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SYMPOSIUM

Hypoxia Tolerance and Metabolic Suppression in Oxygen Minimum Zone Euphausiids: Implications for Ocean Deoxygenation and Biogeochemical Cycles

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Synopsis The effects of regional variations in oxygen and temperature levels with depth were assessed for the metabolism and hypoxia tolerance of dominant euphausiid species. The physiological strategies employed by these species facilitate prediction of changing vertical distributions with expanding oxygen minimum zones and inform estimates of the contribution of vertically migrating species to biogeochemical cycles. The migrating species from the Eastern Tropical Pacific (ETP), *Euphausia eximia* and *Nematoscelis gracilis*, tolerate a Partial Pressure (PO_2) of 0.8 kPa at 10 °C (~15 μM O_2) for at least 12 h without mortality, while the California Current species, *Nematoscelis difficilis*, is incapable of surviving even 2.4 kPa PO_2 (~32 μM O_2) for more than 3 h at that temperature. *Euphausia diomedeae* from the Red Sea migrates into an intermediate oxygen minimum zone, but one in which the temperature at depth remains near 22 °C. *Euphausia diomedeae* survived 1.6 kPa PO_2 (~22 μM O_2) at 22 °C for the duration of six hour respiration experiments. Critical oxygen partial pressures were estimated for each species, and, for *E. eximia*, measured via oxygen consumption (2.1 kPa, 10 °C, $n = 2$) and lactate accumulation (1.1 kPa, 10 °C). A primary mechanism facilitating low oxygen tolerance is an ability to dramatically reduce energy expenditure during daytime forays into low oxygen waters. The ETP and Red Sea species reduced aerobic metabolism by more than 50% during exposure to hypoxia. Anaerobic glycolytic energy production, as indicated by whole-animal lactate accumulation, contributed only modestly to the energy deficit. Thus, the total metabolic rate was suppressed by ~49–64%. Metabolic suppression during diel migrations to depth reduces the metabolic contribution of these species to vertical carbon and nitrogen flux (i.e., the biological pump) by an equivalent amount. Growing evidence suggests that metabolic suppression is a widespread strategy among migrating zooplankton in oxygen minimum zones and may have important implications for the economy and ecology of the oceans. The interacting effects of oxygen and temperature on the metabolism of oceanic species facilitate predictions of changing vertical distribution with climate change.

Introduction

Oxygen concentrations at intermediate depths in upwelling regions are often less than 10 μM and may fall below 2 μM (Paulmier and Ruiz-Pino 2009). Anthropogenic influences are leading to expansion and shoaling of these oxygen minimum zones (OMZs) with a decline in the minimum oxygen concentration (Bograd et al. 2008; Bograd et al. 2008; Stramma et al. 2008; Keeling et al. 2010; Deutsch et al. 2011;). The consequences of such changes in the oxygen environment for oceanic zooplankton likely include altered faunal composition and distribution (Auel and Verheye 2007; Wishner et al. 2013; Maas et al. 2014; Deutsch et al. 2015). Specifically, the vertical habitat of zooplankton may be compressed between relatively well-lit, warming, deoxygenating

and acidifying surface waters and expanding hypoxic zones at intermediate depths, leading to changes in predator–prey interactions that dictate ecosystem dynamics (Chavez and Messié 2009; Bertrand et al. 2011; Koslow et al., 2011, 2014; Seibel 2011, 2015; Bianchi et al. 2013; Brietburg et al. 2015; Netburn and Koslow 2015; Wishner et al. 2013).

The metabolism of diel migrating zooplankton and micronkton also contributes to oxygen depletion in the upper margin of the OMZ (Bianchi et al. 2013), effectively creating a deeper, "oxygen-deficient ecological barrier" (Donoso and Escribano 2014). The low oxygen zones are, in turn, among the largest predictors of deep scattering layer migration depth on a global scale (Bianchi et al. 2013; Klevjer et al. 2016) and the vertical distribution of larger predators is also strongly influenced by oxygen profiles (Prince and Goodyear 2006; Stramma et al. 2011; Seibel 2015). On a more local scale, migration depth often appears to be set by light and predator-avoidance regardless of oxygen content (Wishner et al. 2013; Maas et al. 2014; Cade and Benoit-Bird 2015). Underlying these seemingly contradictory observations is the fact that migration depths are species specific (Antezana 2009; Dypvik and Kaartvedt 2013) and depend on physiological, as well as ecological, considerations (Seibel 2011; Bianchi and Mislan 2016). A physiological understanding of zooplankton hypoxia tolerance is essential for accurate prediction of ecosystem responses to expanding oxygen minimum zones.

