Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones

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Summary

The survival of oceanic organisms in oxygen minimum zones (OMZs) depends on their total oxygen demand and the capacities for oxygen extraction and transport, anaerobic ATP production and metabolic suppression. Anaerobic metabolism and metabolic suppression are required for daytime forays into the most extreme OMZs. Critical oxygen partial pressures are, within a range, evolved to match the minimum oxygen level to which a species is exposed. This fact demands that low oxygen habitats be defined by the biological response to low oxygen rather than by some arbitrary oxygen concentration. A broad comparative analysis of oxygen tolerance facilitates the identification of two oxygen thresholds that may prove useful for policy makers as OMZs expand due to climate change. Between these thresholds, specific physiological adaptations to low oxygen are required of virtually all species. The lower threshold represents a limit to evolved oxygen extraction capacity. Climate change that pushes oxygen concentrations below the lower threshold (~0.8 kPa) will certainly result in a transition from an ecosystem dominated by a diverse midwater fauna to one dominated by diel migrant biota that must return to surface waters at night. Animal physiology and, in particular, the response of animals to expanding hypoxia, is a critical, but understudied, component of biogeochemical cycles and oceanic ecology. Here, I discuss the definition of hypoxia and critical oxygen levels, review adaptations of animals to OMZs and discuss the capacity for, and prevalence of, metabolic suppression as a response to temporary residence in OMZs and the possible consequences of climate change on OMZ ecology.

Key words: *Dosidicus gigas*, climate change, euphausiid, hypoxia, metabolic suppression, oxygen minimum zone.

Introduction

A recent study demonstrated that 50% of coastal marine animal species die after exposure to oxygen levels less than $\sim70\,\mu mol\,kg^{-1}$ (Vaquer-Sunyer and Duarte, 2008). Equivalent oxygen partial pressures (P_{O_2}) are known to cause physiological impairment in humans as well, for example at the tops of mountain peaks (Huey and Eguskitza, 2001). Yet, life thrives in deep-ocean oxygen minimum zones (OMZ), where oxygen values lower than this are pervasive (Levin, 2002; Childress and Seibel, 1998) (Fig. 1). The horizontal and vertical extent of the OMZ, as well as the intensity of the hypoxia found there, varies considerably between ocean basins (Fig. 1, Fig. 2A), but as much as 8% of the volume of the entire ocean contains less than $20\,\mu mol\,kg^{-1}$ oxygen (Paulmier and Ruiz-Pino, 2009). The oxygen content of a water mass is dependent on several factors, including the oxygen content when it was last in contact with air, the time elapsed since that point, and the rate of biological oxygen consumption. These factors, in turn, depend on temperature, air–sea gas exchange, ocean circulation and biology (Keeling et al., 2010; Paulmier and Ruiz-Pino, 2009). OMZs are most pronounced in regions of intense upwelling, such as the Eastern Tropical Pacific (ETP), that support high productivity and high subsurface oxygen demand (Wyrtki, 1962; Kamykowski and Zentara, 1990; Olson et al., 1993; Karstensen et al., 2008).

Substantial loss of dissolved oxygen and shoaling of hypoxia throughout much of the ocean, including the Subarctic and ETP, has been reported (Emerson et al., 2004; Stramma et al., 2008; Bograd et al., 2008; Whitney et al., 2007). For example, Bograd et al. showed that the oxygen content at 200–300m has declined by $11–33\,\mu mol\,kg^{-1}$ in the California Current since 1984 (Bograd et al., 2008). This was predicted to occur with changing climate (e.g. Keeling and Garcia, 2002; Sarmiento et al., 1998; Bopp et al., 2002; Oschlies et al., 2008) because (1) oxygen is less soluble in warm water and (2) global warming is increasing upper ocean stratification, which increases productivity in surface waters, fueling increased oxygen demand at depth and simultaneously reducing oxygen supply to greater depths (Keeling et al., 2010). The hypoxia characterizing the North Pacific is already among the most intense in the global ocean such that further decreases in oxygen content may have severe consequences for resident biota. In some instances, expanding hypoxia (and the associated acidification due to respiratory carbon dioxide production) has encroached onto continental shelves where organisms are not adapted to such conditions (Chan et al., 2008; Grantham et al., 2004; Feely et al., 2008; Bograd et al., 2008).

The adaptations of animals for low oxygen tolerance are well-known and are driven by strong selective pressure to maintain aerobic metabolism *via* enhanced oxygen extraction from hypoxic water (Childress and Seibel, 1998). However, in more intense OMZs, the minimum oxygen level may be less than $5\,\mu mol\,kg^{-1}$ across depths of 100–900m (Fig. 1). Such low values strongly influence the vertical distribution of resident metazoans, with most of them migrating to shallower, more oxygenated waters at night (i.e. diel vertical migration). The physiological adaptations that permit transient occupancy of these intense OMZs have been explored in only a few species but, for these few, it appears that an oxygen limit to the evolution of oxygen extraction capacity is surpassed, and the routine metabolic rate cannot be supported fully by aerobic pathways. These vertical migrators and their metabolic adaptations are of central focus here.
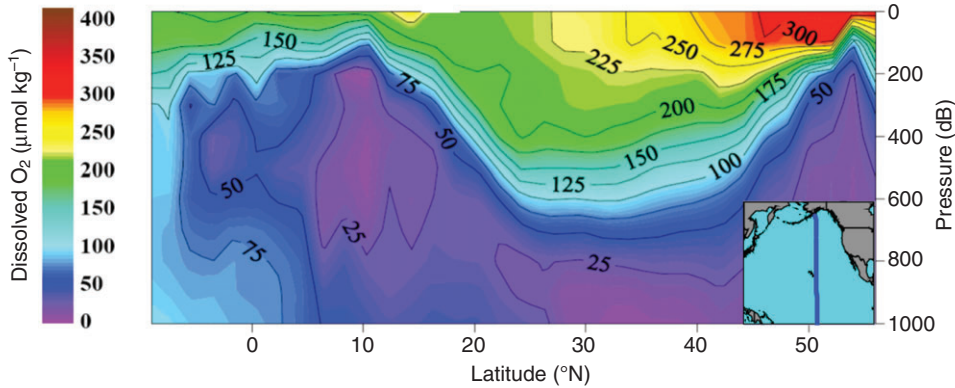
Fig. 1. Depth distribution (1 dB pressure = 1 m depth; right axis) of dissolved O_2 ($\mu mol\,kg^{-1}$; left axis) along the March 2006 P16 transect line (152°W, inset map) in the North Pacific. Reproduced with permission from Fabry et al. (Fabry et al., 2008).

Three prominent strategies for tolerance of hypoxia during daytime forays into oxygen minimum zones are apparent in diel migrators: (1) aerobic metabolic ATP production is enhanced to the extent possible *via* adaptations for oxygen extraction and transport or, more rarely, oxygen storage; (2) anaerobic ATP production is upregulated *via* enhanced glycolytic capacity and alternative pathways with improved efficiency; and (3) total energy consumption is suppressed by shutting down energy-intensive cellular processes including locomotor activity (Childress and Seibel, 1998; Richards, 2011). Many organisms utilize a combination of these strategies to accommodate their highly variable and complicated vertical distribution patterns and local oxygen profiles. The integration of these three strategies is discussed below (Fig. 3). Metabolic suppression by oceanic migrators during daytime excursions into OMZs is a strategy that is little appreciated by oceanographers, yet one that has profound consequences for the ecology of the oceans. Anthropogenic changes in oxygen content may affect the distribution, abundance, performance and survival of marine organisms as well as the biogeochemical cycles dependent on organismal function (Seibel and Dierssen, 2009). This highlights the importance of defining critical oxygen levels based on a mechanistic understanding of organismal tolerance.

