

# Earth's oxygen cycle and the evolution of animal life

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**The emergence and expansion of complex eukaryotic life on Earth is linked at a basic level to the secular evolution of surface oxygen levels. However, the role that planetary redox evolution has played in controlling the timing of metazoan (animal) emergence and diversification, if any, has been intensely debated. Discussion has gravitated toward threshold levels of environmental free oxygen (O<sub>2</sub>) necessary for early evolving animals to survive under controlled conditions. However, defining such thresholds in practice is not straightforward, and environmental O<sub>2</sub> levels can potentially constrain animal life in ways distinct from threshold O<sub>2</sub> tolerance. Herein, we quantitatively explore one aspect of the evolutionary coupling between animal life and Earth's oxygen cycle—the influence of spatial and temporal variability in surface ocean O<sub>2</sub> levels on the ecology of early metazoan organisms. Through the application of a series of quantitative biogeochemical models, we find that large spatiotemporal variations in surface ocean O<sub>2</sub> levels and pervasive benthic anoxia are expected in a world with much lower atmospheric pO<sub>2</sub> than at present, resulting in severe ecological constraints and a challenging evolutionary landscape for early metazoan life. We argue that these effects, when considered in the light of synergistic interactions with other environmental parameters and variable O<sub>2</sub> demand throughout an organism's life history, would have resulted in long-term evolutionary and ecological inhibition of animal life on Earth for much of Middle Proterozoic time (~1.8–0.8 billion years ago).**

oxygen | animals | evolution | Proterozoic

A long-standing and pervasive view is that there have been intimate mechanistic links between the evolution of complex life on Earth—in other words, the emergence and ecological expansion of eukaryotic cells and their aggregation into multicellular organisms—and the secular evolution of ocean–atmosphere oxygen levels (1). Molecular oxygen (O<sub>2</sub>) is by far the most energetic of the abundant terminal oxidants used in biological metabolism (e.g., ref. 2). When this energetic capacity is harnessed by mitochondria in eukaryotic cells, the energy flux supported by a given genome size increases by a factor of ~8,000 (3), potentially paving the way for increased complexity at the cellular level (but see ref. 4). Oxygen is also a crucial component of enzymatic pathways leading to the synthesis of regulatory membrane lipids (5) and structural proteins (6) in eukaryotic organisms and provides a powerful shield against solar UV radiation at Earth's surface through stratospheric ozone production. Furthermore, O<sub>2</sub> is the only respiratory electron acceptor that can meet the metabolic demands required for attaining the large sizes and active lifestyles characteristic of metazoan life (e.g., ref. 7). There is thus ample support for the view that Earth's oxygen cycle provided a crucial evolutionary and ecological constraint on the road to increased biotic complexity, both at the cellular level and on the road to motile multicellular life.

However, it is important to distinguish between the O<sub>2</sub> levels required for: (i) the emergence and ecological expansion of complex (multicellular) eukaryotes, including red/brown algae, land plants, fungi, and animals; (ii) the initial origin of the metazoan (animal) last common ancestor (LCA); (iii) the diversification of basal (e.g., Ctenophora, Cnidaria, Porifera) and more derived (e.g., Bilateria) metazoan clades; and (iv) emergence of the larger body sizes and more complex ecological interactions (such as predation and burrowing) likely to leave robust signals in the fossil record (8).

The requisite environmental O<sub>2</sub> levels for each of these biotic milestones may vary substantially, and some may depend strongly on environmental variability, whereas others may be more directly linked to the environment achieving a discrete “threshold” or range of oxygen levels. Our focus here is on reconstructing an ecological context for the emergence and expansion of early animal life during the Middle to Late Proterozoic [~1.8–0.6 billion years ago (Ga)] in the context of evolving Earth surface O<sub>2</sub> levels.

