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Review Article

Co-evolution of Eukaryotes and Ocean Oxygenation in the Neoproterozoic

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The Neoproterozoic Era (about 1,000 to 542 million years ago) was a time of turbulent environmental change. Large fluctuations in the carbon cycle were associated with at least two severe - possible Snowball Earth - glaciations. There were also massive changes in the redox state of the oceans, culminating in the oxygenation of much of the deep oceans. Amidst this environmental change, increasingly complex life forms evolved. The traditional view is that a rise in atmospheric oxygen concentrations led to the oxygenation of the ocean, thus triggering the evolution of animals. We argue instead that the evolution of increasingly complex eukaryotes, including the first animals, could have oxygenated the ocean without requiring an increase in atmospheric oxygen. We propose that large eukaryotic particles sank quickly through the water column and reduced the consumption of oxygen in the surface waters. Combined with the advent of benthic filter-feeding, this shifted

oxygen demand away from the surface to greater depths and into sediments, allowing oxygen to reach deeper waters. The reduction of deep anoxia would hinder the release of phosphorus from sediments, potentially triggering a potent positive feedback: phosphorus removal from the ocean reduces global productivity and ocean-wide oxygen demand, tending to oxygenate the deep ocean. That in turn would have further reinforced eukaryote evolution, phosphorus removal and ocean oxygenation.

Life and the planet have 'co-evolved' through most of Earth history[1], with the evolution of life affecting the environment and changes in the environment affecting evolution. This two-way feedback coupling became particularly strong during the Neoproterozoic Era[1,2], which saw profound changes in eukaryotic life and the Earth system (Fig. 1). Climate cooled into a series of glaciations including the extreme Sturtian and Marinoan events and the less severe Gaskiers. There were unprecedented fluctuations in the carbon cycle (Fig. 1c), and changes in ocean redox state culminated in the eventual oxygenation of parts of the deep ocean (Fig. 1b).

Previous studies[3-5] have tended to focus on environmental change – especially oxygenation of the atmosphere and oceans – as a pre-requisite for the evolution of complex multicellular life. Some minimum threshold of oxygen is necessary for the existence of animals, and over half a century ago it was argued that a rise in atmospheric oxygen initiated a rapid evolution of metazoans[6]. The argument has reappeared repeatedly since[7,8] and is currently back in vogue[3,9], but several aspects of it can be questioned. Firstly, some simple animals do not need a lot of oxygen[10]. Some sponges[11], for example, require roughly 0.1 mL/L of dissolved O_2, and some bilaterian worms[11] need only around 0.02 mL/L O_2 – equivalent to water equilibrated with roughly 0.0035 of the present atmospheric level (PAL) of O_2. Secondly, there has been sufficient oxygen in the atmosphere to ventilate the surface ocean above these O_2 concentrations[12] since the Great Oxidation ~2400 million years ago (Ma). Before that, once oxygenic photosynthesis had evolved by at latest[1] ~2700 Ma, localised oxygen oases[13,14] with O_2 >0.01 PAL would have existed in the surface ocean. Afterwards, deep convection of oxygenated high-latitude surface waters, could potentially have ventilated parts of the Proterozoic deep ocean[15]. Finally, whilst there is compelling evidence for oxygenation of parts of the deep ocean late in the Neoproterozoic[4,5,16], there is as yet only indirect evidence[17,18] for a rise in atmospheric oxygen at this time.

We thus think it is worth considering if there are mechanisms[19] by which the deep ocean could have been oxygenated in the Neoproterozoic without requiring an increase in atmospheric oxygen. To approach the problem we use a mechanistic framework that links the biogeochemical cycles of phosphorus, carbon and oxygen (and also considers nitrogen, sulphur and iron), focusing on the balance of oxygen supply and demand in ocean waters (Box 1), the processes of weathering on the continents (Box 2), and the redox-sensitive recycling of phosphorus from marine sediments (Fig. 2). We draw upon recent summaries of fossil and molecular clock information for the timing of the radiation of eukaryotes[20] and animals[21], focusing on the evolution of key biological traits, including size, hard parts, motility, and predation. Inspired by earlier work[19], we consider how eukaryote evolution increased the efficiency of 'biological pumping' of carbon in the ocean, and thus shifted oxygen demand to greater depths in the water column. We argue that this increased phosphorus removal from the ocean into sediments, thus reducing deep ocean phosphate concentration, surface productivity and oxygen demand at depth, in a positive feedback process that could have propelled oxygenation of the deep ocean.

Early Neoproterozoic conditions

The early Neoproterozoic was part of the 'boring billion' (circa 1850-850 Ma), so called for the apparent slow pace of biological evolution and a widely-assumed stasis in atmosphere-ocean oxygenation state. The continental crust was assembled in the supercontinent Rodinia in the earliest Neoproterozoic around 1000 Ma. The climate was at least as warm as today, presumably due to increased greenhouse gas concentrations, with no evidence for glaciations between ~2200 Ma and

~715 Ma. Eukaryotes had already evolved, with recent estimates[20] placing their last common ancestor around 1900-1700 Ma, and the divergence of major extant clades before 1200-1000 Ma. Some early eukaryotes were multicellular, with fossils of red algae showing limited cell differentiation by ~1200 Ma[22]. Most of these early fossils occur in shallow marine sediments, but some were living in terrestrial lakes around 1200-1000 Ma[23]. Biomarker evidence suggests the open ocean remained dominated by prokaryotes, although there may be a preservation bias caused by widespread microbial mats[24].

The atmosphere is widely thought to have had around 0.01-0.1 of the present level (PAL) of oxygen through much of the Proterozoic[25]. However, we only really have a reliable lower limit on atmospheric oxygen of 0.01 PAL O_2 from paleosols at around 1.85 Ga and 1.1 Ga[26]. In addition, there are relatively good constraints on oceanic redox state, albeit at sparse intervals[27] (e.g. 1.8, 1.7, 1.64, 1.45, 1.2 Ga), which indicate widespread ocean anoxia (40-100% of seafloor area). The deep ocean was predominantly 'ferruginous' (Fe-rich)[12,27] but with euxinia (anoxic and sulphidic conditions) present at intermediate depths, presumably where highest oxygen demand was concentrated (e.g. in upwelling zones) (Fig. 2a). Euxinic waters were sufficiently widespread to remove most Mo from the oceans[28], but not so extensive as to prevent enrichment of Mo in organic sediments, suggesting euxinic waters covered 1-10% of seafloor area[29,30]. Conceivably there could have been fluctuations between ferruginous and more widespread euxinic conditions[27,31]. Furthermore, some bottom waters[15] may have had a low concentration of oxygen supported by overturning of high-latitude surface waters (Box 1).