Oxygen definiendum

The term 'hypoxia' is most appropriately used to denote an oxygen (O_2) concentration less than some arbitrary value perceived as normal. While hypoxia sometimes specifies a perceived deficiency in O_2 for some physiological or biogeochemical process, evidence of O_2-limited function is usually lacking. Within the oceanographic community, hypoxia is typically defined by O_2 concentration, again usually without knowledge of the effect of that particular O_2 level on organism or ecosystem function. For example, Kamykowski and Zentara define hypoxia as a concentration less than $0.2\,ml\,l^{-1}$ (~9 μmoles) (Kamykowski and Zentara, 1990) while Diaz and Rosenberg define it as less than $2.0\,ml\,l^{-1}$ (90 μmoles) (Diaz and Rosenberg, 1995). Defining hypoxia by O_2 concentration is also problematic because it is the P_{O_2}, not the O_2 concentration, that drives O_2 provision to organismal tissues and determines the effect of hypoxia on organism function. Moreover, organismal O_2 transport systems and biochemical pathways are adapted to manipulate the P_{O_2} internally or to adjust the affinity for O_2 to drive ATP synthesis across the range of ambient O_2 concentrations under which a species has evolved (Connett et al., 1990). Thus, any definition of hypoxia related to the survival or performance of organisms is necessarily species-specific and will

depend on the particular physiological requirements of the species and on the O_2 environment to which the species is adapted.

Brewer and Peltzer recently proposed a 'respiration index' to define critical levels of O_2 and CO_2 (Brewer and Peltzer, 2009). They suggest that the free energy (ΔG) obtainable from the oxidation of organic matter is limited by the ratio of reactants and products (O_2 and CO_2) as if in a closed system moving toward equilibrium (e.g. a test tube). However, they ignore the role of evolution in shaping organismal tolerances to environmental variability (Seibel et al., 2009). Living organisms are open systems that acquire free energy from their surroundings and operate at an approximate steady state. The concentrations of substrates and products in living systems are actively maintained at disequilibrium to ensure a constant ΔG and, thus, thermodynamic drive, for forward flux through the pathways of energy metabolism. Importantly, the concentrations of reactants (e.g. O_2 or CO_2) inside biological cells can be maintained at concentrations far higher than those found in the surrounding seawater (Seibel et al., 2009). Critical gas levels represent species-specific adaptations in O_2 uptake and acid–base regulation that have evolved within specific habitats. Critical gas levels for most species are far from the ultimate limits that can evolve in extreme habitats such as the OMZ. While the physiological community has also struggled with the ambiguity in terminology (Connett et al., 1990; Farrell and Richards, 2009), they recognize that an O_2 concentration that is stressful to one species or under one set of conditions may not be to other species or under other circumstances.

Both oceanographers and physiologists have developed a variety of terms, well-defined only in the unique context of their own disciplines or for specific studies. For example, the terms dysoxia and suboxia are used by oceanographers to refer to particular O_2 concentrations (Levin, 2002) that often, but not always (Canfield and Thamdrup, 2009), correspond to a transition in microbial processes (e.g. denitrification). However, to some physiologists, these terms refer to O_2-limited cytochrome turnover, a state that can only be defined by measuring intracellular P_{O_2} and the production of, and demand for, ATP (Connett et al., 1990). A host of other terms, including microxic, dysaerobic, suboxic, oxic and normoxic, have been inconsistently defined and applied. I believe these terms cannot be defined in a useful way and that inconsistent usage leads to confusion in the literature. The constraints that have been used to bound and define OMZ and O_2 dead zones are also not consistent across studies nor are they biologically relevant in many cases. I recommend abandoning all terms except *hypoxia*, meaning relatively low O_2, and *anoxia*, the complete absence of O_2 (although this term may be merely theoretical as our ability to measure low concentrations of O_2 is imperfect). Similarly, an OMZ should not
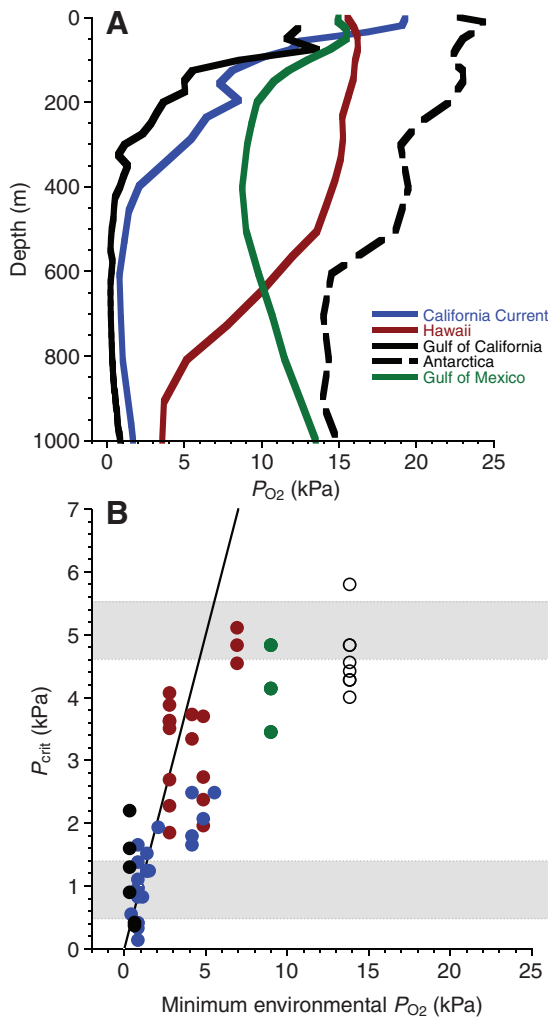
Fig. 2. (A) Oxygen profiles and (B) critical oxygen partial pressures (P_{crit}) of pelagic animals living in the upper water column in regions that vary in minimum O_2 content. The Gulf of California oxygen minimum zone (OMZ) (black) is most pronounced, followed by that in the California Current (blue), Hawaii (red), the Gulf of Mexico (green) and Antarctica (black, dashed). The P_{crit} is the oxygen partial pressure (P_{O_2}) below which the rate of oxygen consumption can no longer be maintained independent of P_{O_2}. In hypoxic regions, a close correspondence between the P_{crit} and the minimum oxygen level experienced by a particular species (see unity line) reflects increasing natural selection for adaptations to low O_2 that may include high gill surface areas, high ventilation rates and blood oxygen-binding proteins with a high binding affinity for O_2. A P_{crit} near the unity line further indicates that a species is living near its evolved physiological limit and may experience stress as OMZs expand. Data to the left of the unity line indicate exposure to oxygen concentrations below the P_{crit} that must be supported in part by anaerobic metabolism or suppressed energy consumption. Gray bars represent two O_2 thresholds that may be useful for putting upper and lower bounds on the oxygen levels tolerated by organisms generally. Above ~5 kPa, P_{crit} plateaus, indicating that further increases in O_2 concentration provide no additional benefit to organisms. In other words, virtually all organisms can tolerate at least this level of hypoxia (depending on measurement temperature). The second threshold is evident where further decreases in O_2 concentration fail to select for lower P_{crit}. This may represent a physiological constraint in O_2 extraction. Reduction in O_2 levels below this threshold will likely result in large changes in species composition, distribution and diversity. Oxygen profiles are from Ocean Atlas (http://www.oceansatlas.org) and P_{crit} data are reviewed in Childress and Seibel (Childress and Seibel, 1998) (B.A.S., unpublished).

be defined by a particular O_2 level but rather, vaguely, as a zone bracketing the depth or depth range of the lowest O_2 concentration in the water column. By this definition, OMZs may be more or less pronounced and occupy more or less of the water column. As long as there is a subsurface minimum O_2 level, there is an OMZ. Whether or not an OMZ has biological relevance depends on the processes and species being addressed. Rather than try to classify O_2 levels, I suggest that authors refer to specific O_2 partial pressures as they influence specific biological processes.

