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Integrative approaches to understanding organismal responses to aquatic deoxygenation

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For *Biological Bulletin*

Integrative approaches to understanding organismal responses to aquatic deoxygenation

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Key words: acclimation, acidification, energetics, estuary, evolution, homeostasis, hypercapnia, hypoxia, metabolism, oxygen, oxygen minimum zone, plasticity, temperature, warming

Abstract

Oxygen bioavailability is declining in aquatic systems worldwide due to climate change and other anthropogenic stressors. For aquatic organisms, the consequences are poorly known but are likely to reflect both direct effects of declining oxygen bioavailability and interactions among oxygen and other stressors, including two – warming and acidification – that have received substantial attention in recent decades. Here, we briefly summarize three unifying themes that underlie this special issue dedicated to the multifaceted effects of oxygen. First, evidence for natural and anthropogenic changes in aquatic oxygen levels: Although organisms are the result of long evolutionary histories during which they were exposed to natural oxygen regimes, superimposed anthropogenic change is now exposing them to more extreme conditions and novel combinations of low oxygen with other stressors. Second, we identify behavioral and physiological mechanisms that underlie the interactive effects of oxygen with other stressors. This synthesis leads to the conclusion that metabolism and energetics provide a powerful and unifying framework. Declining oxygen levels and the other stressors alter not only the bioavailability of oxygen, but also energy requirements and hence metabolic demand for oxygen to cope with stressors, with consequences for how energy as a limiting resource is allocated among competing processes. Third, we assess the range of potential organismal responses, which occur across all levels of biological organization and over multiple time scales. These include rapid behavioral and physiological responses, which can occur on timescales of seconds to minutes, as well as longer-term responses, including acclimation (days-weeks), plasticity (weeks - months), and evolution (years). We conclude by outlining a set of approaches for maximizing the effectiveness of future work, including better reporting of experimental conditions and units, focusing more on long-term experiments using biologically realistic variation in experimentally manipulated factors, and taking truly cross-disciplinary and integrative approaches to understanding and predicting future effects.

Introduction

To humans, the detrimental effects of lack of molecular oxygen (hereafter oxygen) are obvious, as encapsulated by the American Lung Association's adage: "When you can't breathe - nothing else matters" (American Lung Association, 2013). In terrestrial habitats, oxygen typically occurs at high concentrations in a medium (air) that is easily renewed or mixed, and from which oxygen can be obtained relatively easily. In aquatic habitats, by contrast, oxygen is dissolved at low concentrations in a dense, viscous medium (water) from which it is more difficult to extract. In these freshwater and marine systems, oxygen bioavailability can be low and variable, requiring sophisticated morphologies, physiologies, and behaviors for extracting it at rates high enough to support aerobic metabolism.

It is concerning, therefore, that oxygen concentrations in lakes and oceans have declined on average by about 2% in the past 50 years (Schmidtko *et al.*, 2017; Jane *et al.*, 2021), with declines of up to 7% predicted by 2100 (Keeling *et al.*, 2010). Declines are even greater in some parts of the ocean (Schmidtko *et al.*, 2017; Levin, 2018), and extreme hypoxic events are occurring more frequently (Hughes *et al.*, 2020). Here, we use the term *hypoxia* to describe partial pressures of oxygen (PO_2) that are generally lower than 21 kPa, which is the PO_2 of air-saturated water at sea level. Other, more specific meanings of hypoxia may be appropriate in other contexts (Vaquer-Sunyer and Duarte, 2011).

In a recent meta-analysis, Sampaio et al. (2021) wrote "[C]urrent global change-related research efforts should pay far more attention to the role of oxygen concentration as a stressor." Indeed, although two other climate-related stressors – warming and acidification – have received more attention in recent years, the biological effects of deoxygenation may be even more substantial but are rarely addressed. Their meta-analysis (Sampaio *et al.*, 2021), for example, suggests that realistic changes in oxygen availability often have larger effects on biological performance traits, including growth, development, survival, metabolic rates, and reproduction, than do realistic changes in temperature and pH. Likewise, oxygen has more power to explain patterns of species diversity than other conditions (e.g.temperature or CO_2/carbonate chemistry) in coastal ecosystems (Sperling *et al.*, 2016) and high altitude streams (Jacobsen *et al.*, 2003). Moreover, deoxygenation interacts with warming, acidification, and other abiotic and biotic stressors (Reddin *et al.*, 2020). Unfortunately, potential interactions have been understudied, despite widespread recognition that they are important in aquatic ecosystems (Ormerod *et al.*, 2010; Hewitt *et al.*, 2016; Rillig *et al.*, 2019).

This article serves three purposes. First, it introduces the symposium volume. Collectively, the symposium articles examine responses of a diverse set of aquatic systems to declining levels of oxygen. The studies focus on different levels of biological organization and use multiple approaches, including experimental manipulation of oxygen availability in the lab, longer-term observations of correlations among oxygen levels with the distribution and abundance of populations in nature, and historical studies of deep-time correlations among fossil communities and ocean conditions reconstructed from biogeochemical records. Rather than summarizing individual efforts here, we attempt to integrate them into a cohesive perspective that illuminates links among symposium papers and to other recent research.

Second, the article takes an animal-centered point of view. Such a focus facilitates examining mechanistic interactions among deoxygenation and other stressors. It also promotes studying links between temporal and spatial scales of environmental change across a large suite of critical organismal responses, which range from movement to avoid hypoxic zones to the modification of phenotypes via plasticity (Piersma and van Gils, 2010). Ultimately, it is these multiple organism-level effects of deoxygenation that scale up to drive higher-level population and ecosystem effects.

Third, we identify priorities for future research. Like other recent studies (Reddin *et al.*, 2020; Gruber *et al.*, 2021), we call for more research on interactions among deoxygenation (both external and internal) and other stressors. These should include tests of acute environmental hypoxia, but we especially call for longer-term manipulations of oxygen and potential interactors that more closely approximate the magnitudes, variability, and durations of exposure that individuals and communities are experiencing in nature. Such efforts will be time- and resource-intensive yet they are essential to understanding the spectrum of potential responses at timescales relevant to organisms and populations.

Temporal and spatial scales of environmental change

In both lakes and oceans, oxygen levels can be highly variable in space and time, driven primarily by patterns of circulation, solubility, ventilation, photosynthesis, and respiration (Jane *et al.*, 2021; Pitcher *et al.*, 2021; Stramma and Schmidtko, 2021). It is important to distinguish natural patterns of variation – with which populations may have long ecological or evolutionary histories – versus anthropogenic change, which is superimposed onto these natural patterns.

Natural patterns of oxygen variability. Natural processes drive important patterns of oxygen variability in time and space in ways that are often predictable. Changes in surface wind velocity, for example, can trigger upwelling events that transport nutrient-rich, less-oxygenated water from depths to the surface, sometimes causing surface waters to become hypoxic in a matter of hours (Roegner *et al.*, 2011) or stimulating primary production of organic matter whose decay consumes oxygen over days to weeks. Reef structures can restrict flow dynamics, hindering oxygen replenishment and creating steep oxygen gradients on the scale of meters (Hughes *et al.*, 2020). Another major predictable kind of variation is light-driven fluctuations of photosynthetic oxygen production and consumption that occur in almost all shallow aquatic systems (Nelson & Altieri, 2019; Pacella et al., 2018; Saderne et al., 2019; Lucey et al, this issue); oxygen levels can become hyperoxic during the day and hypoxic at night and this fluctuation is amplified in warm, vegetated, or nutrient-rich conditions. Light-driven photosynthetic oxygen production in aquatic systems can, therefore, be broadly predicted from a combination of factors like latitude, depth, and turbidity. Low-oxygen areas can also persist for long durations over both small spatial scales (e.g., low oxygen conditions encountered by aquatic animals living in organic sediments) and very large spatial scales (e.g., profundal zones of stratified lakes and oxygen minimum zones in oceans).

