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SYMPOSIUM

Reconsidering the Oxygen–Temperature Hypothesis of Polar Gigantism: Successes, Failures, and Nuance

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Synopsis "Polar gigantism" describes a biogeographic pattern in which many ectotherms in polar seas are larger than their warmer-water relatives. Although many mechanisms have been proposed, one idea—the oxygen–temperature hypothesis—has received significant attention because it emerges from basic biophysical principles and is appealingly straightforward and testable. Low temperatures depress metabolic demand for oxygen more than supply of oxygen from the environment to the organism. This creates a greater ratio of oxygen supply to demand, releasing polar organisms from oxygen-based constraints on body size. Here we review evidence for and against the oxygen–temperature hypothesis. Some data suggest that larger-bodied taxa live closer to an oxygen limit, or that rising temperatures can challenge oxygen delivery systems; other data provide no evidence for interactions between body size, temperature, and oxygen sufficiency. We propose that these findings can be partially reconciled by recognizing that the oxygen–temperature hypothesis focuses primarily on *passive movement* of oxygen, implicitly ignoring other important processes including ventilation of respiratory surfaces or internal transport of oxygen by distribution systems. Thus, the hypothesis may apply most meaningfully to organisms with poorly developed physiological systems (eggs, embryos, egg masses, juveniles, or adults without mechanisms for ventilating internal or external surfaces). Finally, most tests of the oxygen–temperature hypothesis have involved short-term experiments. Many organisms can mount effective responses to physiological challenges over short time periods; however, the energetic cost of doing so may have impacts that appear only in the longer term. We therefore advocate a renewed focus on long-term studies of oxygen–temperature interactions.

Introduction

Marine invertebrates living in cold, polar waters often have larger body sizes than do related species living in temperate or tropical waters, a pattern systematic enough to have become known as "polar gigantism." Polar gigantism was observed initially by some of the first expeditions to the South Ocean; these expeditions dredged up giant sea spiders, nemertean worms, and isopods. Starting in the mid-1950s, after long-term bases were established in Antarctica, marine biologists gained easier access to near-shore benthic communities, and the extent of gigantism came into clearer focus: in many, but not all, taxonomic groups, Antarctic species are substantially larger-bodied—sometimes many-fold larger—than relatives elsewhere in the world (Moran and Woods 2012). Perhaps the most renowned polar giant is the "colossal squid," *Mesonychoteuthis hamiltoni*. This species, an Antarctic endemic, is not only the largest-bodied species in its taxonomic group, it is the largest invertebrate on earth (Rosa et al. 2017). *Mesonychoteuthis hamiltoni* has captured the public imagination like few other invertebrates, but polar giants also occur within generally small-bodied taxa like sea spiders, amphipods, and isopod crustaceans, and even in single-celled organisms like foraminifera.

Like other biogeographic patterns focused on body size, including Bergmann's Rule (Bergmann 1847; Blackburn et al. 1999), James' Rule (James 1970),

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Thorson's Rule (Thorson 1950; Mileikovsky 1971; Marshall et al. 2012), Cope's Rule (Rensch 1948; Hone and Benton 2005), and the temperature-size rule (Atkinson 1994), polar gigantism has attracted a great deal of attention among biologists, on the grounds that such broad and systematic patterns promise to reveal general principles shaping the evolution of body size. But, like those other rules, polar gigantism has not given up its secrets easily. So far, no clearly dominant, single-factor explanatory mechanism or process has emerged. In an earlier paper, we categorized many hypotheses about polar gigantism into eight groups which invoke, collectively, a diverse set of evolutionary, ecological, physiological, physical, and chemical mechanisms (Moran and Woods 2012). We concluded that the majority of these hypotheses could not be evaluated; in most cases, too little information was available even to begin. We suggested, moreover, that single-factor explanations are unlikely to provide convincing explanations for such a taxonomically pervasive pattern, and that the observed body-size patterns likely emerge from complex interactions among mechanisms playing out in context-specific ways across the globe. For example, cold water may release organisms from constraints on body size arising from the biophysics of oxygen transport and use, but whether individual taxa in fact evolve larger sizes during shifts to colder habitats will depend on an additional suite of temperature-dependent changes to other life history processes, including temperature dependent rates of mortality (Angilletta et al. 2004; Kozłowski et al. 2004). In addition, some of the largest marine animals ever to have lived occurred in warm water, which suggests that higher temperatures are not absolutely constraining and that other factors (like productivity) play important roles in shaping body size (Vermeij 2016).

Of potential explanations for polar gigantism, the oxygen–temperature hypothesis is the most conceptually straightforward and experimentally tractable and, as a result, has received significant attention in recent decades. This idea emerged from the observation that, for ectotherms, changes in temperature alter the ratio of oxygen supply to demand (Woods 1999; Jacobsen et al. 2003; Verberk et al. 2011); in particular, changes in temperature tend to affect oxygen supplies only weakly but oxygen demand from metabolism more strongly. In polar waters, which are extremely cold and thermally stable (Peck 2018) and tend to have high oxygen levels relative to temperate and tropical oceans (Jaccard et al. 2016), metabolic demand for oxygen is very low (because organisms are so cold) and supplies are relatively high (Moran and Woods 2012; Peck 2018). The inference is that oxygen is "easy" to obtain, making the window of feasible body sizes much larger in polar waters than elsewhere in the world. At least some fraction of species have evolved into the upper end of that window, even while others remain small. A recent, novel perspective is that larger body size evolved specifically to help polar ectotherms avoid the toxic effects of oxygen oversupply (Verberk and Atkinson 2013).

More generally, the idea that temperature influences oxygen supply-to-demand ratios has been developed in several other contexts. Deutsch et al. (2015), for example, constructed a simple ratio, φ, in which the numerator is a measure of oxygen supply (concentration) and the denominator is a measure of metabolic demand. By coupling φ to global patterns of ocean temperature and oxygen concentrations, and including additional assumptions about the temperature dependence of supply and demand, they were able to predict that it will be at their equator-ward range edges that marine ectotherms will be most constrained by oxygen and therefore most vulnerable to warming. Jacobsen et al. (2003) developed similar, ratio-based approaches to evaluating oxygen supply-to-demand ratios for high-elevation aquatic insects. Verberk et al. (2011) proposed the "oxygen supply index" (OSI), another ratio-based approach to evaluate supply:demand ratios; the OSI incorporates the joint effects of the solubility, diffusivity, and partial pressure of oxygen. Pörtner (2001) and Pörtner et al. (2017) developed the "oxygen and capacity limited thermal tolerance" (OCLTT) hypothesis, which frames the capacity for aerobic performance as reflecting independent effects of temperature on supply of, and demand for, oxygen. The primary difference between OCLTT and the other ratio-based approaches is that it focuses more explicitly on the internal distribution of oxygen via circulation rather than on external supply via physical processes. Finally, temperature–oxygen interactions have been invoked as a mechanism by which climate warming may result in smaller body sizes via both plasticity and evolution (see the "Discussion" section in Audzijonyte et al. 2019).