In part because of the ambiguity in terminology, a framework for predicting the biological response to changing O_2 levels remains elusive. The critical limitation for developing such a framework is the identification of O_2 thresholds critical (P_{crit}) for organismal survival and performance. Useful criteria for establishing limits for O_2 tolerance must rely on measures of physiological or ecosystem performance and recognize interspecific variation in O_2 tolerance and the capacity of organisms to adjust O_2 provision and demand to compensate for O_2 limitation.

Defining critical O$_2$ levels

The critical P_{O_2} (P_{crit}) may be identified in numerous ways, each relying on a different set of assumptions and with slightly different ecological and physiological implications. Typically, it is defined by (1) the P_{O_2} at which the rate of aerobic metabolism (O_2 consumption) can no longer be maintained independent of P_{O_2} or (2) the onset of anaerobic metabolism, as indicated by the accumulation of metabolic end-products (e.g. lactate) (Hochachka and Somero, 2002). P_{crit} describes the lower O_2 limit to an organism's ability to make physiological adjustments that regulate its metabolic rate at a constant level independent of the ambient P_{O_2} (i.e. oxyregulation) (see Richards, 2011). Possible adjustments include increased heart rate, enhanced gill surface area *via* remodeling (Mitrovic et al., 2009), increased blood pigment levels, adjustment of enzyme systems, and elevated rates of ventilation. Some of these adjustments are triggered by hypoxia-mediated upregulation of gene expression patterns (Flück et al., 2007) while others are regulated physiologically. By contrast, animals whose metabolic rate drops with declining P_{O_2} are termed 'oxyconformers'. Practically, oxyconformation merely describes the metabolic response of organisms to O_2 levels below their P_{crit}. This is because there must always be an upper limit to metabolism beyond which additional O_2 will have no influence. There may be species with very high critical O_2 levels that conceal the upper limit to metabolism.

Connett et al. provide a framework for understanding the influence of P_{O_2} on metabolic rate and the requirement for anaerobic metabolism and metabolic suppression (Connett et al., 1990) (Fig. 3). In their model, the need to actively regulate metabolism, *via* physiological adjustments, as P_{O_2} decreases (arrows in Fig. 3) is influenced by total energy demand (ATP), as is a species' critical P_{O_2} (P_{crit},1; blue line in Fig. 3). As energy demand increases, the P_{crit} also increases, and anaerobic metabolism may be required to contribute to total ATP flux (white region, Fig. 3). For example, an animal operating at its basal (resting, fasted state) level can maintain ATP demand at O_2 levels below the P_{crit} by increasing the rate of anaerobic (e.g. glycolytic) ATP production. At a still lower O_2 level (P_{crit},2), anaerobic metabolism is insufficient to maintain basal ATP flux. Metabolism must be depressed below that point to avoid impaired function [termed 'dysoxia' by Connett et al. (Connett et al., 1990)]. Sustained exposure to O_2 levels below the P_{crit},2 will eventually result in death.
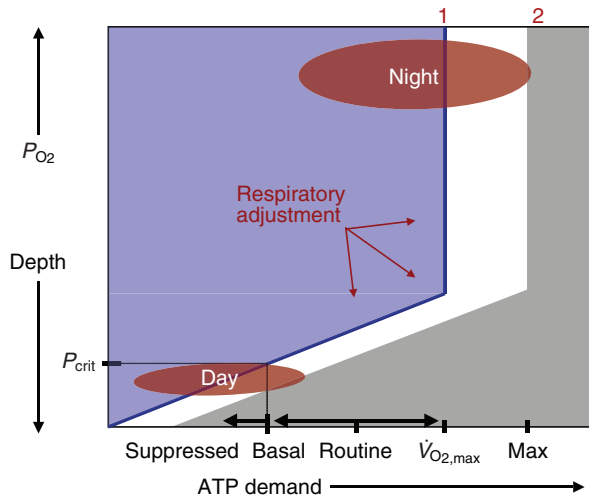
Fig. 3. Total energy (ATP) demand is influenced by seawater oxygen partial pressure (P_{O_2}) below critical values. The solid blue line (1) represents the effect of increasing ATP demand on the critical oxygen partial pressure (P_{crit}). The P_{crit} at a basal rate of metabolism is indicated by the horizontal line. The white region indicates that anaerobic metabolism contributes to maintain total ATP flux. For example, an animal can maintain a basal rate of metabolism even below the P_{crit} by increasing anaerobic glycolysis. Alternatively, total ATP demand could be suppressed. In the gray region anaerobic metabolism is insufficient to maintain ATP flux. Metabolism must be suppressed below that point to avoid impaired function. As P_{O_2} approaches P_{crit}, respiratory adjustments may be made to maintain the rate of O_2 provision to sites of consumption. Adjustments include increased heart rate, enhanced gill surface area, increased blood pigment levels and elevated ventilation. The arrows in either direction from 'routine' metabolism, between 'basal' and '$\dot{V}_{O_2,max}$' (the maximum rate of aerobic metabolism), indicate the maximum aerobic scope for activity. The red ovals indicate the night-time and daytime depth distribution of vertically migrating organisms. The placement of these ovals indicates that daytime forays into the OMZ require metabolic suppression and, to support higher rates, anaerobic metabolism. Figure modified from Connett et al. (Connett et al., 1990).

The critical P_{O_2} is species-specific and depends on the regional O_2 concentration to which a species is adapted as well as the species' O_2 demand. As such, a broad regional comparison of P_{crit} values is required to determine levels of hypoxia that are harmful to ecosystems generally. With some simplifying assumptions, the model of Connett et al. (Connett et al., 1990) can be applied across species to understand the physiological strategies that determine critical O_2 thresholds in species with divergent metabolic rates and from regions with varying O_2 concentration. From Fig. 4, it is apparent that, in a given region, the P_{crit} increases with metabolic rate. Thus, animals with low O_2 demand are more tolerant of hypoxia, all else being equal. It also becomes apparent that, across the range of O_2 demand, organisms from more hypoxic regions have evolved greater hypoxia tolerance due to the mechanisms reviewed below.

