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Oxygen, temperature and the deep-marine stenothermal cradle of Ediacaran evolution

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Ediacaran fossils document the early evolution of complex megascopic life, contemporaneous with geochemical evidence for widespread marine anoxia. These data suggest early animals experienced frequent hypoxia. Research has thus focused on the concentration of molecular oxygen (O_2) required by early animals, while also considering the impacts of climate. One model, the Cold Cradle hypothesis, proposed the Ediacaran biota originated in cold, shallow-water environments owing to increased O_2 solubility. First, we demonstrate using principles of gas exchange that temperature does have a critical role in governing the bioavailability of O_2—but in cooler water the supply of O_2 is actually lower. Second, the fossil record suggests the Ediacara biota initially occur approximately 571 Ma in deep-water facies, before appearing in shelf environments approximately 555 Ma. We propose an ecophysiological underpinning for this pattern. By combining oceanographic data with new respirometry experiments we show that in the shallow mixed layer where seasonal temperatures fluctuate widely, thermal and partial pressure (pO_2) effects are highly synergistic. The result is that temperature change away from species-specific optima impairs tolerance to low pO_2. We hypothesize that deep and particularly stenothermal (narrow temperature range) environments in the Ediacaran ocean were a physiological refuge from the synergistic effects of temperature and low pO_2.

1. Introduction

The role of marine oxygenation as it pertains to early animal evolution is a fundamental question in deep Earth history. Interdisciplinary research spanning palaeontology, geochemistry, and molecular biology increasingly tie changes in Earth's surface environment to the emergence and subsequent radiation of animals across the Neoproterozoic-early Palaeozoic transition approximately 800–500 million years ago (Ma). This interval is marked by evidence for extreme climate fluctuations and biogeochemical perturbations, including two long-lasting glaciation events (i.e. snowball earth glaciations) during the Cryogenian Period *ca* 720–635 Ma [1], and extremely positive carbonate carbon isotope records punctuated by negative excursions [2]. The first large, morphologically complex fossils do not appear in the fossil record until the Ediacaran Period *ca* 635–541 Ma [3]. Multi-proxy geochemical evidence suggests early animal evolution occurred against a backdrop of widespread marine subsurface anoxia [4–6]. While the absolute amount of oxygen (O_2) as a percentage of present atmospheric levels (PAL) through the Neoproterozoic are contentious, researchers have traditionally considered levels of 1–10% PAL to be most likely [7,8]. During the Ediacaran and early Cambrian, redox sensitive trace metal enrichments suggest transient oxygenation events during that time [9,10]. However, regional and stratigraphic inconsistencies in the pattern of these enrichments and several other lines of evidence indicate any oxygenation must have been relatively muted or short-lived [5,6,11–14]. There is therefore emerging consensus that early animals encountered persistent and severely

low O_2 partial pressures (pO_2), which would have had profound effects on the spatial distribution of metabolically-viable habitats [15].

For decades, these low levels of marine O_2 have been opined [16–19], albeit controversially [20], as the environmental barrier to early animal evolution. However, given the striking climatic fluctuations of the Neoproterozoic, O_2 is not the only environmental influence that has been considered. The stratigraphic occurrence of highly diverse, shallow-water shelfal 'White Sea' fossil assemblages *ca* 560–550 Ma [3] in exclusively siliciclastic sediments has been viewed as an indication of habitation in cold-water environments (at latitudes above low-latitude carbonate belts) [21]. To the extent that the Ediacara biota represent animals, this 'Cold Cradle' model of evolution posited that the greater gas solubility of O_2, as well as the sluggish remineralization of nutrients by prokaryotes in cold, shallow, high-latitude waters allowed metazoan ecosystems to flourish in the later Ediacaran.

Detailed palaeontological and geochronological studies now indicate that the oldest non-algal megascopic fossil assemblage is not the White Sea assemblage, but rather is the so-called 'Avalon assemblage' [22–25]. This community is primarily dominated by morphologically complex soft-bodied benthic frondose fossils belonging to two recognized groups, the Arboreomorpha and Rangeomorpha. While there are phylogenetic issues with assigning Ediacaran fronds to the Metazoa ([26], but see [27]), Avalonian assemblages also contain other fossils more likely to be animals, including sponges [28] and body- and trace-fossil evidence for eumetazoan cnidarians [23]. These fossils appear in the middle Ediacaran *ca* 571 Ma in deep-water, aphotic slope and basinal facies [22–24,29], where they are found *in situ*, buried by ash beds or rapidly deposited sediments. The inferred deep-water depositional environment is supported by expansive, kilometre-scale stratigraphic sections of uninterrupted turbidites displaying thick $T_{C\text{-}E}$ and $T_{D\text{-}E}$ Bouma subdivisions, contour-parallel bottom currents, and no evidence for wave-generated sedimentary structures [30]. These stratigraphically oldest deep-water, morphologically complex fossils are found in multiple sedimentary basins worldwide including England, Newfoundland, and the Mackenzie and Wernecke Mountains of northwestern Canada. By contrast, Ediacara biota are absent from shallow-water environments on the shelf until approximately 560–555 Ma, often after the globally recognized Shuram carbon isotope excursion [31–33].