Most work on the coevolution of metazoan life and surface oxygen levels can be characterized as either biological (e.g., attempting to constrain threshold environmental O<sub>2</sub> levels for different organisms), or geochemical (e.g., attempting to constrain environmental O<sub>2</sub> levels before, during, and after the emergence of metazoan life). This discussion is typically framed in terms of a relatively sharp dichotomy—e.g., either environmental oxygen levels have had little to do with the timing of the rise of animal life (e.g., refs. 9 and 10) or oxygenation of Earth's surface has been of primary importance (e.g., ref. 11). Both approaches have emphasized the relationship (or lack thereof) between metazoan evolution and the secular oxygenation of Earth's ocean–atmosphere system, and both provide a critical backdrop for efforts to understand the importance of environmental factors in early animal evolution.

Experimental work with the modern demosponge *Halichondria panacea* suggests an empirical O<sub>2</sub> threshold of ~0.5–4.0% of the present atmospheric level (PAL; corresponding to an equilibrium concentration of ~1–10 μmol·kg<sup>-1</sup> for air-saturated water at 25 °C and a salinity of 35‰) for basal metazoan life (12) and perhaps lower for smaller sponge species. Theoretical calculations for the LCA of bilaterian life indicate an even lower threshold range of ~0.14–0.36% PAL (ref. 13, Fig. 1). In addition, all major eukaryotic lineages contain genes and enzymes allowing for facultatively anaerobic energy metabolism (14), which indicates that either (i) anaerobic pathways of ATP synthesis in primitively aerobic eukaryotes were obtained through interdomain and intradomain

## Significance

Earth is currently the only planet known to harbor complex life. Understanding whether terrestrial biotic complexity is a unique phenomenon or can be expected to be widespread in the universe depends on a mechanistic understanding of the factors that led to the emergence of complex life on Earth. Here, we use geochemical constraints and quantitative models to suggest that marine environments may have been unfavorable for the emergence and large-scale proliferation of motile multicellular life for most of Earth's history. Further, we argue that a holistic evaluation of environmental variability, organismal life history, and spatial ecological dynamics is essential for a full accounting of the factors that have allowed for the emergence of biological complexity on Earth.