Here, I identify two O_2 thresholds that may be important for the development of policy relating to climate-induced changes in O_2 content. In contrast to the two critical O_2 levels discussed above (Fig. 3), these O_2 thresholds are not species or situation specific. They are, however, temperature dependent. The first describes the O_2 level below which specific physiological adjustment or adaptation in O_2 uptake ability is required to sustain routine rates of metabolism in virtually all exposed species. The second describes what may be a physical constraint on O_2 extraction; it is
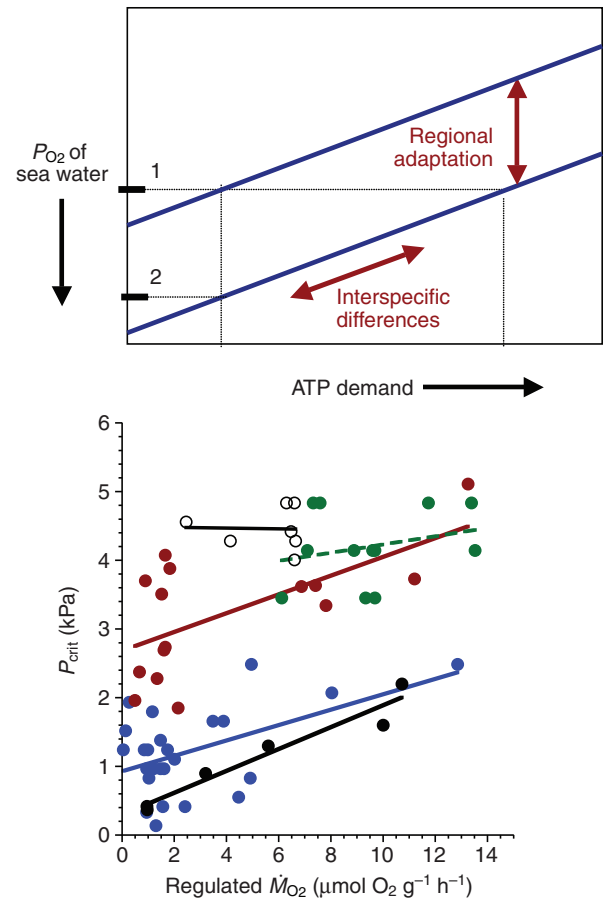

Fig. 4. (A) The relationship between the critical oxygen partial pressure (P_{crit}) and the regulated rate of oxygen consumption (routine rates that are independent of oxygen partial pressure). Variation along the x-axis indicates the effect of evolved metabolic rates on P_{crit}, while variation along the y-axis indicates adaptation to regional differences in O_2 concentration. (B) The relationship between P_{crit} and regulated rates of O_2 consumption ($\dot{M}_{O_2}$) for individuals living in regions of varying oxygen content (colors represent regions as in Fig. 2). The slopes of the regression lines are not significantly different, but the lines for Antarctica, Hawaii and California are significantly different from each other.

the P_{O_2} below which no further adjustment or adaptation in aerobic O_2 utilization appears possible. These thresholds are apparent by plotting P_{crit} for diverse species as a function of the minimum O_2 level to which the species are adapted (Fig. 2B). In this plot, a close correspondence is observed, at least within an intermediate range of P_{O_2} values, between P_{crit} and the minimum P_{O_2} to which a species is exposed. Above this range, P_{crit} plateaus, indicating that further increases in O_2 concentration have no effect on the capacity of animals to regulate metabolism. In other words, virtually all marine animals can regulate their metabolism to at least this O_2 level. Below this threshold, organisms that are not specifically adapted to low O_2 will suffer physiological stress and eventual death. This first threshold occurs at approximately 5 kPa in the present dataset but will increase with temperature. The second threshold is evident where further decreases in O_2 concentration fail to select for lower P_{crit}. It corresponds approximately to O_2 levels below which the biomass of permanent-OMZ residents is reduced and transient residents (e.g. diel migrant biota) reduce metabolic expenditure to facilitate brief (hours) forays into hypoxic water (see 'Metabolic suppression in OMZs').

The first O_2 threshold corresponds closely to the mean LC_{50} (O_2 value resulting in death of 50% of individuals tested) value of $70\,\mu mol\,l^{-1}$ reported for coastal benthic organisms (Vaquer-Sunyer and Duarte, 2008). Higher measurement temperatures, greater activity (metabolic) levels and synergistic stressors will all result in greater sensitivity to hypoxia. Most of the P_{crit} represented in Figs 2 and 4 were measured at 5°C. Regardless, the suggested boundaries put constraints on the expected hypoxia-induced shifts in species' vertical and horizontal distributions and provide a framework within which reductions in biodiversity and expansion of dead zones may be predicted and assessed. For example, the lower O_2 threshold falls just below the minimum O_2 level present along the California coast. Thus, any expansion of the OMZ in this region has the potential to dramatically alter the species composition, vertical distribution of organisms and functioning of the ecosystem as a whole.

Distribution of organisms and vertical migration in relation to the OMZ

The vertical distribution of oceanic organisms is highly complex (Robinson et al., 2010) and difficult to summarize within the scope of this review. However, a few general trends are informative. Firstly, there is little effect of low O_2 levels in the OMZ on the biomass of midwater organisms over a very wide range of O_2 concentrations (down to ~$10\,\mu mol\,l^{-1}$). However, different species have their own unique upper and lower depth limits and unique O_2 signatures. The biomass of permanently deep-living zooplankton and micronekton is quite high throughout the depth range in regions with moderate O_2 minimum zones, such as off the coast of California (Childress and Seibel, 1998) (Figs 1, 2). The modest effect of the OMZ on organismal vertical distribution is especially interesting considering that these O_2 concentrations are far below what is normally considered hypoxic in shallow and coastal habitats (e.g. Vaquer-Sunyer and Duarte, 2008).

At O_2 concentrations below ~$10\,\mu mol\,l^{-1}$, there are pronounced effects on both species' distributions and biomass. Hypoxia of this extreme level characterizes OMZs in the ETP (Fig. 1) and Arabian Sea. Within such regions, there are two dominant patterns of zooplankton distribution. (1) The permanent zooplankton biomass, including copepods, amphipods, chaetognaths and a variety of gelatinous species, is typically reduced within the core of the OMZ, and there is a secondary biomass peak at the lower edge of the OMZ, where O_2 content is rising (Wishner et al., 2008). This group effectively avoids the lowest O_2 concentrations and is adapted to stable hypoxia, like permanent residents in more moderate OMZs (Childress and Seibel, 1998). (2) The few species that do breach the core of the most intense OMZs are typically diurnal vertical migrators that enter shallower, more oxygenated waters at night (for reviews, see Fernandez-Alamo and Färber-Lorda, 2006; Wishner et al., 2008). This group is dominated by myctophid (lantern) fishes, which make up the bulk of the sound-scattering layer in many cases, and euphausiids (krill), although a variety of other taxa are also part of this mass migration. A third group, consisting of highly mobile, nektonic predators such as large squids and fishes, enters the OMZ but is usually tied closely to the surface by physiological constraints (see below).

A large proportion of animal species in all regions of the open ocean migrate on a diel basis between well-lit surface waters and the more dimly lit mesopelagic zone. This is the largest mass animal migration on Earth (Robison, 2009) and is driven predominantly by predator avoidance (although some organisms gain considerable energetic advantage by migrating into cool,

hypoxic waters) (Rosa and Seibel, 2010; Svetltichny et al., 2000). Diel vertical migrators living in areas of oceanic OMZs migrate through a gradient of relatively stable O_2 environments and so possess adaptations for enhanced anaerobic metabolism and metabolic suppression similar to those from unstable O_2 environments (Flück et al., 2007; Richards, 2011). By contrast, organisms from stable O_2 environments tend to rely more on aerobic adaptations for extraction of O_2 sufficient to maintain routine metabolic rates (Childress and Seibel, 1998).

