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Hypothesis Paper

Why O_2 Is Required by Complex Life on Habitable Planets and the Concept of Planetary "Oxygenation Time"

DAVID C. CATLING,[1] CHRISTOPHER R. GLEIN,[1] KEVIN J. ZAHNLE,[2] and CHRISTOPHER P. MCKAY[2]

ABSTRACT

Life is constructed from a limited toolkit: the Periodic Table. The reduction of oxygen provides the largest free energy release per electron transfer, except for the reduction of fluorine and chlorine. However, the bonding of O_2 ensures that it is sufficiently stable to accumulate in a planetary atmosphere, whereas the more weakly bonded halogen gases are far too reactive ever to achieve significant abundance. Consequently, an atmosphere rich in O_2 provides the largest feasible energy source. This universal uniqueness suggests that abundant O_2 is necessary for the high-energy demands of complex life anywhere, *i.e.*, for actively mobile organisms of $\sim 10^{-1}$–10^{0} m size scale with specialized, differentiated anatomy comparable to advanced metazoans. On Earth, aerobic metabolism provides about an order of magnitude more energy for a given intake of food than anaerobic metabolism. As a result, anaerobes do not grow beyond the complexity of uniseriate filaments of cells because of prohibitively low growth efficiencies in a food chain. The biomass cumulative number density, n, at a particular mass, m, scales as $n(>m) \propto m^{-1}$ for aquatic aerobes, and we show that for anaerobes the predicted scaling is $n \propto m^{-1.5}$, close to a growth-limited threshold. Even with aerobic metabolism, the partial pressure of atmospheric O_2 (P_{O_2}) must exceed $\sim 10^{3}$ Pa to allow organisms that rely on O_2 diffusion to evolve to a size $\sim 10^{-3}$ m. P_{O_2} in the range $\sim 10^{3}$–10^{4} Pa is needed to exceed the threshold of $\sim 10^{-2}$ m size for complex life with circulatory physiology. In terrestrial life, O_2 also facilitates hundreds of metabolic pathways, including those that make specialized structural molecules found only in animals. The time scale to reach $P_{O_2} \sim 10^{4}$ Pa, or "oxygenation time," was long on the Earth (~3.9 billion years), within almost a factor of 2 of the Sun's main sequence lifetime. Consequently, we argue that the oxygenation time is likely to be a key rate-limiting step in the evolution of complex life on other habitable planets. The oxygenation time could preclude complex life on Earth-like planets orbiting short-lived stars that end their main sequence lives before planetary oxygenation takes place. Conversely, Earth-like planets orbiting long-lived stars are potentially favorable habitats for complex life. Key Words: Oxygen—Complex life—Atmospheric evolution—Redox—Biomass spectra. Astrobiology 5, 415–438.

[1]Department of Atmospheric Sciences and Astrobiology Program, University of Washington, Seattle, Washington.
[2]Space Science Division, NASA Ames Research Center, Moffett Field, California.

INTRODUCTION

The Earth's biota provides our one example of life. This makes it difficult to determine those properties that are peculiar to terrestrial life and those that are applicable throughout the universe. However, our one case of planetary life ought to provide some clues if we can identify biological properties that derive from unique molecules. When we examine the requirements of terrestrial complex life, such as animals, it is clear that free, diatomic oxygen (O_2) is necessary (see Why terrestrial anaerobic organisms do not grow large). We might expect plentiful O_2 to be unusual in planetary atmospheres, as it is in our Solar System, because relatively reactive O_2 is prone to be removed by combining with reducing gases or surface materials, and its abundance requires a large biological source. Moreover, there was probably insufficient O_2 for animal life until relatively late in Earth history (see The coupled history of biological complexity and atmospheric oxygen). If complex life elsewhere requires abundant O_2 this would suggest that planetary habitats suitable for complex life could be restricted in both their chemical makeup and temporal occurrence. Thus, a key question for astrobiology is whether substantial O_2 is a universal requirement for complex life or merely peculiar to the vagaries of evolution on Earth. Whether or not there is complex life elsewhere impinges upon the relationship between humankind and the cosmos: Are we unique, or are we an insignificant part of a much larger occurrence of complex life? Here, and throughout this paper, we use the term *complex life* to mean actively mobile, multicellular organisms of $\sim 10^{-1}$–10^{0} m scale with specialized, differentiated anatomy comparable to advanced metazoans. We also assume that complex life on a habitable planet would be carbon-based. This assumption is justified in discussions elsewhere (Miller and Orgel, 1974; Wald, 1974; Pace, 2001) and not challenged by speculations of alternative chemistries that can apply only to very simple life (Bains, 2004).

Here, we show how free O_2 provides for the high-energy demands of complex life in a way that is unique to the thermodynamic and chemical properties of oxygen. Because the Periodic Table and thermodynamics are universal, we argue that life elsewhere of comparable complexity to terrestrial metazoa would metabolize O_2. Our deduction that O_2 is necessary for complex life could be criticized as a terrestrial chauvinism, but actually the Periodic Table provides life with a limited chemical toolkit. Unique elements make unique molecules and unique metabolisms. No other element could take the place of oxygen and provide such high-energy metabolism along with oxygen's other advantageous properties. We also show how low energy efficiencies necessarily truncate the food chain and biomass spectrum of anaerobic life. As a consequence of energetic limitations, life without O_2 does not grow large and complex.

If O_2 is a necessary precursor for complex life, the length of time a terrestrial-type extrasolar planet takes to acquire an O_2-rich atmosphere will be a key rate-limiting step for the evolution of complex life elsewhere (as noted previously, *e.g.*, Knoll, 1985; McKay, 1996; Ward and Brownlee, 2000). This "oxygenation time" will then play a critical role in determining the distribution of complex life elsewhere. On Earth, O_2 derives from oxygenic photosynthesis. This metabolism originated in the ancestors of modern cyanobacteria and operates by splitting H_2O and releasing O_2 as a waste product (*e.g.*, Blankenship and Hartman, 1998; Olson and Blankenship, 2004). Generally, the oxygenation time on other planets will depend on the probability of the prior evolution of oxygenic photosynthetic life (the source of O_2) and on geophysical parameters of the planet that affect the buildup of atmospheric O_2. For example, a planet could be endowed with more vigorous tectonism than Earth and a geochemical composition that produces a strongly reducing composition of outgassed volatiles sufficient to prevent the buildup of O_2 throughout a planet's history. Even if photosynthetic microbes arose on such a planet, O_2 consumption by reductants would always outstrip O_2 buildup. For this reason, extrasolar terrestrial-type planets around other stars with less favorable parameters than the Earth may have an oxygenation time that exceeds the main sequence lifetime of their stellar parents. Biology on such planets would never get beyond anything more complex than microbial communities. However, given our enormous ignorance of the variety of planets and their evolution, it could be the case that many Earth-like planets have geophysical parameters highly conducive to O_2 persisting in the atmosphere. For example, planets could start smaller and more oxidized than Earth. Despite being small, such planets might avoid tectonic cessation [which for

Mars prevents recycling of key volatiles like H_2O and CO_2 (*e.g.*, see Kasting and Catling, 2003)] because of more abundant radioactive elements or perhaps tidal heating from a large planetary neighbor. Planets could also orbit stars that are much longer-lived than the Sun, such as K-type or late G-type stars, accommodating an oxygenation time of 5–10 billion years or more. Such planets could be more favorable habitats than the Earth for complex life.

CHEMICAL ENERGETICS AND THE PROPERTIES OF O_2

Key to understanding the differences between aerobic and anaerobic life is that aerobic respiration provides about an order of magnitude more energy for a given intake of food (see Energy of anaerobic versus aerobic metabolism). Throughout this paper, we use the term anaerobic strictly. Microbiologists refer to the metabolism of nitrate reduction as "anaerobic," but really such a metabolism is dependent upon the O_2-rich atmosphere because O_2 is used to oxidize ammonium to nitrate. Consequently, "anaerobic" metabolisms that reduce oxidants ultimately derived from O_2 are irrelevant in a discussion comparing anaerobic and aerobic energetics. Sulfate is a special case we consider later. Today its dominant source is the oxidation of continental sulfides by atmospheric O_2 dissolved in rainwater (Holland, 1978), but it could also be produced as a product of anaerobic photosynthesis. The important question is whether aerobic metabolism is one of several feasible metabolisms of comparable energy release or whether aerobic metabolism is unique. This question we address below. Furthermore, there are two other key factors to consider that bear on the suitability of O_2 for life—namely, the high cosmic abundance of oxygen and the fact that oxygen is a component of liquid water. The chemistry of oxygen has been discussed elsewhere (Lascelles, 1964; George, 1965; Samuel and Steckel, 1974; Gilbert, 1981; Scott, 1991). Here we focus on the relationship of O_2 and complex life.

Why oxygen is a uniquely energetic oxidant

Energetics. Complex extraterrestrial life, like its terrestrial counterpart, would require substantial energy for chemical, electrical, osmotic, and mechanical work. Any organism with differentiated anatomy must require high levels of anabolism, which is the conversion of simple molecules to larger, more complex molecules. In terrestrial life, the synthesis of cellular molecules such as nucleic acids, proteins, and lipids dominates anabolism and involves a large input of free energy. Complex life also expends a considerable amount of mechanical energy in movement, with metabolic rates typically increasing 10-fold during maximum physical activity (Rolfe and Brown, 1997). It is probably by virtue of mobility that complex life on Earth has evolved intelligence because locomotion exerts an evolutionary pressure for developing a central nervous system (ultimately, a brain) for rapid response to external stimuli such as predators (*e.g.*, Albert, 1999). This Darwinian connection between mobility and neurological complexity may plausibly extend to life on other inhabited planets.