The existence of widespread ocean anoxia has been used to infer that atmospheric oxygen was well below the present level for most of the Proterozoic. However, ocean redox state also depends on the limiting nutrient concentration in the ocean, which determines organic carbon production and hence oxygen demand at depth (Box 1). To create widespread deep ocean anoxia (in simple box models) at current concentrations of the ocean macro-nutrients phosphorus and nitrogen only requires[32,33] atmospheric oxygen to drop to 0.5-0.7 PAL O_2. This is well above the 0.01–0.1 PAL O_2 range often given for the Proterozoic[25]. If limiting nutrient levels (and oxygen demand) were lower than present in the Proterozoic, atmospheric oxygen would need to have been lower to produce widespread anoxia. However, if early Proterozoic phosphorus levels were above present[34], and phosphorus was the ultimate limiting nutrient then as it is now, the atmosphere could conceivably have had >0.5-0.7 PAL O_2 and the deep ocean would have remained anoxic (Box 1).

The limited extent of euxinic waters[12,31] in the Proterozoic ocean is consistent with euxinia requiring greater oxygen demand than just anoxia, including the exhaustion of nitrate and iron. Furthermore, the development of euxinia tends to be self-limiting because it requires the localised exhaustion of nitrate[35], which if the resulting water is upwelled can no longer support productivity and oxygen demand. This makes sulphate reduction extremely rare in today's ocean, despite a large reservoir of sulphate. It means that where euxinia did occur in the Proterozoic ocean, oxygen demand must have been fuelled either by nitrogen fixation[35], localised build-up and upwelling of ammonium[36], or a supply of nitrate-rich surface waters from elsewhere. Lower sulphate levels[37] than present (probably 500-3000 μM) can also help explain restricted euxinia in the Proterozoic ocean. Taken together, these considerations mean that limited euxinia cannot immediately rule out high phosphorus levels in the Proterozoic ocean. Therefore, widespread anoxia could have occurred under near-present oxygen levels.

Turning to what could have maintained the Proterozoic ocean in an anoxic and partially euxinic state, it was originally proposed[19] that organic matter derived from single-celled phytoplankton sank slowly through the water column, concentrating oxygen demand near oxygen production. This mechanism[19] can help explain euxinia at shallow depths. However, it would have reduced oxygen demand in deeper waters, making them less prone to anoxia, because their oxygen supply comes from the atmosphere via overturning of high-latitude surface waters (Box 1). To help explain widespread Proterozoic deep ocean anoxia we invoke an additional positive feedback mechanism

(Fig. 2a): More phosphorus is recycled from organic matter in sediments overlain by anoxic water, particularly when fully euxinic[38], and the removal of phosphate by sorption on ferric oxyhydroxides is stopped because those minerals no longer form in the water column or persist during early diagenesis[39]. Crucially, the majority of phosphorus burial and recycling in the ocean (around 80% today) occurs on shallow continental shelves that comprise only a minor fraction (around 10% today) of the sea floor area. Today these shelf sea floors are largely oxygenated, but if much slower sinking of organic material in the Proterozoic ocean created anoxia (and potentially euxinia) at shallow depths – including the bottom of the shelf seas – this could have triggered a large flux of phosphorus recycling from the sediments. This in turn would have increased the concentration of phosphorus in the ocean, fuelling more new production and anoxia[40,41], in a strong positive feedback, which box models[32,41] indicate can tip the open ocean into a globally anoxic state.

Evolution of the biological pump

Shortly before the Neoproterozoic glaciations, there appears to have been a diversification of eukaryotes[42] including a rise in ecological prominence of algae, and the appearance of various protozoans and possibly fungi (Fig. 1a). The Chuar Group (~770-742 Ma) hosts vase-shaped microfossils interpreted as the remains of testate amoebae[43], together with the first substantial expression of eukaryotic biomarkers[44]. Scale microfossils[45] appear in the broadly correlative Fifteenmile Group. This is the first clear evidence of a eukaryote-dominated biological pump.

A shift from a cyanobacteria-dominated biological pump to a eukaryote-dominated one is likely to have led to faster sinking fluxes of organic carbon (Fig. 2b) through an increase in average cell size, greater propensity of such algae to form particulate aggregates, and ballasting due to tests and scales[46-48]. This need not have immediately altered total oxygen demand, which is set by the flux of limiting nutrients into surface waters and how completely they are utilised (Box 1). However, a faster 'biological pump' would have spread the same oxygen demand out over a greater depth of the water column[19], weakening peak oxygen demand, and shifting the zone of maximum oxygen demand from shallow waters to deeper waters and shelf sea sediments (Fig. 2b). If this suppressed anoxia in shelf sea bottom waters (by transferring oxygen demand from the water column to the sediments), it would have led to significantly more efficient phosphorus removal, both in organic matter[38] and adsorbed to iron oxides[39]. The phosphorus concentration of the deep ocean would then drop to a new steady state – where phosphorus sinks again balanced the source from weathering (over 10^4 yr timescales) – causing a concomitant global drop in productivity. This would lower total oxygen demand globally, reducing the volume in which it could support sulphate reduction and euxinic conditions, and thus causing a shift toward more ferruginous conditions.

This mechanism could help explain why there appears to have been a shift away from partially euxinic conditions to more purely ferruginous conditions before the Sturtian glaciation[16], despite a general trend of sulphate accumulating over Proterozoic time[37]. Notably, the Fifteenmile Group shows oxygenated surface waters and mostly ferruginous conditions below storm wave base[11], and the Chuar Group sees widespread ferruginous conditions[16,49] in a basin in contact with the open ocean. The predicted decrease in ocean phosphorus level and change in redox state should in turn have reinforced eukaryote evolution on the (more oxygenated) shelves (Fig. 2b), because it would have stopped sub-surface H_2S production which tends to inhibit (aerobic) eukaryotes.