Anthropogenic changes in oxygen availability. Superimposed on the variation described above are additional effects stemming from human activity, which can exacerbate exposure to low oxygen. Agricultural runoff, for example, increases nutrient levels in streams, lakes and coastal areas. These increased nutrients cause algal blooms, which in turn can cause oxygen minima when the algae die off (Stramma *et al.*, 2012). The extent of 'dead zones' stemming from anthropogenic nutrient inputs are increasing over time and are distributed worldwide (Breitburg *et al.*, 2018).

On longer time scales, total oxygen content is declining and distributions are shifting in oceans and lakes. As we continue to release carbon dioxide and warm the planet, lakes and oceans hold less oxygen due to lower solubility of oxygen in warmer water. Additionally, they become more thermally stratified, and therefore oxygen replenishment is reduced (Reinhard and Planavsky, 2022). Rising temperatures also stimulate metabolic demand by biotic communities throughout the water column, which, together with increased stratification, further reduce oxygen levels. This additional reduction adds up to and frequently exceeds the magnitude of the outgassing of oxygen from its reduced solubility alone (Figure 1). This overall oxygen reduction creates a positive feedback loop that makes acute and localized deoxygenation events even more intense (Breitburg *et al.*, 2018). At the same time, other global-scale effects of climate change are predicted to lengthen the upwelling season and increase upwelling intensity (Wang *et al.*, 2015). Organisms that are adapted to variable oxygen environments generally have physiological mechanisms to increase oxygen uptake or reduce demand in the short term. These mechanisms require energy, which can draw significantly on an organism's energy budget and negatively impact fitness, especially during long-term or repeated exposure to hypoxia (Woods and Moran, 2020). The ability of aquatic organisms to cope with natural fluctuations in oxygen, in both the short and long term, will strongly influence their resilience to climate-change-induced deoxygenation during the Anthropocene. It is likely that organisms that have evolved in environments with natural oxygen fluctuation will be better able to respond to deoxygenation.

Mechanisms underlying the organismal consequences of interactions between low oxygen and other stressors

Hypoxia, acidification (and associated hypercapnia, which refers to elevated levels of CO_2), and warmer temperatures often co-occur, constituting 'the deadly trio' (Figure 1). In combination, these and other stressors can synergistically impact organismal performance and survival (Verberk, Durance, *et al.*, 2016; Howard *et al.*, 2020; Lucey *et al.*, 2020; Tomasetti *et al.*, 2021). One approach to understanding the consequences of stressor combinations, and to predicting their impacts during future climate change, is to better understand the physiological mechanisms by which interactions among stressors are manifest within organisms. Here, as a start, we identify a number of two-way interactions involving oxygen, with the goal of highlighting relevant physiological processes that underlie interaction effects.

Oxygen-temperature interactions. With increasing temperature, metabolic demand for oxygen generally rises. Increases in demand reflect in part the acute effects of temperature on the thermodynamics of chemical reactions, but higher metabolic rates typically are observed even after thermal acclimation (Seebacher *et al.*, 2015). This reflects the pervasive effects of warming on multiple life processes, such as feeding, activity, growth and reproduction, all of which increase with warming up to a point and stimulate energy demands (Clarke and Fraser, 2004).

With increasing temperature, the solubility of oxygen declines (Dejours, 1981), and this is often cited as a source of hypoxic stress for organisms. What is often overlooked, however, is that as solubility decreases with temperature, maximum rates of diffusion increase (Woods, 1999; Verberk *et al.*, 2011; Boag *et al.*, 2018). Warmer water is also less viscous, which thins the boundary layers that act as diffusion barriers and thereby lowers the cost of ventilating respiratory surfaces (Couturier *et al.*, 2007; Verberk and Atkinson, 2013). All else being equal, the net effect is therefore a rise in oxygen bioavailability with temperature (see Figure 1; Atkinson et al., this issue). With increasing temperature, however, rising metabolic demand for oxygen due to increased physiological rates (Q10 ~2-3 even after acclimation (Seebacher *et al.*, 2015)) typically outpaces increases in oxygen bioavailability (Q10 ~1.1-1.4). Depending on organism-specific gas exchange and delivery mechanisms, some aquatic organisms in warmer waters may therefore become oxygen-limited. Such oxygen limitation arises not because of decreasing bioavailability but because the increase in supply cannot keep up with the increase in demand, or the costs of supply are exceedingly high (Verberk and Atkinson, 2013; Audzijonyte *et al.*, 2019; Rubalcaba *et al.*, 2020).

Thermally driven mismatches between oxygen supply and oxygen demand have been proposed as a unifying concept to explain thermal tolerance and geographic patterns of distribution d (Pörtner, 2012; Deutsch *et al.*, 2015). Although detrimental effects of hypoxia are widely acknowledged to be exacerbated by warming, whether or not oxygen becomes limiting under normoxia has been debated (Verberk, Overgaard, *et al.*, 2016; Lefevre *et al.*, 2018; Pauly and Cheung, 2018; Jutfelt *et al.*, 2020; Seibel and Deutsch, 2020; Wootton *et al.*, 2022). A nuance here is that not all organisms are equally at risk from oxygen-temperature interactions (Verberk, Durance, *et al.*, 2016). Differences among organisms in their efficiency and regulation of oxygen uptake suggest that although thermal effects can sometimes be understood from an oxygen perspective, this is not a one-size fits all theory (Verberk *et al.*, 2018; Ern, 2019).

A slightly different take on oxygen-temperature interactions is seen in intertidal systems, in which organisms of marine origin are exposed to aerial temperatures for extended periods. During these periods, oxygen uptake can be inhibited due to a need for water conservation or because animals have aquatic-adapted gas exchange mechanisms that function poorly in air (Barnes *et al.*, 1963; Newell, 1973; Davenport and Irwin, 2003). Many intertidal organisms have evolved behavioral, physiological, and biochemical mechanisms that confer high tolerance to internal hypoxia during these times ((Falfushynska *et al.*, 2020), but warming temperatures are likely to increase the cost of deploying these mechanisms and to exacerbate associated oxygen deficits through increased metabolic rates (Horn *et al.*, 2021).

Interactions between oxygen and hypercapnia/acidification. Increasing levels of atmospheric CO_2 (hypercapnia) alter the carbonate chemistry of seawater and reduce ocean pH (ocean acidification). Locally, acidification and hypoxia often co-occur, since rising rates of biological respiration lead to both increased O_2 consumption and increased CO_2 production, which can accelerate declines in pH in hypoxic areas (Feely *et al.*, 2010). Moreover, oxygen and pH co-vary naturally as both are affected by light and stratification of the water column (see above).

