



SYMPOSIUM

Oxygen and the Energetic Requirements of the First Multicellular Animals

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Synopsis The appearance of multicellular animals during the Neoproterozoic Era is thought to have coincided with oxygenation of the oceans; however, we know little about the physiological needs of early animals or about the environment they lived in. Approaches using biomarkers, fossils, and phylogenomics have provided some hints of the types of animals that may have been present during the Neoproterozoic, but extant animals are our best modern links to the theoretical ancestors of animals. Neoproterozoic oceans were low energy habitats, with low oxygen concentrations and sparse food availability for the first animals. We examined tolerance of extant ctenophores and sponges—as representatives of extant lineages of the earliest known metazoan groups—to feeding and oxygen use. A review of respiration rates in species across several phyla suggests that suspension feeders in general have a wide range of metabolic rates, but sponges have some of the highest of invertebrates and ctenophores some of the lowest. Our own studies on the metabolism of two groups of deep water sponges show that sponges have different approaches to deal with the cost of filtration and low food availability. We also confirmed that deep water sponges tolerate periods of hypoxia, but at the cost of filtration, indicating that normal feeding is energetically expensive. Predictions of oxygen levels in the Neoproterozoic suggest the last common ancestor of multicellular animals was unlikely to have filtered like modern sponges. Getting enough food at low oxygen would have been a more important driver of the evolution of early body plans.

Introduction

No one knows the true environment of the first multicellular animals. While it is generally agreed that oxygen was limited and so the first animals must have tolerated hypoxia, what they fed on is not so clear. We contend that low oxygen was less of a constraint for the earliest animals than getting enough food.

How multicellular animals came about, what triggered the great change from unicellular life to the first animals that functioned with different cells for different tasks, is one of the greatest puzzles today. Hypotheses vary by discipline, but interpretations generally rely on some knowledge of animals living today, more than half a billion years after the event.

For example, biomarkers—organic molecules in the rock record—are thought to have been produced by either microbes, unicellular eukaryotes, or metazoans based on studies on the steroid and other organic content of extant organisms (Love et al. 2009). Other work interprets fossils based on rudimentary comparisons of the rock with structure and ultrastructure of modern animals—sponges or ctenophores often (Maloof et al. 2010; Tang et al. 2011; Yin et al. 2015). All of these studies use molecular clocks to ground their understanding of whether rocks might be expected to have animal remains (Cunningham et al. 2016; 2017).

Phylogenomic studies have produced most of the recent contentious data: these analyses do not agree

about which group of animals—ctenophores or sponges—arose first (Dunn et al. 2008; Philippe et al. 2009; Nosenko et al. 2013; Ryan et al. 2013; Moroz et al. 2014; Whelan et al. 2015; Feuda et al. 2017). We do know however, that genomes of animals did not start small and become larger; in fact the mean genome size for sponges calculated so far, 200 Mb, is larger than the fruit fly *Drosophila melanogaster* genome at 175 Mb (Jeffery et al. 2013). We also know that the shared common toolkit of the ancestor of animals had many of the gene pathways and complex signaling molecules of animals today (Riesgo et al. 2014). Even cell types were complex from what we know of extant groups, but cell type diversification was very likely individualized to particular lineages (Moroz 2009).

This knowledge changes how we think about whether early animals appeared morphologically simple or might have been already quite complex in terms of cell types, tissues, and polarized body plans. In only a few years we have changed our ideas from thinking that animals arose with a stepwise gain in morphological complexity, to now considering that nerves and through guts could have been gained and then lost in sponges or have arisen independently several times (Nielsen 2008; Ryan and Chiodin 2015). These hypotheses are again founded on a rudimentary understanding of the morphology of extant animals, and morphology can be deceptive. Ctenophores appear to have dazzling complexity with iridescent “comb” rows of macrocilia, apical/aboral sensory organs, mouths, and a clear anus (Presnell et al. 2016). Yet no other animal uses macrocilia rather than muscle for propulsion; these are complex structures, but they are no more complex than the changing diameters of the sponge canal system which is able to generate precisely the correct flow just as the most “complex” blood transport systems do in vertebrates. Sponges, which are thought to be morphologically simple in comparison to ctenophores, could have canalized their form to be the most efficient for a filter feeder extracting the smallest particles from the water.

Here we examine what the physiology of modern ctenophores and sponges tells us about what kind of animals the first multicellular stem-group metazoans might have been, and the habitat they might have evolved in.