Given the need for a copious supply of energy, what is the most energetic chemical reaction available? Obviously, more energy is released with more reactants, so comparisons of energy release must be normalized per electron transfer. For any combination of elements in the Periodic Table, H-F and H-OH bonds have the highest bond enthalpies per electron (Table 1), giving the most energetic reactions per electron transfer. Table 1 compares terminal oxidants ranked according to the Gibbs free energy (ΔG^0) for reduction to an aqueous hydride. Because ΔG^0 ($= \Delta H^0 - T\Delta S^0$) is dominated by enthalpy changes (ΔH^0), it generally tracks the hydride bond enthalpy. In the gas phase, oxygen is the next most energetic oxidant after fluorine, although in the aqueous phase (as shown), chlorine is marginally more energetic than oxygen. Thus an extraterrestrial metabolism using fluorine—the most electronegative element in the Periodic Table—would provide the most energy. Indeed, for this reason, rocket scientists have long recognized the hydrogen-fluorine pair as an excellent rocket fuel (Gordon and Huff, 1953), but such a propellant has not been adopted because of the extreme toxicity and corrosive nature of F_2 and HF. Fluorine is also useless as a biological oxidant because it generates an explosion upon contact with organic matter. Fluorine vigorously attacks R-H bonds and reacts with every other element apart from a few of the noble gases. Similarly, in water, chlorine forms highly reactive hypochlorous acid

Table 1. Comparison of Possible Terminal Oxidants for Biology

Atom	Solar system abundance (% atoms)[a]	Redox reaction with hydrogen equivalent	ΔG^0 (kJ)[b]	Hydride bond enthalpy per electron (kJ mol^{-1})[c]	Products in reaction with water	Prohibitive kinetic reactivity with organics?
F	2.7×10^{-6}	$\frac{1}{2}\,F_2 + \frac{1}{2}\,H_2 = HF(aq)$	−296.82	−282.5	O_2, HF	Yes
Cl	1.7×10^{-5}	$\frac{1}{2}\,Cl_2 + \frac{1}{2}\,H_2 = HCl(aq)$	−131.23	−215.5	HOCl	Yes
O	0.078	$\frac{1}{4}\,O_2 + \frac{1}{2}\,H_2 = \frac{1}{2}\,H_2O(l)$	−118.59	−231.5	—	No
Br	3.8×10^{-8}	$\frac{1}{2}\,Br_2 + \frac{1}{2}\,H_2 = HBr(aq)$	−103.96	−183.0	HOBr	Yes
N	0.01	$\frac{1}{6}\,N_2 + \frac{1}{2}\,H_2 + \frac{1}{3}\,H^+ = \frac{1}{3}\,NH_4^+(aq)$	−26.44	−194.0	—	No
S	0.0017	$\frac{1}{2}\,S + \frac{1}{2}\,H_2 = \frac{1}{2}\,H_2S(aq)$	−13.92	−169.0	—	No
C	0.033	$\frac{1}{4}\,C + \frac{1}{2}\,H_2 = \frac{1}{4}\,CH_4(aq)$	−8.62	−206.0	—	No

The list is ranked according to free energy of reduction to the aqueous hydride per electron under standard conditions, ΔG^0 (298.15 K, 1 bar).

Data were obtained as follows: [a]Anders and Grevesse (1989); [b]Lide (1997); [c]Pauling (1960).

(HOCl), and bromine forms reactive hypobromous acid, as follows:

$$Cl_2(g) + H_2O(l) = HOCl(aq) + H^+(aq) + Cl^-(aq) \quad (1)$$

$$Br_2(g) + H_2O(l) = HOBr(aq) + H^+(aq) + Br^-(aq) \quad (2)$$

Both hypochlorous and hypobromous acids are sterilants (bleaches) and highly reactive with a range of organic molecules including thiols, thioesters, amino acids, amines, unsaturated fatty acids, and iron sulfur centers. Aqueous chlorine (as HOCl and OCl^-) also decomposes in sunlight (Nowell and Hoigne, 1992) to release the free radicals $OH\cdot + Cl\cdot$, which attack organic compounds by hydrogen abstraction (Gonenc and Bekbolet, 2001). O_2 is different. O_2 does not react with water but merely dissolves because the character of its bond (O_2 is a triplet diradical) is such that it provides greater stability and kinetic sluggishness than the single bonds in the halogens: The O_2 bond dissociation energy is 498.4 kJ mol^{-1} at 25°C, compared with only 158.8 kJ mol^{-1} for F_2 or 242.6 kJ mol^{-1} for Cl_2 (Lide, 1997). On the other hand, the O_2 bond is relatively weak compared with double bonds, such as C=O (745 kJ mol^{-1}), so that a suitable enzyme can incorporate O atoms in organic matter. The key point is that a halogen-rich planetary atmosphere is ruled out because gases like F_2 or Cl_2 would be removed extremely rapidly by gas-solid, gas-phase, or aqueous reactions. Given that F_2 and Cl_2 could never be abundant gases in a planetary atmosphere or plausible biological oxidants, the presence of free O_2 in a planetary atmosphere allows life to utilize the highest feasible energy source per electron transfer available within the Periodic Table. This is a universal property.

Alternative aqueous oxidants operate at lower energy yield. For example, an anaerobic "sulfuretum" ecosystem could be sustained by sulfate produced by anoxygenic photosynthesis, *i.e.*,

$$2H_2S(g) + CO_2(g) + h\nu = CH_2O(s) + 2S(s) + H_2O(l) \quad (3)$$

$$5H_2O(l) + 2S(s) + 3CO_2(g) = 3CH_2O(s) + 2SO_4^{2-}(aq) + 4H^+(aq) \quad (4)$$

The sulfate would be reduced back to sulfide in a closed cycle. However, the energy release is still meager compared with O_2. The reduction of sulfate by a hydrogen equivalent:

$$1/2H_2(g) + 1/8SO_4^{2-}(aq) = 1/8S^{2-}(aq) + 1/2H_2O(l) \quad (5)$$

yields only −15.6 kJ/mol under standard conditions, almost an order of magnitude less energetic than aerobic metabolism (Table 1).

The "next best" energy source to O_2, if it were possible, would probably involve nitrogen chemistry. (Nitrogen, like oxygen, also has the advantage of high cosmic abundance.) One can hypothesize a closed loop involving nitrate reduction, but this has several problems. In a closed cycle, the oxidation of ammonium to nitrate (nitrification) would require using an oxidant that itself does not ultimately derive from O_2 (thus, so-called anaerobic oxidation with

MnO_2 is excluded). This truly anaerobic oxidation step is prohibitive and unknown on Earth. A hypothetical closed cycle on another world would require anaerobic nitrogen photosynthesis where ammonium, CO_2, and sunlight produce nitrate and organic matter. Again, this is unknown. For the sake of argument, if nitrate were somehow produced in this truly anaerobic fashion, the full reduction of nitrate to ammonium in principle would yield approximately two-thirds of the energy of O_2 respiration. However, a further issue is that a closed anaerobic nitrogen cycle has to contend with kinetically stable dinitrogen. N_2 is the common outgassed form of nitrogen via metamorphism and volcanism, so N_2 would represent the ultimate replenishment of nitrogen lost to long-term geological sinks. Unfortunately, N_2 does not readily accept electrons, and its high activation energy barrier actually requires the input of energy for reduction (about 18–24 ATP molecules per N_2 fixed for terrestrial microbes).

The cosmic abundance and occurrence of oxygen. Another key factor for the biological relevance of oxygen is its abundance. A dependence of complex life on a metabolism that used an extremely rare element would limit its occurrence. However, the element O is third in cosmological abundance behind H and He, and ahead of C (Anders and Grevesse, 1989). Other energetic oxidants, like the halogens, are less abundant than oxygen by many orders of magnitude (Table 1). In comparing the elemental composition of organisms with the inorganic environment, it is tempting to note that oxygen is also the most abundant element by weight in the Earth's crust (47% of the lithosphere) (*e.g.*, Barrow and Tipler, 1986). Indeed, depending on the size of an iron-bearing core, oxygen is generally likely to be the first or second most abundant element in a rocky planet. However, this has no bearing on biology because most of the oxygen is locked up in silicates. Free oxygen never gets released from -SiO_3 or -SiO_4, whether in dissolution, melting, or other chemical change. Also, oxidized crustal species such as ferric iron (Fe_2O_3) and sulfate (SO_4^{2-}) are not primordial but generally thought to have been created from oxidation associated with oxygenic photosynthesis and hydrogen escape to space (*e.g.*, Kasting *et al.*, 1993; Catling *et al.*, 2001).

The importance of the cosmic abundance of oxygen is not that it is plentiful in minerals but that it is found in water, the most common molecular form of O. It is widely believed that liquid water is required for Earth-like life (*e.g.*, Franks, 2000; Chaplin, 2001), and the common definition of a "habitable planet" is one that can sustain substantial liquid water on its surface (Kasting and Catling, 2003). O_2 is congruent with liquid water in two ways. First, O_2 is a gas in the pressure–temperature range of liquid water. A solid terminal oxidant (*e.g.*, sulfur) would have to be consumed as food and would limit habitats. No such restriction applies with O_2. Oxygen can be freed into an atmosphere and is sufficiently soluble (~0.2 m*M* in 25°C seawater) to be distributed throughout an ocean. At the same time, O_2 is sufficiently insoluble that on Earth it partitions between the atmosphere and ocean in a 140:1 ratio, allowing the existence of complex life on land. This partitioning ratio will be modified on different planets in the following way: The O_2 solubility accounts for a factor of $S \sim 42.5$ (at a temperature of 25°C); the amount of O_2 in an atmosphere is proportional to the average scale height ($H_{mean} \sim 12.5$ km on Earth, averaging polar and equatorial values); and the quantity of O_2 in the ocean is proportional to average ocean depth ($z_{ocean} \sim 3.7$ km for Earth). These three factors combine on Earth to give $S \times H_{mean}/z_{ocean} \sim 140$, but variations of H_{mean} and z_{ocean} on other Earth-like planets would change the air–ocean partitioning factor. A second reason that O_2 is congruent with liquid water is that O_2 derives from the splitting of liquid water by photosynthesis. If a planet is habitable, with liquid water and life, it has potential for sustaining an O_2-rich atmosphere if simple life ever evolves oxygenic photosynthesis, the highly desirable capability to extract hydrogen from H_2O. Some bearing on the probability of oxygenic photosynthesis can be gleaned from theories of how it arose on Earth. Terrestrial life may have evolved the basic chemical machinery for oxygenic photosynthesis by first developing defenses against photochemical oxidants produced before Earth had an ozone layer (McKay and Hartman, 1991; Blankenship and Hartman, 1998). Given that ultraviolet (UV) photochemistry is common on planets, oxygenic photosynthesis elsewhere is not necessarily implausible.

ORGANISM SIZE AND O_2

In Why oxygen is a uniquely energetic oxidant, we showed how the energy production of aero-

bic metabolism is as high as possible given the limited toolkit of the Periodic Table. This has implications for how large organisms can grow. When we examine terrestrial life, we find that only aerobic organisms grow large. Anaerobic prokaryotes and anaerobic eukaryotes remain unicellular, or if they become multicellular, the geometry is always sufficiently simple to have all cells in direct contact with the environment (*e.g.*, uniseriate filaments). Let us examine how the size difference between aerobes and anaerobes arises.

