

Supplement S1 (online) to:

Controversial roles of oxygen in organismal responses to climate warming

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Fundamentals of oxygen transfer across respiratory gas exchange surfaces

The fundamental equation that underlies the movement of molecular oxygen (O₂) across a respiratory gas exchange surface is given by:

$$\dot{M}_{O_2}/A = \Delta p_{O_2w-h}/R_{O_2} \quad \text{[Equation S1]}$$

(Hills and Hughes, 1970), where \dot{M}_{O_2}/A is rate of movement of oxygen \dot{M}_{O_2} (uptake, mol per unit time) per unit respiratory gas exchange surface area A , Δp_{O_2w-h} (in atm.) is the oxygen partial pressure difference, or difference of partial pressure of oxygen between water and blood, or hemolymph, on either side of the respiratory exchange surface, and R_{O_2} is the total resistance to oxygen movement in the direction of the partial pressure gradient (units of s. atm. cm². mol⁻¹). Moles are converted to mass using a constant conversion factor, which does not affect the outcome of our analysis. In this equation, the resistance term works in the same way as electrical resistance, while the partial pressure gradient acts as the voltage, the fundamental potential difference driving oxygen movement from high to low partial pressure of oxygen, where oxygen carrying

capacities either side of the gas exchange barrier are different (Dejours, 1981; Piiper *et al.* 1971).

In mass transfer terms, R_{O_2} is the inverse of a conductance term that is itself the product of the capacitance (β_{O_2}) and mass transfer (k_{O_2}) coefficients for oxygen ($R_{O_2} = 1/(\beta_{O_2} \cdot k_{O_2})$) across all relevant mass transfer phases (Hills and Hughes, 1970). Within respiratory gas exchange systems, R_{O_2} is broken down into terms representing the different phases of oxygen movement (Barry and Diamond, 1984; Hughes, 1972). In our amphipod example, oxygen movement, and hence resistance to oxygen movement, will occur across three phases: turbulent water flow in the ventral groove, the static respiratory membrane, and an assumed laminar flow of hemolymph within the gill plate lacunar space:

$$R_{O_2} = R_{wO_2} + R_{mO_2} + R_{hO_2} \quad \text{[Equation S2]}$$

In Eqn. S2, the subscripts w, m and h refer to the resistances provided by the respective phases of oxygen movement (water, membrane, and hemolymph, respectively). Thus, considering that the capacitance coefficient for oxygen in water, β_{O_2} , is equivalent to the solubility coefficient for oxygen, α_{wO_2} , we get for the resistance term R_{wO_2} for movement of oxygen within the turbulent water flow of the amphipod ventral groove:

$$R_{wO_2} = 1/(\alpha_{wO_2} \cdot k_{wO_2}). \quad \text{[Equation S3]}$$

In blood or hemolymph, the solubility coefficient for oxygen, α_{wO_2} , may not only differ from that in external water because of differences in ion concentration but the capacitance coefficient for oxygen in blood or hemolymph (β_{hO_2}) may also include the effects of the concentration of respiratory pigments and the shape of their oxygen equilibrium curves, which will affect the resistance term R_{hO_2} for oxygen movement in the blood or hemolymph phase.

Given the much greater width of the water-flow channel in amphipods relative to respiratory membrane thickness and vascular channels within gill plates, we will assume that most of the resistance to oxygen movement exists in the water phase, that is, in Eqn. S2: $R_{wO_2} \gg R_{mO_2}$ or R_{hO_2} . This point has been argued for the fish gill (Malte and Weber, 1985; Scheid and Piiper, 1971; Hills and Hughes, 1970), however, no such consideration has been given to amphipods before. Applying this assumption to resistance to oxygen movement in amphipods (Eqns. S2 and S3) yields:

$$R_{O_2} \propto 1/(\alpha_{wO_2} \cdot k_{wO_2}) \quad \text{[Equation S4]}$$

The p_{O_2} of inspired water will decline as water passes through the respiratory channel and oxygen is transferred to hemolymph flowing within the gill plates. Assuming no admixing with fully oxygenated water occurs with water in ventilatory groove from inflow through to outflow, the rate of movement of oxygen from water to blood will be expressed using the Fick equation:

$$\dot{M}_{O_2} = \dot{V}_w \cdot \alpha_{wO_2} \cdot (p_{O_2i} - p_{O_2e}) \quad \text{[Equation S5]}$$

where \dot{V}_w is volume flow of water per unit time through the channel, and p_{O_2i} and p_{O_2e} are the partial pressures of oxygen in water entering (inflow) and exiting (outflow) the channel, respectively. Rearranging Eqn. S5 gives:

$$p_{O_2e} = p_{O_2i} - \dot{M}_{O_2} / \dot{V}_w \cdot \alpha_{wO_2} \quad [\text{Equation S6}]$$

In our amphipod example, the variability in p_{O_2} within the hemolymph of individual gill plates is expected to be small relative to the variation from inflow to outflow within the ventilatory water channel, which is supported by the constancy of hemolymph p_{O_2} in a previous (Spicer and McMahon, 1992). Δp_{O_2w-h} in Eqn. S1 will therefore be determined primarily by variations in p_{O_2} within water flowing through the ventral groove; p_{O_2} variation in hemolymph as it flows through the vascular space of gill plates will be considered negligible therefore and a constant hemolymph p_{O_2} of zero will be assumed in our analysis. In analogy to the fish gill and following Randall *et al.* (1967), the partial pressure difference of oxygen (Δp_{O_2i-e}) for water flowing through a gas exchange channel can be expressed as an arithmetic mean of inflow and outflow p_{O_2} values:

$$\Delta p_{O_2i-e} = (p_{O_2i} + p_{O_2e}) / 2 \quad [\text{Equation S7}]$$

Inserting the term for p_{O_2e} from Eqn. S6 into Eqn. S7 and simplifying gives:

$$\Delta p_{O_{2i-e}} = p_{O_{2i}} - \dot{M}_{O_2} / (2 \cdot \dot{V}_w \cdot \alpha_{wO_2}) \quad [\text{Equation S8}]$$

Thus, if we know the values of environmental and hence inspired water p_{O_2} ($p_{O_{2i}}$), \dot{M}_{O_2} , \dot{V}_w and α_{wO_2} , or their normalised values (their factorial deviation from reference values for organisms in a given state), we can establish how these factors influence $\Delta p_{O_{2i-e}}$. An iterative function can then be used to incorporate Eqn. S8 into Eqn. S1 and generate a dynamic model of oxygen movement, which can predict how changes in flow dynamics, body dimensions, and other physical factors directly influence \dot{M}_{O_2} (see Figure 1).

Mass-transfer treatment

In mass-transfer scenarios involving both forced convective flow and diffusion, such as in the case of oxygen movement within water flowing through a gas exchange channel, convective flow is expressed using the Reynolds number (ratio of inertial to viscous forces, which increases with increasing linear water velocity and turbulence), and 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 for oxygen in water (k_{wO_2}), which is expressed within the dimensionless Sherwood number (ratio of convective mass transfer to diffusive mass transport) (Cussler, 2009). Thus:

$$\text{Sherwood number} = k_{wO_2} \cdot h / D_{wO_2}$$

$$= \text{Constant} \cdot (\text{Reynolds number})^x \cdot (\text{Schmidt number})^y$$

[Equation S9]

where h is the width of the channel and D_{wO_2} 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, below).