The ocean setting and the paradigm

While the true constraints of carbon and oxygen concentrations during the Neoproterozoic are not known, ranges of acceptable conditions exist based

on the presence of banded iron formations, carbon isotopes, and other geochemical parameters (Sperling et al. 2013). The transition from the Archaean Eon to the early Proterozoic ~2400 million years ago (Mya) is characterized by the “Great Oxygenation Event” (GOE), a permanent oxygenation of the atmosphere. Atmospheric oxygen concentrations increased 100-fold or more from 0.001% of present atmospheric levels (PALs) to 1–10% PAL (Kump 2008) during the early Proterozoic, although some recent work using chromium isotopes estimates that they could have been as low as 0.1% (Holland 2006; Planavsky et al. 2014). The GOE affected the atmosphere and surface waters but deep ocean basins were still anoxic. It is still debated whether oxygen concentrations remained low throughout the Proterozoic or if they fluctuated until the late Neoproterozoic, but carbon isotopes suggest episodic productivity in surface waters, which would have released some oxygen into the atmosphere and resulted in fallout as marine snow, remineralization, and burial in the oceans (Knoll and Carroll 1999; Riding 2006; Sperling et al. 2015).

Holland (2006) summarizes models of Proterozoic carbon conditions as acritarchs and other protists fed on surface primary productivity (Butterfield and Rainbird 1998), then were remineralized in deep water. This led to episodic anoxic events in deep water throughout the Proterozoic and a growing reservoir of organic carbon many times larger than the present day (Rothman et al. 2003), although this large carbon pool is also debated (Ridgwell and Arndt 2014). At the end of the Neoproterozoic, atmospheric and surface ocean oxygen concentrations rose 10- to 100-fold, and then to nearly present-day levels, and deep ocean basins became oxygenated (Lyons et al. 2014; Sperling et al. 2015) (Fig. 1A). High rates of carbon burial resulted in a reduction of the dissolved organic carbon (DOC) pool, to concentrations similar in quantity and proportion present today (Rothman et al. 2003; Holland 2006) and changing the ocean from a putative green, DOC-rich soup to clear blue oceans, with particulate and inorganic carbon making up a larger proportion of the total carbon pool.

Doushantuo fossil embryos suggest multicellular animals were abundant during the Ediacaran (630 to ~540 Mya, or late Neoproterozoic). Particular 24-isopropylcholestane (24-ipc) sterol biomarkers are suggested to be remnants of the life of demosponges (Love et al. 2009; Gold et al. 2017), which if true, means a substantial population of these animals lived to produce those lipid products 659–645 Mya (Xiao et al. 1998; Xiao and Laflamme 2009; Brocks et al. 2017). This would agree with data pointing to