The Ediacaran fossil record therefore displays a puzzling pattern. For approximately 15 Myr large, morphologically complex eukaryotes and animals only inhabited deep-water settings. This observation is at odds with palaeontological meta-analyses which show that onshore to offshore macroevolutionary patterns predominate across multiple intervals in the later Phanerozoic (541 Ma-present) fossil record [34]. Might deep-water settings have provided a kind of physiological refugia for early metazoans in a generally low pO_2 global ocean? Given that both deep- and high-latitude water masses are colder than shallow-water counterparts at low-latitudes, what ecophysiological or oceanographic differences exist between the two environments, and how do they fit within the context of the Cold Cradle hypothesis? Lastly, as Ediacaran communities did eventually radiate onto the shelf to inhabit shallow-water environments, what stressors might these ecosystems have faced? To shed light on these questions, we apply an oxygen supply index (OSI, table 1) to determine how temperature can govern O_2 supply to animals at oceanographic scales. We then present new experimental respirometry data that illustrate how temperature dynamically affects the absolute pO_2 tolerance of marine ectotherms. Lastly, we integrate these two approaches to re-examine bathymetric patterns within the Ediacaran fossil record in an ecophysiological context.

2. Background and previous work

(a) Oxygen bioavailability in aquatic settings

The challenges of aquatic respiratory gas exchange are well known. In water at standard temperature and pressure there is approximately 30 times less O_2 than in the atmosphere by concentration, and O_2 diffuses approximately 2.4×10^5 times slower through water than air [35]. Furthermore, both temperature and salinity independently impact the solubility of O_2 in water owing to their effects on the Henry gas coefficient. This results in seawater containing on average 25% less O_2 than freshwater at a given temperature [36]. Owing to these physical constraints, limitations in environmental O_2 supply have profound physiological impacts on aquatic animals in the modern ocean.

Respiratory O_2 exchange cannot simply be discussed interchangeably with units of pO_2 or solubility. Instead, the product of pO_2, solubility, and diffusivity potential together represent the flux of O_2 that can be transferred from the environment into a respiring organism. This relationship inherently describes Fick's first law of diffusion, which expresses partial pressure as proportional to its concentration gradient [37]. Putting aside organism-specific differences in surface area to volume ratios, respiratory structures (e.g. gills, pigments), or differences in pumping [38], aquatic animals can only extract O_2 from the water column at an absolute rate proportional to environmental availability. This relationship has been expressed in freshwater environments as the OSI [39] (expressed here in $\mu mol\ kg^{-1}$ $matm\ m^2\ s^{-1} \times 10^{-5}$):

$$OSI \propto \alpha O_2 \cdot DO_2 \cdot pO_2, \tag{2.1}$$

where αO_2 is the solubility of O_2 in water ($mol\ m^{-3}\ Pa^{-1}$), DO_2 is the diffusivity of O_2 in water ($m^2\ s^{-1} \times 10^{-9}$), and pO_2 is the partial pressure of O_2 in water (matm).

(b) Respiration physiology and temperature

The ability to avoid hypoxia in low O_2 conditions represents a physiological balance between O_2 supply and demand. Temperature affects both sides of this equation [40], and for aquatic ectothermic animals, the inability to control body temperature results in metabolic rates which change significantly with ambient temperature. All ectotherms exhibit a Q_{10} rate coefficient in which a 10°C increase in body temperature raises metabolic rate by a factor of approximately 2–3, or about 8% per degree Celsius [41]. Despite numerous processes at the whole-organism, tissue, and enzymatic levels to partially offset this effect, these strategies are invariably not fully effective at countering the effects of temperature on metabolic rate [42]. Marine ectotherms consequently display a thermal performance curve (table 1) which often represents an organism's relative fitness across its natural environmental temperature range [43]. The thermal performance curve reflects the effects of low temperature on O_2 supply and ventilation costs, and high temperatures on

Table 1. Summary definitions of key terms.

term	abbreviation	definition
oxygen supply index	OSI	a term which measures the rate at which oxygen can transfer from the water column into an animal by integrating the partial pressure and diffusivity of oxygen within the water as well as its solubility
thermal performance curve	TPC	the thermal performance curve represents an animal's fitness due in part to thermal tolerance characteristics and temperature dependant effects on physiological and biochemical functions (e.g. fecundacy, growth, metabolic rate). Often it reflects the natural environmental temperature range of the species, that is its thermal window
thermal optimum	T_{opt}	maximum performance occurs here at the peak of the TPC, often at intermediate temperature and represents maximum aerobic scope, that is the greatest difference between MMR and SMR
standard metabolic rate	SMR	the minimum metabolic rate that supports basic maintence requiremens of an organism while at rest and fasting. In ectotherms this rate is temperature sensitive
maximum metabolic rate	MMR	the maximum metabolic rate achieved by an organism during unsustainable physical activity that is limited by aerobic capacity
oxygen- and capacity-limited thermal tolerance	OCLTT	a concept which decribes thermally-induced hypoxemia (low levels of oxygen in blood or tissues) at both ends of an animal's thermal window due to thermal effects imparted on oxygen bioavailability, metabolic demand, and ventilatory capacity to supply enough oxygen to meet these metabolic requirements
critical O_2 level	$[O_2]^{crit}$	this is the critical oxygen level below which standard metabolic rate can no longer be maintained aerobically. At this point, oxygen demand is greater than the animal's capacity to supply oxygen and anaerobic metabolic pathways begin operating

enzyme instability and metabolic demand. In respiratory physiology terms, the thermal performance curve inherently represents aerobic scope, because ATP yield from aerobic respiration is dramatically higher (approx. 15×) than any anaerobic glycolytic pathway. Scope is the instantaneous proportion of metabolic power available to an organism after its basal maintenance costs are met, and can be used to invest in growth, reproduction, predation and defence, and other fitness-related functions [44].