For turbulent water flow through a horizontal channel, as applied here for the amphipod system (Fig. 2), the Sherwood number can be expressed (Cussler, 2009) as:

$$k_w \cdot h / D_w = 0.026 \cdot (h \cdot v_w / \nu_w)^{4/5} \cdot (\nu_w / D_w)^{1/3} \quad \text{[Equation S10]}$$

where v_w is linear water velocity through the channel and ν_w is the kinematic viscosity of water. Rearranging gives the following relationship for the mass transfer coefficient:

$$k_{wO_2} \propto D_{wO_2}^{2/3} \cdot v_w^{4/5} / (\nu_w^{7/15} \cdot h^{1/5}) \quad \text{[Equation S11]}$$

Substituting into Eqn. S4, and then Eqn. S1, gives the rate of movement of oxygen from water to hemolymph within our idealized amphipod as

$$\dot{M}_{O_2} \propto \Delta p_{O_2 w-h} \cdot A / [\nu_w^{7/15} \cdot h^{1/5} / (\alpha_{wO_2} \cdot D_{wO_2}^{2/3} \cdot v_w^{4/5})]$$

or simplified:

$$\dot{M}_{O_2} \propto \Delta p_{O_2 w-h} A \alpha_{wO_2} D_{wO_2}^{2/3} v_w^{4/5} / (v_w^{7/15} h^{1/5}) \quad [\text{Equation S12}]$$

This now allows use of Eqn. S8 to solve Eqn. S12 iteratively (for constant p_{iO_2}).

Energetics of water flow

Incorporating the energetics of water flow through the amphipod ventral groove is considered an essential element of this analysis, because the actual variation in oxygen available to the organism will be the whole-organism \dot{M}_{O_2} minus the aerobic cost in terms of oxygen consumption for generating that \dot{M}_{O_2} , or, put another way, the aerobic cost of physically moving oxygen from water to tissues. Although the aerobic cost of increasing \dot{M}_{O_2} will include aerobic metabolism associated with pumping water by the ventilatory gill pump ($v\dot{M}_{O_2}$) and pumping blood or hemolymph by the circulatory cardiac pump ($c\dot{M}_{O_2}$), our analysis will only consider variation in $v\dot{M}_{O_2}$ and assume this to be a proxy for variation in $c\dot{M}_{O_2}$ as well. We will now predict how \dot{M}_{O_2} varies with changing temperature in our amphipod gas exchange model, if the animal makes no adjustment in the aerobic energy spent delivering oxygen from water to tissues as temperature changes, that is, when $v\dot{M}_{O_2}$ remains constant.

The energy expenditure of the water pump, in terms of power, is expressed in the same fundamental terms as \dot{M}_{O_2} , and therefore the relative variation in power spent

pumping water through the central groove (P_w) and $v\dot{M}_{O_2}$ are directly interchangeable if we assume constancy of aerobic muscle efficiency. We first need to predict how \dot{V}_w varies with temperature, such that P_w is constant; we then observe how this translates into variation in linear water velocity v_w through the ventral groove, and the subsequent impact this has on \dot{M}_{O_2} (Eqn. S12).

The ventral groove of our idealized amphipod (Figure 2) has a width (h) that is much shorter than its depth (d), and length (l). Water is pumped through this groove at a specific rate, in terms of volume per unit time (\dot{V}_w). Linear water velocity (v_w) corresponds to \dot{V}_w divided by the cross-sectional area of the horizontal slit, or $h.d$.

Power spent in ventilation (P_w) is expressed, following Vogel (1994), as:

$$P_w = \dot{V}_w^2 \cdot \theta_w \quad \text{[Equation S13]}$$

where the resistance to water flow (θ_w) within a narrow horizontal channel is expressed (Vogel, 1994) as:

$$\theta_w \propto l \cdot \mu_w / h^3 \cdot d \quad \text{[Equation S14]}$$

Here μ_w is the dynamic viscosity of water. If there is no change in body dimensions (which will be assumed in this analysis), we see that, for P_w to remain constant with changing temperature:

$$\dot{V}_w \propto \mu_w^{-1/2} \quad \text{[Equation S15]}$$

and therefore:

$$v_w \propto \mu_w^{-1/2} \quad \text{[Equation S16]}$$

We can now substitute Eqn. S16 into Eqn. S12, to give us the variation in \dot{M}_{O_2} with temperature such that the aerobic cost of ventilating the ventral groove remains constant. Assuming constant body and gill dimensions, Eqn. S12 is expressed as:

$$\dot{M}_{O_2} \propto \Delta p_{O_2w-h} \cdot \alpha_{wO_2} \cdot D_{wO_2}^{2/3} \cdot v_w^{4/5} / u_w^{7/15} \quad \text{[Equation S17]}$$