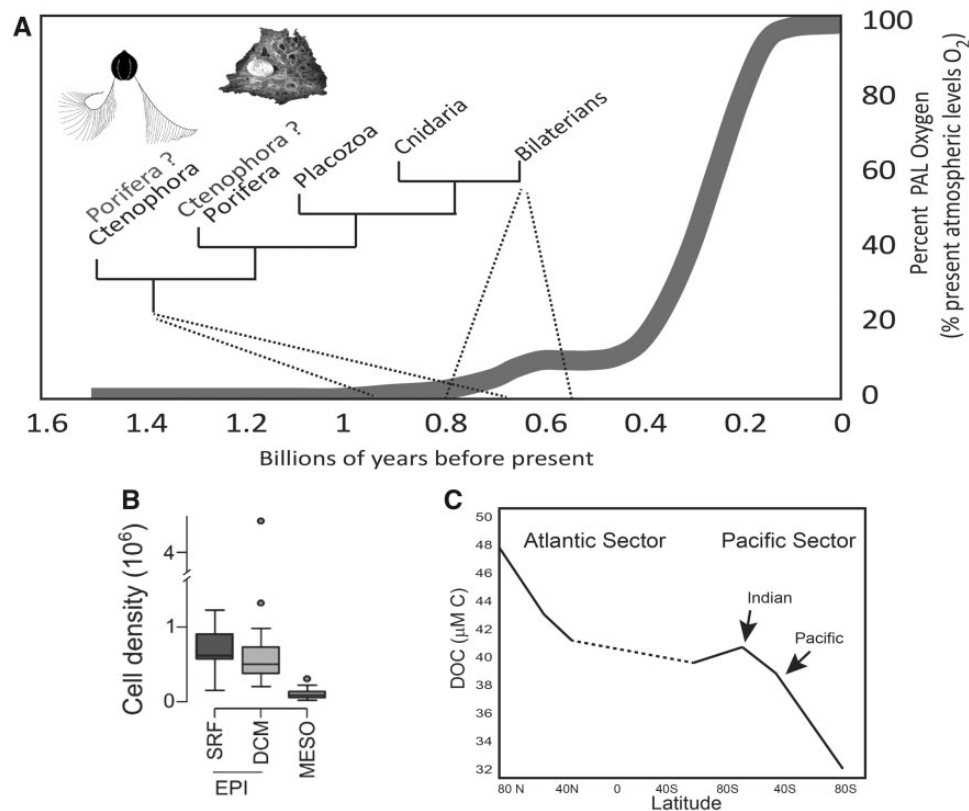


Fig. 1 The ocean setting: conditions contemplated to have existed when the first multicellular animals arose (adapted from Lyons *et al.* 2014). **(A)** Estimated change in oxygenation of the ocean over time and origin of the first animals. The solid line shows oxygen in percent of the PAL and the change that is thought to have occurred during the late Neoproterozoic (800–600 Mya). Scenarios are depicted for both ctenophores and sponges as the earliest evolving group of extant metazoans. Other animal groups could have evolved but have become extinct, and not have left a fossil record. **(B)** Number of unicells (in millions of cells) present at epipelagic (200 m) and mesopelagic (600 m) depths in oceans today from samples collected during the TARA oceans project (from Sunagawa *et al.* 2015). **(C)** DOC in micromoles carbon measured at stations from different latitudes in the Atlantic and Pacific Oceans today (from Hansell and Carlson 1998).

general oxidation of the oceans at that time (Sahoo *et al.* 2012) and provide the link between oxygen and evolution of multicellular animals. However, the question really is whether animals could have evolved earlier, living in much lower oxygen conditions, and have contributed to the oxygenation of the oceans by feeding on material that would otherwise decompose and use up oxygen. If so, interpretations of Ediacaran fossils as stem ctenophores (Tang *et al.* 2011) and sponges (Malooof *et al.* 2010; Yin *et al.* 2015) may not be so far-fetched. The issue is that we do not know ‘how low’ oxygen levels may have been during this period (see Lenton and Daines 2017).

Today’s oceans are oxygen-rich. While oxygen minimum zones are common, they are geographically and vertically localized, although they are anticipated to expand considerably over the next decades due to increases in global temperature and slowing of ventilation (Brandt *et al.* 2010; Stramma

et al. 2010). Many invertebrates use little oxygen and there are many adaptations to low oxygen, for example, during exposure at a long, low tide or in benthic sediments. Furthermore, other large animals including fish, crustaceans, and cephalopods that are highly adapted to life in oxygen minimum zones (OMZ) have a range of metabolic adaptations to low oxygen including high blood volume, thin tissues on gills or elsewhere for oxygen to diffuse across, or respiratory proteins with a high affinity for oxygen (reviewed in Seibel 2011).

Today’s shallow water contains an abundance of food: DOC, zooplankton, phytoplankton, and microbes (which we inclusively define as all bacteria, archaea, and unicellular eukaryotes). The deep sea by contrast is a desert, not entirely depauperate but certainly food-poor, with at least 10-fold fewer bacteria per milliliter compared to surface waters. The recent TARA Oceans project has quantified microbial eukaryotes at different depths throughout parts of

the Pacific and Atlantic Oceans (Kirkham et al. 2013; Sunagawa et al. 2015). What we see from that work is that the mesopelagic zone (600 m depth) is already depleted in unicells by an order of magnitude (Fig. 1B). There is not a lot of food to filter at that depth. But at least in coastal waters there may be mucus houses, and larger clumps of marine snow which these analyses do not include (Alldredge 1998). DOC is also depleted at depth, but more so in the Pacific than Atlantic ocean due to the long time it takes to make its way along the global conveyor belt (Hansell and Carlson 1998) (Fig. 1C).

Despite the range in metabolic rates that we see in animals today, no animal is considered to have tolerated the extremely low oxygen levels that were widespread before the Neoproterozoic oxygenation event which occurred $\sim 635\text{--}630$ Mya (Sahoo et al. 2012). Butterfield (2007) envisioned a world of unicellular organisms (acritarchs) that lived in stasis until some destabilizing event occurred to trigger evolution of a more active predator. This predator was likely another heterotrophic unicell, and the appetite of these unicellular predators may have triggered innovation of gene networks that allowed the plunge into multicellularity. One hypothesis is that the earliest animals tolerated very low oxygen and their activities enabled the oxygenation of deeper waters. Lenton et al. (2014) envision that filtration by multicellular animals may have removed phosphates from deeper waters, thereby reducing oxygen consumption that would have occurred by degradation of the microbial soup. These are stimulating hypotheses, but whether larger, multicellular animals could actually survive that low oxygen depends on their tolerance to low oxygen and also on what they require for food.

