SI Appendix: Physical Limitations on Metabolic Rates Attainable via Breathing

Mechanical oxygen pumping involves certain energy expenditures (breathing cost), which depend on body size and ambient oxygen concentration. Beyond some critical body size and in oxygen-poor environments, maintenance of a high, size-independent metabolic rate appears to be prohibited by energy conservation law due to an overly high breathing cost. We will now show that the larger ectotherms, with their mean metabolic rates deviating consistently from the proposed metabolic optimum (3-9 W kg^{-1}), exist precisely in such prohibitive conditions. This provides further support for the proposed causal coupling between metabolic optimality and animal breathing.

In oxygen balance, when energy consumption is balanced by oxygen delivery,

$$Q = qV\rho = \omega V_T \rho_{O_2} KE, \qquad\qquad [1]$$

where Q (W) is whole-body metabolic rate, q (W kg^{-1}) is mass-specific metabolic rate, V (m^3) is body volume, $\rho = 10^3$ kg m^{-3} is live body density, E is oxygen extraction coefficient (proportion of oxygen that is extracted from the medium during breathing), ω (s^{-1}) is breathing frequency, V_T (m^3) is tidal volume (water or air volume delivered into the body per breath), ρ_{O_2} (kg m^{-3}) is oxygen density in the medium (water or air), and $K = 1.4 \times 10^7$ J (kg of O$_2$)$^{-1}$ is energy conversion coefficient for aerobic metabolism.

Breathing involves periodical movements of some parts of the body that occupy volume V_p (e.g., chest volume in mammals, intraopercular volume in fish, mantle volume in cephalopods) and move there and back along a linear scale $l_T \sim V_T^{1/3}$ at a frequency ω. This corresponds to mean linear velocity $u = 2\omega l_T$, acceleration $a = 4u\omega$, force $F = 8\rho V_p u\omega$ and mechanical power

$$W = Fu = 8\rho V_p l_T^2 \omega^3. \qquad\qquad [2]$$

This is a lower estimate of the mechanical power spent by the organism to make its breathing pump move, because it does not take into account either the work against friction forces, or the work on moving the medium where breathing occurs.

Expressing ω in terms of q using Eq. **1**, one obtains from Eq. **2** that at a size-independent q the mechanical cost $\kappa \equiv W/(\varepsilon Q)$ of oxygen pumping (cost of breathing) grows as squared body length $l = V^{1/3}$:

$$\kappa = \left(\frac{\rho}{\rho_{O_2} EK} \right)^3 \frac{8\beta}{\varepsilon \alpha^{7/3}} q^2 l^2, \qquad\qquad [3]$$

where $\alpha \equiv V_T/V$, $\beta \equiv V_p/V$, and ε is muscle efficiency (the ratio of mechanical work performed by the muscles to the metabolic power of the muscle tissue consumed during this work). In cuttlefish *Sepia officinalis*, for which all parameters entering Eq. **3** were recently measured in detail (1), q and α increased, while E decreased, by 2.9, 3, and 2.3 times, respectively, between 11 and 23°C. The value of κ was measured to increase by 8.3 times (1) compared with 7.9 times predicted from Eq. **3**. At $\beta \sim 1$, Eq. **3** also accurately predicts the absolute magnitude of κ (2.7% vs. the observed 2.9% at $\varepsilon = 0.03$).

After reaching a critical body size for which κ becomes large, further maintenance of a size-independent optimum q is impossible. Putting maximum q_{max} attainable by organisms living

at the basal $q \sim 10$ W kg^{-1} as $q_{max} \sim 10^2$ W kg^{-1}, and assuming $E_{max} \sim 0.8$, $\alpha \sim 10^{-2}$, $\beta \sim 1$, $\varepsilon_{max} \sim$ 0.1 and $\kappa_{max} \sim 0.1$, the upper estimate of critical body length for aquatic animals, where $\rho_{O_2} \approx$ 0.007 (kg of O$_2$) m^{-3} at 25 °C, is

$$l_{cr} = \left(\frac{\kappa_{max} \varepsilon_{max}}{8\beta} \right)^{1/2} \frac{\alpha^{7/6}}{q_{max}} \left(\frac{\rho_{O_2} E_{max} K}{\rho} \right)^{3/2} \sim 10^{-3} \text{ m.} \qquad \textbf{[4]}$$