The combined and interactive effects of hypoxia and acidification remain poorly understood, although they share common links to respiration (Gobler and Baumann, 2016). Low environmental oxygen can directly depress oxygen content of blood and tissues, while high CO_2/low pH in the environment, if directly translated into the interior of the organism, can modulate the oxygen-binding affinity and capacity of oxygen transport proteins (when present). Thus, both stressors can elicit compensatory changes in ventilation, circulation, and acid-base status with associated changes in metabolism (Lehtonen and Burnett, 2016). Models based on bioenergetics and aerobic scope may thus prove useful for understanding and predicting organismal responses to the interactive effects of low oxygen/high CO_2.

Reduced oxygen bioavailability and acidification generally have negative additive or synergistic effects on fitness-related parameters such as survival, growth, activity, and metabolism (Gobler and Baumann, 2016; Sui *et al.*, 2016; Gu *et al.*, 2019), with responses varying among species, life stages, and exposure histories. In contrast, some studies have shown that elevated CO_2 can enhance the oxygen affinity of respiratory pigments (Mangum and Burnett, 1986), improve aerobic performance in hypoxia (Stover *et al.*, 2013; Lehtonen and Burnett, 2016) , and increase hypoxia tolerance (Montgomery *et al.*, 2019). Alone or in combination, low oxygen and acidification can also perturb the complex mechanisms employed by multicellular aquatic organisms to defend themselves against microbial infection by altering key components of the immune system (e.g., the generation of reactive oxygen species (ROS) - unstable molecules containing oxygen that are implicated in cell signaling and cell damage; see Box 1) as well as changing the abundance, distribution, and pathogenicity of microbial populations (Burnett and Burnett, this volume).

Mounting an immune response has been shown to impair metabolism in some crustaceans and to reduce levels of circulating oxygen (Burnett and Burnett, 2015). Fundamental questions remain about how aquatic organisms sense and respond to hypoxia (Sokolova, 2018; de Lima *et al.*, 2021) as well as how and to what extent they balance the competing energetic demands of maintaining immunity and fitness while responding to changes in oxygen, acidification, temperature, and salinity (Lucey *et al.*, 2020).

Oxygen-osmoregulation interactions. Gas-exchange surfaces generally are highly permeable. In fishes and many other water-breathing ectotherms, gills are the sites of both oxygen uptake and of water and ion movement. To maintain oxygen uptake, fishes exposed to aquatic hypoxia compensate by mechanically hyperventilating their gills (Farrell and Richards, 2009) or, over longer periods of time, remodeling gill structures in ways that enhance oxygen uptake, a form of plasticity (Nilsson *et al.*, 2012). Because gills are also a major site of ion and water exchange, hyperventilation also increases the cost of osmoregulation by increasing the diffusive movement of ions across the gills and thus the rate of compensatory active transport required for maintaining blood osmolality (Perry *et al.*, 2003; Evans *et al.*, 2005; Grosell *et al.*, 2010) (increased gill area may also raise the risk of infection; (Nilsson *et al.*, 2012). This trade-off between gill structures optimized for oxygen uptake (i.e., sufficiently high surface area and low diffusion resistance) and for ion regulation (i.e., low surface area and high diffusion resistance) is called the osmorespiratory compromise (Randall *et al.*, 1972; Nilsson *et al.*, 2012). Likewise in aquatic insects, differences in breathing modes (from aquatic gills to aerial gas exchange) are associated with sensitivity to salt (Verberk *et al.*, 2020). In euryhaline fishes, the cost of osmoregulation appears to be species-specific, with estimates ranging from virtually zero to one-third of standard metabolism (reviewed by (Ern *et al.*, 2014); see also (Nilsson *et al.*, 2012)). Furthermore, increasing salinity has been associated with reduced hypoxia tolerance in fish (Rogers *et al.*, 2016). In coastal and estuarine marine invertebrates, similarly high costs of osmoregulation may be incurred, especially when organisms hyperregulate in low-salinity water (Rivera-Ingraham and Lignot, 2017). Aquatic deoxygenation may thus have a broad array of effects on osmoregulatory capacities and energetics.

Oxygen-light interactions. Irradiance can strongly influence levels of dissolved oxygen in aquatic ecosystems due to its effects on photosynthesis (as described above). The intrinsic covariance of light and photosynthetically-produced oxygen affects organisms in both positive and negative ways. As one example, coral reefs may be among the most vulnerable ecosystems to light conditions, especially in combination with the deadly trio of deoxygenation, warming, and acidification. Hermatypic, or calcifying, corals (Scleractinia) can tolerate considerable variation in light and oxygen, which naturally co-vary due to the diel cycle in oxygen availability via photosynthesis during the day and respiration at night. However, excess light (among other factors) can cause corals to expel their symbionts (bleaching), especially when they are (Verberk *et al.*, 2020) thermally stressed (Fitt *et al.*, 2001; Bhagooli and Hidaka, 2004). The reasons for symbiont expulsion remain unresolved, but one prominent hypothesis implicates increased production of ROS by symbionts when they are stressed by too much heat or light, which causes oxidative stress in both coral and symbiont tissues (Downs *et al.*, 2002; Curran and Barnard, 2021).

Via photosynthesis, light can also improve the availability of oxygen to developing embryos in egg masses ((Phillips and Moran, 2015)) and to the tissues of heat-stressed animals that harbor kleptoplastically “stolen” chloroplasts (Laetz et al., this issue). On a broader scale, hyperoxia in surface waters (due to light-driven photosynthesis) may offset some of the increased metabolic costs that come with warmer temperatures in coastal waters (Giomi *et al.*, 2019). Finally, in the water column, light is required for highly visual marine animals, and oxygen stress can disrupt their visual physiology, behavior, and ecology (McCormick & Levin, 2017; McCormick et al., this issue). Hypoxia-impaired vision may impact essential behaviors such as feeding, reproduction, vertical distribution, and predator avoidance in visual organisms.

Metabolism and energetics as central integrators. Although the interactions discussed above emerge from diverse physiological mechanisms, they are linked into a common framework via their effects on energy. Much of the energy that aquatic organisms use to combat stressors comes from aerobic metabolism, which of course depends on adequate supplies of oxygen. The effects of deoxygenation may be manifest not just as direct physiological impacts on the organisms (e.g., anaerobiosis, reduced growth and reproduction), but also in how metabolic energy is allocated among other functions when organisms are under stress, like growth, reproduction, vision, locomotion, anti-predator defense, etc. (e.g., Roman et al., 2019). To be adaptive, organismal responses must not only help the organism tolerate or avoid the stress, but must do so in ways that minimize overall reductions in fitness. For example, if an organism behaviorally responds to oxygen limitation by moving from hypoxic to better-oxygenated areas, oxygen gains may be traded-off against other ecological costs of changing location (e.g., increased risks of predator encounter, Roman et al., 2019).

How often does oxygen rather than energy limit performance or fitness? The answer will depend on the temporal and spatial distribution of metabolic demand relative to the size of safety margins protecting the organism from immediate impacts of resource budget deficits. In the short-term, organisms are expected to die much sooner from suffocation than from starvation, as stores of oxygen typically are negligible compared with stores of energy. On somewhat longer time scales, an important safety margin is the aerobic scope, which is defined as the difference between maximum and standard metabolic rates (the latter is the minimal metabolic rate needed to sustain bodily processes). Via effects on aerobic scope, each leg of the deadly trio can be linked explicitly to energetics (Figure 1; (Jacobsen *et al.*, 2003; Pörtner and Knust, 2007; Verberk, Durance, *et al.*, 2016; Deutsch *et al.*, 2020; Rubalcaba *et al.*, 2020; Seibel and Deutsch, 2020). Each stressor narrows the aerobic scope, either by depressing oxygen supplies (e.g., deoxygenation effect beyond the temperature effect on oxygen solubility) or by increasing metabolic demands (e.g. rising temperature, increases in acidification or pollution, changes in salinity). The narrowing of aerobic scope limits the energy available to organisms for fitness-enhancing activities like growth and reproduction.