The deepest parts of the Proterozoic ocean would likely have had a less negative balance of oxygen supply and demand than intermediate waters above them (Box 1), and those deep waters in closest contact with high-latitude surface waters through ocean overturning would be easiest to oxygenate. Hence if a global reduction in oxygen demand occurred before the Sturtian glaciation, these high-latitude deep waters may have been the first to oxygenate (or if there were already some oxygenated deep ocean waters[15] they would have expanded in volume) (Fig. 2b). Further along the path of the thermohaline circulation, deep waters would have been progressively harder to oxygenate. The most stubbornly anoxic waters would have been at intermediate depths beneath upwelling zones with high nutrient supplies to the surface (i.e. those locations that go anoxic today).

A global decrease in nutrients and new production would have tended to reduce organic carbon burial, but at the same time more efficient transport of organic carbon to depth would have tended to increase it, making the sign of the net effect uncertain. The carbon isotope record of carbonates ($\delta^{13}C_{carb}$) shows relatively stable positive values of around +5‰ through most of the early Neoproterozoic[50] (Fig. 1c). This suggests the proportion of carbon being buried in organic form then was higher than today, consistent with a more efficient biological pump. However, the strontium isotope ($^{87}Sr/^{86}Sr$) record (Fig. 1d) suggests much lower rates of continental erosion at the time, and therefore lower absolute burial fluxes. Hence the burial flux of organic carbon is inferred to have been less than today[51], with no significant changes until much later in the Neoproterozoic[51]. Thus, there seems to be no clear organic carbon burial driver for rising atmospheric oxygen in the early Neoproterozoic.

The shift to a more efficient biological pump before the Cryogenian glaciations has also been proposed as a mechanism to lower atmospheric CO_2 and cool the climate[48]. The argument is that it promoted sulphate reduction, thus making the ocean less acidic and lowering atmospheric CO_2, as long as H_2S was removed by reaction with Fe to form pyrite (FeS_2). However, we argue that spreading oxygen demand over a greater depth of the water column does the opposite of promoting euxinia. The proposal[48] is also at odds with widespread ferruginous ocean conditions at the time[11,16,49] (Fig. 1b).

Weathering into glaciations

The favoured mechanism for climate cooling in the Neoproterozoic is CO_2 drawdown caused by an increase in chemical weathering rate (or weatherability) of the land surface[52-54]. Chemical weathering also affects the oxygenation state of the planet, via the liberation of reactive phosphorus from rocks, which ultimately determines the flux of organic carbon that can be buried in the ocean, and thus the long-term source of atmospheric oxygen. Hence it has been suggested that an increase in bulk silicate weathering rate (continental weatherability) could have caused both global cooling and a rise in atmospheric oxygen in the Neoproterozoic[53]. However, there is no evidence as yet for a significant rise in atmosphere or ocean[16] oxygen prior to the Cryogenian glaciations. This can be explained by the fact that increases in the *flux* of silicate weathering cannot generally be sustained on geologic timescales without a corresponding increase in the supply flux of CO_2 from degassing processes (Box 2). Thus, if an increase in continental weatherability caused CO_2 drawdown and cooling into the Cryogenian glaciations[52-54], this need not have caused a rise in atmospheric oxygen (Box 2).

Early modelling work[52] showed that continental weatherability must have increased by a factor of 3-4 in the early Cryogenian to produce the cooling necessary to account for glaciations. Evolution of the terrestrial biosphere was suggested as a cause[52], but as yet there is no compelling evidence for such developments in the Neoproterozoic. Instead, geological drivers of increased weatherability may have been sufficient to cause Cryogenian glaciations. Widespread rifting of the supercontinent Rodinia began around 825 Ma (or possibly earlier) and produced smaller land masses that ended up in equatorial latitudes. This alone would have tended to increase the weatherability of the land surface, by making it wetter and placing it in the warmest locations. The resulting drawdown of atmospheric CO_2 and cooling is estimated to have been large, but insufficient to have triggered global glaciations[54]. Hence an additional causative factor has been invoked[54]: the emplacement of highly weatherable large basaltic provinces from 825 Ma onwards, based on the idea that a series of mantle super-plumes broke up Rodinia[50,55]. If a large basaltic province erupted in a moist tropical location (or was moved into the tropics by continental drift), it may have been sufficient to trigger extreme glaciations[54]. The Franklin Large Igneous Province is a good candidate, dated[56] at 716.5 Ma around the inception of the Sturtian glaciations – and an outpouring of readily weatherable volcanic rocks is consistent with a decrease in seawater $^{87}Sr/^{86}Sr$ prior to the Sturtian[50,57] (Fig. 1d).

Biological and geological mechanisms for enhancing continental weatherability are complementary, such that any life already on land would accelerate the weathering of basalt provinces. Emplacement of basalt provinces would be expected to lead to some temporary pulses of phosphorus to the ocean as they began to weather, in turn driving intermediate ocean waters toward euxinic conditions. Consistent with this, the upper part of the Chuar Group (>742 Ma) shows a possible short-lived shift from ferruginous to euxinic water conditions[16,49] (Fig. 1b). Changes in molybdenum cycling suggest that there may have been enough sulphidic water (1-4% of the ocean) to remove Mo from the oceans[58], consistent with a short-lived, global pulse of phosphorus weathering[59].

Animal origins and the effects of filter-feeding

Whatever drove the climate to cool culminated in a long and severe interval of glaciations, including the Sturtian and Marinoan postulated 'snowball Earth' events. These glaciations have long been thought to pose a major barrier to the survival – let alone evolution – of eukaryotic life. Yet the pre-glacial fossil record[42] – including red algae, green algae, possible fungi, lobose and euglyphid amoebae – indicates that numerous eukaryote lineages continued through the glaciations (Fig. 1a). Perhaps the glaciations themselves or the refugia for life within them were not so harsh as has sometimes been assumed. After all, extreme cold environments today can harbour diverse prokaryote and eukaryote communities, including animals[60]. Still, compilations of eukaryotic diversity over time[61] do show a significant drop in diversity during the interval of glaciations (which may be a sampling issue), and a major turnover in the taxonomic composition of pre- and post-glacial biotas.