Why terrestrial anaerobic organisms do not grow large

Energy of anaerobic versus aerobic metabolism. In terrestrial life, respiration provides about an order of magnitude more energy than anaerobic metabolisms for a given intake of food. In all cells, energy is conserved first as ATP. ATP is mainly used in the synthesis of macromolecules and to drive active transport across cell membranes. The aerobic degradation of glucose, $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$ ($\Delta G^0 = -2{,}877$ kJ/mol≈the value under physiological conditions), yields 31 mol of ATP/mol of glucose with maximal ATP production (Hinkle *et al*., 1991; Brand, 1994). Free energy is released in the subsequent hydrolysis of ATP to ADP and orthophosphate (P_i) via $ATP + H_2O = ADP + P_i$. The apparent ΔG^0 change of ATP hydrolysis, $\Delta G'^0_{ATP}$, under physiological conditions of 37°C, pH 7.0, free $[Mg^{2+}] = 1.0$ m*M*, and ionic strength = 0.2 *M*, is usually quoted in textbooks as −30.5 kJ/mol (*e.g.*, Berg *et al*., 2002; see also Pänke and Rumberg, 1997). Adjusting the free energy change for cellular product and reactant concentrations via $\Delta G_{ATP} = \Delta G'^0_{ATP} + RT \ln \{([ADP][P_i])/[ATP]\}$, where $R = 8.314$ J/mol and T is temperature (K), gives ΔG_{ATP} of approximately −50 kJ/mol for typical ATP and P_i cellular levels of 2–8 m*M* and ADP about a tenth of this. Thus, roughly half of the aerobic energy is conserved [(∼50 kJ/mol × 31 mol)/2,877 kJ)], while the other half is lost as heat. In contrast, fermentative heterotrophic bacteria yield only ∼1–4 mol of ATP/mol of glucose, with 2 ATP molecules being typical. For example, *Lactobacillus*, which turns milk sour, ferments glucose to lactic acid ($C_6H_{12}O_6 = 2CH_3CHOHCOOH$, $\Delta G^0 = -197$ kJ/mol), yielding 2 mol of ATP/mol of glucose. This implies that about half of the energy is conserved in ATP [(∼50 kJ/mol × 2 mol)/197 kJ)]. Indeed, conversion efficiency to ATP in anaerobic metabolism is generally similar to that in aerobic metabolism, irrespective of the exact anaerobic pathway (Thauer *et al*., 1977), but there is a profound difference in total energy yield. In anaerobic metabolism, only 3–12% (1/31–4/31) of the energy of aerobic metabolism is produced from the same food intake.

The significant lack of energy produced by anaerobic metabolism for a given food intake limits the capability for growing large. Growth requires energy beyond that needed just for maintenance. The growth yield per mole of ATP depends on the species of organism and temperature, but a useful generalization is that the growth per mole of ATP is ∼10 g dry weight of organic matter, which is commonly written as $Y_{ATP} = 10$ g/mol (Bauchop and Elsden, 1960; Russell and Cook, 1995; Desvaux *et al*., 2001). Ignoring losses due to excretion, a theoretical maximum of 310 g (31 mol ATP × Y_{ATP}) of growth can be produced in aerobic metabolism by ingesting 490 g of food (180 g/mol glucose + 310 g). This is a ∼60% (310/490) growth efficiency. In contrast, a typical anaerobic metabolism that produces 2 mol of ATP/mol of glucose would generate only 20 g of growth for an intake of 200 g, which is a 10% growth efficiency. These numbers indicate the general relative difference in growth efficiency between aerobic and anaerobic lifestyles, noting that measurements on higher animals show a range of 30–50% in growth efficiency (Brafield and Llewellyn, 1982). The metabolic difference in growth efficiency offers the most likely explanation for why we observe large aerobes in nature, up to the 2×10^5 kg blue whale, while anaerobes remain unicellular or, at best, uniseriate filaments (Fenchel and Finlay, 1994, 1995). Aerobic respiration is efficient enough to allow aerobic eukaryotes to eat other organisms and grow large and multicellular in a food chain. Conversely, anaerobic organisms are doomed to a unicellular lifestyle and mostly rely on simple diffusion through their cell membranes for their nutrients. Diffusion becomes more inefficient the larger the cell, so it seems likely that the rate of energy intake in anaerobes cannot overcome their low growth efficiency and size limitation. Let us now explore differences between aerobes and anaerobe sizes quantitatively.

The biomass spectrum of aerobic versus anaerobic life. Consider a classical food chain. Sharks eat tuna that eat small crustaceans that eat zooplankton that eat phytoplankton. Could such a

biomass hierarchy exist on an Earth-like planet with solely anaerobic metabolism? The important comparison to make between aerobic and anaerobic lifestyles is the biomass spectrum (number density vs. mass) in the aquatic realm because we assume that life would originate and evolve first in water. Hence for the evolution of large life, life must first be able to reach substantial size in the lower food chain consisting of small heterotrophic organisms feeding on smaller organisms that in turn eat microbes. If the biomass spectrum is truncated in an anaerobic ocean to unicells, complex life would be forestalled. For aerobic life, hundreds of populations from terrestrial lakes and pelagic marine environments show a remarkably consistent power law in which the cumulative number density (*e.g.*, per liter) of aerobic organisms, n, varies as $n(>m) \propto m^{-1.0}$ (Sheldon *et al.*, 1972; Kerr, 1974; Gaedke, 1992; Cyr *et al.*, 1997; Cyr, 2000; Quinones *et al.*, 2003). Such studies cover more than 14 orders of magnitude in body mass from bacteria to crustaceans. The analogous biomass spectrum for purely anaerobic life has not been reported, so here we calculate it theoretically.

Size distributions are the result of energy and mass flows through food webs. Various models have been proposed in the literature for the biomass spectra in aquatic systems (see Kerr and Dickie, 2001, and references therein). Here we use a simplified version of the model of Camacho and Solé (2001). Let us define a number density per unit mass, given by $N(m) = \partial n/\partial m$. Thus $N(m) \sim n(m)/m$. The multiplicative factor from differentiation does not concern us because we are only interested in calculating the slope of the anaerobe biomass distribution to see how it differs from the aerobe distribution. We can write a mass balance equation as follows:

$$\frac{\partial(N(m)\dot{m})}{\partial m} = -N(m)p_m \tag{6}$$

where $\dot{m} = dm/dt$ is the growth rate of an organism of mass m, and p_m is the death rate (in units of time^{-1}) assumed to be due to predation. In Eq. 6, new biomass in the organism as well as reproduction is included in $\dot{m}$. If organisms of mass m are prey for all organisms with mass $m_1 \geq m$, then we can write the death rate for mass m organisms as

$$p_m = \int_m^\infty N(m_1)P(m,m_1)dm_1 \tag{7}$$

where $P(m, m_1)$ is the probability per unit time that an organism of mass m_1 will eat an organism of mass m. The growth rate for organisms that eat organisms with mass $\leq m$ can also be written as:

$$\dot{m} = G\int_{m_0\sim 0}^{m} N(m_1)m_1P(m,m_1)dm_1 \tag{8}$$

where G is the ecological gross efficiency of growth, defined as the ratio of new biomass to the intake of food, and m_0 is the mass of the smallest organism. Following Camacho and Solé (2001), let us assume that the probability of predation varies as $P(m,m_1) \propto m^\beta {m_1}^\beta = k_1 m^\beta {m_1}^\beta$, given that larger organisms require more food and larger prey are more desirable. Here β is a free parameter. Also, we assume that the solution will be a power law of form $N(m) = k_2 m^{-\alpha}$. [Our Eq. 7 departs from Camacho and Solé (2001) in that we do not include an additional constant to further relate $N(m_1)$ to $N(m)$, because this would have to be included in Eq. 8 and would eventually cancel.] By substituting for P and $N(m_1)$, we can integrate Eqs. 7 and 8 as follows:

$$\text{death rate } p_m = k_1k_2m^\beta \int_m^\infty {m_1}^\beta m_1^{-\alpha}dm_1 = k_1k_2 \left(\frac{m^{1+2\beta-\alpha}}{\alpha-1-\beta}\right) \propto m^{-\gamma} \tag{9}$$

$$\text{growth rate } \dot{m} = k_1k_2Gm^\beta \int_0^m m_1^{-\alpha}{m_1}^\beta m_1 dm_1 = k_1k_2G\left(\frac{m^{2+2\beta-\alpha}}{2+\beta-\alpha}\right) \propto m^\delta \tag{10}$$

In integrating, we use the fact that $m^x/x \to 0$ as $m \to \infty$, provided $x < 1$, which sets a convergence requirement $1 + \beta < \alpha < 2 + \beta$. In Eqs. 9 and 10, we also express p_m and $\dot{m}$ as power laws with exponents δ and $-\gamma$ because observations show that $\dot{m} \propto m^{0.75}$ and $p_m \propto m^{-0.25}$ (Calder, 1985). These observations are allometric scaling laws. The most basic allometric relationship, known as Kleiber's Law, is that the whole-organism basal metabolic rate scales as m^a, where exponent a is close to 0.75 (Kleiber, 1932; Savage *et al.*, 2004). Consequently, growth rate, which is proportional to metabolism, also follows Kleiber's Law (*e.g.*, Calder, 1985). Death rate depends on the biomass turnover time τ, such that $p_m \propto 1/\tau \propto$ (biomass/productivity)$^{-1} \propto (m/m^{0.75})^{-1} \propto m^{-0.25}$. Allometric scaling may arise from the fractal nature of life (West *et al.*, 1997; Brown and West, 2000) or simply efficient transport within a three-dimensional organism (Banavar *et al.*, 1999; Dreyer, 2001), but

the issue is not yet settled (*e.g.*, see Kozlowski and Konarzewski, 2004). Nonetheless, Kleiber's Law is well documented and demonstrated for aerobic life from the respiratory complex of mitochondria to blue whales (Peters, 1983; Calder, 1984; West *et al.*, 2002; Savage *et al.*, 2004). Kleiber's Law is usually discussed only within the context of aerobic life. However, Kleiber's Law is independent of the type of metabolism: Measurements of growth rate as a function of size for anaerobic microbes show an $m^{-0.25}$ dependence (Fenchel and Finlay, 1990). Thus, $\delta = 0.75$ and $\gamma = 0.25$ are general in nature and should apply to any metabolism. Equations 9 and 10 allow us to write:

$$-\gamma = 1 + 2\beta - \alpha \tag{11}$$

$$\delta = 2 + 2\beta - \alpha \tag{12}$$

Subtraction of Eq. 11 from Eq. 12 confirms $\delta + \gamma = 1$, as expected. Substitution of Eqs. 9 and 10 into Eq. 6, along with $N(m) = k_2 m^{-\alpha}$, gives a quadratic in α with solution:

$$\alpha = \beta + 1 + \left(\frac{-1 + \sqrt{1 + 8G}}{4G}\right) \tag{13}$$

From Eq. 12, it follows that $\beta = -1 + (\delta + \alpha)/2$. We can substitute β into Eq. 13 to give:

$$\alpha = \delta + \left(\frac{-1 + \sqrt{1 + 8G}}{2G}\right) \tag{14}$$

If we specify G and assume $\delta = 0.75$, we can calculate α from Eq. 14, which is what we seek: the exponent in our number density power law, $N(m) \propto m^{-\alpha}$.

