]: Thanks, very helpful. Accepted.

Commented [WV28]: This seems to suggest that the geometric challenge is an assumption, but later on it is given as a fact.

Commented [AD29R28]: Yes. The topic has not been definitively resolved, and so we keep it as an assumption of the hypothesis, and make small wording changes later to clarify that this is not a demonstrated fact.

Commented [WV30]: Did this study take into account that any decrease in gull surface area would be most pronounced at higher temperatures?

Commented [AD31R30]: They cite just one study (Li et al. 2018), which also does not provide convincing support. Since you asked, and because others may be interested, too, we have now mentioned that work here.

427 temperature; but neither ontogenetic trend was significantly different from zero
428 (Scheuffele *et al*., 2021). Consequently, the available gill surface evidence does not support
429 direct Gill Oxygen Limitation; yet it may not contradict the Ghost of Oxygen Limitation Past,
430 which invokes selection producing phenotypes that have avoided oxygen limitation at all
431 sizes. Thus, the selected fish could have gill surfaces that can normally satisfy oxygen
432 uptake requirements throughout ontogeny *via* a combination of: (i) matching gill surface
433 area and other contributions to oxygen uptake with maximum oxygen demand and (ii)
434 body size reduction in warm water that avoids gill surface limitation of oxygen uptake at
435 the largest sizes, where a geometric challenge of matching gill surface area to demand
436 would become more difficult.
437
438 Testing ultimate explanations or "seeing ghosts" can be challenging. The "Ghost of Oxygen
439 Limitation Past" could be tested using experimental evolution (Atkinson *et al*., 2006;
440 Walczynska and Sobczyk, this volume). Such experiments should decouple the proximate
441 cause or environmental cue (temperature) from the ultimate cause (protecting aerobic
442 scope from oxygen limitation). Thus, each selective regime would simulate fluctuations in
443 both temperature and oxygen partial pressure, with regimes differing by having oxygen
444 partial pressures either predictably positively, negatively or uncorrelated with
445 temperature. Oxygen limitation in the warm would be reduced by a positive temperature-
446 partial pressure correlation and increased by a negative correlation, which is predicted to
447 select for traits that would protect aerobic scope (e.g. a strong reduction in adult size with
448 warming). As such experiments will be tightly controlled, and likely done in the laboratory,
449 they can only demonstrate that the evolution of body size responses to temperature *can* be

Commented [WV32]: This seems to suggest that the geometric challenge is a fact, but previously it is given as an assumption. I remember a discussion with Garrath at the SEB about the leaves of a book analogy of Lefevre et al, and we ended up agreeing that it is flawed since the ventilation/irrigation of the surfaces becomes constraining as the length of the gill filament is increased. A similar idea is presented here with the grove of the amphipod where pO2 is not constant, but declines as length increases. So I agree the geometric challenge is real, but perhaps it is worthwhile to make this point rather than have it as a fact/suggestion?

Commented [AD33R32]: We made the constraint more hypothetical than factual, by changing 'the geometric challenge' to 'a geometric challenge', and 'becomes more difficult' to 'would become more difficult'
We appreciate the wish to clarify the extent to which the surface area constraint is real. However, a discussion of the extent to which different gill structures may lead to a surface area constraint is in our view too big a topic to add to this paper. Garrath has expanded on this topic in other modelling from his PhD, and that will be included in future papers.

generated by selection to protect aerobic scope, but would not demonstrate that this *is* the cause of the warming-induced reductions in late growth and adult size in the field.

A remaining argument is that the "Ghost of Oxygen Limitation Past" may be deemed superfluous if there is little or no evidence for oxygen limitation across a wide range of body sizes and temperatures at field oxygen partial pressures, as described by Seibel and Deutsch (2020). A counter-argument to this is that, within each species, selection from extreme events on the oxygen budget, which may be infrequent, can help to maintain the adaptive match of oxygen supply to demand that is observed most of the time for all sizes, temperatures and activity levels.

In conclusion, evidence does not favour oxygen usually limiting growth directly (proximately) under warming within the physiological range and under typical oxygen partial pressure, as proposed in the Gill Oxygen Limitation hypothesis (Pauly, 2021). But occasional oxygen limitation that produces evolutionary effects on growth rates at large sizes ('the Ghost of Oxygen Limitation Past') cannot be ruled out as an explanation for widespread warming-induced deceleration of growth and reduced adult size in aquatic ectotherms. Moreover, away from the benign conditions of a species' normal oxygen bioavailability and physiological temperatures, environmental warming that places new and increased demands on an animal's oxygen budget, may then lead to oxygen limitation.