Here, we summarize data for and against the oxygen–temperature hypothesis, particularly as it applies to polar gigantism. In effect, this means we are looking for evidence that three-way interactions between oxygen, temperature, and body size affect organismal traits at different levels of biological organization. In practice, many studies

examine two-way interactions lying within the overall three-way framework. For example, they examine interactions between temperature and body size, or between oxygen and body size. Collectively, the data provide significant support for the importance of three-way interactions and for the underlying two-way interactions. But other data appear to be in conflict. After summarizing data for and against, we leverage the conflicting data to develop a refined version of the hypothesis that identifies contexts in which it is more, and less, likely to apply. In particular, we argue that meaningful oxygen–temperature interactions are most likely for taxa and life stages that are geometrically simple and that lack mechanisms for ventilating respiratory surfaces both inside and out. In addition, we develop the idea that even for taxa capable of initiating short-term, emergency measures to ventilate surfaces and relieve oxygen stress, the energetic costs of ventilation may be insurmountably large over longer periods of time. This conclusion suggests that long-term measures of performance will provide more evolutionarily relevant data for evaluating the effects of climate warming on polar marine invertebrates.

Evidence for and against the oxygen–temperature hypothesis

The oxygen–temperature hypothesis provides a framework from which multiple testable predictions can be derived (Table 1). In this section, we briefly summarize key evidence supporting or refuting the predictions; the reader is referred to the cited literature in Table 1 for caveats and fuller discussion of individual studies.

Evidence for the oxygen–temperature hypothesis

Although most specific predictions have not received attention in more than a few studies or focal taxa, the available evidence (Table 1) altogether still provides broad support for the hypothesis. In particular, multiple studies suggest that larger animals allocate more materials and surface area to mechanisms of gas exchange (Lane et al. 2017; Lefevre et al. 2017; Shishido et al. 2019) but that larger animals nevertheless often have lower internal levels of oxygen (Moran and Woods 2007; Peck et al. 2007; Lane et al. 2017). Oxygen also appears to influence upper critical temperatures (CT_{MAX}) of aquatic organisms, and larger-bodied individuals or taxa often but not always have lower CT_{MAX} than do related, smaller-bodied animals (Hutchison et al. 1966; Peck et al. 2007; Verberk and Bilton 2011; Verberk et al. 2013; Di Santo and Lobel 2017). Finally, there is some evidence that whole-animal performance declines in hypoxia or warmer water disproportionately steeply for larger-bodied animals (Peck et al. 2007; Spicer and Morley 2019).

Evidence against the oxygen–temperature hypothesis

By contrast, other predictions from the hypothesis are not supported by data. In Antarctic sea spiders subjected to experimentally decreased levels of oxygen (Woods et al. 2009) or increased temperature (Shishido et al. 2019), whole-organism performance (righting responses) of larger individuals, or larger-bodied species, was not disproportionately depressed. Although this may indicate simply that righting response is a poor proxy for individual performance in this context (Davy et al. 2014), the authors argued that this result reflected that the sea spiders invoked short-term emergency responses, such as increased rates of respiratory gut peristalsis (Woods et al. 2017), or broader size-dependent matching (symmorphosis) of oxygen supply mechanisms to increasing demand at large body sizes. Recent work on Antarctic amphipods (Spicer and Morley 2019) suggests that constraints on body size can be relieved by the evolution of respiratory pigments or changes in gill structure. More broadly damaging to the oxygen–temperature hypothesis is the observation that some large-bodied marine organisms do occur in warm or hypoxic waters. For example, Spicer and Gaston (1999) quote Childress as saying, via a personal communication, that there is no obvious difference in body sizes of midwater fishes and crustaceans across vertical gradients into oxygen minimum zones (OMZs) in the worlds' oceans, in which PO_2 can fall below 1 kPa. Likewise, the very large-bodied jumbo squid, *Dosidicus gigas*, takes frequent, long excursions into the OMZ (Rosa and Seibel 2010). Crustaceans that live within the OMZ frequently show adaptations to low oxygen, including enhanced ventilatory capacity, better oxygen capture from passing water, larger gill surface areas, shorter diffusion distances between water and blood, and respiratory proteins in the blood that capture and concentrate oxygen (Childress and Seibel 1998). These observations all suggest that oxygen–temperature interactions do not represent hard constraints but rather can be overcome by particular combinations of physiology, morphology, and behavior. Recent theoretical work argues more broadly that, among species, oxygen supply capacity generally

Table 1 Predictions derived from the oxygen–temperature hypothesis and studies providing evidence for or against those predictions

	Prediction	Evidence for	Evidence against	Notes
Biogeographic/ evolutionary	Maximum body sizes are larger in aquatic systems with higher oxygen levels or lower temperatures	Chapelle and Peck (1999); Moran and Woods (2012); Rollinson and Rowe (2018b)	See also Moran and Woods (2012) for a list of exceptions, and taxa that show polar nanism (Vermeij 2016)	Vermeij (2016) "[T]he largest animals in all trophic and habitat categories maintain a high body temperature either by producing copious body heat or by living in a warm place." In a large phylogenetic analysis, Rollinson and Rowe (2018b) show that salamanders unable to breathe aerially showed strong relationships between body size and water temperature whereas those that could breathe air did not. See Spicer and Gaston (1999) for interesting critique of Chapelle and Peck (1999) findings.
	Maximum sizes of fossil aquatic organisms are positively correlated with historical levels of atmospheric oxygen	Dudley (1998)	Klug et al. (2015); Vermeij (2016)	
Morphological/ Developmental	Gigantism is more pronounced in taxa or life stages that are geometrically simple or that lack mechanisms for ventilating surfaces inside or out (predictions from this study)	Woods and Moran (2008); Moran and Woods (2010); Marshall et al. (2012)		Little data on gigantism as a function of ventilation capacity
	Large and/or lecithotrophic eggs are disproportionately likely to occur in cold, higher-latitude water	Mileikovsky (1971); Thorson (1950); Moran and Woods (2010); Marshall et al. (2012); Thatje and Hall (2016); Rollinson and Rowe (2018a); Moles et al. (2017)		Consistent with Thorson's rule (see Mileikovsky 1971). Rollinson and Rowe (2018a) show using data on Amphibia that oxygen limitation in warmer water is unlikely to arise in the egg stage *per se* but rather in larvae arising from those eggs.
	Larger-bodied organisms allocate disproportionately large amounts of surface area to structures or mechanisms that support gas exchange	Dexter et al. (2009); Lane et al. (2017); Lefevre et al. (2017); Shishido et al. (2019);		Dexter et al. (2009) study shows ontogenetic patterns within two species of blastoids
	Plastic (i.e., temperature-size rule) or evolved responses of body size to temperature are stronger in water-breathing compared with air-breathing taxa	Forster et al. (2012); Horne et al. (2015); Rollinson and Rowe (2018a, 2018b)		