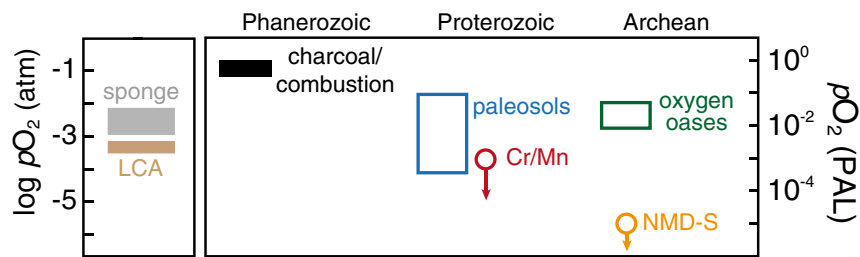
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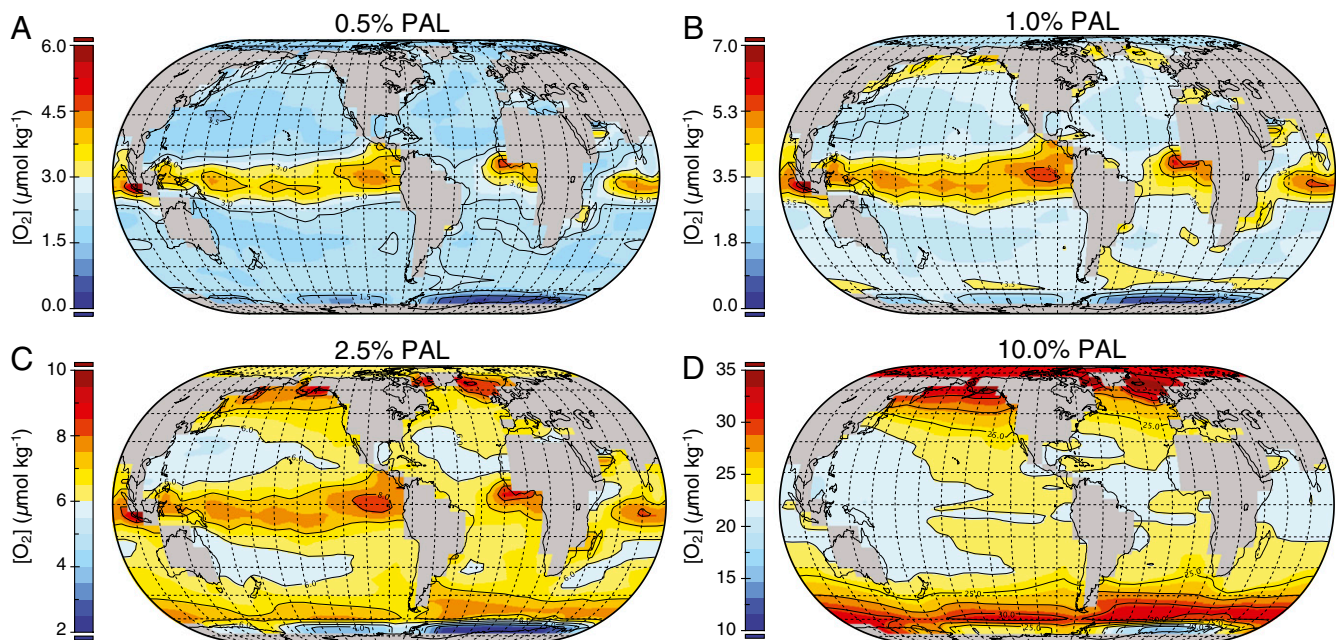
**Fig. 1.** Biological thresholds and geochemical constraints on Earth surface  $O_2$  levels. (Left) Estimated minimum metabolic  $O_2$  requirements of sponges (gray) (12) and the LCA of bilaterian metazoans (brown) (13). (Right) Estimated environmental  $O_2$  levels based on the plant fossil and charcoal record during the Phanerozoic (37); fossilized soil profiles during the Proterozoic (blue), recalculated following ref. 38 according to  $pCO_2$  values in ref. 39; chromium (Cr) and manganese (Mn) systematics of marine chemical sediments and ancient soil profiles (red) (11); non-mass-dependent sulfur (S) isotope fractionation in sedimentary rocks (orange) (40); and 0D and 3D Earth system models for Archean oxygen oasis systems (green) (20–22). Downward arrows represent upper bounds on atmospheric  $O_2$  levels, and the range for oxygen oases refers to the estimated atmospheric  $pO_2$  value at gas exchange equilibrium.

lateral gene transfer (15, 16), or (ii) the earliest eukaryotic organisms contained mitochondria that were facultatively anaerobic (17). In any case, it is possible that early animals were able to respire at very low environmental  $O_2$  levels and possibly even survive periodic episodes of local anoxia.

Geochemical estimates of atmospheric  $pO_2$  levels during the Proterozoic before the emergence and ecological expansion of metazoan life span orders of magnitude, from less than 0.1% PAL (11) to  $\sim 10\%$  PAL (18, 19). Estimates thus span levels well below what active aerobic metazoans would have been able to tolerate on long timescales to values well above those that should be required for animal respiration (Fig. 1). An additional complication is that, after the evolution of oxygenic photosynthesis, disequilibrium between surface marine and atmospheric oxygen levels would have been common, as it is in the modern oceans. A range of biogeochemical models applied to simulate this disequilibrium indicate that bacterial oxygen production in the surface ocean can sustain local dissolved  $O_2$  concentrations of  $\sim 1\text{--}10 \mu\text{mol}\cdot\text{kg}^{-1}$  at steady state (20–22), even at exceptionally low atmospheric  $O_2$  levels (Fig. 1). Thus, local biological  $O_2$  production in some regions of the surface

ocean may have allowed for  $O_2$  levels above the respiratory requirements of basal metazoan organisms regardless of background atmospheric  $pO_2$  (10).