The obtained value of critical body size of the order of 1 mm for aquatic media indicates that the maintenance of optimal value of mass-specific metabolic rate q_{opt} is physically permitted up to body masses of $M_{cr} \sim 1$ mg, as is the case with copepods still featuring optimal q (Table 1). All aquatic organisms with $M > M_{cr}$ do not have the option of maintaining a constant q_{opt}. In such organisms mass-specific metabolic rates should decrease with increasing body size. In agreement with this prediction, the larger aquatic organisms (decapods, cephalopods, fish, as well as the evolutionarily and biochemically closely related amphibians and reptiles, all with mean mass $M \gg M_{cr}$) have mean q several times lower than all the other groups studied (Table 1).

Increase of ambient oxygen concentration significantly reduces the energetic costs of pumping a unit oxygen volume into the body (Eq. **3**). Transition from aquatic medium with $\rho_{O_2 1} \approx 0.007$ (kg of O$_2$) m^{-3} to atmospheric air with $\rho_{O_2 2} = 0.3$ (kg of O$_2$) m^{-3} allows for an increase in metabolic rate q_2 in the air as compared to q_1 in water by as much as $q_2 / q_1 = (\rho_{O_2 2} / \rho_{O_2 1})^{3/2} \sim 280$-fold (see Eq. **4**), all other parameters remaining the same. This explains why, despite their large body size, air-breathing endotherms were able to evolve metabolic rates back to the vicinity of the metabolic optimum, similar to much smaller species both on land and in the sea (Fig. 3). Fig. 3 shows mass-specific metabolic rates of endotherms and of all heterotroph taxa with mean mass M less than, or of the order of, M_{cr} (Table 1). Fig. 3 emphasizes that in all body size intervals occupied by life, from the smallest bacteria to the largest animals, there are taxonomic groups that fit into one and the same optimal range of mass-specific metabolic rates, in terms of similar mean values and confidence intervals (Table 1 and Fig. 2).

The limitation on maximum body size l_{cr}, Eq. **4**, compatible with optimal mass-specific metabolic rate holds independently of the properties of the internal networks distributing oxygen within the body. It does not depend on the type of cardiovascular system, or oxygen carrying capacity of the blood, or any other parameters that determine the process of oxygen distribution within the body after oxygen has been consumed. The estimate of l_{cr} is based, instead, on the physics of the primary process of oxygen intake from the environment. The transition from aquatic to aerial milieu is a necessary, but insufficient, condition for elevation of metabolic rate up to the optimum in large animals. To benefit from the energetically cheap oxygen delivery, the air-breathing animals had first to modify their internal distributive networks and blood and tissue biochemistry. There is no use in high oxygen intake if one cannot properly distribute it within the body. This explains why reptiles and amphibians, whose blood biochemistry is close to that of fish, retain low metabolic rates despite air breathing. And only in endotherms, with their radical evolutionary changes in the cardiovascular system and biochemistry (2), was the elevation of metabolic rates back to the optimum (Figs. 2 and 3) made possible. The analyzed body of evidence is thus consistent with the statement that natural selection favors the optimal metabolic rate in all taxa where this rate is physically achievable.

The proposed theoretical approach, Eq. **4**, opens a wide field for the quantitative analysis, in different groups of organisms, of the numerous organismal parameters that affect breathing costs. The fact that realistic values of these parameters yield an estimate of l_{cr} much smaller than the linear size of species with mean $q \ll q_{opt}$, makes the breathing cost limitation a novel and

numerically competitive explanation of the relatively low metabolic rates observed in the larger ectotherms. This approach unambiguously predicts that for taxa with $l > l_{cr}$ (high breathing cost), metabolic rates *must* decline with growing body size, $q \propto l^{-1}$, if the parameters of Eqs. **3** and **4** are evolutionarily conserved. These fundamental physical considerations show that for such organisms there is no other option than metabolic allometry. In contrast, because breathing cost decline very rapidly with diminishing body length (see Eq. **3**), organisms smaller than l_{cr} have negligible breathing costs and can, at physiological rest, thrive without metabolic scaling. These physical limitations can explain the apparently increasing conspicuousness of scaling patterns with increasing mean body mass of the taxa studied (Table 1 and Fig. 3).

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2. Else PL, Turner N, Hulbert AJ (2004) The evolution of endothermy: Role for membranes and molecular activity. *Phys Biochem Zool* 77:950-958.