Ctenophores

Respiration rates of a broad range of invertebrates show that ctenophores have some of the least expensive metabolic rates of extant animal groups (Thuesen et al. 2005) (Fig. 2; Supplementary Table S1). Ctenophores are extremely tolerant of low oxygen and can live in and move in and out of the lowest regions of estuaries where oxygen often is <1 mg/L ($31\ \mu\text{M O}_2$) (Kolesar et al. 2010) as they hunt ichthyoplankton that avoids anything <2 mg/L ($62\ \mu\text{M O}_2$). Thuesen et al. (2005) found that three species of ctenophores had a similar tolerance of as low as $10\ \mu\text{M O}_2$ and suggested they may also use anaerobic metabolism.

Ctenophores swim by beating rows of macrocilia packed into a structure called a ctene or comb,

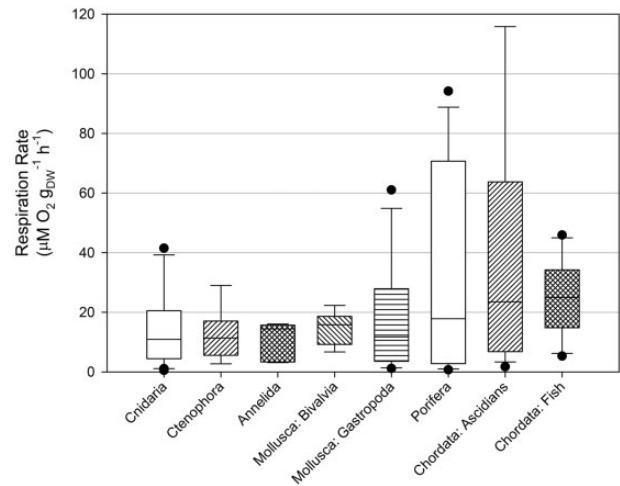


Fig. 2 Respiration rates (in micromoles of oxygen consumed per gram dry weight each hour) of different animal groups taken from numbers in the literature. Original data is shown in Supplementary Fig. S1.

because the macrocilia are lined up so that they look like a comb. An estimated 75% of the respiration in ctenophores is due to beating of the comb rows (Gyllenberg and Greve 1979). Ctenes beat in metachronal waves and are thought to be entrained by viscous coupling (Tamm 1984). Use of metachronal waves in ciliary beating is cost efficient, providing an estimated 10-fold greater efficiency than cilia beating in phase; beat frequency is lower but more water is moved over time (Elgeti and Gompper 2013). The energetics of multiciliated cells is also more cost effective than using unciliated cells (Brooks and Wallingford 2014). The remaining cost of being a ctenophore, after beating the ctenes, must be in feeding, because it has been found that respiration is four-fold higher in feeding than non-feeding specimens (Kremer 1982). However the cost of increased respiration is probably not in digestion, since ctenophore guts were found to be anaerobic and experiments found that the amount of oxygen used in digestion was unchanged in animals treated with antibiotics (Thuesen et al. 2005). Probably then, the cost lies in capturing the prey.

As far as we know, all modern ctenophores are carnivorous (Haddock 2007), but presumably when ctenophores first arose there were no muscular prey to chase. Although some modern day deep-sea ctenophores actively capture larvacean houses and discard them—*Dryodora*, for example, apparently engulfs larvacean houses whole, swallows the larvacean and, spits out the house (Haddock 2007)—this may not have been the case in the past. Instead the oceans must have been, as described above, a

microbial (unicellular) soup. The best way to concentrate a microbial food source might appear to be filtration, and so while it is easy to envision that the evolution of mucus nets and a mechanism to pass the microbial soup through meshes would be favored, we suggest an alternative hypothesis: perhaps the first ctenophores ensnared marine snow and unicells rather than copepods, similar to how the vampire squid uses sticky tentacles to capture marine snow today (Hoving and Robison 2012). Ctenophores either have two long tentacles containing sticky granules that are fired on contact with prey, or they engulf animals through a slit-like mouth that opens to a cavernous interior (Haddock 2007). The ctenophore tentacle is a complicated histological structure with mucus cells, accessory cells, and colloblasts, a highly specialized cell type with adhesive granules; the granules are produced in neighboring cells and moved into the colloblast structure where they are “fired” upon contact with prey (Mackie et al. 1988). There is nothing from studies of modern ctenophores to say that ctenophores would have eaten marine snow, but marine snow is known to be a substantial carbon source for other animals (Alldredge 1998; Robison et al. 2005), could be captured through the feeding means of extant ctenophores, and was likely abundant during the Neoproterozoic (Riding 2006).