(continued)

Table 1 Continued

	Prediction	Evidence for	Evidence against	Notes
Physiological	Larger organisms or structures have lower internal levels of oxygen or disproportionately switch to anaerobic metabolism, especially in warmer temperatures	Peck et al. (2002); Moran and Woods (2007); Lane et al. (2017)		
	Larger organisms have lower critical thermal maxima	Hutchison et al. (1966); Peck et al. (2007); Verberk and Bilton (2011); Verberk et al. (2013); Di Santo and Lobel (2017)	Garten and Gentry (1976); Ospina and Mora (2004)	Clark et al. (2017) ontogenetic support in two species but not a third; Verberk and Bilton (2011) show steeper declines in CT_{max} with body size in experimental hypoxia but not normoxia; see Verberk et al. (2016) for excellent review of potential oxygen limitation of thermal limits in arthropods
Performance	Compared with small organisms, large organisms show steeper declines in performance in hypoxia or in warmer temperatures	Peck et al. (2007); Spicer and Morley (2019)	Woods et al. (2009); Shishido et al. (2019)	See Davy et al. (2014) for critical examination of righting responses as proxies for performance and fitness

Cited studies are restricted to those focusing on aquatic systems (both freshwater and marine); substantially more literature is available for terrestrial systems in the context of several predictions (especially about the oxygen- and size-dependence of critical thermal maxima). References for the table: Thorson (1950); Hutchison et al. (1966); Mileikovsky (1971); Garten and Gentry (1976); Chapelle and Peck (1999); Ospina and Mora (2004); Moran and Woods (2007, 2010, 2012); Peck et al. (2007); Woods and Moran (2008); Woods et al. (2009); Dexter et al. (2009); Verberk and Bilton (2011); Forster et al. (2012); Marshall et al. (2012); Verberk and Atkinson (2013); Horne et al. (2015); Klug et al. (2015); Thatje and Hall (2016); Verberk et al. (2016); Vermeij (2016); Clark et al. (2017); Clarke (2017); Di Santo and Lobel (2017); Lane et al. (2017); Lefevre et al. (2017); Moles et al. (2017); Rollinson and Rowe (2018a, 2018b); and Shishido et al. (2019).

evolves to match demand regardless of body size or temperature (Seibel and Deutsch, 2020).

Over deeper evolutionary time scales, patterns of gigantism in space and time also generally are just partially consistent with the idea that oxygen availability drives or constrains body size. Dudley (1998) argues that hyperoxic atmospheres during the late Paleozoic may have facilitated the evolution of giant insects, as well as other large-bodied terrestrial or semi-terrestrial arthropods and amphibians. Klug et al. (2015), however, found no correlation between Paleozoic levels of atmospheric oxygen and the maximum sizes of marine invertebrates. Similarly, Vermeij (2016) argues that such correlations may have occurred only before the Paleozoic and that other processes have shaped gigantism since then. In particular, gigantism may appear in taxonomic groups undergoing high rates of diversification when ecological conditions are good or when environmental productivity is particularly high (Trübenbach et al. 2013; Klug et al. 2015; Vermeij 2016).

An initial revision of the oxygen–temperature hypothesis

The oxygen–temperature hypothesis emerges from considering relationships among metabolic rate, temperature, body size, and oxygen transport in relatively idealized systems, typically with body shapes assumed to be spheres, cylinders, or sheets and with external oxygen transport driven by physical mechanisms like diffusion or passive transport via water flow. These assumptions result in relatively straightforward predictions about the size-scaling of surface areas, the relative effects of temperature on metabolism and oxygen transport (Verberk et al. 2011; Rubalcaba et al., in review), and the maximum possible sizes of body parts and body size (Harvey 1928; Krogh 1941; Lee and Strathmann 1998; Woods 1999). Other recent models focus more explicitly on internal transport of oxygen via circulation (see Pörtner et al. 2017).

The assumptions of simple models can be unrealistic in several ways. Most multicellular organisms, for example, have multiple mechanisms for enhancing oxygen uptake and delivery relative to what diffusion alone can provide. A non-exhaustive list includes increases in surface area, ventilation of external surfaces, changes in surface conductance to oxygen flux, movement away from hypoxia and toward normoxia, respiratory movements that break up surface boundary layers, and concentrating and carrying oxygen in body fluids using respiratory pigments (see Spicer and Morley 2019). These mechanisms can be so effective that even within characteristically hypoxic environments, such as in the ocean's oxygen minimum layer (OMZ), organisms can sustain constant aerobic metabolic rates even in the lowest oxygen concentrations they are likely to experience (reviewed by Childress and Seibel 1998).

Here we focus on two primary mechanisms for enhancing oxygen uptake and delivery. First, many taxa possess specialized surfaces for respiratory gas exchange (e.g., gills), which can be highly in-folded and therefore have surface areas that scale with organismal body size much more steeply than do the surface areas of simple shapes. Such effects have been discussed recently in the literature on fish gills (Pauly 2010; Cheung et al. 2013; Lefevre et al. 2017). In contrast, other taxa lack specialized respiratory surfaces. Sea spiders take up oxygen directly across the cuticle, and cuticle area scales interspecifically to body mass with an exponent of 0.66 (Woods et al. 2009), as expected from isometric scaling. Hyperallometric scaling of pore area in sea spider cuticle (Lane et al. 2017) partially offsets the surface-area constraint but not to the same degree as is possible in taxa with organs evolved specifically for gas exchange.

Second, many taxa evade the slow pace of oxygen diffusion by actively ventilating respiratory surfaces (respiratory organ or whole body surface) inside and out. External ventilation involves driving flows of water past the surfaces participating in gas exchange. Internal ventilation involves driving blood or other internal fluids past the inner sides of gas-exchange surfaces, where it picks up incoming oxygen and transports it to mitochondria within underlying tissues; this type of internal convective movement has been present since the dawn of the Metazoa and is facilitated by a wide diversity of structures and mechanisms (Monahan-Earley et al. 2013). Compared to pure diffusive transport, ventilation and circulation allow organisms to gather oxygen from greater external volumes of surrounding water and to distribute it throughout greater internal volumes of metabolically-active tissue (LaBarbera 1990). It also increases the oxygen gradient driving transport across the respiratory surface, by continuously flushing the external surface with oxygenated water and the internal surface with oxygen-depleted internal fluids (Fig. 1). Internal circulation can also strongly relieve oxygen-based constraints on the evolution of large body size (Krogh 1941).