Short-term experiments will likely be biased towards finding oxygen rather than food limitation, although the intensity of the challenge will also play a role. This observation, however, does not mean that oxygen is in general more limiting than food. Indeed, low-intensity but chronic increases in metabolic demand (e.g., from a prolonged infection, rising temperatures, or more time away from feeding areas) may result in longer-term limitation of food rather than oxygen.

Time scales and biological levels of response

Potential responses to deoxygenation and associated environmental stressors occur at all levels of biological organization and temporal scales, ranging from rapid molecular and behavioral responses to long-term evolutionary responses. Understanding and predicting future responses will thus likely emerge from integrative studies that work across scales, investigating how interacting effects of oxygen affect the performance of organisms, as well as the physiological mechanisms that give rise to these responses. The effectiveness of different responses likely varies with the temporal scale and intensity of hypoxia. In addition, organisms generally employ a suite of responses (each with their own time scale) which in combination alleviate the worst negative effects of hypoxia. In most cases, no single response can completely alleviate the problems, but even relatively small benefits can add up to large cumulative effects on fitness over time. As a result, it is important to evaluate the chronic, sublethal effects of mild hypoxia and the organismal responses to avoid these effects.

From another perspective, organisms that are part of communities associated with low-oxygen habitats such as oxygen minimum zones often have a broad range of mechanisms and phenotypes for coping with low oxygen levels, and these can act on a wide variety of time scales (see Box 2).

Behavioral responses. Behavioral responses to hypoxia can be employed nearly instantaneously and are often a first line of defense. One example is movement among oxygen microhabitats; different habitats offer gradients in oxygen availability across distinct spatial and temporal scales, which organisms can exploit in different ways depending on their stage, life history, body size and mobility, analogous to how organisms can exploit mosaics of locally available temperatures (Woods *et al.,* 2015). When faced with hypoxia, for example, mobile aquatic organisms may move to areas of higher oxygen, but this can expose them to other stressors such as increased temperature near the surface, higher irradiance, or predation (Koslow *et al.*, 2011; Larsson and Lampert, 2011). Hypoxia may also drive changes in visually-based behaviors, including photobehavior (McCormick et al., this issue), and alter how readily predators detect or capture prey as well as the ability of prey to detect and avoid predators (Breitburg *et al.*, 1994). By contrast, some aquatic organisms move into hypoxic areas to escape from predators that cannot tolerate low oxygen (e.g., *Daphnia pulicaria*, (Larsson and Lampert, 2011).

Another potential behavioral response to hypoxia is reduced feeding activity. Many organisms, for example, including fish (Thetmeyer *et al.*, 1999; Magnoni *et al.*, 2018), crustaceans (e.g., , molluscs (e.g., Das & Stickle, 1993), and annelids (Llanso and Diaz, 1994) feed at lower rates in hypoxia. Physiological effects of low oxygen on reproductive behaviors and investment are also possible (Wu and Or, 2005), although these effects are understudied.

Molecular responses and oxygen transport proteins. Although smaller organisms (<1 mm) can fulfill their metabolic needs by accessing oxygen through diffusion alone, larger organisms require circulatory systems and often oxygen transport proteins (OTPs) (Decker and van Holde, 2010), which enhance oxygen carrying capacity of blood and hemolymph by more than an order of magnitude. The active sites of OTPs contain pro-oxidative metals – either iron (hemoglobins, hemerythrins) or copper (hemocyanins in arthropods and molluscs) – that not only enable the binding and transfer of O_2, but also restrict the production of harmful reactive oxygen species like hydroxyl radicals (·OH; Decker & van Holde, 2010).

How will OTPs interact with and respond to deoxygenation, warming, and acidification? First, OTPs demonstrate impressive resistance to thermal denaturation: hemoglobins and hemocyanins remain undissociated >50 °C and >90 °C, respectively (e.g., (Georgieva *et al.*, 1998; Carvalho *et al.*, 2013). The structure-function relationships of OTPs thus are likely to withstand substantial changes in ocean conditions (see Coates et al., this volume). Second, many organisms can plastically increase OTP expression or alter the molecular structure of OTPs to change oxygen affinity in adaptive ways (Decker and Föll, 2000; Brown-Peterson *et al.*, 2005). These changes typically occur on the time scale of hours to days; for example, Pacific whiteleg shrimp (*Penaeus vannamei*) exposed to low oxygen levels alone or in combination with elevated carbon dioxide for 24 hours altered their mRNA profiles to include a novel hemocyanin isoform among other oxygen-associated regulatory pathways (Johnson *et al.*, 2015). Third, temperature-driven increases in metabolic demand by tissues can lead to rising levels of CO_2 and lactic acid, and the accompanying decreases in pH lowers the oxygen-affinity of OTPs, thereby promoting release of oxygen (the Bohr effect; Weber et al., 2008). Such changes are virtually instantaneous (within minutes), facilitated by the built-in sensitivity of OTPs to pH and the species-specific heterogeneity of OTP subunits that underlie functional adaptations to their environments (e.g., living in the intertidal zone). Long-term increases in water temperature, however, may lead to metabolic fatigue or even derangement (uncontrolled release of oxidizing and nitrosative radicals), which enhances the susceptibility of organisms to disease (reviewed by (Coates and Söderhäll, 2021). OTPs clearly have the capacity to assist organisms in coping with the most extreme predictions of temperature and deoxygenation. What is unclear (and unstudied), however, is how OTPs will contribute intergenerationally, especially in long lived organisms (e.g., decapod crustaceans and shelled molluscs).

Physiological acclimation. On slightly longer timescales, organisms can recruit a variety of physiological mechanisms to help cope with the effects of hypoxia (Fry, 1971; Harrison *et al.*, 2018). In general, these mechanisms fall into three categories: those that increase oxygen uptake and transport, supporting aerobic metabolism by helping to maintain cellular oxygen supply; those that support metabolism by increasing reliance on anaerobic processes; and those that reduce cellular oxygen demand by depressing metabolism (Farrell and Richards, 2009; Richards, 2009). These mechanisms are not mutually exclusive, and a given species will employ one or more at different levels of oxygen stress or over different time-courses of exposure. In addition, species may utilize differing strategies, depending on the duration and intensity of the hypoxic stress naturally experienced (Mandic and Regan, 2018). This suggests some degree of evolutionary matching between environmental conditions and the ability of species to mount effective responses. However, the flip side is that there will be increasing mismatches as organisms encounter increased environmental stressors and novel combinations of stressors as a result of climate change, for which their acclimatory coping responses may no longer be sufficient.