The co-limiting effects of temperature and low pO_2 on aerobic scope can result in the metabolic demands of an animal exceeding its capacity for O_2 supply [45]. This phenomenon is referred to as oxygen- and capacity-limited thermal tolerance (OCLTT, table 1) [46]. At its most basic level, OCLTT represents a reduction of relative aerobic scope at temperatures both above and below T_{opt} (table 1). Above T_{opt}, standard metabolic rate (the lowest rate of O_2 consumption required for maintenance, table 1) makes up a greater proportion of aerobic scope owing to Q_{10} effects (figure 1) [45]. At high enough temperatures, maximum metabolic rate (table 1) can also begin to decrease owing to heat-induced damage to biochemical systems and organ (e.g. heart) failure [47,48]. Combined, warming above T_{opt} causes a decrease in aerobic performance and lowers pO_2 tolerance, causing survivorship to greatly decline [49]. At temperatures below T_{opt}, maximum metabolic rate becomes increasingly inhibited owing to decreasing O_2 bioavailability, and reduced aerobic capacity owing to kinetic reduction in circulatory and ventilatory performance. This results in a net reduction of aerobic scope despite lower metabolic rates, thus limiting low pO_2 tolerance at the cold end of the spectrum as well (figure 1) [47,48,50]. Lastly, at both ends of the thermal performance curve, low ambient pO_2 not only drops maximum metabolic rate (causing a reduction in the range of thermal tolerance by way of reducing overall scope), but also increases the proportion of standard metabolic rate used for ventilation, as animals must exchange more water over their respiratory surfaces in order to extract the same amount of O_2 [51,52].

The OCLTT relationship can be determined for a given animal by measuring what is known as the critical O_2 concentration ($[O_2]^{crit}$, table 1), or alternatively expressed in partial pressure (pO_2^{crit}), across its natural temperature range. $[O_2]^{crit}$ represents the O_2 level below which an organism is no longer able to maintain its standard metabolic rate. At the $[O_2]^{crit}$, aerobic scope is therefore zero, and any further decrease in O_2 saturation, or change in temperature further away from T_{opt}, will result in ATP demand exceeding O_2 supply. At a metabolic level, this deficit in aerobically generated ATP triggers the onset of fermentation. This shift in metabolic pathway dramatically changes respiration rate, allowing $[O_2]^{crit}$ to be calculated using breakpoint analysis on the O_2 draw-down curve generated during closed-system experimental respirometry (electronic supplementary material, figure S9).

3. Results

(a) Oceanographic controls on oxygen supply

OSI has been calculated in freshwater environments, but not in the global ocean. To illustrate the behaviour of OSI in the global ocean for the first time to our knowledge, dissolved O_2, salinity, density, temperature, and pressure data from

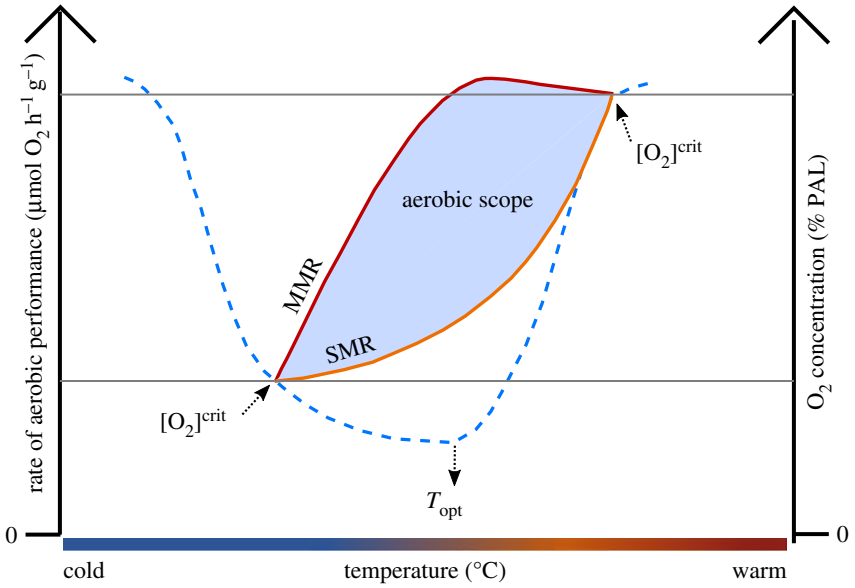

Figure 1. Conceptual model of how temperature and ambient O_2 interact to constrain aerobic performance in ectothermic animals. Aerobic scope is defined as the difference of rates of aerobic performance (left axis), specifically between maximum metabolic rate, MMR, and standard metabolic rate, SMR. Tolerance to low O_2 levels decreases at temperatures both below and above T_{opt}, as O_2 supply capacity falls relative to O_2 demand, resulting in $[O_2]^{crit}$ occurring at progressively higher ambient O_2 tensions (blue dashed line, right axis). Because T_{opt} represents maximum rate of aerobic performance (the greatest distance between MMR and SMR on the left axis), it also corresponds to the $[O_2]^{crit}$ minimum (on the right axis). As low ambient O_2 reduces aerobic scope by lowering MMR, it consequently narrows the thermal window of environmental O_2 tolerance because any temperature-related increase in SMR or reduction in MMR will consequently take up a larger proportion of aerobic scope. Figure adapted from [46].