We thus propose that the oxygen–temperature hypothesis applies most strongly to taxa or life stages

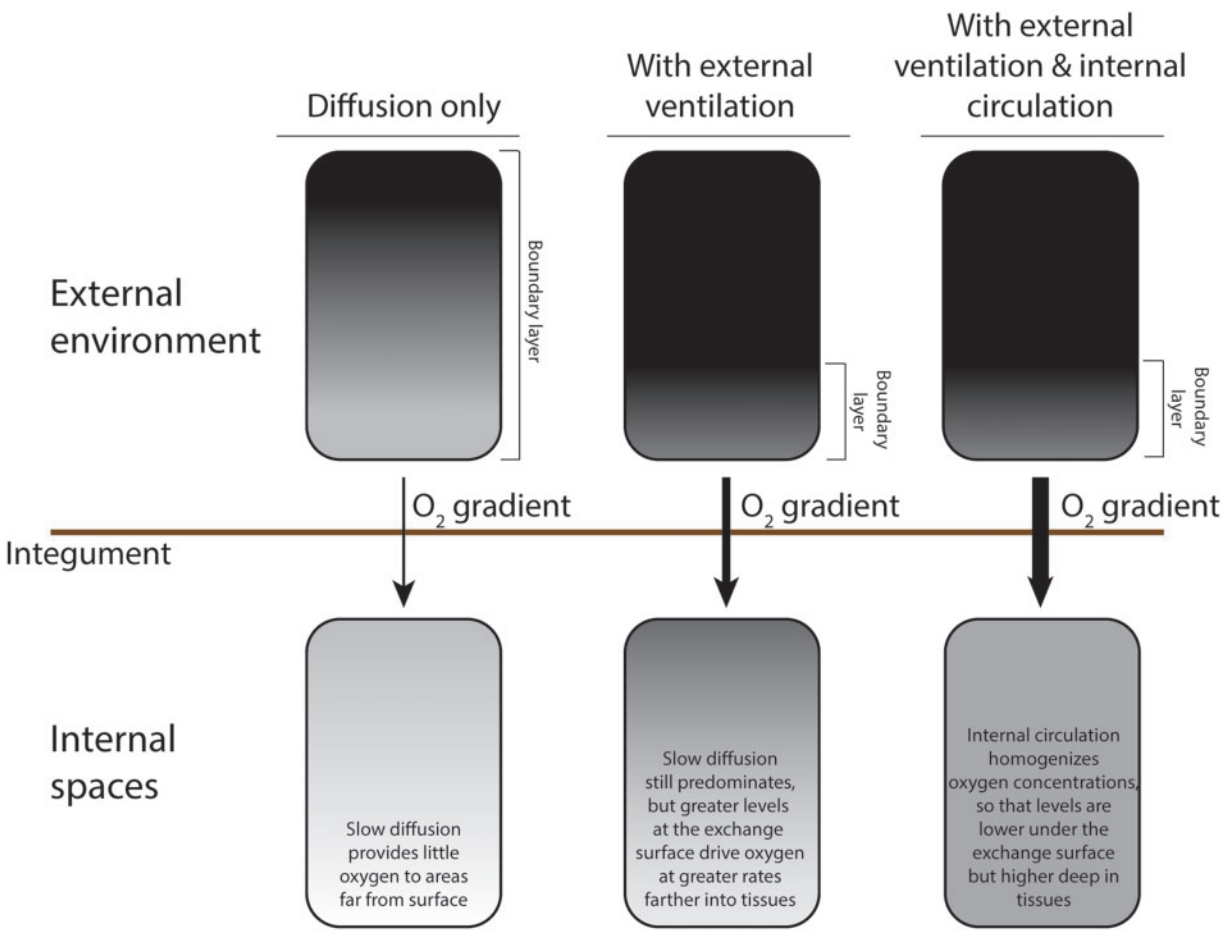

Fig. 1 Effects of external ventilation and internal circulation on oxygen delivery into an aquatic organism. In all three cases, the oxygen concentration is the same far away from the organism (top of the top row of rectangles). If both external delivery is by diffusion alone, an external oxygen gradient will develop across boundary layers. Likewise, oxygen gradients will develop within the organism. As a result, the oxygen gradient across the integument will be very small and rates of oxygen uptake will be correspondingly low. With external ventilation, oxygen-depleted water next to the integument is constantly refreshed with well oxygenated water originating far from the organism, substantially increasing the driving gradient for transport and also raising the local oxygen concentration under the integument. With the addition also of internal circulation, oxygen-depleted body fluids (blood or hemolymph) are circulated under the integument, which further magnifies the driving gradient and thereby overall rates of oxygen transport.

that have geometrically simple respiratory surfaces or that cannot ventilate respiratory surfaces internally or externally (see also Verberk and Atkinson 2013). Which organisms do these conditions describe? It is true that adults of many taxa have simple body forms—spheroidal ctenophores, cylindrical sea spiders and nemerteans, and planar flatworms—that also lack surfaces specialized for gas exchange (they lack gas-exchange organs). They may also, like sea spiders, lack specialized mechanisms for driving external flows past body surfaces. One piece of supporting evidence comes from adult amphibians. In species without lungs, adult body sizes decline with ambient water temperature whereas in species with lungs they do not (Rollinson and Rowe 2018b).

Nevertheless, even taxa with simple shapes can enhance oxygen uptake. The giant Antarctic nemertean worm *Parborlasia corrugatus*, for example, flattens its body in low PO_2, which shortens the distance over which oxygen must diffuse (Davison and Franklin 2002). Many simply-shaped organisms also drive external flows with cilia or muscular contractions of the body wall. For example, the collective flagellar beating by individuals in colonies of unicellular organisms can allow them to escape the "tyranny of transport by diffusion" (Solari et al. 2007; Butterfield 2018). In corals (phylum Cnidaria, class Anthozoa), cilia stir the boundary layer up to 2 mm above the epidermis, which enhances delivery of oxygen by 400% relative to diffusion alone (Shapiro et al. 2014). Similarly-shaped members of another phylum, the ctenophores, swim using "comb rows" made of thousands of fused cilia, which create enough water flow that surface gas

exchange is dominated by advection rather than diffusion (Butterfield 2018). Likewise, with the exception of taxa lacking a circulatory system (e.g., *Planaria*, which nevertheless have highly branching guts), most animals have ways to generate internal flows through respiratory organs or past body surfaces (Monahan-Earley et al. 2013). Such mechanisms include: hearts, which are muscularized parts of the circulatory system that drive hemolymph or blood past gas exchange structures and throughout the body; ciliary or muscular movement of fluid within coelomic compartments in the body; or, as in many echinoderms, ciliary movement of coelomic fluid into and out of thin-walled structures (papulae and tube feet) that extend through the body wall. Even sea spiders, which cannot drive external flows, use both heart-driven flows centrally and gut peristalsis distally to distribute oxygen via flows of hemolymph (Woods et al. 2017). In conclusion, adults of most taxa can ventilate at least one side of their exchange surfaces, and often both, and thus may often be able to evade oxygen insufficiency even if they are large bodied or live at warm temperatures.