More controversially, several recent studies argue for the presence of simple animal life in the form of sponges during and indeed before the interval of glaciations[21,62,63]. The last common ancestor of all extant animals is estimated from molecular clocks[21] to have lived around 800 Ma, prior to the Cryogenian glaciations, with stem group sponges splitting from the rest of the Metazoa roughly 780

Ma and crown group sponges arising roughly 700 Ma. There is argued to be body-fossil evidence of sponges prior to the Marinoan glaciation[62] and steroid biomarkers for sponges[63] before the end of the Marinoan glaciation (at 635 Ma) – but these claims require further assessment[64]. The apparent consistency with molecular clock estimates[21] involves some circular reasoning[64] in that the calibration of the molecular clock assumes biomarker evidence[63] of sponges at 713 Ma.

If sponges were present during the Cryogenian, this would undermine the argument that the appearance of the first metazoans was a response to increasing ocean oxygenation. Between the Cryogenian glaciations there is evidence for persistently ferruginous deep ocean conditions (Fig. 1b), with no clear shifts or trends in deep ocean redox state, and an ongoing mixture of oxygenated and anoxic outer shelf seas[16]. Marginally oxic shelf sea floors would not have prohibited the presence of sponges as some are well adapted[11] to low O_2 (~0.1 mL/L), and actively generate an internal water current through their aquiferous system – a unique mechanism adapted for both feeding and breathing (turbulent-flow gas exchange)[65].

Whenever sponges arose, their filter-feeding activity could have contributed to the ventilation of shelf seas[66] (Fig. 2c). Sponges have a capacity to clear the water column of organic material[67], especially the smallest picoplankton[68] and dissolved organic carbon[69] (DOC), thus transferring oxygen demand from the water column to the sediment interface. The clearing of turbid cyanobacteria-dominated conditions allows light to penetrate deeper in the water column, supporting a shift to eukaryote algae[47]. More importantly, the preferential removal of picoplankton by sponges presents a powerful selective pressure for larger-sized eukaryotic phytoplankton. This leads to more efficient export production, further reducing oxygen demand in shallow waters and transferring it to the sediments and deeper waters. In addition, filter-feeding provided a new pathway of phosphorus removal to sediments (which would also be enhanced by faster sinking algae), thus lowering ocean phosphate concentration and global oxygen demand. The resulting oxygenation would have facilitated the spread and proliferation of higher oxygen-demand Eumetazoa.

Deep ocean oxygenation and its causes

The first suggested evidence for more widespread oxygenation of oceanic waters comes a few million years after the Marinoan glaciation[5]. A plausible mechanism is that glaciation left an easily-weathered, finely-ground rock surface, which in the warm, wet aftermath of glaciation produced a pulse of phosphorus weathering to the ocean[34], consistent with massive phosphorite deposits at the time[70]. Initially, increased phosphorus input to the ocean would have increased new production, oxygen demand and anoxia (10^4 year timescale). Then it would have tended to increase organic carbon burial and atmospheric O_2 (10^6 year timescale), consistent with elevated marine sulphate concentrations[71]. However, the signal [5] of ocean oxygenation (increased Mo, V and U) is short-lived. Hence anoxic and ferruginous conditions may soon have returned to their previous extent, consistent with other analyses[16,71,72] (Fig. 1b). This fits with mechanistic reasoning (Box 2) that once excess CO_2 was removed from the surface Earth system and the silicate weathering flux again balanced degassing, no net increase in phosphorus weathering flux could be sustained – thus causing a return to the original atmosphere and ocean oxygenation state.

An alternative interpretation[5] of the redox proxies is that there was some persistent, widespread oxygenation after the Marinoan, and that local changes explain the drop in trace metal abundances. This might be explained if in the high CO_2 aftermath of a snowball Earth, silicate weathering was limited by the supply of new rock[73]. Then elevated phosphorus weathering fluxes – and with them an increase in atmospheric O_2 – could have been sustained for tens of millions of years[73]. However, the balance of evidence is against persistent, widespread oxygenation after the Marinoan, as deep ocean basins record anoxic and ferruginous conditions[16] leading up to the Gaskiers glaciations (635-580 Ma) (Fig. 1b). Shallow shelves and sometimes outer shelves were oxygenated at this time[16] consistent with the appearance of macroscopic, differentiated eukaryotes upon them[74] (Fig. 1a), but these environments had been oxygenated for long beforehand. The iconic fossils from the Doushantuo Formation (635-551 Ma), including putative animal embryos, come from relatively shallow oxygenated water near a ferruginous deep basin[16,72] with euxinic conditions at intermediate depth[72] (Fig. 1b).

The first compelling evidence for widespread deep ocean oxygenation is found after the Gaskiers glaciation[4,16] (580 Ma), and persisted for tens of millions of years – although other parts of the deep ocean still remained anoxic and ferruginous well into the Palaeozoic (Fig. 1b). Increased nutrient loading to the ocean in the glacial aftermath, fuelling organic carbon burial and a rise in atmospheric oxygen has been invoked to explain persistent ocean oxygenation[4]. However, the Gaskiers is widely thought to have been a 'normal' glaciation and therefore one would not expect especially high CO_2 and temperature driving a large pulse of phosphorus weathering in its aftermath. Instead it is instructive to consider alternative explanations for persistent and widespread (if not global) deep ocean oxygenation.

One option is that there was (finally) a rise in atmospheric oxygen at this time, but not due to glaciation. To generate a sustained increase in phosphorus weathering (and hence atmospheric oxygen) either requires a mechanism to sustain an increase in silicate weathering flux on geologic timescales, or a mechanism to preferentially increase phosphorus weathering relative to bulk silicate weathering (Box 2). Land colonisation driving a shift from seafloor to continental weathering could have allowed a sustained increase in silicate weathering flux and is consistent with increasing seawater $^{87}Sr/^{86}Sr$ both before and after the Gaskiers[57] (Fig. 1d). Life on land would also be expected to preferentially increase phosphorus weathering relative to bulk silicate weathering in pursuit of essential nutrients[53], leading to increased phosphorus burial in the ocean (Box 2), consistent with major phosphorite deposits at the time[70]. However, there is as yet no direct evidence for a phase of land colonisation around the Gaskiers, and rising seawater ^{87}Sr/^{86}Sr can arguably be better explained by changes in the isotopic composition of material undergoing weathering.