To see how this works, let us consider two extreme idealized cases that define the boundaries of possible power law slopes for biomass spectra: (a) an ecosystem with 100% perfect growth efficiency ($G \to 1$) where all food is incorporated as biomass and none is lost to metabolic activity; and (b) an ecosystem with zero growth efficiency. In the first case, setting $G \to 1$ in Eq. 14 gives $\alpha \to 1.75$, in this case, number density per unit mass, $N(m) \propto m^{-1.75}$, and the cumulative number density, $n(m) \propto m^{-0.75}$. This indicates a conservative flux of mass through the food web equal to the net primary productivity, which can be understood as follows. The flux at any mass, m, is the total biomass divided by the turnover time $\propto [n(m)m]/\tau \sim [n(m)m]/m^{0.25} \sim n(m)m^{0.75}$. Consequently, if the flux is conservative, it would be equal to the net primary productivity, F, at the base of the food chain, so $F \propto n(m)m^{0.75}$, giving $n(m) \propto Fm^{-0.75} \propto m^{-0.75}$. Interestingly, since $\beta = 0.25$ (from Eq. 12 with $\alpha = 1.75$ and $\delta = 0.75$), the probability of predation would scale exactly with lifetime, or turnover time, of predator and prey; the longer an organism lives the more likely it would get eaten. We can compare the observed time-averaged exponent of -1.0 for aerobic life to the theoretical upper limit of -0.75. The difference in exponents is expected because the energy flux through the food chain cannot be conservative and must have metabolic dissipation. Now let us consider the second hypothetical case of zero growth efficiency: As $G \to 0$, $n(m) \to n(m) \propto m^{-1.75}$. In this growth-limited case, $\beta \to 0.75$, so the probability of predation depends on productivity (new tissue growth and reproduction) in predator and prey and not on their lifetimes. Essentially, if an organism grows it could eat another organism. Of course, a food chain would no longer be supported before G reaches 0 because some minimum energy is required for reproduction. Aerobic and anaerobic lifestyles lie somewhere in between these two extremes we have considered, but anaerobes are close to the growth-limited case where growth is impossible.

We can estimate the exponent in the power law of the biomass spectrum for aerobic and anaerobic life using empirical data. Measured gross growth efficiencies are somewhat variable (Brafield and Llewellyn, 1982, Chapter 8). But for small aquatic aerobic life that we consider here, we take the estimated gross growth efficiency as ~0.3 (Straile, 1997). With $G_{\text{aerobic}} = 0.3$, the model predicts $n_{\text{aerobic}}(m) \propto m^{-1.1}$, in rough agreement with the observed relationship $n(m) \propto m^{-1.0}$. In Energy of anaerobic versus aerobic metabolism, we showed that there is a theoretical difference of 10% versus 60% in gross growth efficiency between anaerobes and aerobes, *i.e.*, a factor of 1/6, or ~0.17. Limited observations actually show a scaling of ~0.25 (Fig. 3 of Fenchel and Finlay, 1990). Thus, we adopt $G_{\text{anaerobic}} = 0.3 \times 0.25 = 0.075$, which gives $n_{\text{anaerobic}}(m) \propto m^{-1.5}$. This biomass relationship and that for aerobic life are plotted schematically in Fig. 1, along with the theoretical bounds for perfect growth efficiency [$G = 1$, $n(m) \propto m^{-0.75}$] and the breakdown threshold of certain death where a food web can no longer be sustained [$G = 0$, $n(m) \propto m^{-1.75}$].

Figure 1 suggests that anaerobic life is just able to sustain a limited food chain. Generally, preda-

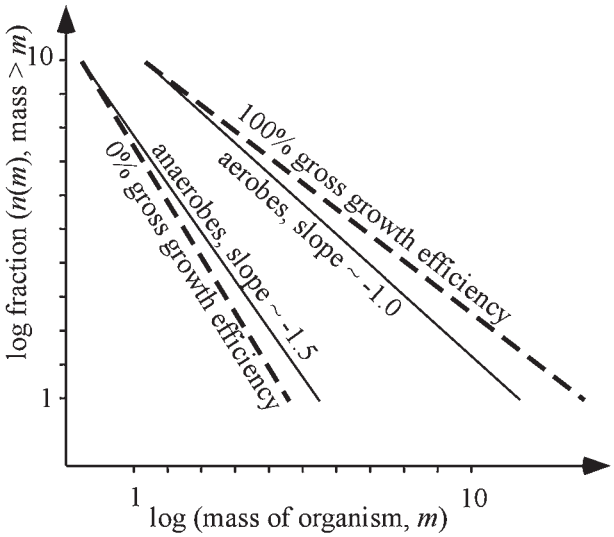


FIG. 1. A diagram showing the relative slopes of biomass spectra of aerobic life (observed) and anaerobic life (calculated). The slopes of biomass spectra are bounded by theoretical lines where the gross growth efficiency is 100% ($G = 1$) and 0% ($G = 0$), respectively. A food web must collapse before the $G = 0$ line because there must be minimum growth for reproduction. Aerobes have a biomass spectral slope that can support large, complex life. However, the anaerobic biomass slope is too steep to support large life forms and close to the $G = 0$ limit.

tors are $\sim 10^3$–10^4 times the mass of prey in aqueous ecosystems (Kerr, 1974), or, equivalently, 10–20 times the size of the prey, given that mass scales with size cubed. If a 2-μm microbe is eaten by a 20-μm organism, the number density of the latter should be 1,000 times lower than for the 2-μm microbe in an aerobic ecosystem where $n(m) \propto m^{-1}$, using $n(m_{\text{small}})/n(m_{\text{large}}) = m_{\text{large}}/m_{\text{small}} = (20\ \mu\text{m}/2\ \mu\text{m})^3 = 1{,}000$. However, in the anaerobic ecosystem, where $n(m) \propto m^{-1.5}$, the 20-μm organisms would be 3.16×10^4 times less abundant, *i.e.*, in this case, $n(m_{\text{small}})/n(m_{\text{large}}) = [(20\ \mu\text{m}/2\ \mu\text{m})^3]^{1.5} = 1{,}000^{1.5} = 3.16 \times 10^4$. Continuing up the food chain, a 200-μm organism would be 10^6 times less abundant than the 2-μm microbe for aerobes or 10^9 less abundant for anaerobes. Because of the difficulty in our oxic world of studying truly anaerobic natural ecosystems, we do not know of any sufficiently comprehensive biomass measurements to verify our theoretical prediction of $n_{\text{anaerobic}}(m) \propto m^{-1.5}$. However, general studies of anoxic ecosystems indicate that the effect of phagotrophs in structuring anaerobic microbial communities is very small relative to aerobic organisms. In particular, anaerobic communities appear limited to protozoa (of varying sizes) that graze on bacteria with relatively little biomass production for larger organisms and a complete absence of small metazoans (Fenchel and Finlay, 1995). A complication here is that eukaryotic anaerobes may be descendants of eukaryotes that were once aerobic but have adapted to anaerobic conditions (Roger, 1999). If that is the case, today's anaerobic ecosystems are not truly representative of biology we would find in a purely anaerobic world.

A possible objection to the above is that some metazoans, such as nematodes or sediment dwellers, live under apparently anaerobic conditions. However, these organisms must complete their life cycles using O_2 (Bryant, 1991). For example, parasitic intestinal nematodes, which mostly live using anaerobic metabolism, have a copious supply of molecules synthesized by their aerobic host, including those made using O_2-dependent pathways. Such parasites are also often exposed to microaerophilic conditions. Similarly, metazoa at hydrothermal vents, such as giant tubeworms, depend on O_2 dissolved in seawater as an electron acceptor. This O_2 ultimately originates with photosynthesis in the surface ocean (Childress and Fisher, 1992; Van Dover, 2000, p. 187).

How much O_2 is needed for aerobic organisms to grow large?

The first aerobic multicellular life perhaps consisted of happenstance aggregates of undifferentiated aerobic cells, when the products of cell division failed to separate because of a simple mutation. This would have conferred a fortuitous evolutionary advantage. Isolation from the outside world would have allowed exclusive use of aerobic metabolism (Pfeiffer *et al.*, 2001) and protection of genes for the next generation. In any case, once Earth's O_2 levels were sufficient, multicellularity was apparently invented more than 10 times (Bonner, 1998), which suggests a definite evolutionary advantage.

