Electronic Supplementary Material

Appendix A1

Contents

Biophysical model parameterization. We processed raw O_2 measurement readings (%O_2 saturation) from individual respirometry chambers to estimate the metabolic rates of eggs within each chamber (μgO_2 h^{-1}). To do this, we first fit cubic splines to individual O_2 traces from each replicate chamber in the dataset. We then took the derivative of each spline to estimate O_2 depletion in chambers in terms of %O_2 saturation h^{-1}. We used replicate chambers with no eggs (blanks) to correct for changes in O_2 due to small fluctuations in temperature in the respirometry chambers during the beginning of the trials. To do this, we calculated the mean rate of % O_2 change for all blank chambers within a replicate used this mean blank rate ($\Delta\bar{O}_{blank}$) to correct for O2 changes in the chambers with eggs not due to metabolism:

$$\Delta O_{egg(corrected)} = \Delta O_{egg(raw)} - \Delta\bar{O}_{blank}\frac{O_{egg}}{\bar{O}_{blank}}$$

Rates of O_2 loss in wells was then converted to units of μgO_2 h^{-1} (dC_o/dt) by accounting for the O_2 concentration of water at saturation, $O_{sat}(T)$, and the volume of water in the egg chamber, V_o:

$$\frac{dC_o}{dt} = \frac{\Delta O_{egg(corrected)}\, O_{sat}(T) V_o}{100}$$

We then extracted estimates of dC_o/dt for each egg in the dataset at O_2 concentrations ranging from 10% to 100% O_2 saturation at intervals of 10%. We used these data to fit our biophysical model.

We fit our four-parameter biophysical model to the dataset described above though non-linear optimization. We did this by integrating Eq. 3. and 6 (main text) using the Euler method with a step size of 12 s. The initial C_o was set to a concentration above O_2 saturation at all temperature (15 μg ml^{-1}), and $C_i(t=0)$ was set at its equilibrium value, C_i^*, at $C_o(t=0)$, which is given by:

$$C_i^* = \frac{-KG-D+GC_o+\sqrt{K^2G^2+(D-GC_o)^2+2KG(D+GC_o)}}{2G}. \qquad \text{(A1)}$$

We integrated Eq. 3 and 6 (main text) for each combination of development day and experimental temperature (n=15). We did not measure live-tissue mass in this study, however we estimated mass from development day using the relationship from [11]:

$\text{Log}(M) = -12.66 + 2.70 \ln(d) + 2.838 \ln(T)$, ($R^2$=0.987, n=67)

where d is days post fertilization, and T is developmental temperature, which was 12 °C for all eggs.

To link model predictions and data, we extracted predicted dC_o/dt values for each treatment at O_2 concentrations ranging from 10% to 100% O_2 saturation at intervals of 10%. Goodness-of-Fit was calculated as the sum-of-squared error between predicted and observed dC_o/dt. We used a Nelder-Mead simplex to search for the parameters that best fit the data. To quantify parameter uncertainty, we used non-parametric bootstrapping. We generated 1000 resampled datasets by sampling with replacement from the 15 treatments. We fit the model to each of the 1000 resampled datasets to generate a probability distribution for each model parameter. Overall the parameters were well constrained by the data (Fig. S2).

We used the biophysical model to predict O_2 limitation in the survival experiments by calculating the expected equilibrium internal O_2 concentration of eggs (Eq. A1) as a function of the exposure period, experimental temperature, and O_2 concentration. C_i^* was then used to calculate O_2 limitation by substituting it into Eq. 2 and 5 (main text). We used the development day on the first day of exposure to quantify metabolic demand, *D*.

Quantifying supply and demand dependence of metabolism. We quantified the relative dependence of embryo metabolism to both supply and demand as the derivative of the realized metabolic rate, B (Fig. 2D), with respect to ambient O_2, C_o (supply sensitivity), and metabolic demand, D (demand sensitivity), at equilibrium in normoxia ($C_o = C_{saturation}$, $dC_o/dt = 0$, $dC_s/dt = 0$):

$$\frac{dB(D)}{dC_o} = \frac{DK\frac{dC_i^*(D)}{dC_o} + KC_i^*(D) + C_i^*(D)^2}{\left(C_i^*(D) + K\right)^2}, \quad \text{(A2)}$$

and

$$\frac{dB(D)}{dD} = \frac{DK\frac{dC_i^*(D)}{dD} + KC_i^*(D) + C_i^*(D)^2}{\left(C_i^*(D) + K\right)^2}. \quad \text{(A3)}$$

Eq. A3 is dimensionless, where 0 and 1 denote complete independence and proportional dependence respectively. To give Eq. A2 the same interpretation we rescaled it by the O_2 conductance of the egg, G.

Computational Fluid Dynamics Model. We simulated random packing of 0.75 cm diameter spheres in Blender [1] using the Bullet physics engine [2]. Spheres which settled in a 7.0 x 3.5 x 3.5 cm egg pocket volume were eggs, spheres outside were stones. This procedure produced 126 eggs. We constructed a rectangular fluid domain around the eggs and stones. We left 0.5 cm of distance between the inlet and first stones to allow the flow to condition. The side boundaries intersected the centers of the outside stones to prevent flow from passing around the stones and eggs. We used the OpenFOAM [3] utilities blockMesh and snappyHexMesh (SHM) to mesh the fluid domain. Our meshing procedures produced a total of 30,892,495 cells. We solved for the steady state velocity field using the simpleFoam solver and then used the velocity field solution for a steady state scalar transport simulation using scalarTransportFoam. The fluid domain had a packing fraction of 0.608, thus to get our desired mean velocity (14 values ranging from 0.0025 to 0.1 cm/s), we set the inlet velocity to 0.392 times the desired mean velocity. We conducted each simulation at five different temperatures (7, 11, and 15 °C).

The boundary condition for the flux of O_2 across each egg's surface depends on its C_i. At equilibrium the influx of oxygen to the egg must balance metabolic losses. To ensure this we iteratively solved for the C_i for each egg that balances Eq. 3 (main text) using Newton's method (tolerance = 0.1%). The boundary condition, β, related the internal O_2 concentration to the concentrations on the cell faces at the surface of the egg by:

$$C_s = \beta C_i + (1 - \beta) C_c$$

$$\beta \equiv \frac{D_{O2}}{\Delta k} + 1,$$

where k is the mass transfer coefficient of O_2 across the egg membrane (G [egg surface area]$^{-1}$); D_{O2} is diffusivity of O_2 in water; Δ is the distance to the center of the cell next to the egg surface;

and C_s, and C_c are the concentrations of O_2 at the surface of the egg, and at the center of the first cell next to the face. Water entering the fluid domain inlet was assumed to be saturated with O_2.

REFERENCES

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