Understanding whether these mechanisms could have allowed a multicellular animal to “make a living” or could have sustained increases in complexity of tissues and functions, involves knowing the energetic costs of producing the material, which means knowing the cost of metabolism. Whether feeding by mucous tentacles or by concentrating individual bacteria on meshes, the last metazoan common ancestor had to trap the food, and the cost of trapping the food must not have exceeded the energy gained in feeding. If gelatinous animals were ctenophore-like and captured mucus-laden marine snow on sticky tentacles as envisioned above, it is possible that muscle arose in those tentacles entirely to draw these toward the mouth. Alternatively, ciliated grooves could have moved the material conveyor-belt wise to the mouth and slowly into the gut, as it occurs in terebellid worms today. Whether modern-day ctenophores can sustain themselves on marine snow is unknown, but perhaps some can. While ctenophores have high clearance rates, from 40 to 200 L/day depending on the size of the animal (Haddock 2007; Granhag et al. 2011), their metabolism is low, and many that are adapted to oligotrophic waters do not do well in high food (Kremer et al. 1986).

Sponges

Sponges do not possess complex tissues like muscles and nerves found in ctenophores; rather they filter water through small pores in their surface, into tube-shaped canals that lead to the spherical chambers fitted with flagellated pump-cells called choanocytes. Here, food—bacteria and/or unicellular eukaryotes—is extracted, wastes are excreted, and the water moves out other canals to a chimney-shaped vent, the osculum. Sponges appear relatively inactive, yet they have a surprisingly high range of respiration rates in comparison to ctenophores. The respiration rates of sponges are as varied as their lifestyles and feeding habits because sponge filtration is for both food and respiration and generally the more a sponge filters, the higher the specific respiration rate (Ludeman et al. 2017). For sponges, an estimated 1–30% of metabolism is related to the cost of filtration (Hadas et al. 2008; Leys et al. 2011; Ludeman et al. 2017) due to the expense of the mechanism of feeding.

Instead of clearing water around them by catching particles on mucus strands away from the body as ctenophores do, sponges draw the water through themselves and pass it over a mucus mesh. Sponges feed on extremely small particles; a typical diet consists of either mostly heterotrophic bacteria supplemented with some eukaryotic unicells, or of DOC. Microbial symbionts process and may transfer DOC to the sponge, or the symbionts are phagocytosed directly by the sponge (Ribes et al. 1999; Yahel et al. 2003; Maldonado et al. 2012; Leys et al. 2018). Sponges are capable of extremely high clearance rates (Kahn et al. 2015) and this comes at a cost which is brought on by pushing the water through the tiny dimensions of the canals and nanometer-sized pores of the mesh covering the collar filter (Leys et al. 2011).

But models of the resistance through the canals show that the cost of filtration depends on the amount of water filtered (Ludeman et al. 2017). Some sponges filter very little and have low respiration rates while others filter a lot of water and have much higher respiration rates (Maldonado et al. 2012). Respiration rates depend on temperature of the habitat (warmer water species have higher respiration rates) and type of food, whether particulate organic carbon (POC) or DOC is abundant, and whether symbionts are present.

Sponges with high microbial abundance (HMA sponges) seem to have a higher energetic cost possibly due to the metabolic load of their symbionts, and yet even some HMA sponges can tolerate prolonged

periods of extremely low oxygen. A common intertidal sponge *Halichondria panicea* survived for 24 days in flow through chambers with 3–4% of the present atmospheric level of oxygen (9–12 $\mu\text{M O}_2$) (Mills et al. 2014). The clearance rate of the sponge was reduced by one-third compared to normal sponges (Mentel et al. 2014), but the sponge grew tendrils, suggesting it was actively seeking a new location and therefore was functioning well (Mills et al. 2014). A different sponge tested in a similar set up (*Tethya wilhelma*) showed tolerance of reduced oxygen, only stopping its typical, repeated, hour-long contractions when oxygen was <4% PAL (Mills et al. 2018). Is this surprising? Not if we take into account that *Tethya* species have one of the lowest filtration and respiration rates of sponges (Reiswig 1975; Ludeman et al. 2017). *Tethya* species have daily and monthly cycles of pumping related to rates of expansion and contraction, but overall their specific respiration rates are extremely low for sponges.