Aerobic adaptations to OMZs

The aerobic adaptations of animals to O_2 minimum layers have been extensively reviewed (Childress and Seibel, 1998) and are summarized here. The problem of hypoxia is one of a reduced gradient to drive diffusion of O_2 from the ambient seawater, across gas-exchange surfaces, to the mitochondria. Because the energy yield of aerobic metabolism is high relative to anaerobic metabolic pathways (Hochachka and Somero, 2002), there is strong selection for mechanisms to take up O_2 effectively from very low ambient partial pressures (i.e. to increase the effective gradient). Accordingly, most permanent residents of moderate OMZs are able to regulate their O_2 consumption rate to the minimum O_2 levels encountered in their environments (Fig. 2) (reviewed in Childress, 1995; Childress and Seibel, 1998). In other words, their P_{crit} are generally lower than the minimum O_2 level they encounter. Specific adaptations for O_2 extraction in crustaceans, fishes and cephalopods living in O_2 minimum layers include: (1) high ventilatory volumes and circulatory capacity (Belman and Childress, 1976); (2) thin blood-to-water diffusion distances across the gills and high gill surface area (e.g. Antezana, 2002; Childress, 1969); and (3) respiratory proteins with a high affinity for O_2 (high affinity=low P_{50}, the O_2 concentration resulting in 50% O_2 saturation of respiratory proteins) (Seibel et al., 1999) and high sensitivity to pH (e.g. a large negative Bohr coefficient) (Seibel and Walsh, 2003). Interestingly, gelatinous zooplankton (e.g. medusae and ctenophores) also appear capable of regulation to very low P_{O_2} despite an apparent lack of O_2 transport systems or specific gas exchange surfaces. In such cases, weak diffusion gradients may simply be sufficient to meet low O_2 demand in species that are 95% water and whose actively metabolizing tissues are located towards the exterior of the animal (Thuesen et al., 2005).

O_2 storage

From a mammalian perspective, 'breath-holding' is an attractive option for periodic forays into hypoxic environments. However, very few, if any, fishes or invertebrates are known to store O_2 in any comparable way. A few possible exceptions have been hypothesized recently. The fish *Bregmacerous cantori* was shown to undergo dramatic changes in the volume and tension of the O_2-filled swimbladder during its vertical migration into the anoxic Cariaco Basin (Love et al., 2004). The conclusion was that the bladder volume decreased at depth as the fish utilized the O_2 stored within to support aerobic metabolism in otherwise anoxic waters. O_2 stored for buoyancy in the chambers of the *Nautilus* shell (Cephalopoda) may be used to support a suppressed rate of aerobic metabolism during exposure to hypoxia (Boutilier et al., 1996). Thuesen et al. recently demonstrated that gelatinous organisms (e.g. medusae) take up O_2 (passively, as far as is known) into the mesogleal gel that can later be used to support aerobic metabolism in active tissues when ambient O_2 levels are too low (Thuesen et al., 2005). It was shown that this O_2 storage is sufficient to support short migrations into subsurface hypoxia in shallow estuaries. The

utility of this strategy for O_2 provision during migrations over greater depths into OMZs would be limited.

Are high-performance animals excluded from the OMZ?

The ability of a species to regulate its rate of O_2 consumption to low P_{O_2} is facilitated by low O_2 demand. The evolved demand for O_2 (energy) varies by nearly 1000-fold, independent of body size and temperature, among species across a depth range. Efforts to explain this variation typically invoke limitations on resource availability (e.g. O_2 or food) in the deep sea. This implies that deep-living species would evolve a higher energy consumption rate if only more resources were available. However, without specific energetic demand, an increase in consumption is wasteful and presumably not favored by natural selection. In other words, organisms do not consume O_2 and produce ATP they don't need. Thus, reduced metabolic rates of permanently deep-living and gelatinous species do not result from resource limitation. Regional comparisons have shown that deep-sea species of some taxa have low metabolic rates even in regions with higher O_2 concentrations. Furthermore, species from high O_2 regions have higher P_{crit} despite metabolic rates as low as those from hypoxic regions (Fig. 4) (Childress and Seibel, 1998). Thus, low rates are not a specific adaptation to hypoxia. Rather, low rates reflect sluggish habits that result from relaxed selection for strong locomotory abilities for visual predator–prey interactions in the poorly lit bathypelagic waters (Childress, 1995; Seibel and Drazen, 2007).

Nevertheless, many vertically migrating zooplankton living in OMZs cannot support their routine energy demands aerobically at depths across the entire O_2 gradient encountered (Fig. 2). For example, the large copepod *Gaussia princeps* has a P_{crit} of 1.6 kPa but lives part of the time at P_{O_2} values as low as 0.8 kPa (off the Californian Coast). It has a substantial capacity to survive entirely without O_2 (10–14 h) (Childress, 1977) whereas some other copepod species are excluded from intense OMZs (Auel and Verheye, 2007). Similar strategies have been described for the vertically migrating cephalopod *Gonatus onyx* (Hunt and Seibel, 2000) and the vertically migrating fish *Stenobrachius leucopsaurus* (Torres et al., 1979). *Gonatus onyx* has a P_{crit} of only 4.0 kPa but spends its daytime at a P_{O_2} of <0.8 kPa. Similarly, *S. leucopsaurus* has a P_{crit} of 1.6 kPa despite spending its daytime hours in relative hypoxia. The jumbo squid, *Dosidicus gigas*, has among the highest metabolic rates of any animal on the planet, yet is closely tied to regions of low O_2 (Seibel, 2007). Despite respiratory adaptations for hypoxia tolerance, *D. gigas* cannot support its routine metabolic rate across the entire depth range it occupies (Gily et al., 2006; Rosa and Seibel, 2010). *D. gigas* has a P_{crit} of 1.6 kPa but routinely experiences a P_{O_2} below 0.4 kPa (L. Trueblood and B.A.S., unpublished data).

The elevated metabolic rates of vertically migrating and high-performance nektonic species preclude aerobic survival in the most pronounced OMZs by virtue of the extraordinary ventilation volumes required to meet routine O_2 demand. In the absence of hypoxia-induced metabolic suppression (see below), a 100 g *D. gigas* must pass more than 2 liters of OMZ core water, at 10 μmol l^{-1} O_2 and assuming complete extraction of all available O_2, across its gills every minute to meets routine needs (Rosa and Seibel, 2010). Because ventilation and locomotion are tied in many active squids and fishes (Wells, 1988), the demand for O_2 increases as ventilation rates increase. This positive feedback effectively precludes most top predators from OMZs. As a result, the depth distribution of most high-performance fishes, such as tunas and swordfish, is limited by O_2 concentrations less than ~150 μmol l^{-1} (Brill, 1994).

In fact, swordfish and marlin were shown to reduce the extent of their vertical excursions by more than 500 m off the Californian Coast relative to more oxygenated waters in the Atlantic (Carey and Robison, 1980; Prince and Goodyear, 2006). Both white sharks and mako sharks appear to be limited in waters with O_2 concentrations less than ~50 μmol l^{-1} (Vetter et al., 2008; Nasby-Lucas et al., 2010) although both occasionally experience waters with as little as 20 μmol l^{-1} O_2. The great white shark, *Carchardodon carcharias*, was similarly shown to limit its depth range in hypoxic regions and to make shorter and shallower dives where O_2 was low (Nasby-Lucas et al., 2010).

Epipelagic predators appear to have reached an upper limit to O_2 extraction. Increased metabolism and decreased P_{O_2} both select for increased gill diffusion capacity. Permanent residents of the OMZ already have gill diffusion capacities similar to those of active fishes and squids (Childress and Seibel, 1998; Wegner et al., 2010) despite a demand for O_2 that is orders of magnitude lower than that of shallower-living species. Presumably, both groups are near a lower limit for gill membrane thickness and perhaps an upper limit for gill surface area. Thus, organisms with higher metabolic rates may be constrained in terms of the structural and biochemical capacities required for O_2 extraction in the OMZ.