the National Oceanic and Atmospheric Administration World Ocean Atlas (NOAA WOA13 V2) were used to calculate *in situ* pO_2, diffusivity and solubility (electronic supplementary material). Temperature and salinity independently affect solubility. It is therefore possible for solubility to change dissolved O_2 concentrations in the ocean without any adjustment in pO_2, such as across salinity or temperature gradients. Ultimately, the total amount of O_2 that is present at a given location is dependent on not just solubility, but also on atmospheric pO_2 and the saturation state of the water column (electronic supplementary material, figures S1–S3). At the sea surface, there is little variation in the pO_2 of seawater with latitude owing to the limited change in saturation water vapour pressure across the normal range of marine water temperatures combined with the near constant atmospheric pO_2 at sea level (figure 2; electronic supplementary material, figure S7; [53]). At depth though, increases in pO_2 of approximately 14% per 1000 m depth occur owing to large increases in hydrostatic pressure (electronic supplementary material, figures S4–S6 and S8; [54]). However, because pressure increases the fugacity of O_2 exponentially, it also has a reciprocal effect on the solubility coefficient in water owing to increased outgassing tendency (electronic supplementary material, figure S2a). This leads to little change in OSI past the thermocline unless the water mass is undersaturated with respect to O_2 (electronic supplementary material, figures S1d and S4d). For instance, at depths between 200–1000 m in the modern ocean, undersaturation (lower than equilibrium dissolved O_2 concentrations) driven by remineralization of organic matter plays a significant role in developing oxygen minimum zones (OMZs) on upwelling margins (electronic supplementary material, figure S6d; [55]).

Finally, the third component of OSI, diffusivity, is largely controlled by Brownian motion, as molecular O_2 exists as a dissolved gas within seawater. As such, the capacity of O_2 to diffuse through the liquid medium is heavily dependent on the ratio of temperature to density of seawater, which together govern its kinematic viscosity (also known as momentum diffusivity) [56]. Given the wide thermal ranges and saline nature of seawater, it is critical to take diffusivity into account in studies of respiration physiology. However, this is not commonly done. Within the ocean, increases in viscosity owing to colder water temperatures at higher latitudes or greater depths overcome increases in solubility on OSI (electronic supplementary material, figures S4–S7). The key point here regarding O_2 supply for respiration as determined from OSI is that cold marine waters at depth or high-latitudes in equilibrium saturation conditions have only 60–70% of the O_2 supply available in shallow low-latitude regions (figure 2; electronic supplementary material, figures S4d and S7d). The physiological implications of this novel result are immediately apparent and probably far reaching, as cold waters in the global ocean are therefore much more difficult to respire in, despite having greater O_2 solubility [39,57].

(b) Aerobic respiration and temperature

To illustrate how $[O_2]^{crit}$ varies with temperature, we conducted 86 $[O_2]^{crit}$ measurements on the intertidal anthozoan cnidarian *Diadumene lineata* (figure 3). This species of sea anemone was selected as intertidal organisms regularly encounter significant diurnal and seasonal temperature and pO_2 fluctuations [58]. Furthermore, anemones possess a diploblastic body plan and therefore rely entirely on cutaneous diffusion of O_2 into two epithelial tissue layers—the external ectoderm, and internal endoderm—for aerobic respiration. These layers are supported by a hydrostatic skeleton constructed of a gelatinous, metabolically-inert tissue called mesoglea. This makes the anemone body plan at least physiologically analogous, if not necessarily homologous, to many