In principle, one could compare a geochemical estimate of atmospheric  $pO_2$  to an estimate of the dissolved  $O_2$  level required for respiration in a particular metazoan organism and use this to infer whether there would have been any respiratory “metabolic barrier” to animal emergence and ecological expansion during a given period in Earth’s history (e.g., refs. 11 and 23). However, this basic approach has a number of drawbacks. First, it neglects spatial heterogeneity in oceanic  $O_2$  levels—for example, decoupling between atmospheric  $pO_2$  levels and oxygen availability in benthic habitats or the existence of surface ocean regions with relatively high dissolved  $O_2$  as a result of local  $O_2$  production. Second, a system with an average steady-state  $O_2$  level above that required for a given metabolism could nonetheless be inhospitable overall from an evolutionary perspective if  $O_2$  levels oscillate significantly on timescales that are unfavorable for the long-term persistence of robust populations (e.g., refs. 24 and 25). Finally, the comparative threshold approach ignores variable metabolic  $O_2$  demand during



**Fig. 2.** Results from the EMIC [Grid Enabled Integrated Earth System Model [GENIE]]. Steady-state dissolved oxygen concentrations in the surface layer of the ocean after 20-thousand-year spinup for atmospheric  $pO_2$  values of (A) 0.5%, (B) 1.0%, (C) 2.5%, and (D) 10.0% of the PAL. Note the differing scales for  $[O_2]$  for each background  $pO_2$  level.

an organism's life history, the ecology of the organism, and the potential synergistic interactions between  $O_2$  and other key environmental variables, such as temperature and pH.

Here we explore a series of quantitative biogeochemical models with the goal of providing a new framework for understanding the constraints posed by a relatively low oxygen Earth system on early animal ecology and evolution. First, we use a 3D Earth system model to assess spatial variability in surface ocean  $O_2$  and surface–benthic decoupling at a range of atmospheric  $pO_2$  values relevant for the Late Proterozoic Earth [ $\sim 800$ – $600$  million years ago (Ma)]. We then use a regional time-dependent box model of the surface ocean to evaluate the potential for temporal variability in  $O_2$  levels within productive regions of the surface ocean. Finally, we build on these perspectives to propose a simple conceptual model that frames the links between Earth's oxygen cycle and the early evolution of animal life as a question of habitat viability, highlighting the stability of environmental  $O_2$  levels (e.g., ref. 25) against the backdrop of evolving baseline  $pO_2$  (11, 26), rather than the attainment of threshold  $O_2$  levels for a single life history stage (10, 11, 23). We suggest that this view provides a more realistic and powerful framework for understanding the links between environmental stress and the early evolution of complex metazoan life on Earth. Finally, we argue that spatiotemporal  $O_2$  variability during Middle Proterozoic time would have resulted in a challenging evolutionary and ecological landscape for early animal life, even at relatively high background atmospheric  $pO_2$ .

### Oceanic Oxygen Landscapes

We evaluate steady-state spatial heterogeneity in surface ocean and benthic  $O_2$  levels by using a 3D Earth system model of intermediate complexity (EMIC) configured for a low  $pO_2$  world (see ref. 22). The model ocean is integrated to steady state at a range of imposed atmospheric  $pO_2$  values, and the resulting distribution of dissolved oxygen concentrations at the surface and ocean interior is evaluated. We explore a range of atmospheric  $pO_2$  levels over two orders of magnitude (0.1–10% PAL), meant to be broadly inclusive of estimates for the Mid- and Late-Proterozoic Earth system (Fig. 1), and we focus our analysis on the spatial arrangement and concentration distributions of both surface and benthic grid cells at steady state.