There are some high-performance predators that have managed to minimize the trade-offs inherent between high performance and hypoxia tolerance. In bigeye tuna (*Thunnus obesus*), for example, the onset of cardio-respiratory adjustments during acute hypoxia occurs at lower ambient O_2 levels (~40 μmol l^{-1}) than in other species from more oxygenated regions (Lowe et al., 2000). In this case, greater hypoxia tolerance appears to be due to a hemoglobin with a high affinity for O_2. However, the utility of a high-affinity respiratory protein is constrained in active animals by the need to release O_2 at high rates to metabolically active tissues. Even in sluggish permanent residents of OMZs, an enhanced pH sensitivity (e.g. Bohr coefficient) is required to facilitate the release of O_2 to the tissues at rates sufficient to support even a low O_2 demand (Childress and Seibel, 1998). In the case of *T. obesus*, this constraint is overcome because the enhanced Bohr effect is coupled with unique adaptations that facilitate greater off-loading as blood is warmed in the core of the tuna's heterothermic body (Lowe et al., 2000). Similarly, *D. gigas* and other vertical migrators have high-affinity respiratory proteins that are both temperature and pH sensitive, facilitating O_2 uptake in cold, deep waters where O_2 is scarce and enhancing O_2 release in warmer surface waters where O_2 and O_2 demand are elevated. However, even these adaptations are insufficient for survival in the core of the OMZ. Blood hemocyanin–O_2 saturation falls below 20% at around 300 m depth in *D. gigas* (Fig. 5). The P_{crit} corresponds to ~60% hemocyanin–O_2 saturation at *in situ* temperatures. This level is reached as shallow as 160 m throughout much of the squid's natural range. If not for an upregulation of anaerobic ATP production and the pronounced metabolic suppression described below, vertical migrators would still largely be excluded from well-developed OMZs.

Anaerobic metabolism in OMZs

Aerobic metabolism may be supplemented with anaerobic metabolic ATP production (e.g. fermentation) in order to maintain a routine metabolic rate under hypoxic conditions in the OMZ. However, the yield of ATP per mole of fermentable substrate is always modest during anaerobic glycolysis compared with oxidative metabolism (Hochachka and Somero, 2002). Some anaerobic metabolic pathways (those resulting in succinate and propionate accumulation, for example) are more efficient than
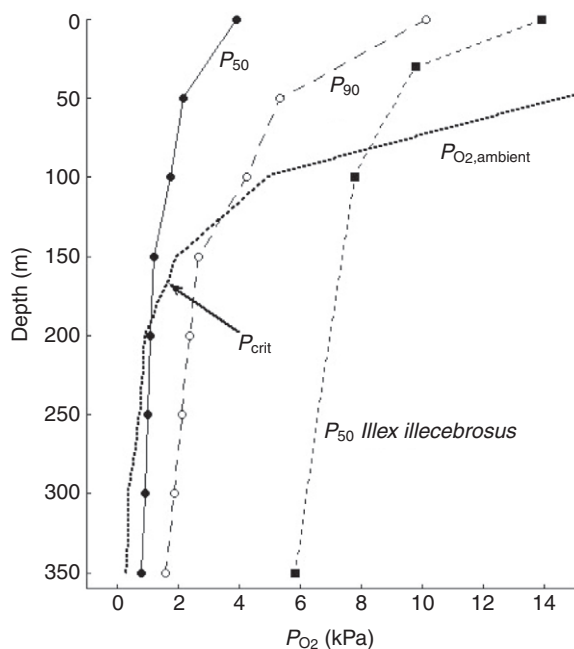
Fig. 5. Relationship between blood–O$_2$ saturation and depth in the jumbo squid, *Dosidicus gigas* (B.A.S., unpublished data). The ambient O$_2$ in the Gulf of California decreases as a function of depth, resulting in reduced O$_2$ saturation of the blood. The oxygen partial pressure resulting in 50 and 90% saturation of hemocyanin with oxygen in *D. gigas* (P_{50} and P_{90}, respectively) decreases with depth (i.e. the affinity of hemocyanin for oxygen increases due to reduced temperature at depth). By comparison, the P_{50} for another ommastrephid squid, *Illex illecebrosus*, is shown from the more oxygenated Atlantic Ocean. The lower P_{50} of *D. gigas* reflects adaptation to the pronounced oxygen minimum zone (OMZ) in the Eastern Pacific. At depths above ~200 m, the blood of *D. gigas* is at least 50% saturated. At depths shallower than ~130 m, its blood is at least 90% saturated. By contrast, *I. illecebrosus* would have to stay shallower than 100 m to achieve even 50% saturation if it lived in the Eastern Pacific. The critical oxygen partial pressure (P_{crit}) occurs near 160 m for *D. gigas*.

others, although still nowhere nearly as efficient as oxidative metabolism. Therefore, utilization of anaerobic metabolic pathways for anything but short bursts of activity requires a substantial supply of food or large stores of fermentable substrate (i.e. glycogen). Depletion of reserves during anaerobic metabolism minimizes the advantages gained by migration to the rich food supplies in shallow water. A further consequence of anaerobic metabolism is the accumulation of deleterious end-products. Anaerobic glycolysis results in the accumulation of protons as well as organic compounds such as lactate and octopine (Hochachka and Somero, 2002), so consideration must be given to acid–base disturbances (Hochachka and Mommsen, 1983). Some anaerobic pathways also produce volatile end-products that can be excreted directly across the gills, allowing their continued use without risk of acid–base disturbance (Stecyk et al., 2004). However, the remaining energy contained in the volatile end-products (e.g. ethanol) is lost and cannot be recycled. Despite these disadvantages, there is evidence for elevated anaerobic metabolic capacity as well as the accumulation of anaerobic end-products following hypoxic exposure in some diel vertical migrators living in regions with pronounced OMZs.

Torres et al. (J. J. Torres, M. D. Grigsby and M. E. Clarke, manuscript in preparation) demonstrated higher activities of alcohol dehydrogenase, an enzyme involved in glycolytic ATP production with the concomitant formation of ethanol, in myctophid fishes from the Arabian Sea compared with regions with higher O$_2$ concentrations. This exciting finding suggests that cyprinids are not the only fishes that produce ethanol as an anaerobic end-product and represents a novel physiological adaptation to OMZs. Ethanol is a volatile end-product that can be excreted directly across the gills, along with the CO$_2$ that is simultaneously produced, thereby avoiding several of the disadvantages of anaerobic metabolism (Stecyk et al., 2004). However, the energy contained in the excreted ethanol is lost. Similarly, Gonzales and Quinones showed that krill that migrate into an OMZ have very high activities of lactate dehydrogenase compared with a copepod that lives permanently in the well-oxygenated waters above (Gonzales and Quinones, 2002). However, this comparison is confounded by body size and phylogeny and the conclusions are in contrast to most studies of enzymatic activities in OMZs. For example, most studies find no suggestion of increased anaerobic capacity in the enzymatic profiles of fishes, cephalopods or copepods living in OMZs (Childress and Somero, 1979; Vetter and Lynn, 1997; Thuesen et al., 1998; Seibel et al., 2000). Vetter et al. actually found that lactate dehydrogenase (LDH) activity in the Dover sole (*Microstomus pacificus*) decreased in response to low O$_2$ (Vetter et al., 1994).