Most unicells, including those with sophisticated morphologies, physiologies, and behaviors, do not have truly effective mechanisms for ventilating surfaces (although those with cilia or flagellae may do so, or may be able to move to locations of higher local oxygen levels; see Solari et al. [2007] for an interesting discussion of the roles of ventilation in evolutionary transitions between uni- and multicellularity). Unicells typically also lack mechanisms for high-speed internal transport, although cytoplasmic streaming may effectively distribute metabolites and oxygen throughout cellular spaces (Hochachka 1999). Once every generation, all sexual lineages are reduced transiently to a state of unicellularity—in the form of a zygote that lacks cilia or flagellae as well as the sensory, morphological, or muscular machinery needed for driving external and possibly internal flows (Woods 2020). That flow machinery becomes functional only gradually through development, and often continuing to emerge well into the juvenile phase. The diverse, ubiquitous mechanisms used by metazoans to enhance oxygen uptake lead us to predict that the oxygen–temperature hypothesis will apply most strongly to organisms or life stages that lack such mechanisms. These include early embryos, organisms embedded in other organisms (parasites), or embryos and larvae embedded in gelatinous matrices, such as the egg masses of nudibranchs and worms. These may also include early juvenile stages that have significantly higher metabolic rates while still not possessing fully-developed adult-like systems (Rollinson and Rowe 2018a).

Two paradoxes and their potential resolutions

The revised hypothesis suggests, paradoxically, that it is the smallest taxa (unicells) and life stages (zygotes, embryos) that are disproportionately prone to oxygen–temperature interactions, even though they have the lowest overall demand for oxygen and the largest surface-area-to-volume ratios for oxygen uptake. Are such small structures ever constrained by oxygen insufficiency, regardless of size and temperature? Second, the revised hypothesis suggests that adults—by ventilating surfaces, driving circulatory flows, or evolving other oxygen handling mechanisms such as respiratory pigments—should be able to evade oxygen insufficiency. Yet it is global patterns of *adult body size*, and in particular the observation of giants at the poles, that has motivated much of the discussion of the oxygen–temperature hypothesis.

Does the oxygen–temperature hypothesis apply to eggs and embryos?

Here, we further decompose this question into two parts. First, is there evidence that eggs or embryos of aquatic taxa are ever limited by oxygen, and, if so, in a size- or temperature-dependent way? Second, is there evidence for polar gigantism of eggs and embryos?

Evidence for oxygen limitation in embryos is clearest from studies on species that encapsulate eggs or lay egg masses (Chaffee and Strathmann 1984; Booth 1995; Seymour and Bradford 1995; Cohen and Strathmann 1996; Lee and Strathmann 1998; Woods 1999; Fernández et al. 2002; Moran and Woods 2007; Woods and Podolsky 2007). Multiple studies have shown that oxygen levels in these structures can fall far below ambient values, and usually are lower as embryos advance through development (and have higher metabolic rates). Low oxygen can delay embryonic development (Chaffee and Strathmann 1984; Moran and Woods 2007), and higher experimental temperatures or lower external flows exacerbate oxygen shortages (Cohen and Strathmann 1996; Lee and Strathmann 1998; Woods and Moran 2008; Moran and Woods 2010). Solutions abound; eggs can be actively ventilated by parents (Fernández and Brante 2003), many egg masses are laid as thin ribbons or sheets (Hurst 1967) that minimize diffusion distances, and in other species

the capsule or egg mass structure changes to accommodate higher oxygen fluxes toward the end of development (Seymour 1994; Seymour and Bradford 1995; Lee and Strathmann 1998; Mills and Barnhart 1999; Mueller et al. 2011). Larvae in egg masses of arenicolid polychaete worms provide another example. Once larvae become mobile, they swim through the gel of the egg mass and position themselves around the periphery where oxygen availability is higher (Strathmann 2005).

What about oxygen limitation in eggs and embryos that are free living, that is, not protected by layers of capsule or gel or aggregated into masses? Detailed studies of this question have been carried out by Kranenbarg et al. (2000, 2003), who developed diffusion models to understand oxygen fluxes to and within fish embryos. Typically, fish embryos do not have active blood flow in their circulatory systems until about 40% of the way through development. Before that point oxygen moves through embryonic tissues by diffusion only. The authors' numerical models indicated that maximum diameters of fish embryos are about 0.4–1.8 mm in flowing water and 0.2–1.4 mm in stagnant; as elucidated for egg masses, sizes are smaller in stagnant water because oxygen is impeded from moving to the embryo surface by external boundary layers. The actual diameters of fish embryos were in the range of 0.28–0.72 mm, which is smaller than the predicted maxima but still close to sizes that might result in oxygen limitation. Additional data obtained by pushing oxygen electrodes through zebrafish embryos showed that even over distances of about 0.5 mm, oxygen levels could drop in excess of 5 kPa. As fish embryos, including zebrafish, continue to develop, metabolic demand by embryonic tissues eventually rises high enough that diffusive supply alone becomes inadequate, at which point the embryonic cardiovascular system starts to transport oxygen by convection. These results are broadly consistent with others studies showing that even modestly lowered levels of oxygen in water can depress rates of development and metabolism in fish (Alderdice et al. 1958; Garside 1959; Hamor and Garside 1976; Gruber and Wieser 1983; Mueller et al. 2011) and amphibians (Mills and Barnhart 1999). These results are inconsistent, however, with other work on salmonid eggs (Einum et al. 2002; Rombough 2007) showing that large eggs fare better in hypoxia than do small eggs and that embryonic metabolic rates increase more slowly with size than does egg surface area.