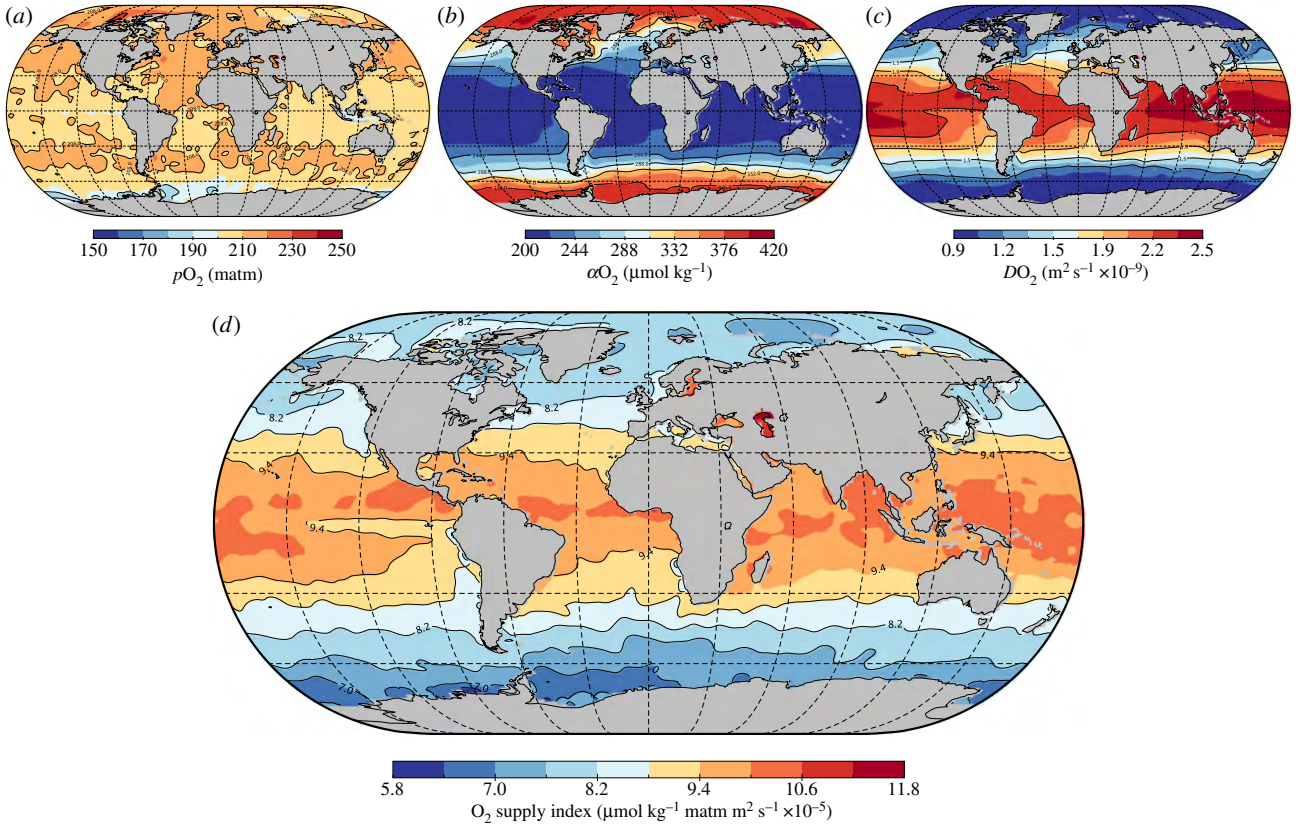

Figure 2. Factors governing oxygen supply to animals. (*a*) Average annual partial pressure of O_2 (pO_2) in the global ocean at surface. (*b*) Average annual solubility of O_2 (αO_2) in the global ocean at surface. Values increase with latitude owing to the thermal effects on Henry's solubility coefficient. (*c*) Average annual diffusivity of O_2 (DO_2) in the global ocean at surface. (*d*) Average annual bioavailability of O_2 in the global ocean at surface, expressed using the oxygen supply index (OSI) [39]. Despite the increased solubility of O_2 in cold water, the kinematic viscosity also increases substantially, reducing the diffusivity of O_2 at a rate greater than the offsetting effect on solubility. As a result, the supply of O_2 to respiratory surfaces actually decreases approximately linearly as water becomes colder.