(ii) Avoiding critical flaws in growth models

Several models that explore how oxygen limits growth and which quantitatively predict the Temperature-size rule (TSR) have greatly overestimated warming-induced reductions in body size. For instance, the magnitude of warming-induced body size reduction in an aquatic crustacean, predicted from a model that assumed oxygen limitation, overestimated body size reductions by about an order of magnitude (Einum *et al*., 2021). Similar overestimation of body size reduction occurs in fish (Cheung *et al*., 2013; Lefevre *et al*., 2018). The quantitative mismatch between observed and predicted size reduction recorded by Einum *et al*., (2021) occurred even when they avoided problems of comparing demand with passive oxygen uptake by instead using their measure of oxygen supply capacity that accounts for improvements in uptake with warming (Kielland *et al*., 2019).

One source of error comes from the choice of growth models used. For over a century, classic models of organism growth and metabolism have been used to predict reduced adult or final body size at increased temperatures (Pütter, 1920, von Bertalanffy, 1960; Cheung *et al*. 2013, Kearney 2021). These models express growth rate as the difference between the rate of surface-related acquisition of resources available to supply growth and other metabolic activities (variously considered as 'anabolism' (Pütter, 1920, von Bertalanffy, 1960) or 'assimilation' (first term on right side of Eqn. 4) and the rate of metabolic breakdown of organic resources (second term on right side of Eqn.4) - characterized as 'catabolism' (Pütter, 1920; von Bertalanffy, 1960) or 'maintenance metabolism' (Cheung *et al*., 2013; Kearney, 2021).

$$dm/dt = Hm^{a} - Km^{c} \qquad \text{[Equation 4]}$$

Commented [WV34]: Note that the recent PNAS paper by Deutsch et al does predict size reductions in the appropriate size range. This could be used to highlight how mass transfer models will help get a better view of oxygen supply capacity (and how it changes with body size and temperature). For example, in the paper by Einum et al (2021), uptake capacity was assumed to scale with surface area (exponent around 0.67). However, if one takes into account boundary layers and relative roles of convection and diffusion with mass transfer relationships, the scaling exponent is much lower (exponent around 0.3). As a consequence, smaller size reductions are predicted (as with a decrease in body size, demand drops more quicly than the fall in supply capacity).

Commented [AD35R34]: Thanks for these good comments. We deal later with Deutsch et al. (2022), as at this point we are just providing examples of overestimation. At the end of this section, we will mention the point about this benefit of mass-transfer models.

where m is mass, t is time, H and K are coefficients of anabolism (or assimilation) and catabolism (or maintenance metabolism) respectively, and a and c are their respective exponents. In these models, relative to the rates of anabolism (assimilation), the rate of catabolism (maintenance metabolism) scales more steeply with increasing body mass ($c > a$) and the coefficient for catabolism is more sensitive to temperature ($dK/dT > dH/dT$, where T is temperature) (Atkinson and Sibly, 1997; Kearney, 2021; Pauly, 2021). These models can predict the observed increase in initial growth rate with warming (Atkinson and Sibly, 1997) as well as the decrease in growth rate with warming later in ontogeny.

However, it is important that any modeled decline in growth should allow for individuals to have sufficient capacity for oxygen uptake and energy acquisition in order to complete reproduction (Kozłowski *et al*., 2004; Kearney, 2019; Pauly, 2021). Moreover, growth models should incorporate the amount of resources diverted from late growth towards reproduction (Day and Taylor, 1997; Kozłowski *et al*., 2004; Kearney, 2019; Marshall and White, 2019), which is predicted by life-history optimization to arise from selection by external mortality (Kozłowski *et al*., 2004). We suggest that part of the ongoing controversy about what determines the shape of growth curves when resources are abundant (Pauly, 2019; Marshall and White, 2019; Kearney, 2019; White and Marshall, 2019) may be reduced if: (i) explanations incorporating the ultimate (selective) effect of oxygen or other resource limitation (Section 2i, above) are prioritized over proximate constraints of oxygen or energy shortage on growth, which we have shown is unlikely to apply widely in favorable conditions, and (ii) the debate is not polarized as selection either on increased

Commented [WV36]: Good addition? Or I guess if one chooses the exponents correctly, it could explain decreases in growth rate….right?