Substituting Eqn. S16 into Eqn. S17 gives:

$$\dot{M}_{O_2} \propto \Delta p_{O_2w-h} \cdot \alpha_{wO_2} \cdot D_{wO_2}^{2/3} / (\mu_w^{2/5} \cdot v_w^{7/15}) \quad \text{[Equation S18]}$$

Kinematic viscosity ν_w is the ratio of dynamic viscosity μ_w and the density of water. Therefore Eqn. S18 could be further simplified by combining the dynamic viscosity terms, however this would have then meant that we could not clearly distinguish the effect of variation in linear water velocity v_w at neutral cost to the organism. For this

reason, Eqn. S18 has not been simplified further. However, the overall effect is that \dot{M}_{O_2} will vary with dynamic viscosity to the power $-13/15$ and density to the power $7/15$.

Normalising temperature effects on total and residual \dot{M}_{O_2} to a 0°C reference

When measuring effects that are normalised to a reference value (in this case 0°C), the relative variation in \dot{M}_{O_2} available for residual tissues not involved in the physical process of oxygen movement (${}_r\dot{M}_{O_2}$) will also be dependent on a reference fraction of whole organism metabolism (\dot{M}_{O_2}) dedicated to ${}_r\dot{M}_{O_2}$ (f_r). Thus:

$$f_r = {}_r\dot{M}_{O_2} / \dot{M}_{O_2} \quad \text{[Equation S19]}$$

Therefore:

$${}_r\dot{M}_{O_2} = f_r \cdot \dot{M}_{O_2} \quad \text{[Equation S20]}$$

Given f_r is the remainder of \dot{M}_{O_2} after ${}_v\dot{M}_{O_2}$ is subtracted:

$$f_r = 1 - f_v \quad \text{[Equation S21]}$$

where f_v is the fraction of \dot{M}_{O_2} dedicated to oxygen movement (${}_v\dot{M}_{O_2} / \dot{M}_{O_2}$). Therefore:

$${}_r\dot{M}_{O_2} = (1 - r_v) \cdot \dot{M}_{O_2} \quad [\text{Equation S22}]$$

Thus, if we can predict variation in \dot{M}_{O_2} for neutral variation in ${}_v\dot{M}_{O_2}$ (Eqn. S17), Eqn. S22 can be used to estimate normalised variation in ${}_r\dot{M}_{O_2}$, the real metric of interest (Figure 3).

Physical variables contributing to thermal sensitivity of total and residual \dot{M}_{O_2} in the amphipod gill model

Values important to the modeling of variation in both \dot{M}_{O_2} and ${}_r\dot{M}_{O_2}$ are the 0°C values of the fraction of \dot{M}_{O_2} dedicated to oxygen movement r_v (assumed to be 0.2, given this includes aerobic costs of pumping water externally and hemolymph internally) and p_{O_2e} , which was assumed to be 75% of p_{O_2i} given the arrangement of the amphipod gills within the water channel is unlikely to result in a large fractional extraction of oxygen from the water current. The gills of amphipods were suggested to be low efficiency by Chapelle & Peck (1999), however this was contested by Spicer & Gaston (1999); no data exists for O_2 extraction from the water current in amphipods. Environmental p_{O_2} (p_{O_2i}) has been assumed to be constant with changing temperature, and all variation is estimated for air-equilibrated seawater (Salinity= 35 practical salinity units, p.s.u.) at atmospheric gas pressure.

Table S1 presents thermal sensitivity relations of the physical variables that contribute to variation in \dot{M}_{O_2} within the amphipod ventral groove with changing temperature

(Eqn. S17; Fig. 3), and the sources used to calculate their variation. Figure 4 contrasts the thermal sensitivities of uptake that relies on solubility alone, the combination of solubility, diffusivity and p_{O_2i} (the latter being constant) in the OSI (Verberk *et al.*, 2011) and the cost neutral variation in $r\dot{M}_{O_2}$ modelled in the present work.