Aggregates of aerobic cells that rely solely on diffusion of O_2 are limited in size by the rate at which O_2 can diffuse to the innermost cell. This problem was first noted by Warburg (1923), Fenn (1927), and Newton-Harvey (1928), and was later applied to the fossil record to infer a possible connection between animal size and the history of atmospheric O_2 (Raff and Raff, 1970; Runnegar,

1982, 1991b). A maximum size limit can be derived by assuming that the metabolism of the innermost cell consumes O_2 at the rate that O_2 reaches it by diffusion so that the inner partial pressure of O_2 drops to 0. Appendix 1 gives the mathematical derivation for the case of a spherical organism, and the solution for other geometries can be derived analogously. Table 2 gives the resulting mathematical expressions for size limits on aerobic life for different external partial pressure of atmospheric O_2 (P_{O_2}) values and different organism shapes. A typical value around room temperature for the O_2 consumption rate of small animals, such as roundworms (*e.g.*, *Ascaridia galli*), flatworms (*e.g.*, *Crenobia alpina*), snails (*e.g.*, *Helix pomatia*), and slugs (*e.g.*, *Limax flavus*) is $M \sim 0.1$ cm^3 of O_2 h^{-1} cm^{-3} of tissue (Altman and Dittmer, 1968). However, smaller life forms can persist on lower O_2 consumption rates, *e.g.*, the giant unicellular green alga *Acetabularia mediterranea* and the sea anemone *Metridium senile* have $M \sim 0.03$ cm^3 of O_2 h^{-1} cm^{-3} of tissue (Runnegar, 1991b, and references therein). The lower metabolic rate arises because of Kleiber's Law (Hemmingsen, 1960; West *et al.*, 2000). Metabolic rate also has a temperature dependence (Gillooly *et al.*, 2001). However, even if these corrections are taken into account, in conditions of $O_2 \sim 1\%$ of present atmospheric level (PAL), aerobic creatures are very small, limited by a diffusion rate proportional to the O_2 gradient from the exterior. A maximum size of $\sim 10^2$ μm at 1% PAL (Table 2) is about the size of a single eukaryotic cell or a few microbial cells. A size of ~ 1 mm is not reached until ~ 0.1 PAL. At this size, a cilia–muscle transition would become possible (Sleigh and Blake, 1977) and could lead to pumped tubes or rudimentary circulation. Table 2 also includes calculations for organisms that take up O_2 on their entire outer surface by diffusion and then distribute O_2 with blood circulation. For such organisms, a size of ~ 1 cm can be reached for O_2 at ~ 0.1–1 PAL. Modern examples of "cylindrical" organisms that breathe through vascularized skin (and also oral mucosa) include the lungless salamanders of the family Plethodontidae, which are typically ~ 1 cm or less in width (Bishop, 1994). The relevance of high O_2 to body size in diffusion-dependent organisms is confirmed by Carboniferous fossils. High O_2 lev-

Table 2. Size Limitations of Diffusion-Based Aerobic Life

	Organism shape and physiology					
	Organisms with diffusion only			*Organisms with blood circulation*		
Parameter	*Cylinder of radius* a	*Sphere of radius* a	*Disk of thickness* a	*Cylinder of radius* a	*Sphere of radius* a	*Disk of thickness* a
Minimum P_{ex} for given size, a	$\frac{Ma^2}{4K}$	$\frac{Ma^2}{6K}$	$\frac{Ma^2}{8K}$	$\frac{aMd}{2K} + P_b$	$\frac{aMd}{3K} + P_b$	$\frac{aMd}{2K} + P_b$
Theoretical maximum size	$\sqrt{\frac{4K}{M}P_{ex}}$	$\sqrt{\frac{6K}{M}P_{ex}}$	$\sqrt{\frac{8K}{M}P_{ex}}$	$\frac{2K}{Md}(P_{ex} - P_b)$	$\frac{3K}{Md}(P_{ex} - P_b)$	$\frac{2K}{Md}(P_{ex} - P_b)$
Maximum size at P_{ex} of						
0.21 atm	$\sim$1.5 mm	$\sim$1.8 mm	$\sim$2.1 mm	9–28 mm	13–43 mm	$\sim$9–28 mm
0.021 atm (10% PAL)	$\sim$0.5 mm	$\sim$0.6 mm	$\sim$0.7 mm	0.8–3 mm	1.2–4 mm	0.8–3 mm
0.002 atm (1% PAL)	$\sim$150 μm	$\sim$180 μm	$\sim$200 μm	—	—	—

The first three columns are for organisms relying solely on diffusion of O_2 to all internal cells. The other columns are for creatures with blood circulation that rely on diffusion only through an epidermal layer. Here, M is the metabolic rate of O_2 consumption (in cm^3 of O_2 h^{-1} cm^{-3} of tissue), K is a permeability constant for O_2 through tissue (in cm^2 of O_2 atm^{-1} h^{-1}), P_{ex} is the external P_{O_2} (in atm), and P_b is the average P_{O_2} in the blood (in atm). We assume that $M = 0.03$ cm^3 of O_2 h^{-1} cm^{-3} of tissue (see text) for the first three organisms and $M = 0.03$ and $M = 0.01$ cm^3 of O_2 h^{-1} cm^{-3} of tissue for organisms with circulation. The latter represents the empirical range of metabolic rate for the organism size. Maximum size is calculated assuming P_b is an average of arteries $\sim P_{ex}/2$ and veins ~ 0 atm, and $K = 8 \times 10^{-4}$ cm^2 of O_2 atm^{-1} h^{-1} (Weisfogh, 1964; Brown, 1984). Epidermal layers vary from about 10 to 30 μm. For the animals with circulation, we assume an epidermal layer of thickness $d = 30$ μm. PAL of $O_2 = 0.21$ atm.

els are inferred from geochemical data (Berner *et al*., 2000) when contemporaneous gigantism is evident in insect fossils, including 0.7–m-wingspan dragonflies, 1–m-long millipedes, and giant spiders (Dudley, 1998).

Obviously, calculations in Table 2 do not apply to animals with respiratory organs, such as lungs or gills; these features would develop after simple diffusion-based animals first evolved. Respiratory organs enormously increase the area available for diffusion [*e.g*., the alveolar surface area in an adult human is ~100 m^2 (Colebatch and Ng, 1992)]. Indeed, the fact that folds in the skin can greatly increase the surface area means that the diffusion-limited size calculations are only crude guidelines. Organisms with complex surfaces can be larger. Corals and sponges are obvious examples. Another example is the frog *Telmatobius culeus*, which has poorly developed lungs, but has adapted to a high-altitude (3,812 m) lake (Titicaca, Peru) with highly vascularized skin flaps through which it absorbs O_2 (Hutchinson *et al*., 1976; Weber *et al*., 2002).

It is interesting to consider how high the O_2 level has to be for animals with closed blood circulation to become possible. A closed circulation system is a pumped system with a continuous series of vessels to circulate blood to all cells (found in most vertebrates); in contrast, an open circulatory system, found in some invertebrate phyla, directly bathes internal organs in blood in a body cavity or hemocoel (although in insects this is generally used to supply nutrients rather than O_2). The physics of blood flow must limit the minimum size for an organism with a pulsed circulatory system. Organisms with blood circulation must have sufficiently wide arteries branching from the aorta so that the heart pulse is not completely viscously damped. It can be shown that this requires viscous impedance matching at branch points, so that the cross-sectional area of a parent artery is the sum of the two daughter arteries into which it divides (West *et al*., 1997). At the other end of the system, capillaries are dominated by viscosity because they are only ~10 μm or less in diameter, so when a capillary divides, the sum of the daughter capillary cross-sectional areas must exceed that of the parent capillary for optimal flow energetics. As an organism decreases in size, eventually a point is reached at which even the arteries are too constricted to permit area-preserving branching. In this case, an aorta would branch immediately to capillaries, and the heart pulse wave would not extend far beyond the aorta. The minimum organism mass when this crossover point occurs is ~3 g (West *et al*., 2002). For an organism with density ~1 g cm^{-3}, this corresponds to a radial dimension of ~9 mm. West *et al*. (2002) have suggested that this is applicable to the mass of the smallest mammal, the Etruscan pygmy shrew (*Suncus etruscus*), which has mean adult mass ~2 g (Jurgens, 2002) and 0.1-mm-radius aorta (Dobson, 2003). This mass limit also happens to be similar to the smallest flying mammal (2–3 g), the bumblebee bat (*Craseonycteris thonglongyai*), as well as the smallest bird (~3 g), the Cuban bee hummingbird (*Mellisuga helenae*). This lower mass limit for closed circulation would be commensurate with the maximum size ~1 cm in Table 2, which was derived with a completely different line of physical reasoning. Thus, closed blood circulation may have become possible when P_{O_2} was high enough to allow an organism of sufficient size to support pulsatile blood flow. This could occur for O_2 ~0.1 PAL, if we generously assume a favorably small epidermal layer ~10 μm and low metabolic rate, M ~0.03 cm^3 of O_2 h^{-1} cm^{-3} of tissue. No doubt, organisms would first have had passive fluid-filled vessels before evolving tubes pumped by muscles, respiratory transport proteins, and a localized muscular pump. Given that disk-shaped organisms would have been the largest organisms to evolve under purely diffusion-based circumstances (Table 2), these might be favored as the first organisms to reach sufficient size for circulatory systems. This would be consistent with flat, frond-like shapes of some Ediacaran fauna such as *Dickinsonia* (Runnegar, 1991a).

As a caveat, we note that the P_{O_2} available to an organism is not necessarily the same as the atmospheric P_{O_2}. Photosynthetic organisms produce O_2 inside their cells and so would not be subject to external diffusion constraints. This is possibly relevant to fossil algae from the Proterozoic (2.5–0.543 Ga), when P_{O_2} was likely only a few percent PAL (see The coupled history of biological complexity and atmospheric oxygen). Furthermore, some animals exist in symbiosis with algae, so early animal life may have also relied upon symbiotic algae (Fisher, 1965). Another caveat is that anaerobic life could become macroscopic and passively mobile but not complex. An analog can be found in jellyfish, which essentially consist of two layers of cells separated by a buoy-

ant, metabolically inert mass of jelly, or mesogloea. Consequently, *Cyanea capillata* (the lion's mane jellyfish), the largest example, grows up to 2.5 m across with tentacles >30 m. It is conceivable that a colony of anaerobic cells could grow macroscopic in a similar manner. Such an entity, passively mobile, mostly dead, and with relatively simple differentiation, does not fall within our definition of complex life given in the first paragraph of the Introduction. In summary, we conclude that an O_2 level ~0.1 PAL is needed to grow as large as approximately millimeter scale on the basis of diffusion and that an O_2 level ~0.1–1 PAL is a prerequisite to cross the threshold for organisms of size ~1 cm with closed, pulsatile circulatory systems.

These deductions about P_{O_2} and organism size should also apply to life on other habitable planets if we accept that O_2 is required for complex life (see Chemical Energetics and the Properties of O_2) along with two further postulates. The first postulate is that Kleiber's Law applies to life on other planets as it does to life on Earth. If the basis for Kleiber's Law is fractal geometry [self-similar transport systems for mass and energy, combined with an evolutionary drive to minimize energy dissipation (West *et al*., 2000)] or simply a result of efficient transport in a three-dimensional organism (Banavar *et al*., 1999; Dreyer, 2001), then its extension to life elsewhere is entirely plausible. The second postulate is that evolution on other planets would achieve similar aerobic metabolic efficiency as evolution has done on Earth. With these postulates, the relationship between diffusion and metabolic rates for life elsewhere would scale similarly to that for terrestrial life. If so, similar gradients in P_{O_2} would be required to grow large and complex because we have framed the calculated diffusion limits generally in terms of P_{O_2} in equilibrium with water. A planet with a higher total atmospheric pressure would change the density of an organism's tissue, which we assume to be similar to water, and change the O_2 diffusion coefficient K. However, the effect is small. Liquid density, ρ, as a function of pressure, P, is given by $\rho = \rho_0(1 + \beta P)$, where β is compressibility. Given $\beta = 0.459$ GPa^{-1} for water (at 20°C), there would only be a 0.5% increase in density for an extrasolar planetary atmospheric pressure of 100 bar (0.01 GPa).