The few studies that have measured accumulation of anaerobic end-products under hypoxic conditions suggest that glycolysis is upregulated in the OMZ but that it accounts for only a small fraction of the total routine energy budget. *Dosidicus gigas* spends hours each day at O$_2$ levels below its P_{crit} (Rosa and Seibel, 2008; Rosa and Seibel, 2010). Under laboratory conditions that mimic its daytime habitat (10°C, ~10 µmol l^{-1} O$_2$), octopine accumulated to a level that amounts to only 7% of the total ATP demand under those conditions (Rosa and Seibel, 2010). Similarly, the euphausiids *Euphausia eximia* (Fig. 6B) and *Meganyctiphanes norvegica* both accumulated lactate under hypoxic conditions. However, *E. eximia*, which lives in an extreme OMZ in the ETP, survived treatment with 0.8 kPa P_{O_2} (J. Schneider and B.A.S., unpublished observation) whereas O$_2$ below 1.8 kPa resulted in 100% mortality in *M. norvegica* (Spicer et al., 1999), a species that lives in the North Atlantic but sometimes experiences moderate hypoxia in fjords. Anaerobic metabolic ATP production may simply support activity levels above the basal metabolic rate for short periods of time, as opposed to wholly supporting metabolic expenditure throughout the period of hypoxic exposure.

Metabolic suppression in OMZs

Suppression of total metabolism appears to be a prerequisite for the survival of prolonged bouts of O$_2$ limitation (Hochachka and Somero, 2002; Bickler and Buck, 2007; Guppy and Withers, 1999). Total metabolism consists of the energy produced *via* oxidative (aerobic) metabolic pathways (estimated from the O$_2$ consumption rate, $\dot{M}_{O_2}$) and that produced by anaerobic metabolic pathways (McDonald et al., 1998), typically estimated from the accumulation of anaerobic end-products. Metabolic suppression is achieved primarily by shutting down expensive cellular activities (Hand, 1998), such as biosynthesis and ion pumping (Buck and Hochachka, 1993). The resulting decrease in ATP consumption is matched by a decrease in ATP production primarily achieved through down-regulation of glycolytic enzymes (Lutz and Nilsson, 1997; Brooks and Storey, 1997). Thus, a ubiquitous feature of hypoxia- and anoxia-tolerant organisms is the ability to maintain

nearly constant levels of ATP (Hochachka and McClelland, 1997; Suarez et al., 1987).
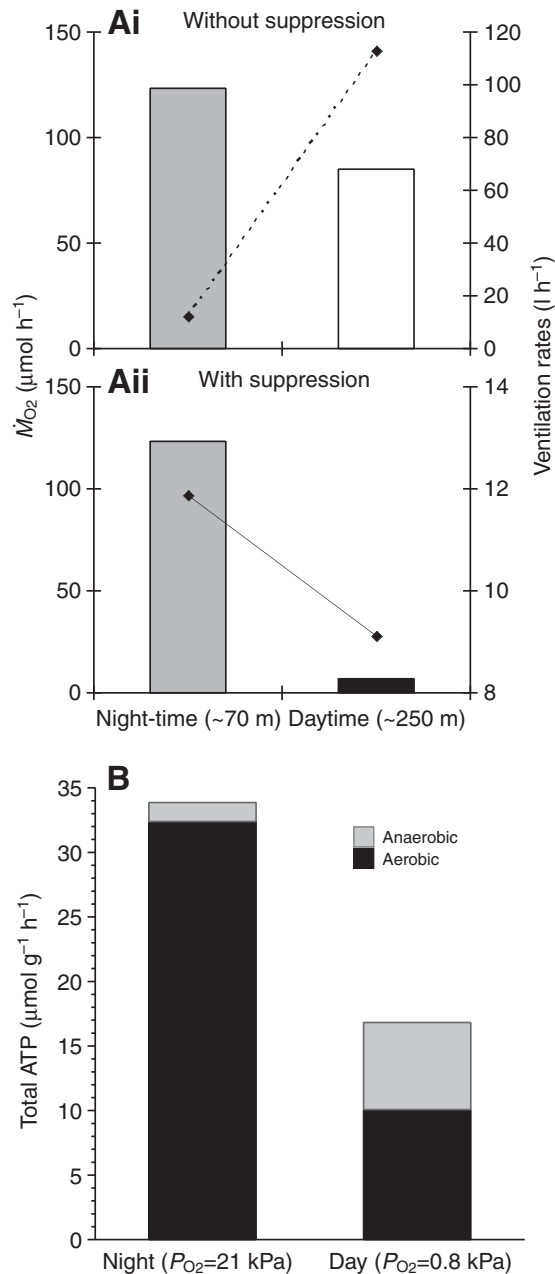

Fig. 6. (A) Projected ventilation rates (connected black diamonds; l h⁻¹) and routine metabolic rates (bars; μmol O₂ h⁻¹) for a 10 g squid (*Dosidicus gigas*) during daytime and night-time depth distribution in the Guaymas Basin in the Gulf of California, with (ii) and without (i) the occurrence of metabolic suppression (Rosa and Seibel, 2010). Ventilation rate equals the water volume from which all O₂ must be extracted per hour to support routine metabolism. The oxygen consumption rate ($\dot{M}_{O_2}$) was measured under conditions that mimic the squid's daytime and night-time distributions (21 kPa P_{O_2}, 20°C and 0.8 kPa P_{O_2}, 10°C, respectively). (B) Total ATP demand in the krill *Euphausia eximia* under conditions that mimic its daytime and night-time depth distributions in the Eastern Tropical Pacific oxygen minimum zone (OMZ). The gray bars represent the anaerobic contribution to ATP production as measured by lactate accumulation. The black bars represent the aerobic contribution measured by oxygen consumption. The total ATP demand is suppressed by about 50% in this species (J. Schneider and B.A.S., manuscript in preparation).

Not surprisingly, the duration of anoxia/hypoxia tolerance among organisms is inversely related to the extent of their metabolic suppression (Hand, 1998). Brine shrimp (*Artemia* spp.) can shut down metabolism to immeasurable levels and survive complete anoxia for at least 4 years (Hand, 1998). Such complete metabolic suppression has not been reported in marine species, but extreme suppression is thought to occur during diapause in some oceanic copepods (Auel et al., 2005). More commonly, organisms suppress metabolism by 50–95% and supplement the remaining energy demand using a combination of available O₂ and anaerobic metabolic pathways. Such organisms survive anoxia for hours or days.

Metabolic suppression during diel migrations in the OMZ has been demonstrated in only a few oceanic species. *Dosidicus gigas* maintains its rate of energy consumption independent of the ambient P_{O_2} until a critically low level (P_{crit}=1.6 kPa) is encountered (Gilly et al., 2006) (L. Trueblood and B.A.S., unpublished data). Below the P_{crit} (at depths below ~150 m), the $\dot{M}_{O_2}$ of *D. gigas* sharply declines and anaerobic metabolism is enhanced to partially offset the reduction in aerobic ATP synthesis. Squids use the glucose–opine anaerobic pathway, which entails a reductive condensation of pyruvate to form octopine with the net synthesis of three ATPs per unit glycogen catabolized (1.5 units ATP per unit octopine produced). Octopine is accumulated to levels 10-fold higher under hypoxic conditions relative to controls. Despite this dramatic upregulation, glycolytic flux accounts for only 7.0% and 2.8% of the energy deficit at 10°C and 20°C, respectively. This represents a substantial suppression of total ATP production in *D. gigas* (~87% at 10°C, under the hypoxic conditions characteristic of its daytime habitat depth) (Rosa and Seibel, 2010) (Fig. 6A). Some vertically migrating euphausids, which are a common prey item for *D. gigas*, similarly suppress total ATP production (~40%) under hypoxic conditions characteristic of their deeper daytime habitat. The accumulation of lactate in euphausiids (see 'Anaerobic adaptations to the OMZ') is significant but, as in *D. gigas*, represents a fairly small fraction of the total metabolic demand (Fig. 6B).