The capacity for acclimation to hypoxia also varies substantially among species, with some species showing little capacity and others demonstrating much greater plasticity (Borowiec *et al.*, 2020). The process of acclimation to higher temperatures may also be relevant to an organism's ability to cope with subsequent exposure to hypoxia. Across a range of taxa, acclimation to moderately increased temperature generally tends to either have no effect on or to improve performance in moderate hypoxia and often increases tolerance of subsequent extreme hypoxia (Collins *et al.*, 2021). However, relatively few studies have used designs suitable for rigorously assessing these patterns (Collins *et al.*, 2021). There are even fewer studies that examine the capacity for acclimation to combinations of increased temperature and reduced oxygen. This is an area that is ripe for further investigation.

In addition, much of what we know about the ability of organisms to adjust to hypoxia comes from laboratory studies that use constant rather than fluctuating oxygen conditions. In nature, fluctuating oxygen conditions are common, yet much less is known about the capacity for physiological plasticity in this type of exposure (Remen *et al.*, 2012; Williams *et al.*, 2019). The relatively limited data that exist suggest that different mechanisms may be recruited depending on the pattern of exposure, and thus that the strategies for coping with constant and intermittent hypoxia may differ. For example, in Atlantic killifish (*Fundulus heteroclitus*), acclimation to constant, but not intermittent, hypoxia was associated with physiological changes that may reduce the costs of ionoregulation, suggesting an overall strategy of metabolic suppression. In contrast, acclimation to intermittent hypoxia was associated with physiological changes that improved the capacity to recover from hypoxia during intervening normoxic periods (Borowiec *et al.*, 2015, 2018).

Acclimation and evolution of body size. Higher developmental temperatures generally cause ectotherms to grow faster but to mature earlier at smaller sizes (Ray, 1960; Atkinson, 1994; Ohlberger, 2013), a phenomenon known as the temperature-size rule, or TSR (Atkinson, 1996). Support for a role for oxygen comes from the observation that TSR is more common and pronounced in aquatic ectotherms (Forster and Hirst, 2012; Horne *et al.*, 2015), for which oxygen bioavailability is substantially lower than in aerial systems (Verberk & Atkinson, 2013), and that experimental reduction in oxygen availability can lead to a more pronounced TSR (Frazier *et al.*, 2001; Hoefnagel *et al.*, 2018). However, evidence from experimental studies is still limited and connections to oxygen are debated (Audzijonyte *et al.*, 2019; Verberk *et al.*, 2021). One interesting experimental example is *Lecane inermis*, a rotifer, for which, like most animals, fecundity increases with body size. However, under a combination of high temperature and low oxygen conditions, smaller individuals were more fecund than larger ones, indicating that size-reductions with warming constituted an adaptive response to temperature-dependent oxygen conditions (Walczyńska *et al.*, 2015). Temperature-dependent changes in body size involved changes in cell size (Walczyńska *et al.*, 2015). However, changes in cell size do not fully explain the suite of TSR-like phenotypes (Angilletta *et al.*, 2004), and it remains unclear how well the mechanisms for TSR observed in rotifers translate to those in other organisms.

Traditional explanations for the TSR invoke proximate mechanisms such as physiological constraints on the uptake of oxygen or other resources, which can be modeled with supply and demand-type growth models (Pütter, 1920). The TSR, however, can also occur in the absence of changes in baseline metabolic rate or in experiments with ample oxygen and food, suggesting that explanations need not invoke temperature-driven oxygen or food deficits (Hoefnagel *et al.*, 2018; Wootton *et al.*, 2022). Similarly, supply-and-demand models do not account for processes such as maturation and allocation of resources to reproduction versus other functions (Audzijonyte et al., 2019; Marshall & White, 2019; Verberk et al., 2021). Moreover, cause and effect can be difficult to disentangle. For example, debate around the role of oxygen in the TSR in fish revolves around the question of whether the relatively smaller gill surface areas of larger fish is a physiological constraint on oxygen uptake (i.e., a cause) or whether it reflects lower oxygen requirements in larger fish (i.e., a consequence). Organisms generally have multiple mechanisms to enhance oxygen uptake, and these can confound experimental tests of oxygen-limited performance at high temperatures and large body sizes (e.g.,(Shishido *et al.*, 2019).

Ultimate explanations rooted in life-history theory suggest instead that changes in body size should be viewed from the perspective of optimizing growth, reproduction, and fitness. For the TSR, this could be related not just to constraints but also to temperature-dependent rates of mortality, relationships between size and fecundity, or costs of reproduction (Kozłowski *et al.*, 2004; Arendt, 2011). Indeed, temperature-driven changes in oxygen demand and supply could themselves select for optimal reaction norms as encapsulated by the Ghost-of-Oxygen-

Limitation past hypothesis, which postulates that organisms have evolved to maintain oxygen provisioning under a range of conditions by different means (reducing activity, reducing growth, reducing cell size, early maturation at a smaller size) and that past phenotypes that did not accurately predict and prepare for oxygen limitation would be selected against (Verberk *et al.*, 2021). The life-history optimization model presented in this issue (Audzijonyte et al.) shows that neither life-history optimization nor physiological changes alone are likely to explain TSR responses, but that both are operating simultaneously. These results suggest that although oxygen constraints may shape adult body size in warmer conditions, they operate within a set of life-history processes and growth and reproduction trade-offs.

Similar questions arise on longer time scales. The role of oxygen in the evolution of body size was first suggested by comparative analyses of arthropods achieving larger (even giant) size whenever available levels of oxygen are high. One example is paleogigantism of dragonflies and other insect species during the Carboniferous, when oxygen levels in the atmosphere may have exceeded 31% (Berner *et al.*, 2007). Another (contemporary) example is polar gigantism, which aligns with generally greater oxygen availability in water toward the poles (Chapelle and Peck, 1999; Moran and Woods, 2012; Lane *et al.*, 2017). Although the physiological mechanisms underlying the two patterns are not completely analogous (Verberk and Atkinson, 2013), together they have led to broader analyses of correlations between body size and oxygen availability.

Evolution may be tracked as either a process or a product; the former can reveal mechanisms while the latter shows their context-dependent outputs. From the process perspective, evolution of smaller body size under hypoxia has been experimentally demonstrated in some taxa (Zhou *et al.*, 2007; Klok and Harrison, 2009)Walczyńska and Sobczyk, this issue). Natural experiments (product) have also shown correlations between environmental oxygen levels and body size within taxa (Chapelle & Peck, 2004) (Chapelle & Peck, 2004; Walczyńska et al., 2021), and, in intense oxygen minimum zones (see Box 2), benthic metazoan meiofauna and small macrofauna dominate whereas larger invertebrate taxa are rare, creating body size gradients (Levin, 2003; Gooday *et al.*, 2010). These experiments and patterns suggest that, at least from the perspective of small ectotherms, observed global declines in body size, which increasingly are thought to be driven by climate warming (Daufresne *et al.*, 2009), reflect functional oxygen deficiencies or functional hypoxia (Harrison *et al.*, 2018). So far, no data for evolutionary patterns are available for larger ectotherms, which prevents developing more universal conclusions.