Moreover, the transfer of carbon and nutrients from surface to depth by diel migrators can comprise a substantial fraction of the total vertical flux (Longhurst and Harrison 1989; Longhurst et al. 1990; Dam et al. 1995; Zhang and Dam 1997; Hays et al. 1997; Steinberg et al. 2000, 2008a, 2008b; Buesseler et al. 2008; Stukel et al. 2013). However, estimates of this contribution are calculated based on the assumption that metabolic rates of migrating zooplankton at depth are equivalent to those measured at the surface, save for the effect of temperature on metabolism. This assumption may be met in some regions of the ocean, but extreme hypoxia in pronounced oxygen minimum zones (OMZ) has a profound effect on the metabolic rates of zooplankton and micronekton (Seibel 2011; Elder and Seibel 2015; Kiko et al. 2015; Auel et al. 2005). In these regions, oxygen concentrations drop within intermediate water depths to a threshold level beyond which further adaptation for oxygen provision appears constrained (Seibel 2011). Below this "adaptation threshold", vertical migrators often reduce their oxygen consumption rates by 40–80% (Seibel 2011; Maas et al. 2012; Seibel et al. 2014; Elder and Seibel 2015; Svetlichny et al. 2000; Kiko et al. 2015; Auel et al. 2005). Such a strategy limits the consumption, repackaging, and excretion of carbon and nutrients by vertical migrators while at depth in pronounced OMZs, thereby impacting biogeochemical cycling in OMZs.

Euphausiids are critical components of pelagic ecosystems worldwide (Ikeda 2013) and can comprise up to 50% of the total zooplankton biomass in the eastern tropical Pacific (Brinton 1979 and see Fernández-Älamo and Färber-Lorda, 2006, for review). While their vertical migration patterns are diverse, many species are known to migrate into the core of pronounced OMZs (Brinton 1979; Sameoto et al. 1987; Escribano et al., 2000; Antezana 2009; Maas et al. 2014). However, the hypoxia tolerance of euphausiids is highly variable and poorly constrained (Teal and Carey 1967; Childress 1975; Spicer et al. 1999; Strömberg and Spicer 2000; Antezana 2002; Tremblay et al. 2010; Huenerlage and Bucholz 2013; Werner and Buchholz 2013). Here, we assess the metabolic strategies of several dominant euphausiids that reside in regions with variably developed OMZs to test the hypothesis that total metabolism is suppressed during daytime forays into low oxygen. We further estimate the depths at which the oxygen partial pressure (PO_2) becomes critical for maintenance of routine metabolism and begins to constrain euphausiid vertical distributions.

Methods

Oxygen definiendum

Oxygen partial pressure (PO_2): expressed in units of kilopascals (kPa), represents the portion of the pressure exerted by gas in seawater that is attributed to oxygen. At 100% air saturation, the PO_2 is 21% of the total or ~21 kPa. Other units, 760 torr (mmHg) = 101 kPa = 1 atm.

Oxygen concentration [O_2]: expressed in units of μmoles kg^{-1} (μM), depends on oxygen solubility, which depends on the temperature and salinity. At air saturation, 33 ppt salinity and 10 °C, seawater contains 282 μM oxygen. Note: 1 mole O_2 = 22.4 liters = 32 grams. Thus, 282 μM = 6.3 ml kg^{-1}.

Unit conversion: The PO_2 of seawater is expressed relative to what that seawater would hold at a given temperature in equilibrium with air. Seawater at 10 °C holds 282 μM when air-saturated (at PO_2 = 21 kPa). An [O_2] of 28 μM at 10 °C, thus has a partial pressure of 2.1kPa (10% of 21 kPa). That same [O_2] will exert a greater relative pressure at

higher temperature because gases are less soluble. Air-saturated seawater at 20 °C holds only 233 μM. Thus, 28 μM O_2 in seawater at 20 °C exerts a PO_2 of 2.5 kPa.

Hypoxia: characterized by low PO_2. This is a relative term with no strict definition or associated value.

Critical PO_2 (P_{crit}): The PO_2 at which the rate of oxygen consumption is no longer independent of PO_2. P_{crit} may also be defined as the PO_2 beyond which anaerobic metabolic end-products (e.g., lactate) are accumulated.

Specimen collection and environmental sampling

Nematoscelis gracilis and *Euphausia eximia* were collected in the Tehuantepec Bowl (13°N, 105°W) and Costa Rica Dome region (9°N; 90°W) on two research expeditions to the Eastern Tropical Pacific (ETP) that took place October 18 to November 17, 2007 aboard the *R/V Seward Johnson*, and December 7, 2008 to January 6, 2009 aboard the *R/V Knorr*. *Nematoscelis difficilis* was collected aboard the *R/V New Horizon* in the California Current (San Clemente Basin, California, 32.5°N, 118°W) in October 2010. *Euphausia diomedeae* was collected aboard the *R/V Thuwal* in the Red Sea (22°29' N, 30°2' E) in spring 2014. CTD-DO casts were conducted daily.