An alternative explanation is that the appearance of Eumetazoa triggered co-evolutionary feedbacks within the ocean that oxygenated it, without any change in atmospheric oxygen[47]. Cnidarians have an estimated[21] crown group origin around 700 Ma, and probably had benthic ancestral forms. Both bottom-dwelling soft corals[75] and cnidarian medusa[76] are capable of pumping water for active ventilation and suspension feeding. Together with sponges, these could have augmented the removal of DOC and smaller phytoplankton from the water column, tending to oxygenate it (Fig. 2c).

In addition, the first zooplankton – possessing both guts and the capacity to swim – would have introduced a powerful new clearing agent into the oceans, further oxygenating the water column and triggering a shift from cyanobacteria toward eukaryote dominance of the surface ocean[2] (Fig. 2d). Whenever the first jelly-forms appeared in the plankton – either ctenophore 'comb jellies' or cnidarian jellyfish – they could have increased the efficiency of the biological pump, as jelly biomass can sink very rapidly[46]. The seminal 'oxygen happens' hypothesis[19] suggested that the key event oxygenating the water column was when the first bilaterian zooplankton evolved, producing rapidly sinking faecal pellets[46]. However, meso-zooplankton appear to post-date the Gaskiers aftermath by tens of millions of years.

Whether the precise mechanism involved benthic filter-feeding or planktonic grazing, the evolution of Eumetazoa should have shifted oxygen demand to shelf-sea sediments and to deeper waters in the open ocean. This would have reduced total phosphorus recycling from sediments, thus reinforcing through positive feedback the shift to a more oxygenated ocean state (Fig. 2d). The resulting changes could have fed back to facilitate the rise of more mobile and predatory animal forms[77]. The efficiency of organic carbon burial should have increased and this would have counteracted an overall reduction in organic carbon production due to reduced nutrients. An overall increase in organic carbon burial[51] might be consistent with a positive excursion in the $\delta^{13}C_{carb}$ record

(Fig. 1c) and increased continental erosion indicated in the ^{87}Sr/^{86}Sr record[57] (Fig. 1d), in the aftermath of the Gaskiers glaciations.

The ensuing 'Shuram' extreme negative $\delta^{13}C_{carb}$ anomaly, ending[78] 551 Ma (Fig. 1c), with its decoupled $\delta^{13}C_{org}$ variations, is unlikely to have been triggered by the onset of filter feeding or planktonic grazing oxidising a massive pool of dissolved organic carbon that had accumulated in the ocean[79], because this would have rapidly drained the atmosphere of oxygen[80]. The $\delta^{13}C_{carb}$ anomaly has been interpreted as due to burial diagenesis[81], requiring a mechanism for globally synchronous alteration[82], such as a flux of isotopically light terrestrial organic material from land colonisation[83]. However, a lack of co-variation between $\delta^{13}C_{carb}$ and $\delta^{18}O_{carb}$ rules out a diagenetic origin for the anomaly[84]. Self-sustaining, massive release of methane from hydrates might explain it without draining the atmosphere of oxygen[85], but at this stage we leave the Shuram as an ongoing puzzle – reviewed elsewhere[82].

Testing models for deep ocean oxygenation

The timing of key evolutionary events and geochemical changes in the Neoproterozoic remain uncertain. We have reviewed two main groups of hypotheses: those involving changes in weathering affecting phosphorus input to the ocean (hence organic carbon burial and atmospheric oxygen); and those involving evolutionary innovation affecting phosphorus removal/recycling within the ocean (hence oxygen demand and ocean oxygenation state). Both sets of hypotheses could be better quantified in more detailed models, and potentially falsified with new data.

Three-dimensional ocean models can be used to establish what combinations of atmospheric oxygen concentration, marine phosphorus concentration, and remineralisation profile with depth can reproduce available proxy data for ocean redox state at different times. By opening the phosphorus cycle in such models – i.e. simulating inputs to the ocean, and interactive removal processes – they could then be used to examine whether changes in the biological pump can indeed change ocean oxygenation state, and by how much. They could also be used to examine the effect of changing the input of phosphorus from weathering on ocean redox state, and identify which observations can distinguish between the different hypotheses.

Hypothesised changes in silicate weathering fluxes could be tested with new lithium and osmium isotope proxies, and a proxy for phosphorus weathering could also be sought. A sequential extraction method for phosphorus in marine sediments could be used to test for predicted changes in phosphorus removal/recycling with depth, and this could be complemented by assessment of changes in the depth (and/or form) of phosphorite deposition. Finally, predictions for changes in organic carbon burial could be tested against the δ^{13}C record, and fed into more traditional box models to estimate the effects on atmospheric oxygen over multi-million year timescales.

Such tests would test our argument that the evolution of complex eukaryotes could have caused ocean ventilation. Instead, ocean oxygenation did not require a rise in atmospheric oxygen and its net effect on atmospheric oxygen is uncertain. Furthermore, we argue that oxygen was not a key culprit in the origin of animals[11] (alternative explanations for which are explored in Box 3). Instead, early animals may have played a key role in the widespread (though not universal) Ediacaran oxygenation of the deep oceans, which in turn may have facilitated the advent of many subsequent animal traits including mobility and carnivory[77].

The arrival of bilaterians and the evolution of a through-gut marked the beginning of an essentially modern marine biosphere, with structurally familiar food webs[2]. This had several profound impacts on the Earth system. In particular, mobile animals began to mix and oxygenate the sediments (bioturbation). There is evidence for locomotion on the seafloor[86] at ~565 Ma and for feeding on the sediment surface[87] >555 Ma, with 3-dimensional burrowing appearing before the base of the

Cambrian[88]. Oxygenation of the upper sediments by bioturbation would have added sulphate to the ocean[89], lessening the role of methanogenesis in organic decomposition, but crucially would also have removed phosphorus[90]. The resulting reduction in phosphate concentration and organic carbon burial would ultimately have caused a decrease in atmospheric O_2 (Boyle, R.A. *et al.*, Evolution of bioturbation stabilised atmospheric oxygen. Submitted). These predicted effects of bioturbation could help explain the return of widespread anoxic and sometimes euxinic conditions in the early-mid Paleozoic ocean. Thus, animal evolution, far from being a response to rising atmospheric oxygen might actually have sent oxygen in the opposite direction.

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T.M.L. wrote the paper with input from all co-authors.