Although developmental information is lacking for most Antarctic invertebrates, enough data exist to suggest that, within taxa, Antarctic embryos generally have much larger egg sizes than their temperate relatives (Moran and Woods 2010; Peck 2018; Figs. 2 and 3). For example, the largest molluscan embryo, over 4 cm long, was recently described from the Antarctic nudibranch, *Bathydoris hodgsoni* (Moles et al. 2017). The metabolic rates of large Antarctic eggs and larvae are unknown for most species, but their sizes are similar to or larger than the fish eggs and embryos studied by Kranenbarg et al. (2000, 2003). Another database of georeferenced embryo sizes published by Marshall et al. (2012) reveals patterns that are consistent with predictions from the oxygen–temperature hypothesis (Fig. 4). In particular, they plotted embryo size as a function of absolute latitude, with embryos divided into three developmental types: feeding planktonic, non-feeding planktonic, and aplanktonic. Embryo sizes differed systematically across developmental type, with feeding planktonic being the smallest (mean diameter approximately 0.1 mm, with range 0.05–0.3 mm), non-feeding planktonic intermediate (mean diameter ~0.4 mm, range 0.05–1.5 mm), and aplanktonic the largest (mean ~1 mm, range 0.1–3.5 mm). These differences among developmental types likely reflect where the nutrients and energy come from to support development; in non-feeding

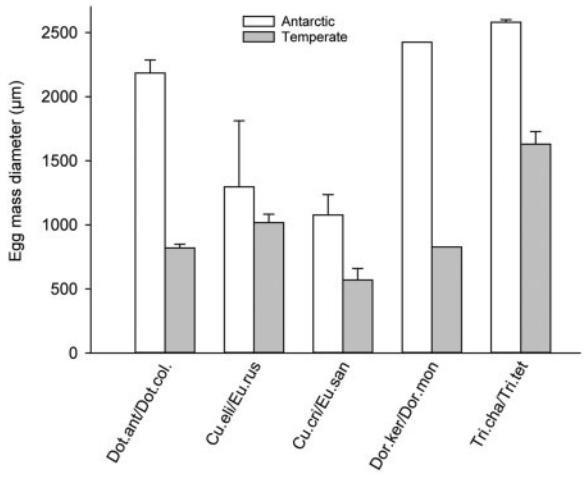

Fig. 2 Egg mass widths of Antarctic and temperate nudibranch species paired by phylogenetic relatedness, as determined from COI barcoding of egg masses and adults collected by the authors. Sheet-like masses were measured across the narrow width of the sheet; spheroid or strand-like masses were measured across the short diameter of the spheroid or strand. Error bars are standard errors from the width of n egg masses. Sample sizes were $n = 7$ for *Doto antarctica*, 4 for *Doto columbiana*, 3 for *Cuthona elioti*, 6 for *Eubranchus rustyus*, 5 for *Cuthona crinita*, 3 for *Eubranchus sanjuanensis*, 1 for *Doris kerguelenensis*, 1 for *Doris montereyensis*, 9 for *Tritonia challengeriana*, and 4 for *Tritonia tetraquita*. Data from Woods and Moran (2008), Moran and Woods (2010), and the authors' unpublished data.

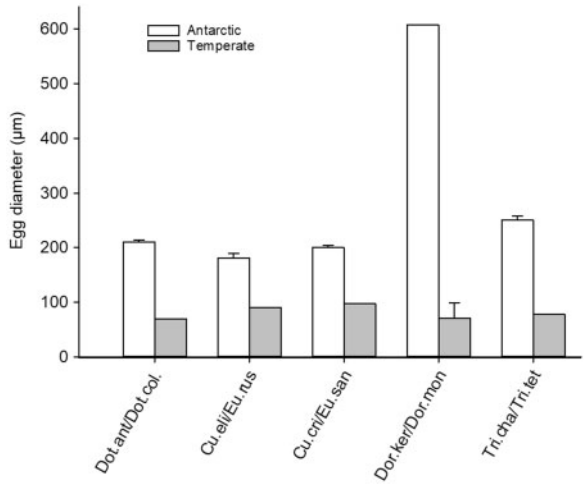

Fig. 3 Egg sizes of Antarctic and temperate nudibranch species paired by phylogenetic relatedness, as determined from COI barcoding of egg masses and adults collected by the authors. Size was measured as the shortest diameter across an egg or early-stage embryo. Error bars are standard errors of the mean from *n* egg masses. Sample sizes were $n = 7$ for *D. antarctica*, 1 for *D. columbiana*, 3 for *C. elioti*, 1 for *E. rustyus* (egg size reported in Goddard 1984), 5 for *C. crinita*, 1 for *E. sanjuanensis*, 1 for *D. kerguelenensis*, 1 for *D. montereyensis*, 15 for *T. challengeriana*, and 4 for *T. tetraquita*. Except where noted, measurements are from the author's unpublished data, largely taken from the same egg masses as in Fig. 2.

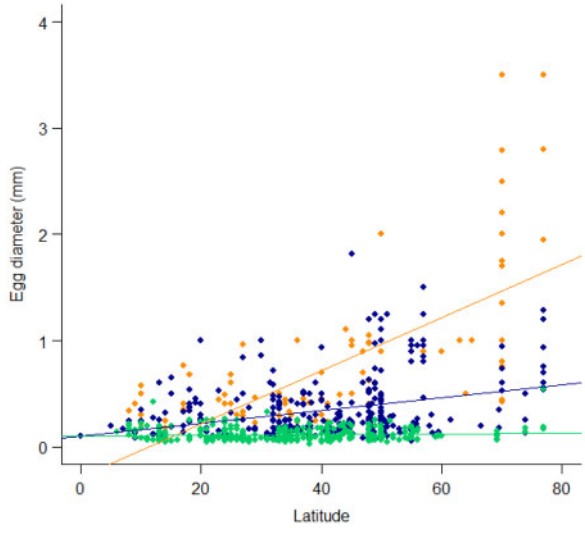

Fig. 4 Egg diameters ($N = 992$) as a function of absolute latitude from five phyla of marine invertebrates, replotted from Supplementary Data provided by Marshall et al. (2012). Species are coded by developmental mode—with green denoting feeding planktonic larvae, blue denoting nonfeeding planktonic larvae, and orange denoting aplanktonic (nonfeeding) larvae. Lines are ordinary least squares, and all groups show a significant positive relationship with latitude. See Marshall et al. (2012) for additional analysis and discussion of these results.

types, those have to be put into the egg by the mother. There are, however, significant increases in egg size with latitude in all three developmental types, and those increases are steepest in the largest size-class, the aplanktonic eggs. In other words, in the developmental class most likely to be oxygen-limited (because of large overall size), the latitudinal variation is greatest—likely reflecting that the largest embryos (in excess of 3 mm diameter) are possible only in very cold, polar waters. There are numerous hypotheses about the mechanisms underlying geographic patterns in offspring size (Peck 2018), of which increased oxygen availability is only one. Nevertheless, the implication of the data and models described above is that eggs and embryos may be oxygen limited, at least for some taxa in some ecological contexts. Missing in most cases is convincing experimental evidence that larger embryos are more likely than smaller ones to be limited by oxygen, that embryos in general are more oxygen limited when temperatures rise, or that larger embryos are disproportionately affected by high temperatures.