Commented [AD37R36]: The models also can predict the growth decrease later in ontogeny, so I've changed your added text accordingly. But other problems occur, which are described later.

Commented [WV38]: This needs a better transition as the previous sentence talks about increase in growth. I have tried to add a transition.

Commented [AD39R38]: We have adapted your transition (see above response), and used it.

Commented [WV40]: This is central also to DEB.

Commented [AD41R40]: Agreed. This is covered by Kearney 2019, from which more DEB papers can be accessed.

reproduction or on avoiding oxygen (or other resource) limitation; both may need to be considered.

Additionally, a particularly crucial flaw in these classic models, including the variant by Einum *et al*. (2021), is that they predict a reduction in growth efficiency with warming; this runs counter to the typically increased efficiency observed under benign conditions in the physiological temperature range (Angilletta and Dunham, 2003). However, it would be informative to investigate whether warming enhances efficiency at small but not at large sizes during ontogeny - which would mirror the observed effects of temperature on growth rate during ontogeny.

It remains to be determined whether this failure to avoid unrealistic reductions in warming-induced growth efficiency applies also to a recent model that quantitatively predicts the TSR across a range of sizes from microbes to large fish (Deutsch *et al*., 2022). Observations used to parameterize this model indicate that hypoxia tolerance typically declines with size during growth - slightly in fish and more strongly in smaller species - and also with increased temperature. This model did not examine growth trajectories explicitly, though it is worth exploring whether its predicted warming-induced body size reduction arises from assumptions that match those used in the classic growth models. Specifically, does the greater temperature dependence and shallower mass-scaling of metabolic or oxygen demand than of oxygen supply efficacy also predict a warming-induced reduction in growth efficiency at all sizes? Such a finding would run counter to the observed warming-enhanced growth efficiency under conditions relevant to the TSR (Angilletta and Dunham,

Commented [WV42]: This increased efficiency is likely also size dependent and may in fact be the same problem of trying to explain both faster initial growth and slower final growth?

Commented [AD43R42]: Yes. I agree. I tried not to make the arguments more complex, but now that you mention it, I have now added a new sentence after (Angilletta and Dunham, 2003).

Commented [AD44]: Have used comments from the guest editor and information from Deutsch et al. 2022 to explain the issue more fully in this paragraph.

2003). Unlike the previous models of the TSR, that of Deutsch *et al.* (2022) predicts well the interspecific mean and variability of body size reduction with warming. It is therefore important to know whether unrealistic warming-induced reductions in growth efficiency are mostly avoided in this model, or whether the improved predictions of TSR emerge despite an unrealistic formulation.

This problem with how the growth models are applied to predicting the TSR (Angilletta and Dunham, 2003) is not new, but the continued use of such models requires measures to overcome the problem. More generally, the models poorly capture effects of size and temperature on metabolism. Thus, instead of a simple difference in temperature-dependence of assimilation and maintenance metabolism in the classic growth model, a more realistic growth model would capture changes in multiple costs, efficiencies and resource allocations with: (i) temperature (e.g. enzyme titers and efficiencies; viscosity effects on movement in water; Verberk and Atkinson, 2013); (ii) body size (e.g. size-scaling of costs of locomotion and ventilation); and (iii) combined size and temperature (e.g. viscosity effects on scaling of ventilation; Section 1; Verberk and Atkinson, 2013; Verberk *et al*., 2021). At different sizes and temperatures, adjustments may be made to the capacity for uptake of limiting resources (e.g. Sollid *et al*., 2005; Sollid and Nilsson, 2006) (the upper metabolic limit, considered the upper boundary of the oxygen supply safety margin above routine expenditure), to the size of the safety margin, and to the amount of other routine investment, which is not just tissue maintenance but may also include routine costs of locomotion and resource capture (Verberk *et al*., 2021). Not accounting for these adjustments and how they vary between species is likely to produce erroneous predictions

of warming-induced shrinkage in adult body size. However, modeling all these adjustments individually will likely be prohibitive, and therefore alternative ways of capturing size-dependent responses to temperature will be required. One step towards resolving the problem could include allowing not just the coefficients to be temperature-dependent, but also the exponents, *a* and *c* as individuals grow larger (Kozłowski et al., 2004), which is more likely to reflect observed metabolic responses (e.g. Rubalcaba *et al*., 2020). It is also important to correctly characterize empirically the two terms on the right side of Equation 4, so that their difference equates to growth and nothing else. For instance, if standard metabolic rate is used as a measure of maintenance metabolism or catabolism, its growth overheads will need to be accounted for (Rosenfeld *et al*., 2015). Moreover, an assumption of isometric scaling is not often supported for metabolism (hence catabolism) at various levels of activity including standard, resting and routine (Glazier, 2005.