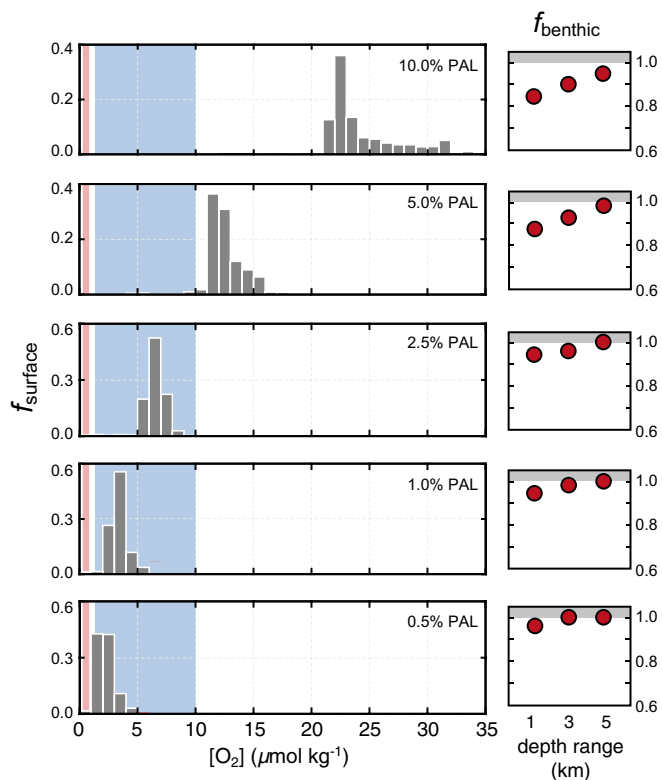
Although we use an effectively modern continental distribution, we do not mean to suggest that large-scale ocean circulation during the Proterozoic was necessarily similar to that characteristic of modern Earth. However, we consider it likely that the basic range of large-scale “circulatory settings”—low-latitude to midlatitude equatorial upwelling due to Hadley cell convergence, high-latitude deep convection, eastern boundary upwelling—is well represented by the zonal and meridional circulation patterns of modern Earth. This assumption should be particularly true with respect to nutrient recharge to the surface ocean (of primary importance for our simulations here). Although more accurately diagnosing patterns of large-scale ocean circulation under different continental configurations and explicitly linking these to the fossil record of early metazoan life is a crucially important topic for future research, we consider it unlikely that changes in large-scale ocean circulation would dramatically alter our primary conclusions.

Consistent with previous work (20–22), we find that broad regions of the ocean can be characterized by dissolved  $O_2$  levels significantly above ambient gas exchange equilibrium at steady state as a result of localized biological  $O_2$  release (Fig. 2). At atmospheric  $pO_2$  levels below  $\sim 2.5\%$  PAL, the distribution of surface ocean  $O_2$  is controlled principally by the biosphere, with the highest dissolved  $O_2$  levels located primarily in areas of high productivity (and  $O_2$  release) along eastern boundaries and along regions of equatorial divergence (Fig. 2A and B). However, a critical transition occurs at  $pO_2$  levels above  $\sim 2.5\%$  PAL, at which point surface  $O_2$  distributions are controlled by the atmosphere rather than local  $O_2$  release in the surface ocean. In this regime, high  $O_2$  levels are