For deep-water sponges, there is a huge difference in budget depending on whether particulate or dissolved carbon makes up the main energy source. The deep-water glass sponge *Aphrocallistes vastus* removes less oxygen and carbon (0.5 $\mu\text{Mol O}_2$; 2.9 $\mu\text{g C}$) than *Geodia barretti* (20 $\mu\text{Mol O}_2$; 10.5 $\mu\text{g C}$) which inhabits similarly deep water on the Norwegian continental shelf (Fig. 3). It is assumed that the relatively high respiration rate of *G. barretti* is due to the aerobic fixation of carbon and nitrogen by its symbionts (Radax et al. 2012), and sections of the tissue studied by transmission electron microscopy show that *G. barretti* phagocytoses its microbial symbionts (Leys et al. 2018).

These two species can provide insight into the oxygen needs of the different types of sponge-grade metabolisms that might have existed in early oceans. Since *G. barretti* lives in well oxygenated water (minimum 245 μM) of the North Atlantic, we thought that reduced oxygen would compromise its energetic needs and if so then perhaps symbioses were not possible until oceans were well-oxygenated. To test this we used helium to reduce ambient oxygen in flow-through chambers to 7% air saturation (20 $\mu\text{M O}_2$) while maintaining flow rates, temperature, and salinity unchanged. We found that while filtration was reduced by one-third as in the case of *H. panicea* (Mills et al. 2014), the respiration rate remained largely unchanged even at the lowest ambient oxygen levels (Fig. 4). Filtration rates dropped almost immediately once oxygen was reduced, which indicated an ability to detect either reduced oxygen, change in gas mixture (helium for air), or possibly

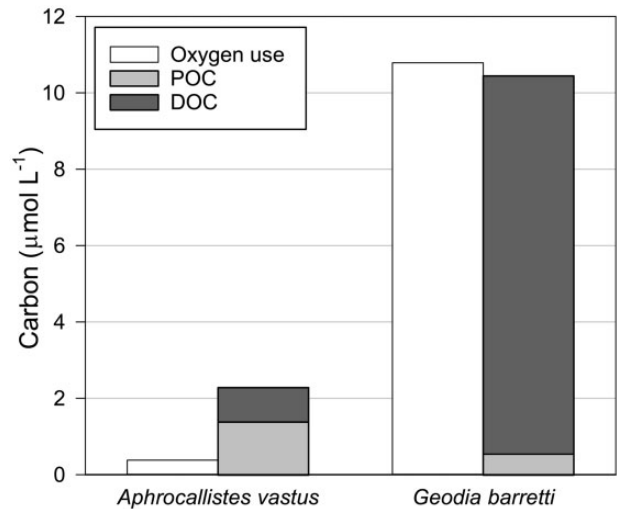


Fig. 3 Energy budget for two deep water sponges, *A. vastus* and *G. barretti* plotted with data from Leys et al. (2018) and Yahel et al. (2007). Oxygen consumed by filtration and removal of particulate and DOC (measured by the difference in incident and excurrent samples) shows two very different strategies.

some other trigger that came with the change in gas mixtures since we found the sponge was highly sensitive to the smallest perturbations. But, most significantly, respiration was unchanged even when there was <15 μM of oxygen available (Fig. 4C). When ambient oxygen became lower than that, filtration ceased, and at any time filtration stopped the tissues immediately became anoxic. Because sponge tissues are riddled with water canals, it is not possible to insert sensors only into mesohyl to know oxygen levels actually in the sponge tissues, and so we do not know the true measure of oxygen in the sponge tissue. However, even after 3 days without pumping, when ambient oxygen levels returned to normal, the sponge resumed filtration and respiration at normal levels. It is not known how long a sponge could survive with no exchange of water.

Many sponges stop filtering for periods; Annandale (1912) called it the sponge “siesta.” Many intertidal species cease filtration when they are either in small tide pools or if exposed to air. It is generally assumed that sponges exposed to air can get sufficient oxygen across their epithelia as long as they are moist, although it is likely they too are anoxic internally when not filtering. But it would be expected these species may be adapted to a range of temperatures, salinities, and oxygen levels over tide cycles, seasons, and weather. Deepwater sponges should experience more stable conditions, and so *G. barretti*—which lives in normally well-oxygenated water—shows an impressive resilience to low oxygen conditions.