One potential solution to prevent overestimating warming-induced reductions of body size may be to incorporate mass-transfer dynamics. Einum *et al*. (2021) did not use such dynamics in their model that overestimated size reduction, and assumed that uptake capacity scaled with surface area (exponent around 0.67). However, Deutsch *et al*. (2022), used mass-transfer modeling to compare responses of species across the size range from aerobic microbes to large metazoans, examining the relative roles of convection and diffusion on oxygen movement through the stagnant boundary layer surrounding respiratory exchange surfaces. They predicted a much lower scaling exponent of around 0.3 for microbes, leading to a lesser warming-induced body size reduction compared with that of larger metazoans, as is generally observed (Forster *et al*., 2012).

Commented [WV45]: I feel there are two points here, which deserve to be disentangled:
1.Isometric scaling (exponent of 1) is inappropriate, given the overwhelming evidence of allometric scaling
2. Allometric scaling of SMR may not reflect the catabolism term as it includes not only maintenance metabolism, but also growth overheads and other overheads.

Commented [AD46R45]: We have re-written the sentence prior to the text highlighted by the guest editor to make the general point that a good empirical application of the model would generate just growth as the difference between the two terms in the growth equation. We then attempt to address the specific concerns of the guest editor.

Commented [AD47]: This paragraph in response to Wilco's earlier comments about the use of mass-transfer improving predictions in Deutsch et al. (2022).

Conclusion

To improve estimates of oxygen bioavailability and to provide mechanistic models of oxygen limitation, we have applied mass-transfer modeling to quantify how water flow regime at external gas exchange surfaces affects oxygen bioavailability. Applying this modeling framework to amphipods, we found a greater increase in oxygen bioavailability with warming ($Q_{10} \sim 1.24$) when costs of moving oxygen are kept constant, compared with using the Oxygen Supply Index (Verberk *et al.*, 2011) ($Q_{10} \sim 1.05$-1.16). Unlike the OSI, our measure of oxygen bioavailability is not a single generic index, but can be customized to particular respiratory systems (e.g. laminar *versus* turbulent flow through open *versus* closed channels or along differently shaped surfaces) and levels of respiratory activity. The framework therefore provides a more complete mechanistic approach to understanding variation in oxygen bioavailability among diverse water-breathers. However, a full exploration of impacts of different flow regimes and respiratory structures will be the subject of future work. A major further benefit from such modelling arises from its potential to generate realistic estimates of both the rate of oxygen uptake, and the aerobic cost of achieving such uptake. Thus, maximizing the oxygen available for all metabolic processes not associated with oxygen movement (e.g. maintenance, reproduction and growth) ($_{\mathrm{r}}\dot{M}_{\mathrm{O2}}$) depends on how much oxygen is devoted to moving oxygen from the environment to tissues. A consequence of this distinction between types of metabolic cost is that we predict an increase in costs of moving water and oxygen while $_{\mathrm{r}}\dot{M}_{\mathrm{O2}}$ decreases as pO_2 is reduced towards $p_{\mathrm{crit}O2}$. This prediction challenges the idea that oxygen limitation of metabolic rate at $p_{\mathrm{crit}O2}$ reflects the start of oxygen limitation on fitness. Instead, we predict

Commented [WV48]: So vMO2 is ventilation?

Commented [LG49R48]: This is highlighted a few times by Wilco. See my response in Section vi.

Commented [AD50R48]: Changed text to' moving oxygen from environment to tissues, which in principle can include both ventilation and circulation. In section 1.iv, we discuss how in practice, for the amphipod model, we had to use ventilation costs as an index of these costs.

Commented [WV51]: Maybe add a sentence on how that is relevant to the reader? I guess it challenges the idea of Pcrit estimations which is defined as the point where oxygen consumption equals the minimal maintenance costs and these are assumed to be constant, but this may not be the case as you have argued here, This implication may not be obvious for the reader

Commented [AD52R51]: Now tried to spell out the relevance more fully.