To put the egg–oxygen problem in context, we consider two broader ideas that emerge from recent literature. The first focuses on the stage-specificity of potential oxygen constraints during ontogeny. In a study of egg sizes in relation to habitat temperature of several hundred species of Anura (frogs and toads) and Caudata (salamanders), Rollinson and Rowe (2018a) showed that egg diameters decreased consistently with increasing habitat temperatures for species with aquatic larvae but *increased* with temperature for species with terrestrial larvae or no larvae (direct developing). In addition, within the subset of species having aquatic larvae, the negative slope of egg diameter on habitat temperature was the same regardless of whether eggs were laid in water or on land. Based on these patterns, the authors suggest that oxygen constraints indeed shape the evolution of egg size—but not directly via oxygen shortage in the egg stage *per se*; rather, oxygen shortages arise most critically in the subsequent larval stages, in which warm water selects for smaller individuals. Although compared with the eggs from which they came, larvae typically have access to more sophisticated behaviors and physiological systems—they still are very small, live in aquatic worlds dominated by viscous forces and relatively thick boundary layers, and often have much higher metabolic demand for oxygen than they did as an embryo. These ideas about relative oxygen constraints in egg versus juveniles have yet to be applied to polar marine invertebrates.

A second, very different interpretation of Antarctic gigantism among eggs, embryos, and egg masses is that it may have evolved to help maintain internal levels of hypoxia required for normal development. Evidence that regional hypoxia is important in embryos comes largely from mammals, in which embryonic success is higher under the comparatively hypoxic conditions in the uterus (in the range of 1.5–8%, Ma et al. 2017); and from *Drosophila*, in which hypoxic microenvironments inside developing embryos regulate the differentiation of stem and progenitor cells and are essential to the development of the tracheal system (reviewed in Simon and Keith 2008), which is the main mechanism for oxygen uptake and distribution. If the same is broadly true for other, non-model Metazoa, then the large size of eggs, embryos, and egg masses of invertebrates in the Southern Ocean may reflect selection to *limit* diffusive supply and maintain key hypoxic microenvironments in developing stages. However, one might expect selection for hypoxia in early stages to be reduced compared with warmer-water regions, because polar larvae and juveniles might not need fully-developed or elaborate mechanisms for oxygen uptake and transport where oxygen supply is high and demand is comparatively low.

Hampering our ability to test this opposing hypothesis is that almost nothing is known about internal embryonic oxygen levels or their roles in development of any marine invertebrates, let alone polar ones. If the "maintaining hypoxia" hypothesis is correct, however, we predict that (1) internal levels of oxygen should be similar within eggs and embryos of related invertebrate taxa across broad geographic temperature gradients, (2) the sizes of eggs, embryos, and egg masses should be optimized for maintaining these internal levels while allowing in sufficient O_2 to support development and metabolism, and/or (3) polar larvae and juveniles should have comparatively less developed mechanisms for oxygen uptake and transport than embryos from warmer regions. For egg masses in particular, the prediction is that their size and shape should reflect the need to deliver sufficient oxygen to internal embryos for development, while maintaining developmentally important levels of hypoxia in embryos. Egg masses of Antarctic nudibranchs were indeed much larger than the masses of their temperate relatives (Moran and Woods 2010). Oxygen levels, however, were also higher in the Antarctic masses, suggesting they were "overconstructed" for oxygen supply; that is, they could have been considerably larger before internal oxygen levels were reduced to the levels routinely reached in temperate relatives. One (speculative) interpretation is that Antarctic embryos may need higher oxygen overall than temperate ones (Moran and Woods 2010); another (even more speculative) possibility, however, is that optimal egg mass size is not set by oxygen supply *per se*, but instead by optimization of diffusive oxygen transport to balance embryonic requirements for oxygen with the need for essential areas of hypoxia inside embryos in the mass. Under this scenario, if larvae and juveniles of Antarctic species do not need highly developed gas exchange systems (because the high ratio of oxygen supply to demand), then Antarctic masses could "tolerate"—and benefit from—higher levels of oxygenation than egg masses of their warmer-water relatives.

Does the oxygen–temperature hypothesis apply to juveniles and adults? The energetic costs of ventilation

The second paradox is that adult organisms usually can solve oxygen problems, at least in the short term, by driving flows of water and of blood (or hemolymph) past external and internal surfaces, respectively (see Fig. 1). If so, how could oxygen–temperature interactions ever drive global patterns of body size? Two potential resolutions are that external ventilation and internal circulation both incur energetic costs that become unsustainable if used over long periods of time and that larger organisms may pay proportionately smaller metabolic costs to drive flows, especially in polar waters. A related issue is that organisms may be able to sustain metabolic energy supplies by switching partially to anaerobic processes; although such measures may be sustainable for hours or a few days, the accumulation of anaerobic end products may limit their longer-term utility (Peck et al. 2002; Verberk et al. 2013). We examine these problems by briefly summarizing empirical data on costs and then by examining their size- and temperature-dependence.

Costs of ventilation have been controversial because they are difficult to measure independently of other processes, including movement, internal circulation, and digestion (Jørgensen et al. 1986). In general, the medium—air or water—affects costs significantly, as water is much denser, has higher viscosity, and typically contains much lower concentrations of oxygen (Kramer 1983). Using fishes engaged in water breathing, depending on species, method, and assumptions, different research groups have estimated costs as low as a few percent of the total metabolic rate up to 40% or more, with a consensus range of 5–15% (Holeton 1980). As a

percentage, costs typically are higher whenever environmental oxygen availability is lower or activity or temperature is higher (Kramer 1983; Fernandes and Rantin 1994), with the exception that costs can sometimes be lowered, for example, by switching from branchial to ram ventilation as swimming speeds increase (Farrell and Steffensen 1987). In marine invertebrates, costs are less well known but may be lower, reflecting lower levels of activity, lower mass-specific metabolic rates, and alternative ways of pumping water. In cuttlefish, costs of ventilation were quite low (<1.5% of resting metabolic rate, r.m.r.) within the species' typical temperature range and rose to 8.6% of r.m.r. at warmer temperatures (Melzner et al. 2006). In general, the costs of driving flow with cilia appear low (Jørgensen et al. 1986). One associated problem arises for filter feeders, which often use cilia to drive flow. In these taxa, water flow has the dual functions of ventilating surfaces and carrying small food particles to the filters—that is, in filter feeders, water flows and therefore pumping costs can be much higher than needed strictly for oxygen delivery (Riisgård and Larsen 1995); the costs of ventilation *per se* may amount to a small fraction of the overall pumping costs. Likewise for internal circulation of fluids: a detailed analysis of rainbow trout, *Salmo gairdneri*, estimated that the costs of the cardiac pump amounted to <5% of the total metabolic rate at rest to <2% at higher swimming speeds (Farrell and Steffensen 1987).