Finally, we mention in passing that the importance of large size for complexity is an obvious precursor for life that has the potential to develop technology. First, small life (~10^{-3} m) cannot have enough cells for neurological complexity. Second, physics limits the abilities of small organisms. This is best exemplified in a famous essay by Went (1968) that compares ants with humans. Went (1968) noted how ants cannot use tools because a miniature hammer has too little kinetic energy, how ants cannot pour liquids because of surface tension, and how ants cannot use ant-sized books because intermolecular forces would stick the pages together.

THE BIOCHEMICAL REQUIREMENT OF O_2 BY THE METAZOA

In Why oxygen is a uniquely energetic oxidant, we considered the uniqueness of O_2-utilizing metabolism in a general context. There are two further aspects of O_2 to consider with respect to its biological necessity for complex life on Earth. One is the unique role of O_2 for the synthesis of various biochemicals used in complex life. The other concerns the apparent influence of historic increases in atmospheric O_2 in facilitating increased biological complexity. The idea that O_2 triggered the sudden development of complex life at the end of the Precambrian is met with skepticism by some paleontologists (*e.g*., Budd and Jensen, 2000), while largely embraced by others (Runnegar, 1991a; Knoll and Carroll, 1999). However, sufficient O_2 must at least have been a precursor to advanced metazoans.

The biochemical role of O_2 in terrestrial complex life

There are two functions of O_2 in aerobic life. The first, which accounts for most O_2 use [~90% in mammals (Rolfe and Brown, 1997)], is to serve as a terminal electron acceptor in the energy production of aerobic metabolism. The second, less widely recognized, role for O_2 is as an obligatory oxidant in the biosynthesis of numerous specialized molecules (Bloch, 1962; Margulis *et al*., 1976; Towe, 1981). More than 200 enzymes are known to use O_2 as a substrate, although not all of these biochemical pathways are fully understood (Malmstrom, 1982). "Oxygenases" catalyze reactions in which one or both atoms of O_2 are incorporated into organic molecules, while "oxidases" catalyze reactions in which O_2 serves as electron acceptor only (*e.g*., Chapman and Schopf,

1983). Some molecules, such as the amino acid tyrosine, are synthesized anaerobically in microbes and plants, whereas in animals an O_2-dependent pathway is used. Other molecules, such as sterols, polyunsaturated fatty acids, and, with certain qualifications, carotenes and respiratory pigments, are produced only in O_2-dependent reactions (Bloch, 1962). Table 3 shows a few prominent biochemicals that use O_2 as an obligatory oxidant in biosynthesis. Because pathways are O_2-dependent, even animals that inhabit apparently anoxic environments need O_2 for growth and biosynthesis. For example, the nematode *Ascaris*, which infests a billion people's intestines worldwide, has evolved unique hemoglobin that binds O_2 25,000 times more tightly than its mammalian homolog. Thus, despite its low-O_2 environment, *Ascaris* females extract enough O_2 to biosynthesize ~12 mg of cholesterol in daily egg production (Sherman *et al*., 1992). Clearly, free O_2 greatly diversifies and extends an organism's capacity for organic synthesis. Oxygen-dependent biosynthesis of the structurally unusual amino acid hydroxyproline is an important example because collagen, a hydroxyproline-containing protein, is essential for the structure of animals (Towe, 1981). Hydroxyproline allows collagen to possess a hydrogen-bonded helical structure that is strong enough to form the structural foundations of the most familiar trappings of biological complexity: blood vessels, tendons, cartilage, and skin (*e.g*., Darnell, 1995, pp. 1126–1134). Oxygen-dependent biochemicals are also used in sclerotization, the process that hardens cuticles in arthropods (Mason, 1965; Brunet, 1967). In sclerotization, protein chains become cross-linked by hydrogen bonds through the action of highly reactive quinones produced by phenol oxidase, which promotes the oxidation of catechols (compounds with a benzene ring and two hydroxyls) (Hopkins and Kramer, 1992). Indeed, the emergence of unique phenol oxidases may have led to hard exoskeletons at the end of the Precambrian, with an associated increase in animal size and fossil preservation (Burmester, 2002). In the same vein, Rhoads and Morse (1971) and Lutz and Rhoads (1977) have noted that organic acids produced from anaerobic metabolism tend to dissolve extracellular minerals, which explains why calcerous skeletons are excluded from present-day benthic environments in equilibrium with O_2 <0.2 PAL. Thus the appearance of calcified skeletal fossils at 549 Ma in the Ediacaran Period (Martin *et al*., 2000) may also be linked to the role of O_2 in facilitating skeletal biochemistry.

Generally, it is reasonable to conclude that the presence of O_2 confers an evolutionary advantage in opening up new metabolic pathways for syn-

Table 3. Important Metazoan Biochemicals that Use O_2 in Their Biosyntheses

Biochemical	*Biological role*	*Synthesis step using O_2*
Sterols and their derivatives	Cell membranes (*e.g*., cholesterol); precursor to steroid hormones (*e.g*., testosterone and estradiol) and bile acids for fat digestion	Squalene → squalene-2,3-epoxide[1]
Collagen	Structural support and connective tissues	Proline → hydroxyproline[2]
Polyunsaturated fatty acids	Cell membranes	Desaturation of Δ^9-monounsaturated fatty acids[1]
Tyrosine	Protein structure; precursor to thyroid hormones, pigments, and several neurotransmitters	Phenylalanine → tyrosine[1]
Retinol (vitamin A)	Visual process in higher animals; cell growth and differentiation; reproduction	Beta-carotene → retinal[3]
Ubiquinone	Mitochondrial respiratory chain; antioxidant	4-Hydroxybenzoate → ubiquinone[4]

[1]Bloch (1962).
[2]Fujimoto and Tamiya (1962).
[3]Leuenberger *et al*. (2001).
[4]Alexander and Young (1978).

thesizing specialized structural molecules. However, we are circumspect in generalizing this to animal-like life elsewhere; it is uncertain whether structural molecules produced in O_2-dependent pathways would necessarily be explicitly required for complex life elsewhere. Nonetheless, all of the above indicates that O_2 is essential in the biosynthetic pathways of terrestrial animals.

The coupled history of biological complexity and atmospheric oxygen

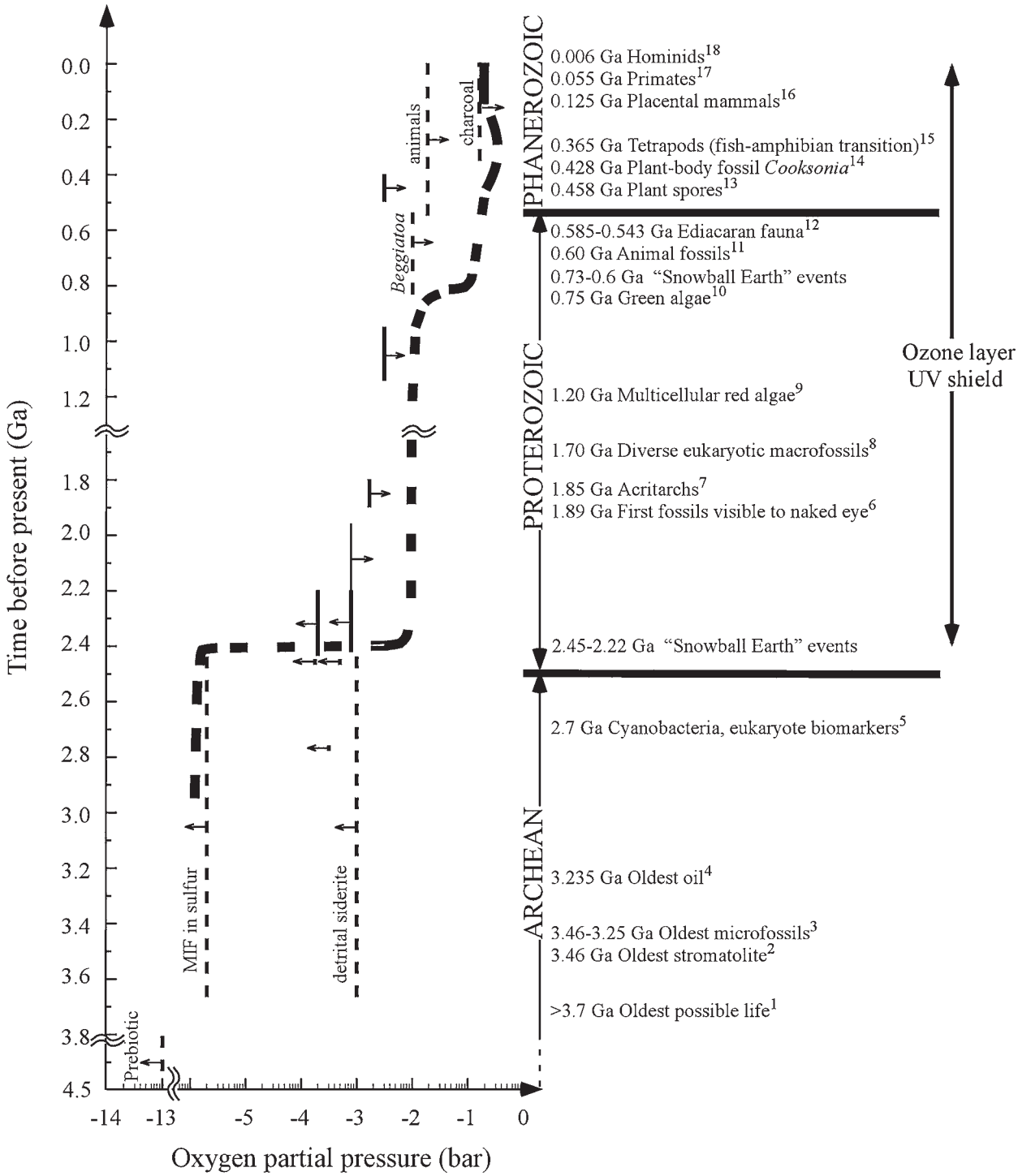
Darwin (1859) famously noted the paradox of a long Precambrian history without obvious fossils followed by an eon of relatively abundant fossils. If atmospheric O_2 was an environmental constraint on organism size, this paradox is resolved. Nursall (1959) first suggested that the time scale to build up O_2 was a rate-limiting step for the appearance of metazoans, a view later championed by Cloud (1968, 1976, 1988). Modern data suggest that there were two major increases in Earth's atmospheric O_2. These occurred near the beginning and end of the Proterozoic (2.5–0.543 Ma), respectively. The first increase occurred around 2.4–2.3 Ga in the Paleoproterozoic when the atmosphere became redox-dominated by O_2 (Holland, 1999). Then in the Neoproterozoic, about 1.0–0.6 Ga, O_2 increased a second time, as evidenced by increased sulfate levels in the ocean (Canfield and Teske, 1996; Canfield, 1998; Hurtgen *et al*., 2005). Both O_2 increases appear to have been followed by substantial changes in the Earth's climate and biota. In particular, both the Neoproterozoic and Paleoproterozoic eras are characterized by tropical glaciations (Evans *et al*., 1997; Hoffman *et al*., 1998) and large oscillations of sedimentary carbon isotopes that may follow increases in O_2 (Knoll, 1986; Kaufman, 1997; Melezhik *et al*., 1999; Lindsay and Brasier, 2002; Bekker *et al*., 2004).