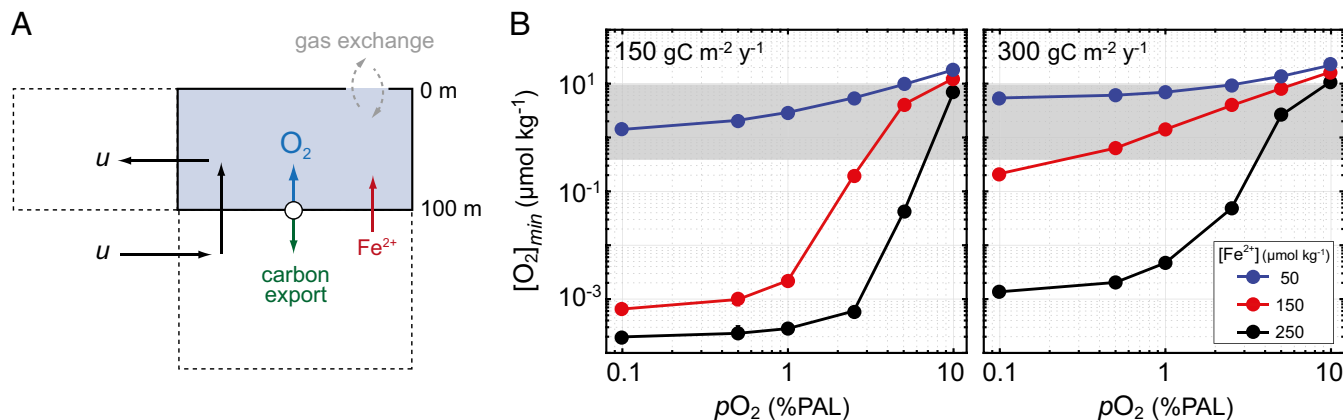
more commonly observed in colder, high-latitude surface waters where aqueous  $O_2$  solubility is relatively high (Fig. 2D).

The precise atmospheric  $pO_2$  threshold at which this transition occurs will ultimately depend on a number of factors, but the presence of this transition and its functional relationship with atmospheric  $pO_2$  is unlikely to be dramatically different from that observed here (Supporting Information and Fig. S1). At  $pO_2$  levels above this transition point, most of the surface ocean will be near gas exchange equilibrium with atmospheric  $pO_2$  (Fig. 2D). Below this transitional atmospheric  $pO_2$ , surface ocean dissolved  $O_2$  levels are expected to be extremely variable in time and space and may often have been well below levels that would be expected to challenge aerobic metazoan organisms (Fig. 2A and B), despite locally elevated values in productive regions of the surface ocean.

Perhaps more importantly, we observe strong decoupling between atmospheric  $pO_2$ , surface ocean  $O_2$  distributions, and  $O_2$  availability in the benthic realm. On an ocean scale, benthic grid cells are almost entirely devoid of  $O_2$  at steady state at  $pO_2$  levels below  $\sim 2$ – $3\%$  PAL, and, even above this level, the seafloor is dominated by anoxic conditions in our model (Fig. 3). The pervasiveness of benthic anoxia in shallow settings may also have been exacerbated by nutrient limitation of biological oxygen production in the surface ocean during much of Proterozoic time (refs. 27 and 28, Supporting Information). The upper ocean grid size in our model does not fully resolve the shallowest of continental margin settings (29), and it will be important to leverage high-resolution models of the upper ocean (e.g., ref. 30) in future research. Nevertheless,



**Fig. 3.** (Left) Relative frequency distributions for dissolved oxygen concentration in surface ocean grid cells ( $f_{\text{surface}}$ ) and (Right) the fraction of benthic grid cells that are anoxic ( $f_{\text{benthic}}$ ) for a range of atmospheric  $pO_2$  levels. Relative fraction of benthic grid cells that are anoxic are calculated for three different cumulative depth ranges, the upper 1 km, upper 3 km, and upper 5 km of the ocean. Shaded blue boxes denote estimated ranges for basal metazoan organisms derived from laboratory experiments (12), and red boxes show lower limits for bilaterian organisms estimated from theoretical calculations (13).