Table S1. Q_{10} values for a range of physical variables relevant to the modeling of respiratory gas transfer presented in this study. Solubility and diffusivity variation is provided for oxygen in water. All values are for air equilibrated seawater (Salinity = 35 p.s.u.) at atmospheric gas pressure.

Physical variable	Q_{10} (0-30°C)	Q_{10} (0-15°C)	Q_{10} (15-30°C)	References
Solubility (mol m ⁻³ Pa ⁻¹)	0.83	0.80	0.85	Benson and Krause (1984)
Diffusivity (m ² s ⁻¹)	1.35	1.39	1.30	St Denis and Fell (1971)
Dynamic viscosity (kg m ⁻¹ s ⁻¹)	0.77	0.74	0.79	Patek <i>et al.</i> (2009), Siedler and Peters (1986)
Density (kg m ⁻³)	1.00	1.00	1.00	Siedler and Peters (1986)
Kinematic viscosity (m ² s ⁻¹)	0.77	0.75	0.80	(ratio of dynamic viscosity and density of water, see above)

Table S2. Abbreviations for terms used in modeling in this study.

Abbreviation	Definition
<i>A</i>	Gas exchange surface area

a	Exponent of the coefficient of anabolism (or assimilation) H
α_{wO_2}	Solubility coefficient for oxygen in water
β_{hO_2}	Capacitance coefficient for oxygen in hemolymph
c	Exponent of the coefficient of catabolism (or maintenance metabolism) K
c_{critO_2}	Oxygen concentration below which oxygen uptake decreases
$c\dot{M}_{O_2}$	\dot{M}_{O_2} associated with pumping hemolymph around the body
c_{O_2}	Concentration of oxygen
d	Depth of the ventral groove
$\Delta p_{O_{2i-e}}$	Partial pressure difference of oxygen for water flowing through a gas exchange channel from inflow to outflow
$\Delta p_{O_{2w-h}}$	Partial pressure difference of oxygen between water and blood, or hemolymph, on either side of the respiratory exchange surface
D_{wO_2}	Diffusion coefficient of oxygen in water
r_r	Residual fraction of \dot{M}_{O_2} when cost of oxygen movement removed
r_v	Fraction of \dot{M}_{O_2} dedicated to ventilation and circulation
h	Width of the ventral groove
H	Coefficient of anabolism (or assimilation)
K	Coefficient of catabolism (or maintenance metabolism)
k_{wO_2}	Mass transfer coefficient for oxygen in water
l	Length of the ventral groove
m	Mass
\dot{M}_{O_2}	Rate of movement of oxygen from water to hemolymph
μ_w	Dynamic viscosity of water
p_{critO_2}	Oxygen partial pressures below which oxygen uptake decreases
p_{O_2}	Partial pressure of oxygen
$p_{O_{2e}}$	Outflow partial pressure of oxygen
$p_{O_{2i}}$	Inflow partial pressure of oxygen
P_w	Power expenditure associated with ventilating the ventral groove
R_{bO_2}	Resistance to oxygen movement within gill hemolymph
R_{mO_2}	Resistance to oxygen movement within the gill membrane
$r\dot{M}_{O_2}$	Residual \dot{M}_{O_2} when cost of oxygen movement removed
R_{O_2}	Total resistance to oxygen movement from water to hemolymph
R_{wO_2}	Resistance to oxygen movement within water
t	Time

T	Temperature
θ_w	Resistance to water flow within the ventral groove
u_w	Kinematic viscosity of water
v_w	Linear water velocity through the ventral groove
\dot{V}_w	Volume flow rate of water through the amphipod ventral groove
x	Empirical exponent of the Reynolds number in mass transfer relations
y	Empirical exponent of the Schmidt number in mass transfer relations

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