Figure 2 shows how the P_{O_2} through time is correlated with the evolution of life. The atmosphere started out with virtually no oxygen ($P_{O_2} \sim 10^{-13}$ bar) before life existed (Kasting, 1993), but now contains 21% O_2 by volume ($P_{O_2} \sim 0.21$ bar). The rise of O_2 at 2.4–2.3 Ga would have provided a global source of O_2 for respiration and created an ozone layer, shielding surface life from biologically harmful UV radiation. Photochemical models show that harmful UV in the 200–280 nm range is mostly absorbed with an ozone layer that is well established at $P_{O_2} \sim 0.002$ bar (Kasting and Donahue, 1980; Kasting, 1987). Paleontological evidence is consistent with the hypothesis that the rise in O_2 at 2.4–2.3 Ga invoked biological evolution. The oldest known fossils that are visible to the naked eye are found in 1.89-Ga shales from Michigan: spirally coiled organisms (*Grypania spiralis*), which were probably filamentous algae (Han and Runnegar, 1992; Schneider *et al*., 2002). If *Grypania* respired, it would have needed P_{O_2} ~0.02 bar or more to survive, unless it were photosynthetic, in which case sufficient P_{O_2} could have been generated internally (Runnegar, 1991a). Acritarchs, which are fossilized tests of eukaryotic organisms, are first found in 1.85-Ga rocks in China (Zhang, 1997) and become abundant in younger Proterozoic rocks, generally in the size range 40–200 μm (Knoll, 1992). The rise of O_2 may also be linked to the emergence of relatively large filamentous life. Diverse fossils up to 4–5 mm long are found in northern China at 1.7 Ga, and may be multicellular (Shixing and

FIG. 2. The thick dashed line shows a possible evolutionary path for atmospheric O_2 that satisfies biogeochemical data. Dotted vertical lines show the duration of biogeochemical constraints, such as the occurrence of detrital siderite ($FeCO_3$) in ancient riverbeds (Rasmussen and Buick, 1999). Left-pointing arrows indicate upper bounds on the P_{O_2}, whereas right-pointing arrows indicate lower bounds. Unlabeled solid vertical lines indicate the occurrence of particular paleosols, with the length of each line showing the uncertainty in the age of each paleosol. Inferences of P_{O_2} from paleosols are taken from Rye and Holland (1998). An upper bound on the level of P_{O_2} in the prebiotic atmosphere at ~4.4 Ga (shortly after the Earth had differentiated into a core, mantle, and crust) is based on photochemical calculations. MIF is "mass-independent isotope fractionation," which in sulfur is caused by photochemistry in an O_2-poor atmosphere. The P_{O_2} level inferred from MIF observed in pre-2.4 Ga sulfur isotopes is based on the photochemical model of Pavlov and Kasting (2002). Biological lower limits on P_{O_2} are based on the O_2 requirements of (1) the marine sulfur-oxidizing bacterium, *Beggiatoa* (Canfield and Teske, 1996), (2) Ediacaran animals, and (3) charcoal production in the geologic record. A "bump" in the O_2 curve around ~300 Ma, in the Carboniferous, is based on the interpretation of Phanerozoic carbon and sulfur geologic data by Berner *et al*. (2000). Data are from the following references: [1]Rosing (1999), [2]Buick *et al*. (1981), [3]Walsh (1992), [4]Rasmussen and Buick (2000), [5]Brocks *et al*. (1999), [6]Han and Runnegar (1992) and Schneider *et al*. (2002), [7]Zhang (1997), [8]Zhu and Chen (1995), [9]Butterfield (2000), [10]Butterfield *et al*. (1994), [11]Xiao and Knoll (2000), [12]Narbonne and Gehling (2003), [13]Gray (1993), [14]Kenrick and Crane (1997), [15]Clack (2000), [16]Ji *et al*. (2002), [17]Ni *et al*. (2004), [18]Brunet *et al*. (2002).

Huineng, 1995; Zhu and Chen, 1995). Then by 1.4 Ga, similar, often larger, carbonaceous fossils become abundant worldwide in marine sedimentary rocks (Knoll, 1992).

The inferred second rise in O_2 in the Neoproterozoic may have led to a decrease in atmospheric methane (Pavlov *et al.*, 2003), subsequent glacial periods associated with the loss of greenhouse warming (Hoffman and Schrag, 2002), and a drastic change in Earth's biota. The pivotal event for complex life was the appearance of macroscopic animal fossils. The oldest known radially symmetric impressions of animal embryos, eggs, and possible sponges date from ~600 Ma



(Li *et al*., 1998; Xiao and Knoll, 2000). These follow the Marinoan glaciation (~610 Ma), which is the last of between two and four Neoproterozoic glaciations (Hoffman and Schrag, 2002). Subsequent soft-bodied fossils were initially identified in the Ediacaran hills in southern Australia, and similar Ediacaran fossils appear on six continents. The oldest Ediacaran fossils are ~575 Ma (Narbonne and Gehling, 2003). Shortly afterward, fossil evidence for animals with bilateral symmetry is found at 555 Ma (Martin *et al*., 2000). Molecular evidence suggests that the metazoa originated earlier at ~1,000 Ma, with modern phyla such as arthropods, molluscs, brachiopods, and echindoderms diverging ~600–800 Ma (Benton and Ayala, 2003). Actually, molecular clock dates for the early diversification of animals vary by as much as 1 billion years, but all require at least some interval of animal evolution that precedes the entrance of animals into the fossil record. The appearance of animals is consistent with a second rise in O_2 around 0.8–0.6 Ga (Canfield and Teske, 1996; Knoll and Holland, 1995; Knoll and Carroll, 1999; Hurtgen *et al*., 2005). Certainly P_{O_2} levels must have been at least 0.1 PAL to support large animal metabolism (see How much O_2 is needed for aerobic organisms to grow large?).

Finally, in the Phanerozoic eon (0.543 Ga to present), P_{O_2} has probably always been 0.2 ± 0.1 bar, sufficient to support large life forms. The colonization of the land by plants began around ~450 Ma, fish evolved into amphibians by 365 Ma, and placental mammals were present by 125 Ma (Fig. 2). Charcoal is found in continental rocks from 350 Ma onwards, which indicates O_2 exceeding an ~15% mixing ratio for all recent epochs because wood cannot burn below this level (Lenton and Watson, 2000).

THE OXYGENATION TIME ON EXTRASOLAR HABITABLE PLANETS

The previous sections provide a case that sufficient O_2 is necessary for complex life, and that on Earth, it took ~3.9 billion years to reach the required level of O_2. By extension, on other inhabited planets, the appearance of complex life would be mainly rate-limited by the oxygenation time. What sets this time scale?

The factors that controlled the oxygenation time on Earth are still an issue of debate, though oxygenic photosynthesis is the only plausible source of O_2 that could account for Earth's O_2 (Walker, 1977). In photosynthesis, the production of 1 mol of organic carbon ("CH_2O") generates 1 mol of O_2 via $CO_2 + H_2O \rightarrow CH_2O + O_2$. Burial of organic matter in sediments is the source of O_2 because otherwise respiration or decay reverses the reaction. However, the fate of this O_2 depends on the magnitude of the various sinks for O_2; there is no reason for the O_2 to accumulate or persist in the atmosphere, as is often stated (*e.g*., Lane, 2002, p. 24). Plausible evidence for the earliest oxygenic photosynthesis comes from 2-α-methylhopane biomarkers derived from cyanobacteria and steranes derived from eukaryotic sterols at 2.7 Ga (Brocks *et al*., 1999, 2003; Summons *et al*., 1999). Thus, oxygenic photosynthesis likely existed long before detectable O_2 in the atmosphere. This means that either the O_2 source increased or the O_2 sink decreased. Some workers have favored the former idea, arguing for an increase in organic burial rates (Des Marais *et al*., 1992; Godderis and Veizer, 2000; Bjerrum and Canfield, 2002). Others dispute the increased O_2 source on the basis of the constancy of the carbon isotope record and suggest a decrease in the O_2 sink from reducing gases that emanate from the solid Earth (Kasting *et al*., 1993; Kump *et al*., 2001; Catling *et al*., 2001; Holland, 2002). Proponents of the latter hypothesis note how excess hydrogen (or hydrogen-bearing reducing gases) in the pre-oxygenated atmosphere would necessarily promote hydrogen to escape to space and irreversibly oxidize the Earth.

On other habitable planets, the level of O_2 sinks and sources would also be the important factors for setting the oxygenation time. Assuming oxygenic photosynthesis arises, tectonic activity, which produces a flux of reduced species (hydrothermal aqueous ions, volcanic gases, and metamorphic gases), would have to be sufficiently low to allow a chemical state where O_2 production from organic burial exceeds reduced sinks. However, the possible controls on the build up of O_2 are intricate. To illustrate this, we briefly discuss possible planetary parameters that could affect oxygenation:

1. *Planet size*. Although the rocky, inner planets of the Solar System are all Earth-sized or smaller, this may not be typical. A stellar nebula that condenses to a disk with a larger surface density than that of the solar nebula would produce much larger inner planets (J.