Population ecology. Organismal-level effects that stem from rising temperatures and deoxygenation readily scale up to influence the population ecology and ranges of both pelagic and benthic species (Levin *et al.*, 2009; Cheung *et al.*, 2013; Roman *et al.*, 2019; Pinsky *et al.*, 2020; McGinty *et al.*, 2021). Shifts in population structure and ecology may result from multiple factors, including altered patterns of migration and dispersal, changes to timing of reproduction and allocation of resources among offspring (with tradeoffs between size and number), and biotic interactions among community members. As one example, warmer, hypoxic waters typically depress the body sizes of zooplankton and fish (Pörtner and Knust, 2007), driving corresponding decreases in fecundity (Blueweiss *et al.*, 1978). Likewise, non-lethal but warmer, hypoxic waters can depress the efficiency of prey capture, growth, and reproduction, which together impact the productivity of populations. Recent evidence and models suggest that ocean warming and deoxygenation will progressively move and compress suitable habitats for marine organisms, with strong impacts on future species distributions (Deutsch *et al.*, 2015; Pinsky *et al.*, 2020).

Recommendations for future studies

Report measured conditions more comprehensively and in standard units. One problem impeding progress is lack of standardized approaches to reporting units and experimental approaches (Hofmann *et al.*, 2011). Some fields such as physics, biogeochemistry, and ecology tend to work in terms of oxygen concentrations, whereas others, such as physiology and organismal biology, use percent air saturation or partial pressures. Oxygen concentration is less useful for the purposes of predicting organismal performance, because the bioavailability of oxygen is affected not just by concentration but also by factors like solubility, viscosity, and temperature (Denny, 1993; Woods, 1999; Verberk *et al.*, 2011) and because oxygen moves into organisms down gradients of partial pressure. Likewise, bioavailability is distinct from concentration because it also involves the interplay between diffusion and water convection (Rubalcaba *et al.*, 2020; Frakes *et al.*, 2021) (Atkinson et al., this issue). As a result, data on environmental oxygen should be reported with associated parameters (temperature, atmospheric and hydrostatic pressure/depth, salinity, and flow fields if available) so that values can be compared across locations, units, and organisms. More broadly, we need more comprehensive reporting of environmental conditions in space and time. Such approaches will better facilitate meaningful meta-analyses of biological data.

Do longer, multifactorial experiments using realistic environmental conditions. This synthesis leads to two conclusions about the kinds of studies needed going forward. Echoing other authors working on multifactorial approaches (Boyd *et al.,* 2018; Wootton *et al.,* 2022), we recommend that studies consistently build in interactions among oxygen and other relevant stressors. Such an approach rapidly expands the necessary number of treatment groups, although there are ways to avoid full-factorial experiments using, e.g., experimental versions of a Morris analysis (implementing just subsets of all possible factorial treatments (Morris, 1991) or other ways of rationalizing and implementing fewer treatments (Boyd *et al.,* 2018).

Second, we need more long-term experiments, especially those that address inter-generational acclimation across at least 3-4 generations. Short-term experiments are easier to carry out and typically less expensive. Nonetheless, it is critical to carry out experiments that mimic the sorts of long-term changes we see in nature (Brown *et al.,* 2001; Kuebbing *et al.,* 2018) to determine not just whether organisms can tolerate or compensate for short-term environmental change, but also the long-term effects of small, long-lasting changes within an organisms' short-term tolerance range. For example, emergency physiological responses to hypoxia may allow organisms to withstand short-term exposures but may become energetically unsustainable over longer periods of time (Woods and Moran, 2020). Also important is understanding the effects of increasing *frequency* of moderate or extreme stressors like marine heat waves and hypoxic events. One potential approach would be to adopt for oxygen (and its interactions) the tolerance landscapes approach used for temperature (Rezende *et al.,* 2014), in which one analyzes responses to progressively longer exposures and then estimates responses to even longer exposures by projection (Figure 3). Note also that longer-term experiments can more easily accommodate ranges of experimental hypoxia that are relevant to the system in question. Clearly, such efforts will require more time and money, and we urge funding agencies to prioritize this sort of work.

Need for integrative work across levels of biological organization.

"Integrative biology is both an approach to and an attitude about the practice of science." (Wake, 2003)

The importance of integrative biology, as proposed in this paper, is widely recognized; yet, the term has diverse interpretations. Integrative biology can mean a multidisciplinary approach that incorporates diverse fields of science or subdisciplines of biology, or a multilevel approach that bridges different levels of biological organization (Wake, 2003). Similarly, research can integrate across time scales or life history stages (Wake, 2008; Lailvaux and Husak, 2017). The basic, essential need of organisms for oxygen means that to fully understand its effects on biological systems, oxygen effects must be approached integratively from every angle. This is particularly important since other major environmental drivers, like temperature and CO_2, influence oxygen dynamics at all levels.

Today, society seeks to understand the effects of climate change on emergent ecosystem properties such as fish biomass or production, biodiversity, or phenology. Several integrative indices and concepts have been proposed to address the effects of deoxygenation and associated warming on these issues. These include the metabolic index (Deutsch *et al.,* 2015), the aerobic growth index (Clarke *et al.,* 2021), and the oxygen and capacity-limited thermal tolerance model (Pörtner *et al.,* 2017) as well as varied ecosystem models. Application of most of these indices requires process measurements on individual organisms, sometimes at different life stages or temperatures, and thus integration across levels is inherent to addressing many scientific questions and societal needs (Pörtner, 2021). We suggest that there remains significant scope for developing even broader indices derived from more diverse perspectives.

The papers in this volume highlight the importance of oxygen interactions, including their effects on the function of oxygen transport proteins (Coates et al., this issue), immune defense (Burnett and Burnett, this issue), organismal performance and survival (Laetz et al., Lucey et al., this issue), life-history optimization (Audzijonyte et al., Atkinson et al., this issue) and plastic and evolutionary responses over multiple timescales (Roman & Pierson; Walczyńska and Sobczyk, Schulte et al., Sperling et al., Borges et al., this issue). Other factors less often considered, like light, can also interact with oxygen availability to affect organismal performance in surprising ways (Laetz et al., McCormick et al., this issue).

Integrative biology comes with substantial logistical hurdles. Experiments that measure the physiological and energetic effects of oxygen interactions, particularly among the “deadly trio,” are essential to making general predictions about climate change effects at higher levels; yet, these types of integrative projects require substantially more time, effort, and resources than single-stressor science. Effectively working across levels of organization (e.g. molecular, cell, organism, ecosystem) requires both broad and deep expertise in different fields of science. In addressing ocean ecosystems, assessments by the Intergovernmental Panel on Climate Change (IPCC) are increasingly recognizing the importance of understanding combined effects of multiple stressors, and integration of those effects across life stages, different levels of biological organization and trophic levels (Cooley et al. in press). Our hope is that, as happened with carbon dioxide and ocean acidification in the beginning of the 21st century, the fundamental importance of oxygen in driving biological responses to climate change will eventually inform multiple levels of management and policy.

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Box 1: Causes and consequences of Reactive Oxygen Species (ROS)

Reactive Oxygen Species (ROS) are small molecules and free radicals derived from molecular oxygen, e.g., the superoxide anion ($\bullet O_2^-$) and hydrogen peroxide (H_2O_2). ROS are highly reactive, reacting with the first molecule with which they come into contact and thus causing damage to proteins, lipids and DNA, a situation referred to as oxidative stress. Although low levels of ROS may be necessary for normal functioning as regulatory mediators in signaling processes (Dröge, 2002; Boardman *et al.*, 2012), oxidative stress contributes to senescence and ultimately death (Fenn *et al.*, 1967; Fridovich, 1998; Lane, 2002).