609 that reductions in metabolic activities contributing to fitness occur before p_{crit02} is reached.
610 The appearance of an approximately invariant total metabolic rate as oxygen partial
611 pressure is reduced below normoxia results from a simultaneous increase in investment in
612 generating water and oxygen movement. The modeling framework can be developed
613 further to provide mechanistic predictions of oxygen limitation and life history (e.g. body
614 size). Thus, by incorporating into the models the costs of moving water and oxygen, and
615 how these costs vary with size and temperature, oxygen limitation at different body sizes
616 and temperatures can help predict combinations of body size and temperature that avoid
617 oxygen limitation.
618
619 We argue that a physiological understanding should be combined with evolutionary
620 principles to clearly distinguish between oxygen as a proximate and as an ultimate factor.
621 We describe how experimental treatments that increase rather than reduce oxygen tension
622 should be used to test for proximate oxygen limitation. Under benign growth conditions
623 within the normal range of oxygen tensions and physiological temperatures used to
624 describe the Temperature-Size Rule, experimental hyperoxia provided little evidence that
625 oxygen normally limited growth and adult size. Overall, we found little support for
626 warming-induced oxygen limitation usually decelerating growth and producing small
627 adults. However, evidence was consistent with oxygen as an ultimate or evolutionary factor
628 leading to slowed growth and smaller size at increased temperatures that avoided oxygen
629 limitation – termed the Ghost of Oxygen Limitation Past. We proposed that experiments to
630 test this idea should decouple the proximate cause or environmental cue (temperature)
631 from the ultimate cause (protecting aerobic scope from oxygen limitation). We suggest that

part of the ongoing controversy about what determines the shape of growth curves when resources are abundant may be reduced if: (i) explanations incorporating the ultimate (selective) effect of oxygen or other resource limitation are prioritized over proximate constraints of oxygen or energy shortage on growth, which we showed was unlikely to apply widely in favorable conditions, and (ii) the debate is not polarized by arguing for selection just on increased reproduction or just on avoiding oxygen (or other resource) limitation; both may need to be considered. Finally, we highlight a crucial flaw in classic growth models that led to a predicted reduction in growth efficiency with warming, which runs counter to the typically increased efficiency observed under benign conditions in the physiological temperature range. Overall, this paper shows the importance of a multi-disciplinary approach, as advocated by Verberk *et al.* (2016a) and Audzijonyte *et al.*, (2019), which here combines perspectives from evolutionary ecology and physiology.

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Figure legends

Figure 1. A schematic of the change in oxygen partial pressure through the amphipod ventral groove (arrow indicates direction of water flow), assuming the partial pressure of oxygen in hemolymph is constant. As oxygen is extracted at the gill plates, the partial pressure of oxygen at the inflow (p_{O2i}) falls in line with the decline in the mass of dissolved oxygen (solid line). At lower solubilities (such as at higher temperature, which also causes a much stronger increase metabolic oxygen demand), p_{O2} (dashed line) will fall more rapidly for a fixed water volume flow rate ($\dot{V}_w$) and rate of movement of oxygen from water to blood ($\dot{M}_{O2}$). This effect cannot occur in isolation however, because the decline in the average partial pressure in water along the gill plates from inflow to outflow $p_{O2w} = (p_{O2i} + p_{O2e})/2$ and hence the partial pressure difference Δp_{O2w-h} between water and hemolymph across the gill diffusion barrier also reduces $\dot{M}_{O2}$, which will have a corresponding effect on variation in Δp_{O2i-e}.

Figure 2. A schematic of the idealised amphipod respiratory gas exchange system. Turbulent water flow is generated by beating abdominal limbs (pleopods, not shown) and passes posteriorly (arrow denotes direction of flow) through a narrow channel of width *h*, within the lateral walls formed by 6 pairs of gill plates. In our idealized amphipod gas exchange channel, and in order to simplify the mass transfer modelling, we treat the 6 gill plates flanking one side of the channel as a single liquid-solid interface where gas exchange occurs over the surface of area *l.d*. Although the system is open anatomically, it is treated physiologically as a closed gas exchange space, with no oxygen diffusion or admixture of

Commented [WV53]: Does it need to be zero? Seems a bit extreme. I also think the model will werk when partial pressure is uch lower than that of the water.