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Figure legends

Figure 1. Timeline of biological and environmental change spanning the Cryogenian, Ediacaran and Cambrian periods. a, Compilation of biological evidence, updated from refs. [42,61]: key animal traits (red dots); estimated contributions to marine export production inferred from biomarker and microfossil data (blue diamond marks first substantial occurrence of eukaryotic biomarkers[44]); first appearance of key eukaryotic phenomena, traits and clades (dashed lines indicate more debatable evidence). **b**, Summary of ocean redox conditions at different depths, updated from ref. [16] (question mark indicates possible intervals of intermediate-water euxinia). **c**, Secular variation in the carbon isotope composition of marine carbonates. **d**, Strontium isotope composition of seawater. Figure adapted from ref. [57].

Figure 2. Key steps and feedbacks in the co-evolution of eukaryotes and ocean redox state. Schematic diagrams of a generic shelf sea and upwelling ocean margin, showing redox state (O_2 = oxygenated, Fe^{2+} = anoxic ferruginous, H_2S = anoxic euxinic), key interactions (solid arrows indicate direct relationships, dashed arrows indicate inverse relationships), and sinking rates. Four stages are depicted: **a**, Background Proterozoic ocean state. **b**, Onset of eukaryotic algae. **c**, Onset of benthic filter-feeding animals . **d**, Onset of swimming animals with guts. A key positive feedback is that anoxia supports phosphorus recycling from sediments, increasing productivity and oxygen demand, whereas oxygenation of bottom waters increases phosphorus removal into sediments, decreasing productivity and oxygen demand.

Box 1 – Controls on ocean oxygenation state

Oxygen concentration in a water parcel below the depth of wind-driven mixing depends on the balance of oxygen supply and demand fluxes. Oxygen supply flux is determined by the concentration of oxygen in the surface waters that exchange with this location (in turn equilibrated with the atmosphere above), the rate of mixing or advection of oxygen to the parcel at depth, and any removal of oxygen on the way there (by remineralisation). The oxygen demand flux in the water parcel is set by the supply flux of limiting nutrient to the surface photic zone above, the fraction of this which goes into export production, the stoichiometry of oxygen demand to nutrient in the resulting sinking organic material, and the rate of sinking of the organic matter through the water parcel (determining the amount of remineralisation that occurs within it). If oxygen is exhausted in a water parcel, bacterial respiration will preferentially use the electron acceptor that gives the highest free energy yield per unit of organic carbon, following a well established hierarchy; NO_3, Fe^{3+}, SO_4, methanogenesis. Phosphorus is the ultimate limiting nutrient determining oxygen demand over long timescales, even though nitrogen is often proximately limiting (i.e. runs out first), as long as biological nitrogen fixation tends to counteract any deficit of nitrogen due to denitrification.

In a simple 3-box ocean model[91], the water parcel at depth becomes the entire deep ocean (d) and warm, low-latitude surface waters (l) where nutrients are fully utilised are distinguished from cold, high-latitude surface waters (h) where nutrients are incompletely utilised but oxygen enters to ventilate the deep ocean. All sinking material is assumed to be remineralised in the water column, leading to a relationship for oxygen concentration in the deep ocean[33,91]:

$$O_2(d) = O_2(h) - r[P(d) - P(h)]$$

Where $O_2(h)$ (~ 325 μM today) is the oxygen concentration in high-latitude surface waters where deep water formation occurs, $[P(d) - P(h)]$ (= 0.91 μM today) represents the amount of phosphorus in upwelling deep water that is used to generate export production in high-latitude surface waters , and $r = 170$ is the Redfield ratio of O_2 consumption to phosphate production during remineralisation.

Using these values, the deep ocean goes anoxic, O_2(d) = 0, when O_2(h) and therefore atmospheric O_2 ≈ 0.5 PAL. However, even under today's atmospheric O_2 = 1 PAL, if all the upwelling P(d) = 2.15 μM were utilised (i.e. P(h) = 0) the deep ocean would go anoxic.

The 3-box model[91] shows no dependence of O_2(d) on either thermohaline circulation ($d \rightarrow l \rightarrow h \rightarrow d$) or high-latitude overturning ($d \leftrightarrow h$), nor can it explore the effect of changes in remineralisation depth. However, a 4-box model[92] which distinguishes intermediate-depth waters, shows that intermediate and deep water O_2 are sensitive to all of these factors. Deep waters generally have higher O_2 (hence are harder to turn anoxic) than intermediate waters because (i) thermohaline and overturning circulations ventilate them first, and (ii) more remineralisation happens per unit volume in the shallower intermediate waters. A multi-box model[32] captures these effects and also incorporates positive feedback between bottom water anoxia and phosphorus recycling from sediments. This creates a threshold at O_2 ≈ 0.7 PAL, when anoxic waters expand vertically onto the bottom of the continental shelves and phosphorus recycling from shelf sea sediments tips the deep ocean into a globally anoxic state[32].

Box 2 Weathering controls on carbon dioxide, phosphorus and oxygen

To illustrate the controls on carbon dioxide in the atmosphere and ocean (*C*), phosphorus in the ocean (*P*), and oxygen in the atmosphere (O_2), we consider a simplified system of these three coupled reservoirs. This extends previous work on the Neoproterozoic[53] to include a crucial constraint from balancing the CO_2 cycle. CO_2 is supplied to the atmosphere-ocean by degassing (*D*) and removed by silicate weathering (where $F_C \approx 7\times10^{12}$ mol yr^{-1} and $C \approx 3.2\times10^{12}$ mol, giving a residence time of $\tau_C \approx 5\times10^5$ yr today). Silicate weathering depends on uplift (*U*), any changes in weatherability (*W*) – due to biotic amplification of weathering, location of the continents or outpouring of large igneous provinces – and is an increasing function of CO_2, *f*(*C*) – directly and via the effect of CO_2 on temperature (which is implicit here):

$$\frac{dC}{dt} = F_C[D - UWf(C)]$$

At steady state, which is achieved on a $\tau_C \approx 5\times10^5$ yr timescale, the silicate weathering flux must balance the degassing flux; $UWf(C) = D$. Therefore any changes in uplift (*U*) or weatherability (*W*) are counteracted by changes in *C* on this timescale, and only changes in degassing (*D*) alter the absolute flux of silicate weathering. Crucially, bulk silicate weathering is also the key source of phosphorus to the ocean (where $F_P \approx 3.6\times10^{10}$ mol yr^{-1} and $P \approx 3.1\times10^{15}$ mol, giving a residence time of $\tau_P \approx 9\times10^4$ yr today). We include the biological possibility of selective weathering of phosphorus relative to bulk silicate rock as a factor, *S*. Phosphorus is removed from the ocean by burial, which is an increasing function of phosphorus concentration, *b*(*P*):