ROS can come from exogenous or endogenous sources. A primary exogenous source is high light intensities, especially in the ultraviolet part of the spectrum. Primary endogenous sources are mitochondria and chloroplasts (Murphy, 2009; Foyer, 2018). During the normal process of oxidative phosphorylation by mitochondria, for example, electrons leak and interact with molecular oxygen to form ROS. Rates of ROS production increase with mitochondrial membrane potential and are therefore minimized when rates of oxygen delivery to mitochondria (O_2 supply) match rates of oxygen utilization (O_2 demand). Consequently, oxygen delivery and utilization have to be tightly regulated to balance the generation of energy with the production of toxic oxidants. This is seen as a primary reason that oxygen delivery is regulated at many levels (e.g. reversible oxygen binding with hemoglobin, dynamic regulation of both blood flow and ventilation).

Due to the cytotoxic nature of ROS, many organisms have evolved mechanisms to prevent or neutralize them, and to repair the damage they cause. For example, many organisms produce pigments that absorb and then dissipate excess light energy to prevent exogenous ROS production (i.e. melanin, xanthophyll; (Goss and Latowski, 2020). Similarly, uncoupling proteins reduce mitochondrial membrane potential and ROS production. Once ROS are formed, cells can utilize antioxidant enzymes such as superoxide dismutase, peroxidase and catalase, transforming them into less reactive molecules (Zelko *et al.*, 2002). Cells also have mechanisms to limit or repair the damage that occurs due to oxidative stress, including the mobilization of molecular chaperones, hydrolyzed nucleotides and DNA repair enzymes (Nakabeppu *et al.*, 2004).

Although a direct link between the deoxygenation of aquatic ecosystems and oxidative stress in aquatic organisms has not been established, both deoxygenation and oxidative stress are increasing due to global warming (Paital, 2016), meaning that aquatic organisms will increasingly face each of these stressors and their compound effects as global temperatures rise.

Box 2: An integrative example: multiple responses by pelagic organisms in oxygen minimum zones

Organisms that inhabit oxygen minimum zones (OMZs) for much or all of their lives typically have morphological and physiological adaptations allowing them to maximize O_2 extraction, including enhanced capacity for ventilation and circulation, high gill surface area, high O_2 binding capacity and affinity of oxygen transport proteins, and a large Bohr effect (Childress and Seibel, 1998). Moreover, organisms that move transiently into OMZs typically are oxyregulators that maintain O_2 consumption rates when ambient levels of oxygen remain above some critical partial pressure (P_{crit}). A well-documented example is the jumbo squid, *Dosidicus gigas*, which migrates vertically on a daily basis into the OMZ in the Eastern Tropical Pacific (Gilly *et al.,* 2006). It copes with hypoxia by suppressing its metabolism and changing its swimming behavior (Rosa and Seibel, 2008). At environmental PO_2 (below 1.6 kPa; at depths below 150 m), *D. gigas* switches from aerobic to anaerobic pathways (Rosa and Seibel, 2008). Similar responses have been described in other vertically migrating organisms. An additional challenge for diel migrators is the generation of reactive oxygen species (see Box 1), promoted by reoxygenation as they move into surface waters at night. Jumbo squid minimize damage from ROS by enhancing the heat shock protein response and the activity of key antioxidant enzymes (Trübenbach *et al.,* 2013).

Figure legends

Figure 1. Schematic showing how components of the deadly trio – deoxygenation, warming, and acidification – combine to affect oxygen supply:demand relationships. (A) Aerobic organisms use oxygen to support their metabolisms, and demand for oxygen typically rises rapidly with warming over the short term, at least prior to acclimation or evolutionary adaptation. Simultaneously, the physical processes affecting rates of oxygen supply (solubility and diffusion coefficient of oxygen, shown as dotted lines; additional effects of temperature on viscosity of water are not shown) combine to cause rates of supply to increase approximately linearly with temperature but often much less steeply than demand. The aerobic scope (difference between resting metabolic demand and potential rate of oxygen supply, green line) defines the amount of metabolic power that can be devoted to tasks other than basal maintenance. Here, the historical range of temperatures is marked in blue, corresponding to a large aerobic scope. (B) Climate change is causing oxygen levels in oceans to decline, both from temperature-related declines in solubility but also shifts in respiration by community members and changes in patterns of stratification and oxygen transport. Combined, these effects are depressing functional rates of oxygen supply to organisms. In addition, warmer temperatures (range indicated in orange) are stimulating short-term demand for oxygen more than they are increasing supplies. Finally, acidification has a wide range of direct effects but likely also is raising metabolic demand for oxygen by increasing costs of pH homeostasis and calcification. Organisms experiencing one or more conditions in the deadly trio may engage in greater overall levels of activity in attempts to find better conditions locally. Together, these effects may strongly narrow the aerobic scope.

Figure 2. Natural oxygen regime is hypothesized to shape oxygen performance curves and vulnerability to deoxygenation. Reductions in performance likely differ for species that have evolved under variable oxygen conditions (a) and those form more stable conditions (b). In both panels, historic variability in oxygen conditions is indicated in green, while red indicates the new oxygen regime, which has the same fluctuation but a lower mean. Owing to differences in the breadth of the oxygen performance curve, loss of performance is less pronounced in species from variable oxygen conditions. Performance in hyperoxia (oxygen levels higher than normoxic) has been explored less than in hypoxia, and can be highly variable among organisms and situations, with some organisms performing well and others showing performance decrements.

Figure 3. Physiological performance and temporal scale. Effects of oxygen on performance are modulated by temporal scale, with specific physiological processes changing on specific time-scales. (A) For example, anaerobic metabolism will be used most at low PO_2 and can supplement energy budgets on short temporal scales only. (B) In addition, a given physiological process (e.g., maximum metabolic rate) may be affected by oxygen availability differently when it has to be sustained for minutes, hours, or weeks and long-term exposure to hyperoxia could even reduce performance due to toxicity effects (Box 1).

Figure 1

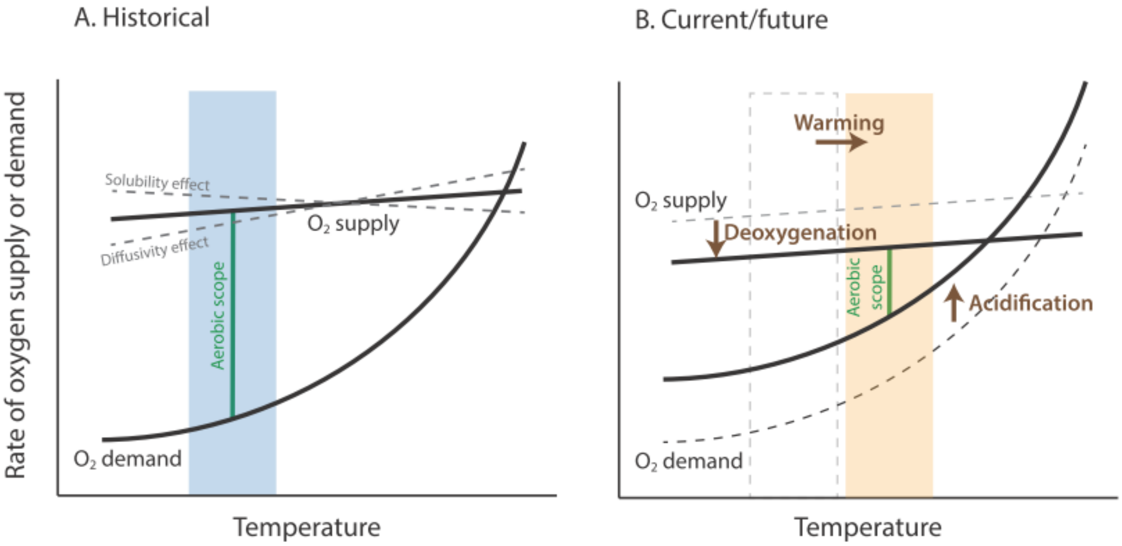

A. Historical
B. Current/future
Rate of oxygen supply or demand
Solubility effect
Diffusivity effect
O_2 supply
Aerobic scope
O_2 demand
Temperature
Warming
O_2 supply
Deoxygenation
Aerobic scope
Acidification
O_2 demand
Temperature