In summary, costs of ventilation and circulation typically are small when viewed as a percentage of overall metabolic expenditures, but they may nevertheless be large enough to impact energy budgets when paid over extended periods of time.

Missing from these point estimates of costs, however, is a framework for predicting how costs vary as functions of body size and temperature. Increasing body size clearly affects multiple aspects of oxygen supply and demand. Mass-specific metabolic rates (metabolic density) typically decline with size, as do ratios of surface-area-to-volume across which respiratory gases are exchanged. Cardiac output typically scales like overall metabolic rate, with an exponent near 0.75 (Peters 1983; Calder 1996). In addition, larger animals dissipate energy relatively less rapidly in their vascular networks (West and Brown 2005), so that from a hydrodynamic perspective larger animals pay lower relative costs to circulate fluids. More generally, larger-bodied organisms live in worlds characterized inside and out by higher Reynolds numbers (Verberk and Atkinson 2013), meaning that viscous damping forces become increasingly weak compared with the inertial forces that keep fluids moving. Larger organisms likely therefore spend proportionately less energy moving external and internal fluids.

Like body size, temperature also has multifaceted effects on oxygen supply and demand, and potentially on the costs of moving fluids. In polar waters, metabolic demand typically is low and external concentrations of oxygen relatively high. However, cold water is also more viscous, which makes driving external flows more difficult, especially for small-bodied organisms. The viscosity problem may also play a role in internal rates of liquid circulation (blood and hemolymph), although this problem is potentially offset by higher solubility of oxygen in these fluids (Mark et al. 2002). Indeed, some Antarctic fishes have lower costs of circulation because they can dispense with red blood cells entirely, which contribute significantly to blood viscosity (Davison et al. 1997).

Large body size among polar ectotherms may thus reflect the joint influences of oxygen and temperature in two distinct ways. Large size is *possible* because cold temperatures significantly elevate the ratio of oxygen supply to demand (Woods 1999; Moran and Woods 2012). This conceptual framework assumes that oxygen is transported by diffusion alone, whereas in reality, as summarized above, this assumption applies to only a limited number of morphologies, taxa, and life stages. Most metazoans violate the assumptions of the oxygen–temperature hypothesis by driving external or internal flows, building complex respiratory surfaces, deploying oxygen-carrying pigments, etc. These mechanisms for enhancing oxygen uptake and transport incur substantial energetic costs if deployed over long periods, diverting energy, which could be used for other fitness-enhancing functions, to respiratory functions like building, maintenance, and ventilation of gas exchange surfaces. Organisms can avoid many of these costs by simply not ventilating, but we suggest these cost-savings may be possible only in cold, highly-oxygenated waters. Thus, paradoxically, short term experiments testing the oxygen–temperature hypothesis may find no support for it, even though in the long term, large body size is in fact enabled by cold temperatures.

Second, when organisms *must* drive flows (e.g., during seasonal increases in temperature or increased levels of locomotion), larger animals may have a proportional advantage because they naturally experience higher Re and pay proportionately smaller energetic costs to drive flows. From this perspective, cold, polar waters may promote the evolution of

large size as a way to minimize relative energy costs. By contrast, large size is difficult to evolve in warmer waters because diffusion alone never suffices; large, warm organisms always have to pay the costs of driving external and internal flows. Interestingly, some marine giants do live in warm water (McClain et al. 2015): *Tridacna gigas*, the largest bivalve that ever lived, inhabits shallow tropical seas; the largest poriferan is *Xestospongia muta*, the Caribbean Barrel Sponge; and the largest shelled gastropod, the Australian Trumpet (*Syrinx aruanus*) inhabits the tropical Indo-Pacific. In warmer waters, giants may evolve mostly under conditions of high food availability in which large size and comparatively high metabolic rates confer selective advantages in acquiring resources (Vermeij 2016). In this situation, the costs of meeting the long-term oxygen demands of a larger body are compensated by the additional energy that larger organisms can acquire. The high frequency and dramatic scope of gigantism in polar regions may reflect that in a cold, highly oxygenated environment, there are fewer energetic barriers to selection for larger size. A productive way to analyze this idea would be to construct both meta-analyses and models of the size- and temperature-dependence of the costs of driving external and internal flows.

Conclusion

Across Metazoa, the evolution of large body size has gone hand-in-hand with the emergence of more complex, powerful mechanisms for enhancing oxygen uptake into and transport within the body. Thus, it is perhaps not surprising that even for the largest-bodied organisms, it is difficult to experimentally demonstrate links between body size and oxygen limitation. In most environments, it may instead be most productive to look for this pattern in early life history stages, or in the comparatively small number of organisms or life stages that depend on oxygen diffusion alone. A key observation, however, is that in later life-history stages, the mechanical and physiological mechanisms used to obtain and distribute oxygen incur energetic costs. Although costs may typically represent a small fraction of the overall energy budget, their cumulative impacts over time may be significant—a worrisome observation given that most global change models predict that marine and aquatic organisms will be subjected to both higher temperatures and lower oxygen availability in the future.

Compared with warm-water regions, the cold, oxygen-rich water of the Southern Ocean may allow gigantism both because organisms live in conditions promoting higher ratios of oxygen supply-to-demand and because, under those conditions, the energetic costs of both short- and long-term mechanisms for enhancing oxygen supply are lower for any given mechanism or body size. These energetic savings can then be allocated to other fitness-related processes, enabling the evolution of greater body size in environments where large size is a selective advantage. These ideas could be tested in several ways: (1) directly, by comparing the energy required for ventilation of surfaces in related, similarly-sized organisms from tropical versus polar environments, and (2) indirectly, by comparing similarly-sized animals within taxa to see whether animals from cold environments have fewer or less elaborate mechanisms for gas exchange. Finally, a particularly valuable path will involve shifting from measuring responses to acute or short-term manipulations of temperature and oxygen levels to carrying out much longer-term experiments that focus on assessing performance and fitness in the novel conditions we expect to occur during climate change. Doing so will be logistically and financially challenging, but such experiments will be essential if we are to understand both the fundamental processes shaping the evolution of body size and the opportunities and risks of climate change.

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