$$\frac{dP}{dt} = F_P[SUWf(C) - b(P)]$$

Steady state for the phosphorus cycle is achieved faster than CO_2 on a $\tau_P \approx 9\times10^4$ yr timescale, with burial balancing inputs from weathering; $b(P) = SUWf(C)$. Therefore transient changes in weathering inputs will lead to transient changes in *P*. However, on the $\tau_C \approx 5\times10^5$ yr timescale of CO_2 steady state, silicate weathering flux is set by degassing, therefore $b(P) = SD$ and only selective weathering of phosphorus by biology can decouple phosphorus burial from degassing. This in turn has implications for atmospheric oxygen, because oxygen is supplied to the atmosphere by organic carbon burial, which depends on phosphorus burial and on the C/P burial ratio, which is a declining function of oxygen concentration[38,40], $r(O_2)$. Oxygen is removed by oxidative weathering which here depends only on the supply of new rock via uplift (because it only becomes sensitive to O_2 at very low concentrations):

$$\frac{dO_2}{dt} = F_O[b(P)r(O_2) - U]$$

Today $F_O \approx 7.8\times10^{12}$ mol yr^{-1} and $O_2 \approx 3.7\times10^{19}$ mol, giving a residence time of $\tau_O \approx 5\times10^6$ yr much longer than that of CO_2, however if O_2~0.1 PAL in the early Neoproterozoic, it could have had a comparable residence time to CO_2 and they may have reached steady state together. Either way, at steady state $SDr(O_2) = U$ and only changes in uplift, degassing or selective weathering of phosphorus by biology could have driven changes in atmospheric oxygen. Therefore, increases in the overall weatherability of the land surface (*W*) either due to biology, tropical location of the continents, or the outpouring of large igneous provinces should not have altered atmospheric oxygen.

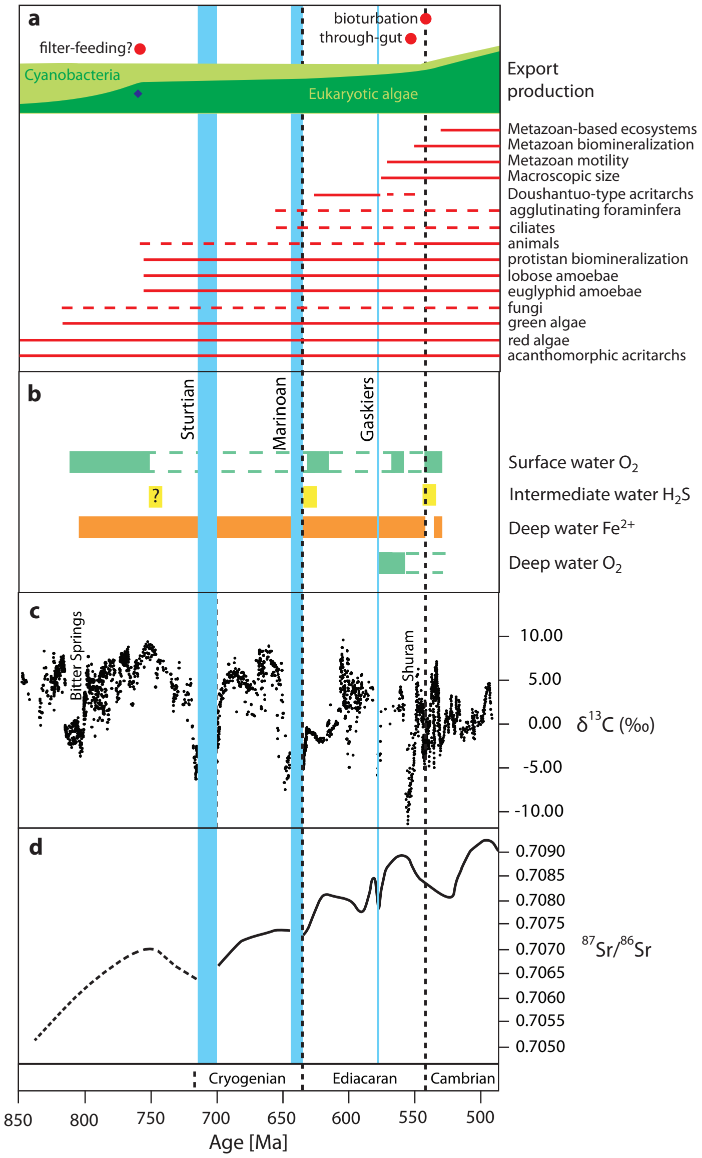
Box 3 – Mechanisms for the evolution of Eumetazoa