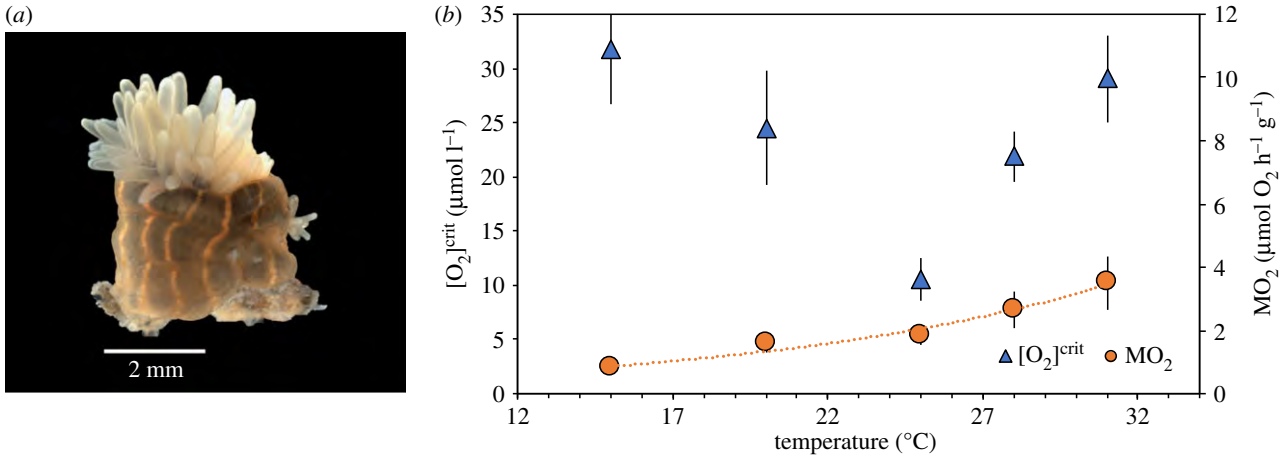

Figure 3. Variation of pO_2 tolerance with temperature. (*a*) Intertidal anthozoan cnidarian *Diadumene lineata* (YPM IZ 077401) from the New England region of the western Atlantic. Image courtesy of E.A. Lazo-Wasem. (*b*) $[O_2]^{crit}$ and MO_2 data for 86 individuals of *D. lineata* binned into five experimental temperatures (±0.3°C). Mean (±s.d.) standard O_2 consumption rate (MO_2) increases consistently with temperature owing to the Arrhenius relationship ($Q_{10} = 2.50$, $n = 70$, $R^2 = 0.96$). Absolute environmental O_2 tolerance ($[O_2]^{crit}$) displays a bidirectional relationship ($n = 86$, $R^2 = 0.75$). Triangles represent average mean values and whiskers represent standard error. These data provide, to our knowledge, the first experimental support for physiological principles hypothesized to be universal for marine ectotherms [45,46], indicating these principles are applicable to questions regarding the deep-water origin of Ediacaran organisms. (Online version in colour.)

Avalonian Ediacaran organisms without circulatory or respiratory systems. The comparison may be even closer than analogue, as many workers interpret some morphologically complex Ediacaran fossils to in fact be total-group actinarian cnidarians [59–61].

Results of respirometry experiments demonstrate this taxon displays mass-normalized standard metabolic rates (MO_2) that increase predictably with temperature ($Q_{10} = 2.50$, figure 3). For $[O_2]^{crit}$, mean values take the form of a concave-up parabola, demonstrating the predicted bidirectional effects that temperature has on environmental O_2 tolerance. At a T_{opt} of approximately 24°C, aerobic scope is maximal and *D. lineata* is able to respire aerobically well into low pO_2 levels ($[O_2]^{crit}$ of 10.4 µmol l^{-1} or 4.7% PAL). However, upon warming to

28°C, pO_2 tolerance decreases significantly ($[O_2]^{crit}$ of 21.9 µmol l^{-1} or 10.4% PAL). Cooling to 20°C produces a similar decrease in tolerance ($[O_2]^{crit}$ of 24.5 µmol l^{-1} or 10.2% PAL). Such bi-directionality has been studied at the molecular level and predicted to occur at the organismal level [46–48,50,62], yet despite its purported generality as a physiological principle that affects all aquatic ectotherms [46], this relationship has not previously been demonstrated experimentally using respirometry. While the exact shape of the concave-up parabola and position of the thermal optimum almost certainly differs between organismal lineages in different environments and over evolutionary timescales, the bi-directional effects seen in these *D. lineata* respiratory data are likely to be universal. These novel experimental results demonstrate that hypoxia tolerance is not a single threshold value in dynamic, shallow-water marine environments, but rather is determined jointly from the effects of temperature on OSI and on an animal's supply capacity over metabolic demand.

4. Discussion

(a) Seasonality and thermal tolerance in low pO_2 oceans

These physiological principles have significant use for understanding how low ambient O_2 levels and climate might have synergistically affected early animal life. While focus on the absolute lower O_2 limits for metazoan aerobic respiration [63,64] or critical thresholds on carnivory [65] provide important constraints on early animal ecosystems, the exogenous thermal environment in combination with pO_2 probably governed the metabolic viability of habitats for early animal ecosystems. Critically, because ambient pO_2 governs the size of aerobic scope, low pO_2 narrows the thermal range over which aerobic respiration can occur. Mathematically, this can be thought of in terms of the area above a polynomial regression run through a plot of $[O_2]^{crit}$ with respect to temperature for a given species, expressed as:

$$A = \left(\frac{2}{3}\right) \cdot b \cdot h, \tag{4.1}$$

where A is the area enclosed between the parabola and a chord intersecting the y-axis at an environmental O_2 level. The length of this chord between the intercepts with the parabola is b, which represents the thermal range of aerobic respiration at a given O_2 concentration. h is the height from the parabola vertex ($[O_2]^{crit}$ at T_{opt}) to the chord, and represents aerobic scope (figure 4*a*). Because A in equation (4.1) is related to the product of both O_2 and temperature, any decrease in aerobic scope (h) caused by lower ambient O_2 concentrations will also narrow the thermal range of aerobic respiration (b). For *D. lineata*, ambient pO_2 at 14.5% PAL corresponds to a 16°C allowable range for aerobic respiration which meets minimum requirements for maintenance, but ambient pO_2 at 10% PAL results in only a 9°C allowable range (figure 4). Thermal range continues decreasing to zero at a pO_2 of approximately 5% PAL, where the animal can only respire aerobically at its T_{opt} of approximately 24°C. This relationship has significant implications when considering the impact of thermal variability in the Ediacaran ocean. pO_2 levels of 10% PAL are on the high end of estimates for this interval, although in reality very little is concretely known about exact atmospheric O_2 levels during this time [5,7,8]. If pO_2 were closer to the lower-end estimate of 1% PAL, then presumably all megascopic metazoans would be driven deep into the respective vertices of their OCLTT parabola-space (figure 4*a*). This is if any could have survived at all; while metazoan macrofauna (0.3 to approximately 50 mm in size) can often have lower O_2 requirements [63], theoretical, experimental, and oceanographic evidence suggest minimum pO_2 levels of 1–4% PAL are needed for non-bilaterian megafauna in the absence of thermal variability [64,68,69]. Furthermore, marine animals require sustained metabolic rates to be a factor of approximately 2 to 5 greater than resting demand in order to sustain ecological activity [40]. Thus, the thermal range of low pO_2 tolerance in the Ediacaran may have been considerably narrow. We note that although this discussion is couched in terms of traditional Ediacaran O_2 estimates, the general principle of lower thermal range holds for any degree of low-oxygen Earth system.