Figure 2

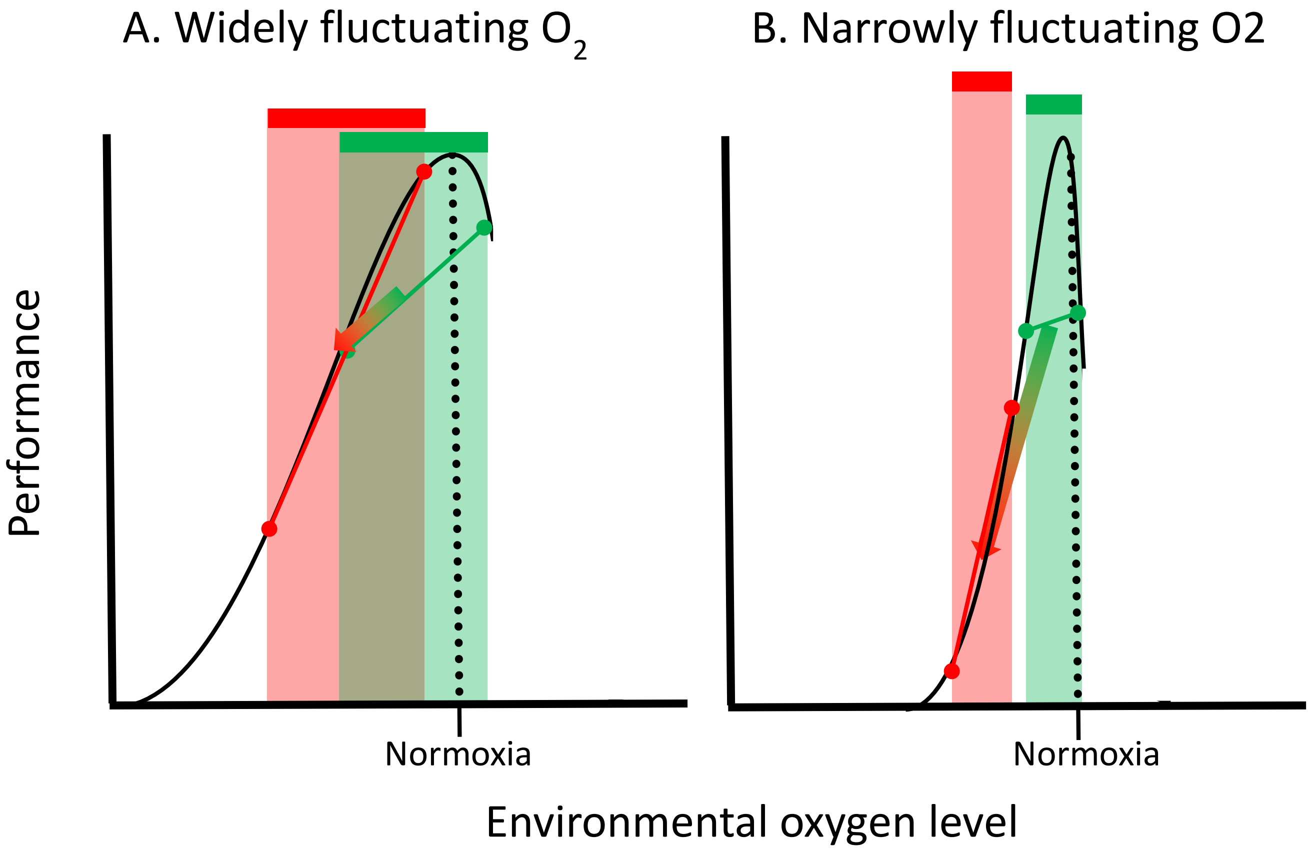
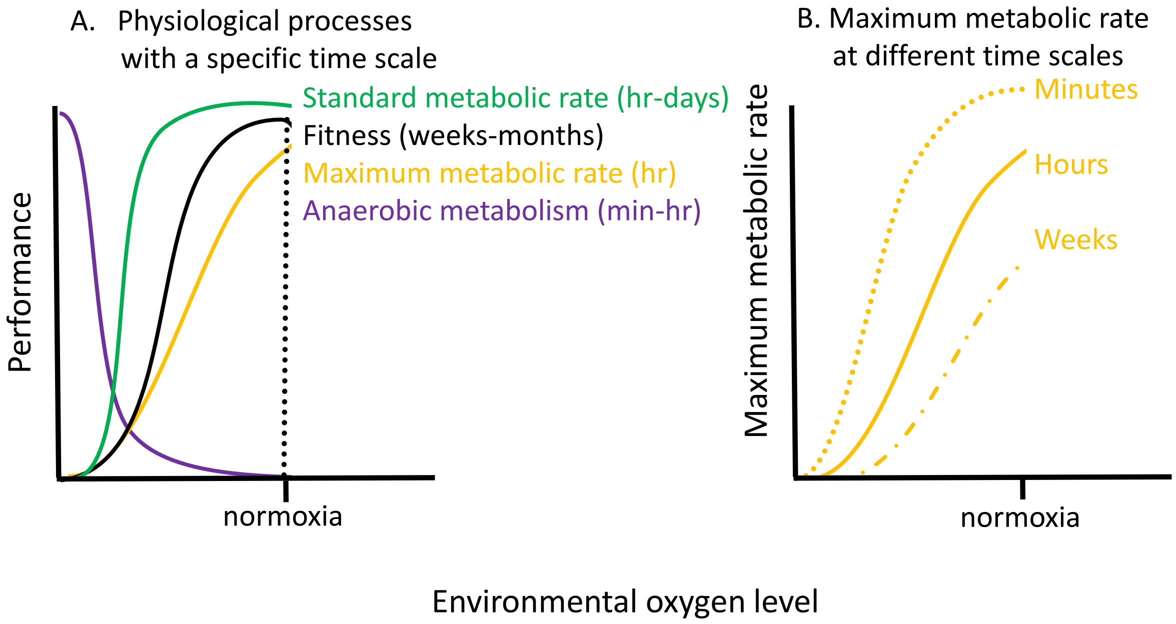

A. Widely fluctuating O_2
B. Narrowly fluctuating O2
Performance
Normoxia
Normoxia
Environmental oxygen level

Figure 3


A. Physiological processes with a specific time scale
Standard metabolic rate (hr-days)
Fitness (weeks-months)
Maximum metabolic rate (hr)
Anaerobic metabolism (min-hr)
Performance
normoxia
B. Maximum metabolic rate at different time scales
Minutes
Hours
Weeks
Maximum metabolic rate
normoxia
Environmental oxygen level