Specimens used for live animal experiments were collected using three different nets. In the ETP and California Current, the primary net was a modified, opening-closing Tucker Trawl (Childress et al. 1978) with a 10 m^2 mouth fitted with a 30-l insulated closing cod end. Trawls were conducted both day and night. The insulated cod end minimizes temperature changes during recovery to the surface and protects specimens from physical damage due to water turbulence. Depth, temperature, and conductivity were recorded continuously during each trawl. The trawl opening-–closing was triggered electronically using a Multiple Opening/Closing Net and Environmental Sensing System (MOCNESS) mechanism (Biological Environmental Sampling Systems). Ship speed was maintained between 1 and 2 knots. Specific Tucker trawls were conducted to capture euphausiids at their day (300 m, 12 pm) and nighttime (50 m, 12 am) depths. Both trawls were at the target depth for 30 min. *Nematoscelis gracilis* from these trawls were frozen immediately upon capture for subsequent lactate measurement and were used to assess *in situ* capture and hypoxic stress as well as recovery from stress prior to laboratory measurement. Additional ETP specimens were collected using a bongo net deployed vertically to ~100 m. In the Red Sea, individuals for experiments were captured in repetitive vertical tows using a simple ring net (WP3) in the upper 100 m at night.

Only specimens that showed no visible signs of damage and were capable of active swimming were selected for study. Upon recovery, animals were transferred to air-saturated, filtered seawater and held for a 12 h (4–6 h in the Red Sea) period in plastic containers at densities less than $10 l^{-1}$. This minimized metabolism associated with digestion (specific dynamic action) and ensured that animals had recovered from the stress of capture. The temperature during this acclimation period was consistent with subsequent experimental temperatures.

Oxygen consumption measurement and analysis

Oxygen consumption rates (MO_2) were measured using two primary methods. With the exception of three individuals each of *N. gracilis* and *N. difficilis* (see below), all measurements were end-point determinations of oxygen content in closed chambers. Individual krill were placed in 50 ml gas-tight glass syringe respirometers. Syringes without animals served as controls and provided the starting oxygen concentration for oxygen consumption rate calculations. Seawater samples were withdrawn from the syringe using a 50 μl Hamilton gas-tight syringe and injected past a water-jacketed oxygen-electrode connected to an oxygen meter (Strathkelvin Instruments). The electrode was calibrated using air- and nitrogen-saturated seawater. Respirometry chambers were placed inside a waterbath that was connected to the electrode, ensuring that both animal and electrode were maintained at the same temperature. The difference in oxygen concentration between animal and control chambers represented the oxygen consumed by the animal. For hypoxia experiments, the chambers were filled with seawater that had been previously equilibrated with a certified gas mixture containing the experimental oxygen level. Respiration runs lasted either 12 (normoxia) or 6 (hypoxia) h. Hypoxia experiments were necessarily shorter in duration to avoid large changes in the oxygen content in the chambers. Acclimation and measurement were at 10 °C except for *E. diomedeae*, which was measured at 22 °C. In both regions, the experimental temperature was representative of the base of the upper oxycline and the upper depths of the oxygen minimum zone.

A second set of experiments was conducted on *N. gracilis* and *N. difficilis* using flow-through

respirometry. Individual krill ($n = 3$ for each species) were placed in cylindrical chambers with inlet and outlet ports (Loligo Systems). Water was treated in a water-jacketed gas-equilibration column (Radnotti Glass) by bubbling with a certified gas mixture containing 1% oxygen (balance nitrogen). Water was pumped from the gas-equilibration column, past an oxygen electrode, through the animal chamber and then past a second electrode. The difference in oxygen concentration between the two electrodes, normalized to the flow rate (controlled via peristaltic pump), was used to calculate the oxygen consumption rate. Using this method, animals could be maintained at a constant oxygen concentration throughout the experiment. No difference (*t*-test, $P < 0.05$) in mean oxygen consumption rates was found between the two methods for *N. gracilis* and, therefore, these data were pooled with the endpoint measurements for all analyses.

Oxygen consumption rates (MO_2) are presented as a function of body mass (M), according to $MO_2 = aM^b$ where a is a normalization constant and b is a scaling coefficient (slope), and as means ($\pm$ SE) corrected to a common wet mass of 20 mg. Although the scaling relationships were significant for two species, the sample sizes and size range available within each species was insufficient to provide confidence in the scaling relationships. Scaling relationships with limited sample size and size range typically produce slopes that are highly variable, while larger size ranges and sample sizes provide scaling relationships with slopes that converge toward a range of -0.33 to -0.1 for most animal groups studied (Glazier 2006; Seibel 2007). Therefore, correction for body size was accomplished assuming a scaling coefficient of -0.25, consistent with the cross-species analysis for euphausiids and for many species (Ikeda 2013). Correction using the scaling relationships measured here would not have changed our conclusions. A mass of 20 mg was chosen for normalization, producing the smallest possible error. Once each individual measurement was corrected for body mass in this fashion, the species could be compared using *t*-tests.