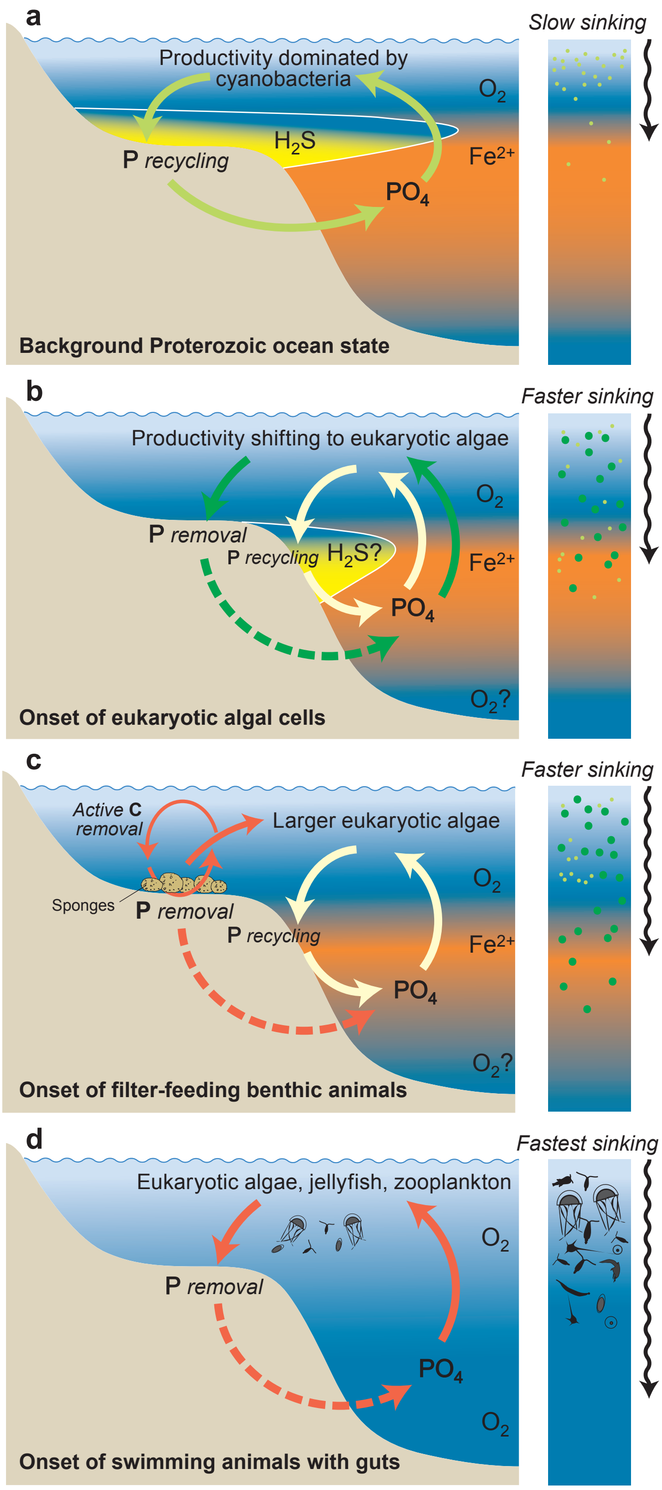
Given that oxygen availability was not a significant constraint on animal evolution[11,47], what else can explain the delayed and dramatic appearance of this singularly influential clade? A null hypothesis is that there was no external impetus for animal proliferation. It simply took a long time to evolve the uniquely complex gene regulatory networks necessary to build tissue- and organ-grade animals[47]. However, the developmental evolution required involves significant changes in cellular differentiation, and an alternative perspective is that exceptional selection pressures were necessary to explain the origin of Eumetazoa.

Animal development requires the evolution of altruistic somatic cells with irreversible differentiation, which forgo their own long-term reproduction to facilitate that of separate reproductive cells. This contrasts with plants and fungi where most somatic cells are capable of de-differentiation and independent replication. A key breakthrough was likely the evolution of a cellular differentiation hierarchy[93,94] between a proto-germline retaining infinite division potential, the reproduction of which is facilitated by differentiation in other somatic cell lineages. This required multi-level selection for conflict mediation[95], whereby the opportunity for independent cell division by non-reproductive cells is restricted (e.g. by limiting the number of somatic cell divisions) when the evolutionary interests of the cell and the organism conflict. This in turn was facilitated by sophisticated gene regulatory circuitry[94] with conflict modifier loci[95], probably involving a juvenile larval stage[94], and utilization of ancestral[96] cellular adhesion functions.

Kin selection[97] gives a mechanism by which somatic cells can evolve to not reproduce – if the soma and germline share genetic material. This will happen if both the germline and the soma are descended from an obligate single cell bottleneck and are thus closely genetically related. But for a single cell bottleneck to consistently occur in an ordered manner, a tightly controlled gene regulation program is necessary in the first place – requiring altruistic somatic cells that obey developmental instructions to facilitate the bottleneck. This ‘catch 22’ situation is the essence of the problem of animal origins.

To explain the altruistic origins of Eumetazoa, a direct causal link with extreme glaciations has been hypothesised[98]: In the kind of localised refugia[60] where eukaryotic cells likely survived Neoproterozoic glaciations, fluctuations in temperature, water potential and resource availability led to extreme and repeated founder effects, raising genetic relatedness and promoting kin selection for inter-cellular cooperation. Founder effects and restricted dispersal has been linked to the evolution of altruism both theoretically[99] and empirically[100]. High genetic relatedness (and low probability of successful independent reproduction) lifted a constraint on the evolution of an altruistic soma for the first time in Earth's history. This permitted the evolution, by kin selection, of an obligate separation between undifferentiated reproductive cells and a terminally differentiated soma. Once this type of development had become “locked in”, the increased degree of specialisation permitted by terminal differentiation allowed the explosive exploration of form seen in Ediacaran (and Cambrian) fossils.


a
filter-feeding?
bioturbation
through-gut
Cyanobacteria
Eukaryotic algae
Export production
Metazoan-based ecosystems
Metazoan biomineralization
Metazoan motility
Macroscopic size
Doushantuo-type acritarchs
agglutinating foraminfera
ciliates
animals
protistan biomineralization
lobose amoebae
euglyphid amoebae
fungi
green algae
red algae
acanthomorphic acritarchs
b
Sturtian
Marinoan
Gaskiers
Surface water O_2
?
Intermediate water H_2S
Deep water Fe^{2+}
Deep water O_2
c
Bitter Springs
Shuram
10.00
5.00
0.00
-5.00
-10.00
$\delta^{13}C$ (‰)
d
0.7090
0.7085
0.7080
0.7075
0.7070
0.7065
0.7060
0.7055
0.7050
$^{87}Sr/^{86}Sr$
Cryogenian
Ediacaran
Cambrian
850
800
750
700
650
600
550
500
Age [Ma]


a
Slow sinking
Productivity dominated by cyanobacteria
O_2
H_2S
P *recycling*
Fe^{2+}
PO_4
Background Proterozoic ocean state
b
Faster sinking
Productivity shifting to eukaryotic algae
O_2
P *removal*
P *recycling*
H_2S?
Fe^{2+}
PO_4
O_2?
Onset of eukaryotic algal cells
c
Faster sinking
Active **C** *removal*
Larger eukaryotic algae
O_2
Sponges
P *removal*
P *recycling*
Fe^{2+}
PO_4
O_2?
Onset of filter-feeding benthic animals
d
Fastest sinking
Eukaryotic algae, jellyfish, zooplankton
O_2
P *removal*
PO_4
O_2
Onset of swimming animals with guts