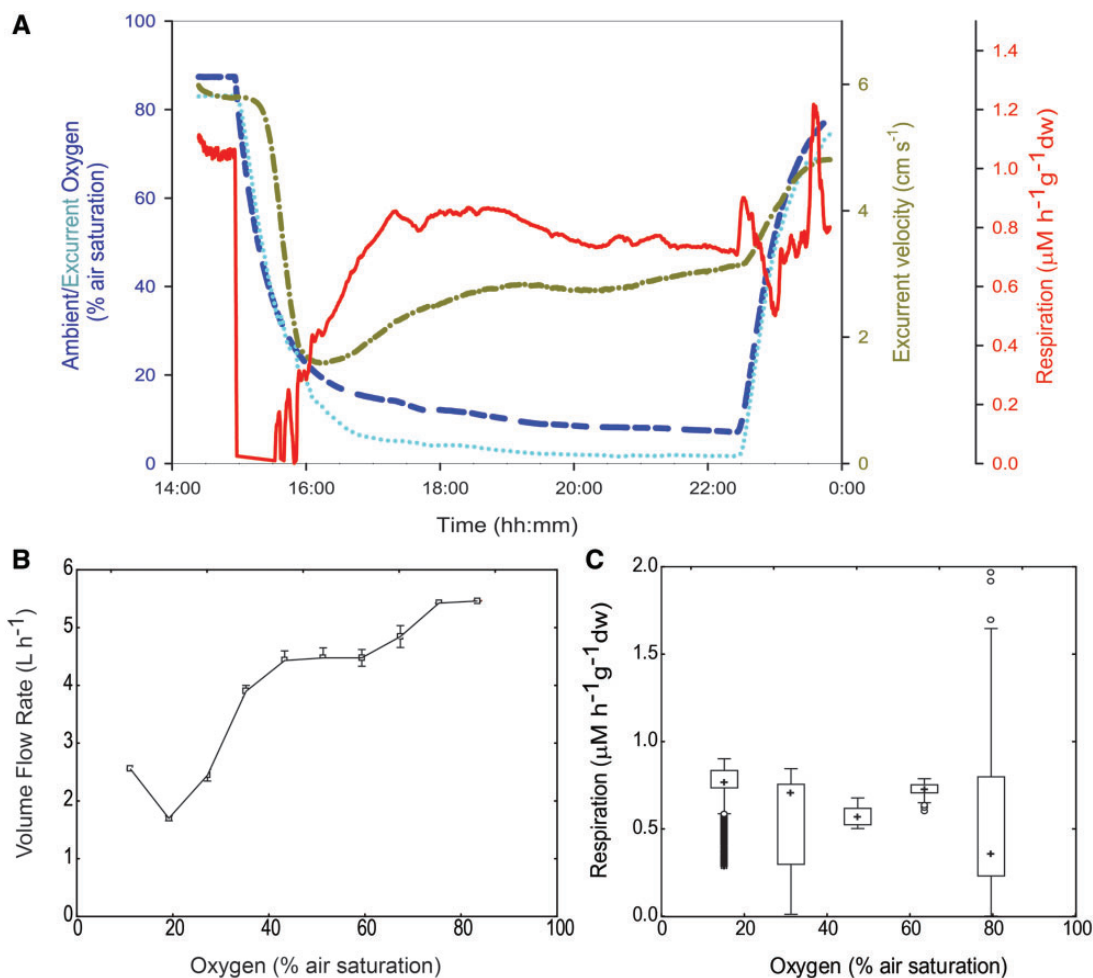


Fig. 4 Effect of hypoxia on filtration and respiration in *G. barretti*. **(A)** Exchange of helium for oxygen in water flowing through a chamber with *G. barretti* shows a rapid reduction in ambient oxygen (dark blue, long dash) and a concurrent reduction in the excurrent oxygen (light blue, dotted), left hand y axis. Excurrent velocity (brown, dash-dot, first right-hand y axis from the sponge measured with a thermistor-based flow sensor shows a rapid reduction in filtration rate until ~16:00 h when the sponge returned to filter at about one-half to two-thirds the normal filtration rate. The respiration rate (red, solid line, second right-hand y axis) at two-thirds the filtration rate is relatively stable. **(B)** The volumetric flow rate is reduced at lower oxygen levels. **(C)** Respiration rates are relatively unchanged at all ambient oxygen levels.

What about glass sponges? We might expect that *A. vastus* with such a low metabolic rate would be indifferent to greatly reduced oxygen levels. Glass sponges are more difficult to maintain in flow-through aquaria; one reason might be that the energy budget is more precarious because a reduction in filtration would be a large loss in daily carbon. In earlier work we found that *A. vastus* uses induced current (Leys et al. 2011), and subsequent work shows that up to one-third more water is filtered during periods of increased flow due to tidal currents, but using less oxygen, indicating that this sponge must use induced current.