Several behavioral observations are consistent with the idea that vertically migrating species suppress metabolism during daytime forays into OMZs. In one of the earliest reports of midwater animal behavior *in situ*, Barham described the orientation and activity levels of several species of vertically migrating midwater fishes in the California Current (Barham, 1971). At their deeper, daytime depths, most individuals were 'passively drifting' whereas at shallower, night-time depths, they were 'actively swimming'. Barham attributed this 'lethargy' to the low O₂ levels in the deep-sea (Barham, 1971). When alerted to the submersible, all fishes swam quickly away. Similar *in situ* behavior was reported for the vertically migrating mesopelagic squid *G. onyx*. Hunt and Seibel observed that individuals in shallow water were quick to dart away when approached by the submersible whereas individuals in deeper water held their positions and escaped only after persistent harassment by the investigators (Hunt and Seibel, 2000). Some species are quick to escape from submersibles while others are attracted to submersible lights. Thus, *in situ* determinations of activity levels can be misleading. For example, Cowles reports equal activity levels during both day and night off the Californian Coast for the vertically migrating shrimp *Sergestes similis* (Cowles, 1994). However, he also reported that shrimps observed by the submersible were always swimming directly downward. This was explained as positive geotaxis in response to the lights of the submersible. His laboratory analysis, by contrast, revealed a

reduction in O_2 consumption and swimming speed with declining O_2 concentrations (Cowles, 2001).

Svetlichny et al. found that the copepod *Calanus euxinus* reduced its basal metabolism, as well as its scope for activity, under laboratory conditions that mimic its daytime habitat in the Black Sea (P_{O_2} ~1.5 kPa, 8°C) relative to air-saturated conditions at 18°C (Svetlichny et al., 2000). The frequency of locomotory acts decreased more than 3-fold. Hypoxia was shown to provide an important energetic advantage during diel migrations in this species.

Antezana found no difference in $\dot{M}_{O_2}$, swimming or feeding activity under low O_2 conditions in the vertically migrating krill *Euphausia mucronata*, living in the OMZ in the ETP (Antezana, 2002). This finding contrasts with those for other krill species (J. Schneider and B.A.S., unpublished) (Spicer et al., 1999; Teal and Carey, 1967) (see above). Antezana did find elevated gill surface area relative to species living in more oxygenated regions, suggesting enhanced O_2 extraction capabilities in *E. mucronata* (Antezana, 2002).

I suggest that metabolic suppression is a common strategy for survival during daytime forays into pronounced OMZs. The prevalence of suppression within the vertically migrating community in any given region will depend on the local O_2 distribution as well as temperature and the particular species complex. The extent of suppression for any particular species will depend on its metabolic demand, phylogenetically available biochemical pathways, its vertical distribution and capacity for O_2 transport. Thus, the consequences for organismal contributions to biogeochemical cycles and ecosystem dynamics (see below) will be difficult to predict and requires direct measurement for the dominant species.

Potential consequences of expanding OMZs

The survival of oceanic organisms in OMZs depends on total O_2 demand and the capacities for O_2 extraction and transport, anaerobic ATP production and metabolic suppression. These parameters are, within a range of P_{O_2}, closely tuned to the O_2 level to which a species has evolved. In other words, the minimum O_2 level experienced by a particular species is often very close to its evolved P_{crit}. This fact facilitates the identification of two O_2 thresholds, as discussed above, that may prove useful for policy makers in the near future. Between these thresholds, specific physiological adaptations are required of virtually all species. Climate-induced changes in O_2 concentrations may dramatically alter the ecosystem, especially if O_2 concentrations are pushed below the lower threshold. This threshold represents a limit to adaptation that will certainly result in an ecological transition, from one dominated by a diverse midwater fauna to one dominated by diel migrant biota that must return to surface waters at night.

In the event of such a transition, the ecology of the region will be fundamentally altered. For example, interactions between top predators and their prey may change as the habitable night-time depth range is compressed. Anaerobic metabolism and metabolic suppression are not sustainable due to substrate limitation, end-product accumulation (i.e. O_2 debt) and the likely constraint imposed on foraging at depth by metabolic suppression. These factors dictate a return to surface waters during some portion of the day. If deep-water hypoxia expands due to climate change, the depth to which migrating organisms must return to burn off an O_2 debt at night will shoal. However, surface waters also impose O_2 stress due to elevated O_2 demand at high temperature and constrained O_2 transport due to ocean acidification (Rosa and
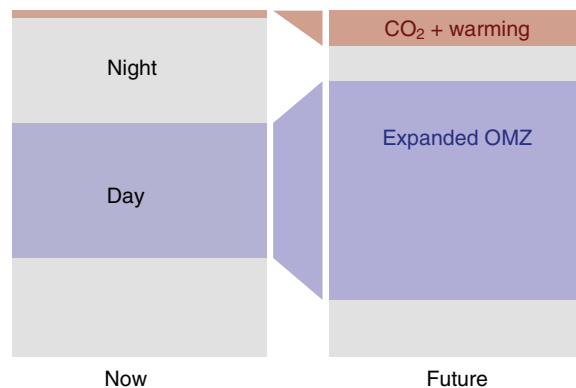

Fig. 7. Schematic illustrating compression of the night-time habitat range (gray) for vertically migrating species from the present (left) to a future ocean impacted by global warming, ocean acidification and expanding oxygen minimum zones (OMZs). Warm surface waters (red) are stressful to some species, and expansion of this zone will restrict the depth to which organisms can retreat to burn off an oxygen debt accrued during night-time forays into OMZs (blue).

Seibel, 2008) and high temperature (Ekau et al., 2010). Thus, the synergistic impact of these three climate-related variables (temperature, ocean acidification and declining O_2) is to compress the habitable night-time depth range of vertically migrating species (Fig. 7). Because vertical distributions and environmental tolerances are species-specific, habitat compression will affect each species differently and may result in altered ecological interactions. Some species may additionally alter their geographical range as a result of climate variation. For example, a recently reported invasive expansion of the range of *D. gigas* in the Eastern North Pacific (Zeidberg and Robison, 2007) has been attributed to, among other things, a climate-induced shoaling of the OMZ within the California Current System (Bograd et al., 2008; Vetter et al., 2008).

Expanding OMZs will also affect biogeochemical cycles. Of particular interest here is the influence of OMZs on the contribution of mesopelagic animals to respiratory carbon flux. Diel migrators consume carbon near the surface and then transport it to depth, where it is excreted as CO_2 (Angel 1989; Longhurst et al., 1990). This process is responsible for a large fraction of the carbon exported from surface waters in some regions (Ducklow et al., 2001). The OMZ influences this flux by altering the biomass, vertical distribution and physiology of resident organisms. In particular, if most migrators are suppressing metabolism at depth, then the amount of CO_2 released is similarly suppressed, thus decreasing the efficiency of this 'biological carbon pump'. In other cases, such as for the myctophid fishes discussed above, a reliance on anaerobic ethanol production may contribute novel substrates for microbial metabolism. These considerations have not been considered in any quantitative analysis of carbon flux. Animal physiology and, in particular, the response of animals to expanding hypoxia, is a critical, but understudied, component of biogeochemical cycles and oceanic ecology (Seibel and Dierssen, 2009).

List of abbreviations

ETP	Eastern Tropical Pacific
$\dot{M}_{O_2}$	oxygen consumption rate in molar units
OMZ	oxygen minimum zone
P_{50}	oxygen partial pressure resulting in 50% saturation of respiratory proteins
P_{90}	oxygen partial pressure resulting in 90% saturation of respiratory proteins

P_{crit} critical oxygen partial pressure
P_{O_2} oxygen partial pressure
ΔG free energy

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