**Fig. 4.** Results of the time-dependent biogeochemical model. (A) A schematic depiction of the main processes represented by the model. (B) Minimum seasonal dissolved oxygen concentrations in the surface ocean as a function of background atmospheric  $pO_2$  value for two different baseline carbon export rates (*Left* and *Right*), and at deep  $[Fe^{2+}]$  levels of 50  $\mu\text{mol}\cdot\text{kg}^{-1}$  (blue), 150  $\mu\text{mol}\cdot\text{kg}^{-1}$  (red), and 250  $\mu\text{mol}\cdot\text{kg}^{-1}$  (black). Gray shaded box encompasses the biological limits denoted in Fig. 1.

our results imply the potential for a severe O<sub>2</sub>-based ecological bottleneck for the large-scale dispersal and expansion of basal metazoan organisms with a benthic life stage (e.g., ref. 31), despite background atmospheric  $pO_2$  above the levels currently considered to be required for respiration (Fig. 2). We emphasize that this decoupling is a robust feature and arises as a natural consequence of the ocean's biological pump—for example, any changes to the nutrient status of the ocean interior that favor greater local dissolved O<sub>2</sub> accumulation in the surface ocean will tend to exacerbate O<sub>2</sub> deficiency in the benthic realm.

In summary, our 3D model results suggest a complex interplay between atmospheric  $pO_2$ , surface ocean dissolved O<sub>2</sub> distributions, and O<sub>2</sub> availability in the benthic marine realm over the range of atmospheric  $pO_2$  values envisioned for the Late Proterozoic Earth system. Surface ocean dissolved O<sub>2</sub> levels would have been spatially variable and, in many cases, well above those predicted based on ambient atmospheric  $pO_2$ . At the same time, O<sub>2</sub> availability in benthic habitats would have been largely insulated from surface ocean–atmosphere O<sub>2</sub> dynamics, making dispersal and long-term survival of metazoans with a benthic life stage extremely difficult—even at background atmospheric  $pO_2$  levels well above those conventionally assumed to be challenging for basal metazoan life.

### Temporal Variability in Surface O<sub>2</sub> Levels

Although the Earth system model results provide insight into the long-term mean state of the ocean's oxygen landscape, we can also diagnose temporal variability in surface O<sub>2</sub> levels using a simpler regional model (21) that tracks dissolved O<sub>2</sub> concentrations in a generalized coastal surface ocean environment as governed by physical transport, gas exchange, and biogeochemical processes. This approach has a number of advantages for our purposes. First, it allows us to apply dynamic wind stress on diurnal and seasonal timescales such that air–sea gas exchange can be periodically forced. Second, it allows us to explicitly explore the role of externally sourced deep ocean reductants (e.g., Fe<sup>2+</sup>) on regionally large O<sub>2</sub> production fluxes.

Briefly, our model assumes that a fraction of primary production in surface waters escapes respiration and becomes exported beneath the photic zone. This exported carbon is offset by a stoichiometric production of O<sub>2</sub>. Oxygen that accumulates in the surface ocean is transported by upwelling circulation and equilibrates with the atmosphere through air–sea gas exchange on a timescale governed by temperature, salinity, and surface wind speed (21). The upwelling circulation also injects ferrous iron (Fe<sup>2+</sup>) into surface waters, which

can react with O<sub>2</sub> produced by photosynthesis and thus represents a potential sink term for O<sub>2</sub>. Ferrous iron concentrations were kept constant in the simulations explored here. It is likely that pulsed benthic and/or hydrothermal sources of Fe<sup>2+</sup> would have resulted in temporally variable bottom water ferrous Fe concentrations, leading to periodic anoxia. Given poor empirical constraints on local and global variability in Fe<sup>2+</sup> fluxes, we do not explicitly model these processes here, rendering our conclusions conservative.

Seasonal (monthly) oscillations in biological carbon fluxes and air–sea gas exchange result in oscillatory changes in dissolved [O<sub>2</sub>] that are, in some cases, very large (*Supporting Information* and Fig. S2). To diagnose the potential biological consequences of this temporal variability, we evaluate the minimum surface O<sub>2</sub> level attained during a seasonal cycle at a range of atmospheric  $pO_2$  values similar to those explored above and assuming a range of deep water dissolved [Fe<sup>2+</sup>] levels (Fig. 4). Relatively modest [Fe<sup>2+</sup>] levels well below some recent predictions for ferruginous Proterozoic oceans (32) can significantly depress seasonal minima and maxima in dissolved [O<sub>2</sub>], even at very high rates of biological productivity (Fig. 4B). Indeed, under most conditions, the estimated tolerance range for basal metazoan organisms (12) is only safely exceeded throughout the year at  $pO_2$  on the order of ~5–10% PAL.