Geodia barretti does not use induced current, and so it appears that temporarily anoxic tissues (2–

3 days) and long-term hypoxic conditions allowing filtration at a reduced rate, do not disturb its budget. Both habitats have high DOC (70–100 μM in the Norwegian fjords, and 50–70 μM in the Strait of Georgia, British Columbia) but the glass sponges we have analyzed do not remove a measureable amount of DOC from the water they filter (Yahel et al. 2007) and they have no microbial symbionts in their mesohyl or in their syncytia (Leys 1999). The data suggest that sponges tolerate hypoxia by reducing feeding activity. They also tolerate extended periods of low food availability by reducing maintenance costs: rates of cell replacement were negligible in temperate sponges in winter months (Kahn and Leys 2016). Altogether, filtration in sponges seems to be costly and

mechanisms to reduce that cost have driven innovations in their morphology and physiology.

Adaptations to a food poor environment

The focus on oxygen limitations and its challenge to animals in early oceans has obscured the problem of carbon. Although sponges are highly effective at removing particulate carbon as bacteria and unicellular eukaryotes, filtration is costly due to the resistance generated by the small dimensions of the filter. Adaptations to reduce the energy spent in ciliary and flagella beating in both ctenophores and sponges indicate that it represents a significant portion of their energy budget. But whereas in ctenophores, the cost of the ciliary beat is the same proportion of the budget regardless of feeding habit (tentaculate or lobate) (Thuesen et al. 2005) and the total budget is very low; in sponges, the cost of filtration depends upon the amount filtered, and whether symbionts are present or not.

Symbioses may have been effective for taking advantage of DOC in Neoproterozoic oceans. Some estimates indicate there was a vast amount of DOC in Precambrian seas (Tahata et al. 2015), but use of DOC by microbial symbionts would come with the energetic cost of ventilating the tissues; anaerobic respiration is possible but produces substantially less energy. And so while symbioses may have been an expensive “next step,” due to the need for oxygen it is less likely they would have been affordable to the “first” filter feeding animals.

There are other clues from modern poriferans that sponge habits are generally oxygen and carbon expensive, and that catching food is energetically expensive. Deep-sea sponges live in a low food environment and have very distinct morphological adaptations. Carnivorous sponges are common in deep sea environments. They are demosponges that have lost the aquiferous system and instead trap crustaceans using hook-shaped spicules. And larvae of the two solely deep sea groups—hexactinellids and carnivorous sponges (in two distinct Classes)—are the only larvae with multiciliated cells (Boury-Esnault and Vacelet 1994; Leys et al. 2006; Riesgo et al. 2007). Both the hexactinellid *Oopsacas minuta* and the carnivorous sponge *Lycopodina* (formerly *Asbestopluma*) *hypogea* have multiciliated cells in their epithelium; all other sponge larvae have uniciliated cells. Multiciliated cells are typical of metazoans and are potentially more energy efficient (Brooks and Wallingford 2014). Energy saving adaptations seem to be used in the deep sea where food is more scarce.

Even glass sponge syncytia may have arisen as an adaptation to life in a food poor environment. Glass sponge embryos become syncytial during early development by the fusion of blastomeres; the resulting multinucleated tissue forms long strands that envelop the entire animal (Leys et al. 2006). As a result glass sponge canals are vast (up to 0.5 mm in diameter) and flagellated chambers are large (60–100 μm in diameter); more open space reduces the resistance to water flowing through and allows glass sponges to use induced current (Leys et al. 2011). Moreover, glass sponges do not use choanocytes; rather their flagella pumps are on enucleate “collar bodies” that bud off stolons from a central, recessed nucleated choanoblast (Mackie and Singla 1983). The choanoblast is hidden under the syncytial reticular tissue where it is protected from potential damage—much as turbellarians can recess their nuclei in the epithelium to protect them from damage (Tyler 1984). That choanoblasts are valuable and expensive to replace is also suggested by the lack of new nuclei formed (“turnover”) seen in the glass sponge choanosome; all new cells formed appear at the growing edge or lip of the sponge (Kahn and Leys 2016). We suggest that glass sponge syncytia, and the electrical signaling it enables, are most likely an adaptation to the deep, food poor habitat.

Conclusions

Glass sponges and ctenophores have very low specific respiration rates. Both groups show very specific adaptations to oxygen poor and carbon poor ocean habitats. The lifestyle of modern ctenophores as active predators is not likely to have occurred in early oceans but the unusual tissues and low metabolic rates of extant animals make it easy to envision a drifting collector of marine snow from the water column. Likewise, many modes of sponge function today are energetically expensive and would not work in early ocean conditions, but other adaptations such as the thin tissues and syncytia of modern glass sponges could reflect the lifestyles of the earliest sponges. These adaptations allow minimal energy used (as oxygen required) to enable the capture of as much food as possible. Low oxygen is clearly not such a problem, and for this reason we think that availability of food rather than of oxygen would have been the greatest limiter of the evolution of complex multicellular animals.

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Supplementary Data

Supplementary data available at *ICB* online.

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