With low ambient O_2 narrowing the thermal range of taxa, seasonal temperature extremes in the shallow Ediacaran surface ocean probably had a significant impact on the aerobic respiration of metazoans. The effects of seasonality in the modern ocean can be used as an example. In the mid-latitudes, average annual sea surface temperature ranges greater than 10°C at 40° N. By contrast, seasonal temperature variation in the deep ocean is minimal (less than 1°C) across almost all latitudes at 1000 m depth (figure 4*b*). We propose this difference ultimately governed metabolically viable habitat for early animals in the Ediacaran.

(b) A stenothermal origin for the Ediacara biota

The Cold Cradle hypothesis [21] originally posited that the Ediacara biota evolved in shallow, cold-water environments owing to increased O_2 solubility. However, we have shown that O_2 bioavailability in the global ocean maintains a positive linear relationship with temperature (electronic supplementary material, figure S7d). Furthermore, although cold-water has been theorized to increase aerobic scope by reducing standard metabolic rate in well-oxygenated conditions, low pO_2 environments negate any benefit that this provides [39]. At a whole-organism scale in the Ediacaran, aerobic scope throughout the global ocean was likely to be very limited if estimates for a lower O_2 Earth system are correct. Given the interaction of temperature on O_2 supply and aerobic demand, shallow-water environments would not have had the ideal ecophysiological conditions needed to establish diverse ecosystems given seasonal temperature fluctuations.

We hypothesize it was the lack of thermal variability, and not necessarily cold conditions, that can best explain the deep-water origin of Ediacara biota. These environments would have been far below the thermocline (roughly 200 m in the modern ocean), where the global ocean is cold and nearly isothermal (figure 4*b*). In such conditions, the synergistic effects of temperature and low environmental pO_2 on aerobic scope are muted and organisms can instead optimize their biochemical functions through compensatory adaptation to a significant degree [41,70]. Despite the lower bioavailability of O_2 in cold water, this compensatory capability allows modern animals to inhabit sub-zero waters in Antarctic habitats of the Southern Ocean [42], and severely undersaturated hypoxic deep-water OMZs on continental