Chambers, personal communication). In turn, planetary size would affect the surface heat flux, which drives tectonic activity. Another effect of planetary size is its influence on hydrogen escape. In the case of diffusion-limited escape, the rate scales with planetary size because the diffusion length, set by the atmospheric scale height, gets smaller, all other things being equal. On the other hand, if the planet is sufficiently large, hydrogen would be gravitationally bound.

2. *Planetary continents*. The presence or absence of continents would affect the oceanic heat flux, total continental weathering, and organic carbon burial. Granite production is caused by tectonism, and the presence of oceans appears critical (Meissner, 1986; Campbell and Taylor, 1993). A habitable (ocean-bearing) planet may grow continents also, but the exact mechanisms for continent formation even for Earth are still debated (Taylor and McLennan, 1995, 1996).
3. *Planet composition*. More oxidized or more reducing compositions should give rise to more oxidized or reducing gases, respectively, in volcanism and metamorphism. An insight into extrasolar planet composition could be determined by spectroscopy of the parent star's photosphere because photospheres track CI chrondritic composition, the primordial material from which planets form (Taylor, 2001, p. 75). There could also be a notable tectonic sensitivity to mean planetary density and initial specific radioactivity, which changes principally because of radioactive potassium abundance.
4. *Planet biospheres*. Some anaerobes remove mobile reductants and oxidize an atmosphere [*i.e.*, $CO_2 + 2H_2 = CH_2O$ (buried) + H_2O; $CO + H_2 = CH_2O$ (buried)]. This is counteracted by fermenters, which consume organic matter and release gases such as methane. The efficiency with which reductants are buried could limit hydrogen escape and planetary oxidation. Also, we have little grasp of how the source of free O_2, the burial of photosynthesized organic carbon, may differ on other planets. When we look at the Earth, carbon that fluxes into the ocean–atmosphere system fluxes out into sediments as organic carbon and inorganic carbonate carbon in an ~1:4 ratio (Schidlowski, 1988). Consequently, this ratio is tied to O_2 production. However, currently there is no adequate explanation for this carbon ratio so its value on other planets with different biospheres is guesswork.

Knoll (1985) and McKay (1996) have also argued that the oxygenation time would be the rate-limiting step in the development of advanced extraterrestrial life. This is in contrast to others who, neglecting the co-evolution of the environment, have thought biological factors to be important. For example, Carter (1983) considered the rate-limiting steps for the development of metazoa to be the evolution of the genetic code and the organization of the nervous system, but the fossil record (Fig. 2) does not support this. One possible biological factor that may be important for the development of metazoa is cell organization, in addition to a physical constraint from oxygen. Almost all anaerobes are prokaryotes, and aerobic prokaryotes are also tiny. So it follows that in terrestrial life the eukaryotic cell organization was an important prerequisite for multicellularity. However, we note that eukaryotic life appears to have existed long before animals (Brocks *et al*., 1999), so that oxygen is a more convincing ultimate rate-limiting factor for life that is explicitly animal-like rather than merely multicellular. Clearly, the factors that go in to the oxygenation time, as discussed above, are complicated and, for the most part, scientifically undeveloped. But even if the oxygenation time is comparable on other planets, we note that many stars are much longer lived than the Sun. The Sun, a G2–type star, has a 10 billion year lifetime on the main sequence. But G5, K0, or K5 stars would last 13, 18.4, and 45.7 billion years, respectively, and could accommodate long planetary oxygenation times. Given how little we know about the occurrence of Earth-sized planets and their planetary evolution, we cannot reasonably say whether habitable planets elsewhere are going to be more or less likely than Earth to harbor high O_2 atmospheres and complex life.

CONCLUSIONS

In summary, oxygen is the most feasible, most energetic source for driving the metabolism and growth of advanced life. Thus, in our view, an O_2-rich atmosphere is very likely a necessary precursor to metazoan-like life on planets elsewhere.

The salient points related to this conclusion and its consequences are as follows:

1. The reduction of O_2 provides the largest feasible free energy release per electron transfer in the Periodic Table, except for F_2, which exceeds oxygen in energy release, and Cl_2, which is comparable. However, fluorine and chlorine are orders of magnitude less cosmically abundant than oxygen and in any case cannot accumulate in a planetary atmosphere because of their extreme reactivity. O_2 is more stable because the triplet character of dioxygen imposes a kinetic barrier.
2. Aerobic metabolism provides about an order of magnitude greater energy release for a given intake of food than anaerobic metabolism. This means that in aerobic organisms more food intake can be converted to new biomass (rather than used in maintenance or reproduction), which results in higher growth efficiency. We derive a model for the biomass spectra of aerobic versus anaerobic life, which suggests that the size distribution in anaerobic life is close to the growth-limited threshold and cannot support large organisms. The biomass number density, n, at a particular mass, m, is observed to scale as $n\ (>m) \propto m^{-1}$ for aerobes. This compares with our calculated dependency of $n \propto m^{-1.5}$ for anaerobes, which is close to the limit $n \propto m^{-1.75}$ when food chains cannot exist. The biomass dependency for anaerobes is a new theoretical prediction that perhaps could be verified with appropriate data from laboratory anaerobic ecosystems.
3. Even with aerobic life, the first macroscopic forms would rely on diffusion, which would limit organism size as a function of P_{O_2}. P_{O_2} must exceed $\sim 10^3$ Pa to allow organisms that rely on O_2 diffusion to evolve to a size $\sim 10^{-3}$ m. At this size, muscles may develop from cilia, facilitating pumped tubes and rudimentary circulation.
4. We assume that simple circulation systems would evolve from organisms that grow large under diffusion-limited circumstances. Physical limits on pulsatile blood flow in a closed circulation system suggest that a size threshold of $\sim 10^{-2}$ m is needed. A P_{O_2} in the range $\sim 10^3$–10^4 Pa is needed to exceed this size threshold for complex life with circulatory physiology.
5. We postulate that Kleiber's Allometric Law relating metabolic rate and body mass applies to life on other planets as well as life on Earth. This law appears independent of metabolism type, given that it is observed in both terrestrial anaerobic and aerobic life. Given this postulate, conclusions 2, 3, and 4 would also apply to life on other planets.
6. Large life on Earth appears to have co-evolved with the history of atmospheric O_2. Combined with the above considerations, this suggests that the key rate-limiting step for planets to evolve complex life is the time that it takes to reach a sufficient P_{O_2}. However, there is no *a priori* reason to accept the "Rare Earth" view of Ward and Brownlee (2000) that the oxygenation time on inhabited planets elsewhere will be about the same as the Earth's or prohibitively longer so that habitable planets are generally baked by their parent star's red giant phase before complex life gets the chance to arise. We could imagine that some planetary parameters elsewhere may be even more favorable than Earth's for allowing the accumulation of atmospheric O_2.
7. Oxygen is a component of liquid water and derives from it. Liquid water is generally considered a necessary precursor for Earth-like life, so habitable planets with oceans and life have the potential to develop O_2-rich atmospheres if oxygenic photosynthesis evolves.

APPENDIX 1: ORGANISM SIZE AND O_2 DIFFUSION

Newton-Harvey (1928) and Alexander (1971) both considered a cylindrical aerobic organism relying on O_2 diffusion. Here we give the mathematics relating an organism's size to P_{O_2} gradi-

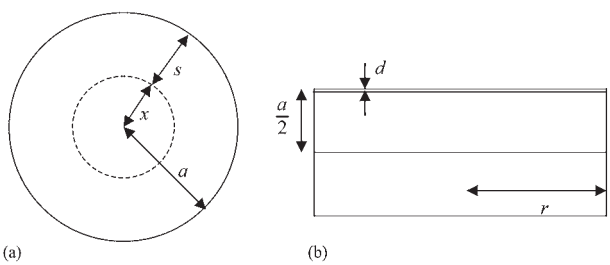


FIG. A1. Cross-sectional geometry of different organisms: (a) spherical and (b) disk-shaped.

ent for a spherical organism relying on diffusion. This methodology can easily be extended to the other geometries to obtain all the expressions of Table 2. We then give the mathematics relating the organism size to P_{O_2} gradient for a disk-shaped organism with blood circulation (Fig. A1b). Consider first Fig. A1a, a spherical organism of radius a. Consider an inner sphere of radius x separated from the exterior by a membrane of thickness s. The volume of the inner sphere is $V = (4/3)\pi x^3$, and its area for O_2 to diffuse inwards is $A = 4\pi x^2$. If the metabolic rate of O_2 consumption is M cm^3 of O_2 cm^{-3} of tissue per unit time, then the required rate of O_2 diffusion to support metabolism inside the inner sphere is $J = VM$ cm^3 of O_2 per unit time. This expression for J can be equated to that given by Fick's first law of diffusion, as follows:

$$-AK\frac{dP}{ds} = J = VM$$

Given that $s = a - x$, it follows that $ds/dx = -1$ and $dP/dx = (dP/ds)/(ds/dx) = -dP/ds$. Upon substitution for $-dP/ds$, A, and M we obtain $dP/dx = Mx/3K$. The required gradient in P_{O_2} to support metabolic rate M is then given by

$$P_{ex} - P_{in} = \int_0^a \frac{Mx}{3K}dx = \frac{Ma^2}{6K}$$

where P_{ex} is external P_{O_2} and P_{in} is internal P_{O_2} at the center. The limiting condition $P_{in} = 0$ gives the maximum radius, a (Table 2).

For a disk-shaped animal (Fig. A1b) with blood circulation, we follow the method of Alexander (1971), who considered a cylindrical animal. Assume that O_2 can enter from both upper and lower surfaces of the disk, and neglect the sides. The organism is assumed to have an epidermal thickness, d, through which O_2 diffuses into the bloodstream over area $A = \pi r^2$. The volume of the upper half of the animal is $V = (a/2)(\pi r^2)$, and the required rate of O_2 input to support metabolism is $J = VM$ cm^3 of O_2 per unit time. Fick's law of diffusion gives $J = -AK(P_b - P_{ex})/d = VM$, where P_{ex} is the external P_{O_2} and P_b is the average P_{O_2} in the blood. Upon substitution for V and A, this gives the result shown in Table 2.

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ABBREVIATIONS

ΔG^0, Gibbs free energy; PAL, present atmospheric level; P_i, orthophosphate; P_{O_2}, partial pressure of atmospheric oxygen; UV, ultraviolet.

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Address reprint requests to:
David C. Catling
Department of Earth Sciences
University of Bristol
Wills Memorial Building
Queen's Road
Bristol B58 1RJ
United Kingdom

E-mail: David.Catling@bristol.ac.uk