Commented [AD54R53]: Yes, it is just constant. Zero has been removed.

Commented [WV55]: The effect of temperature on the rate of oxygen extraction will be much greater than that on solubility…might be worth mentioning oxygen demand too?

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866 water assumed to occur from outside the groove. Although there is evidence of a role for
867 extra-branchial gas exchange in some amphipod species (Spicer and Gaston, 1999) and life
868 stages (Spicer and McMahon, 1992), this is not incorporated into our modelling, which
869 assumes the gills are the principal site of respiratory gas exchange.
870
871 **Figure 3**. A semi-$\log_{10}$ plot of variation in the rate of movement of oxygen ($\dot{M}_{O2}$; dotted)
872 from water to hemolymph in our amphipod gas transfer model, where the aerobic cost to
873 the animal of pumping water through the channel remains constant and the initial fraction
874 of oxygen extraction from the water current is 25%; all values are normalised to 0°C.
875 Physical variables are predicted to drive variation in $\dot{M}_{O2}$ with temperature, according to
876 the equation $\dot{M}_{O2} \propto \Delta p_{O2w\text{-}h}.A.\alpha_{wO2}.D_{wO2}^{2/3}.\nu_w^{4/5}/(\upsilon_w^{7/15}.h^{1/5})$ (Eqn. 3). These variables are
877 diffusivity of oxygen in water ($\dot{M}_{O2} \propto D_{wO2}^{2/3}$; fine dash), kinematic viscosity of water ($\dot{M}_{O2} \propto$
878 $\upsilon_w^{-7/15}$; coarse dash), the partial pressure gradient of oxygen ($\dot{M}_{O2} \propto \Delta p_{O2w\text{-}h}$; dash dot dash),
879 solubility of oxygen in water ($\dot{M}_{O2} \propto \alpha_{wO2}$; dash dot dot dash), and linear water velocity
880 through the channel ($\dot{M}_{O2} \propto \nu_w^{4/5}$; medium dash). Linear water velocity varies proportional
881 to dynamic viscosity of water (μ_w) to the power -1/2 when assuming a constant power
882 spent in ventilation. These physical variables combine to produce an increase in $\dot{M}_{O2}$ with
883 increasing temperature. Residual $\dot{M}_{O2}$ ($_r\dot{M}_{O2}$; solid) represents the oxygen bioavailability for
884 functions not involved in the physical process of oxygen movement (i.e. those pumping
885 external water and hemolymph) and shows an approximate Q_{10} of 1.24.
886
887 **Figure 4**. Variation in three different proposed metrics of oxygen bioavailability with
888 changing temperature, normalised to 0°C and displayed on a semi-$\log_{10}$ plot. All models

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assume constant environmental partial pressure of oxygen (p_{O2i} in the analysis presented here). Water oxygen content will decline in line with the solubility coefficient of oxygen in water (α_{wO2}; dash dot dot dash); $Q_{10} \simeq 0.83$. The Oxygen Supply Index, or OSI (product of solubility and diffusivity coefficient variation; dash), increases slightly with temperature; $Q_{10} \simeq 1.11$. Our definition of oxygen bioavailability, as mass-transfer-derived variation in the rate of oxygen movement ($\dot{M}_{O2}$), when costs of oxygen movement are constant and subtracted from whole organism $\dot{M}_{O2}$ ($_{r}\dot{M}_{O2}$; solid) and initial fraction of oxygen extraction from the water current is 25%, also increases with temperature in amphipods, but to a greater extent than the OSI; $Q_{10} \simeq 1.24$.

Legend for Supplement S1, available online.

Oxygen bioavailability and its response to warming is modelled using a mass-transfer approach. We illustrate the modeling using an amphipod model of respiration. The model is described step-by-step under the following headings: Fundamentals of oxygen transfer across respiratory gas exchange surfaces; Mass-transfer treatment; Energetics of water flow; Normalising temperature effects on total and residual $\dot{M}_{O2}$ to a 0°C reference; Physical variables contributing to thermal sensitivity of total and residual $\dot{M}_{O2}$ in the amphipod gill model; Table S1. Q_{10} values for a range of physical variables relevant to the modelling of respiratory gas transfer presented in this study; Table S2. Abbreviations for terms used in modeling in this study. Supplementary references.