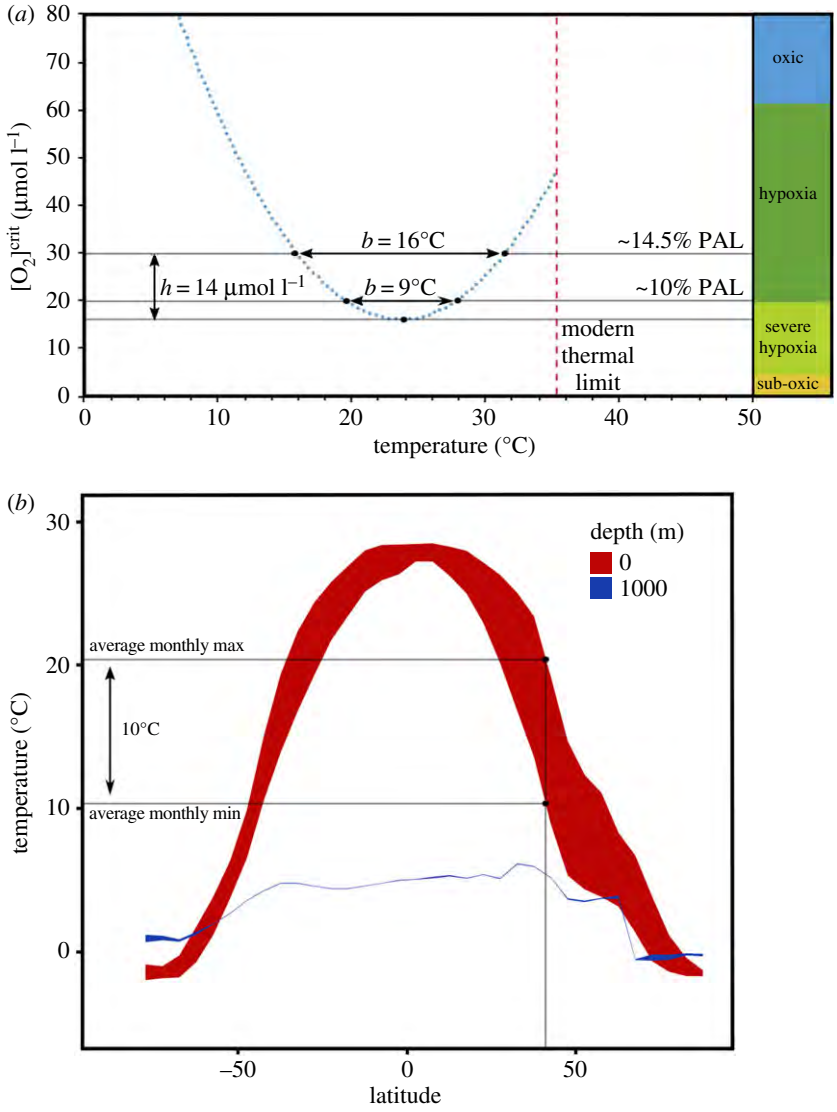

Figure 4. Impact of seasonal temperature variation on aerobic respiration in low pO_2 conditions. (*a*) Polynomial regression run through the $[O_2]^{crit}$ field of *D. lineata* ($R^2 = 0.75$). The region above this blue dashed line represents the temperature and O_2 conditions in which this species can maintain aerobic respiration (note that the regression vertex does not perfectly correspond with the lowermost measured $[O_2]^{crit}$ value). While the exact equation of the polynomial probably differs between species, the bidirectional nature of O_2 tolerance with respect to temperature is probably universal [46]. Because the area of this aerobic field (*A*) is dependent on the severity of hypoxia *h*, and the thermal range of the environment *b*, lower ambient concentrations of dissolved O_2 result in a narrower thermal range of aerobic respiration. Black horizontal lines represent O_2 levels at 14.5% PAL, 10% PAL, and the vertex of the parabola. The red dashed line is approximately 35°C, the average upper critical (lethal) long-term thermal limit of many shallow-water ectotherms living in modern tropical environments [66]. Descriptive low-O_2 state boundaries are adapted from [67]. (*b*) Average seasonal temperature ranges across all latitudes of the global ocean at surface and 1000 m depths. Envelope height (shown here for 40°N) represents the difference between maximum and minimum mean monthly temperatures for a given latitude. Data from NOAA WOA13 V2. (Online version in colour.)

slopes [55]. In an ecophysiological context, this means that while ambient pO_2 (*h* in figure 4*a*) governs the thermal range of aerobic tolerance (width of *b* within the parabola, figure 4*a*), animals are able to shift their thermal optimum, T_{opt} (vertex of figure 4*a*), to cooler temperatures via long-term adaptation [41]. The global distribution of Ediacaran genera [71], combined with the fact that multiple taxa were capable of inhabiting both deep- and shallow-water environments in the later Ediacaran, suggests that thermal acclimation capacity was unlikely to be an issue [33,72,73].

It would seem that stenothermal habitats (areas which experience only a narrow range of temperatures) at depth would be a key environmental attribute needed for animals evolving in a low pO_2 world. While we hypothesize temperature variability may be the most important factor, the fact that such deep-water environments are colder than the surface ocean is not inconsequential: this would be particularly beneficial for viable aerobic habitat if the global mid-Ediacaran upper ocean was significantly warmer than the modern. Today, animals in the tropics are already near their upper thermal limits that at ecological timescales are commonly approximately 35°C [43,45,66]. In other words, though warmer waters increases O_2 bioavailability approximately linearly, the increase in metabolic rates and thus O_2 demand with temperature is exponential, and in such conditions this quickly leads to temperature-induced hypoxia [40,45]. In this light, if there was an environmental control on the stratigraphic appearance of Ediacaran organisms, the up-slope movement onto the shelf and eventually into littoral habitats observed between the Avalon–White Sea and White Sea–Nama assemblages [31–33] could be read as either increased ambient pO_2 or global cooling. A further

expectation of this model would be that if middle Ediacaran (*ca* 570 Ma) shallow-water fossil occurrences are discovered, we expect them to be much lower in abundance and diversity (i.e. stressed communities) than temporally equivalent deep-water communities.

5. Conclusion

The Ediacara biota appear to have originated in deep-water slope and basinal settings in a global ocean that was still dominated by widespread low pO_2 conditions [5,12,33]. In marine settings, total dissolved O_2 concentration is a linchpin for metazoan life, but the critical threshold values at which point it imposes limits on physiological function can vary dramatically depending on multiple environmental factors such as primary productivity and organic carbon respiration, pH, salinity, hydrostatic pressure, and especially temperature. As we demonstrate here for the first time to our knowledge, in marine environments, the bioavailability of O_2 (OSI) cannot simply be inferred from absolute environmental O_2 concentrations alone, but rather it is the product of solubility, partial pressure, and diffusivity together. This has important implications for previous hypotheses that have considered the effect of water temperature on O_2 solubility in Ediacaran oceans [21], as diffusivity is actually a stronger lever on O_2 bioavailability, and works in the opposite direction. As a result, it is energetically more costly for animals to respire in cold, viscous water, despite its greater O_2 solubility [39]. Furthermore, with new experimental physiology data, we show that temperature and O_2 are synergistic and together govern the aerobic scope of marine ectothermic animals. The effects of temperature on O_2 supply and demand cause O_2 tolerance to vary widely as temperature fluctuates. As a result, Ediacaran environments which experienced a wide range of temperatures, such as shallow-water littoral zones and microbial bioherms, would have physiologically challenged early animal communities already living in low ambient O_2 conditions. We hypothesize that the apparent deep-marine origin of the Ediacara biota in cold, stenothermal environments may be the evolutionary solution to O_2 and temperature co-limitation.

Data accessibility. Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.bf43443 [74].

Authors' contributions. T.H.B. and E.A.S. designed the study. T.H.B. and L.E.E. contributed physiological data to the analysis. R.G.S. and T.H.B. performed the analysis of oceanographic data and T.H.B. analysed the results. T.H.B. wrote the manuscript with input from R.G.S., L.E.E., P.M.H. and E.A.S.

Competing interest. We declare no competing interests.

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