In summary, seasonal variability in biological O<sub>2</sub> production, carbon export, and surface winds would have resulted in significant temporal variability in surface O<sub>2</sub> within productive regions of the surface ocean and in shallow continental margin settings. In addition, even modest amounts of Fe<sup>2+</sup> relative to some predictions for the Proterozoic ocean interior (18, 32) would have been sufficient to depress seasonal minima in dissolved O<sub>2</sub> to levels that would be expected to have inhibitory metabolic effects on emerging metazoan organisms for much of Proterozoic time. Such temporal variability in redox structure, particularly in light of the spatial complexity of the long-term mean state oxygen landscape discussed above, would have provided an additional environmental constraint on the early evolution of animal life.

### Conclusions and Future Directions

Taken together, our results imply a long-term mean surface ocean oxygen landscape during the Proterozoic that would have been characterized by significant spatial variability (Fig. 2). Superimposed on this long-term mean state were surface ocean dissolved O<sub>2</sub> distributions that would have varied significantly on seasonal timescales (Fig. 4), all against a backdrop of consistently low benthic O<sub>2</sub> levels (Fig. 3). Although it should be stressed that many

key biological innovations must have preceded the Late Proterozoic emergence of basal metazoan clades (33), we suggest that early multicellular organisms would have had to contend with a “patchy” and evolutionarily restrictive oxygen landscape for much of Proterozoic time, despite background local  $O_2$  levels that may, in some cases, have been sufficient to fuel their metabolic needs. Although our discussion here focuses on metazoans, if periodic anoxia was common throughout shallow oceans during the Neoproterozoic, all eukaryotic life may have been oxygen-stressed on evolutionary timescales. This relationship may, in part, explain the early rise but protracted diversification of eukaryotes (34).

More broadly, we stress that geochemical reconstructions of background atmospheric  $pO_2$  (11, 26) and experimental/theoretical reconstructions of resting  $O_2$  demand in basal animal organisms (12, 13), although critical, only provide part of the picture. Proterozoic marine environments would have been characterized by  $O_2$  levels that departed strongly from background mean  $pO_2$ , and the oxygen demand and marine habitat of different life history stages could present physiological bottlenecks that are currently poorly known for basal metazoan organisms. Indeed, microfossil evidence for metazoan life stages adapted for large-scale dispersal across inhospitable environments may represent a paleontological signal for such ecosystem oxygen stress during the Late Proterozoic (24). In this light, our results strongly suggest that the distribution

of  $O_2$  within the ocean–atmosphere system played a significant role in structuring evolutionary innovation and ecological success among early animals.

Finally, we hypothesize that the interplay between long-term changes in base-level  $pO_2$  and some degree of spatiotemporal oxygen variability may promote rather than inhibit biological and ecological innovation. For example, theory predicts that environmental fluctuation can often reinforce evolutionary transitions in individuality (35), such as multicellularity. A critical combination of baseline  $pO_2$  and spatiotemporal variability may thus have ratcheted the evolution of more advanced forms of multicellularity in some eukaryotic lineages. In addition, variability in the marine oxygen landscape against the backdrop of increasing baseline  $O_2$  during the Late Proterozoic (36) may have acted as a creative evolutionary force, ultimately favoring rapid phylogenetic diversification and ecological expansion among early animal lineages. Future work pinpointing when and how this transition occurred will be essential for fully understanding how changes in surface  $O_2$  levels have affected the tempo and mode of early metazoan evolution.

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