Mortality

Mortality was determined in separate experiments in which 5–7 krill were placed in 300 ml biological oxygen demand (BOD) bottles with seawater that had been equilibrated with the desired gas concentration, as in the respiration experiments described above. Experiments lasted from 3 to 6 h.

Lactate measurement

Following respiration and mortality experiments, animals were weighed on a motion-compensated shipboard balance system (Childress and Mickel 1980), frozen in liquid nitrogen and stored at $-80\,^{\circ}C$ for subsequent analysis of lactate accumulation. In the Red Sea, animals were frozen on dry ice and subsequently weighed ashore. L-Lactate was measured on whole-animal homogenates using a hand-held lactate meter (Accutrend Lactate-Plus). The animals were ground under liquid nitrogen in a saline solution containing 500 mM sodium, 50 mM magnesium, 20 mM potassium, 12 mM calcium, and 20 mM Tris buffer at pH 7.4. A standard curve was run with known concentrations of L-Lactate (Sigma Chemicals).

Critical oxygen partial pressure (P_{crit})

Critical oxygen partial pressures (P_{crit}, defined above) is typically indicated by a dramatic drop in the rate of oxygen consumption and by an accumulation of anaerobic end-products (e.g., lactate). This was measured for a limited number of *Euphausia eximia* using oxygen consumption regulation ($n = 2$) and also via lactate accumulation as described below. P_{crit} values for the other ETP and Red Sea species were estimated based on the adaptation threshold illustrated in Fig. 4A (redrawn from Seibel 2011) and described in detail below (“Discussion” section). P_{crit} for *N. difficilis* in the California Current was estimated based on measurements for species living in similar oxygen environments (Childress 1975; Kiko et al. 2016). Normalization of these P_{crit} values to changing temperature with depth was accomplished using a Q_{10} of 2.5 (Deutsch et al. 2015; Ikeda 2013; Fig. 5).

For *E. eximia*, four specimens were placed in 4 ml vials at $10\,^{\circ}C$ and allowed to deplete all available oxygen to determine a critical oxygen partial pressure (P_{crit}). The P_{crit} was defined as the oxygen partial pressure below which the regulated routine metabolic rate could no longer be maintained independent of the oxygen partial pressure. The P_{crit} was determined by regression analysis comparing the rate between a PO_2 of 4 and 15 kPa (regulated rate) and between 1 and 2 kPa (oxygen-limited rate). The P_{crit} was the point at which the regressions intersected. The rate above 15 kPa was discarded as a period of recovery from stress and handling. The 4-ml chambers were slightly too small to allow adequate length of trials to minimize the contribution of the acclimation phase to the rate. The reported P_{crits} are thus considered conservative (high) estimates. Two of the measured

specimens were larger and consumed the oxygen very quickly (in less than 3 h). The oxygen-limited phase of the regulation could not be distinguished clearly from the recovery phase and these two runs were discarded. The two smaller specimens required ~ 6 h to consume the oxygen entirely and a distinct regulated phase was observed.

In an additional determination of a critical oxygen partial pressure, 2–3 individual *E. eximia* were placed in each of 4–300 ml BOD bottles filled with seawater at oxygen partial pressures ranging from air saturation to anoxia. Specimens were removed following 12-h incubation, quickly weighed and frozen in liquid nitrogen. The final oxygen concentration in the bottles was measured using the endpoint analysis described above. L-Lactate was measured as above.

Total metabolism calculation

The calculation of total metabolic rate, consisting of the combined energy production by aerobic and anaerobic pathways, was modified from McDonald et al. (1998). Aerobic production of energy, in ATP equivalents, is 6 ATPs per O_2 molecule consumed. Anaerobic production was estimated by the increase in anaerobic end-products. In crustaceans, including euphausiids, lactate is known to be the principal anaerobic end-product (Spicer et al. 1999; Holman and Hand 2009). The present analysis quantified anaerobic metabolism as ATP = 1.5*(Δlactate), assuming glycogen is the primary substrate for anaerobic glycolysis. Total metabolic rate is calculated as the sum of ATP produced aerobically and anaerobically.

Results

Oxygen consumption rates

The mean oxygen consumption rates (MO_2, μmole O_2 $g^{-1}h^{-1}$) recorded for four species of euphausiids are shown in Table 1. The temperature- and mass-corrected normoxic rates measured for *N. difficilis* and *E. diomedeae* were significantly higher (*t*-test, $P = 0.001$) than for either *N. gracilis* or *E. eximia* (Fig. 1A). Although the regressions of oxygen consumption versus body mass for *E. eximia* ($MO_2 = 55.8\ M^{-0.66}$) and *E. diomedeae* ($MO_2 = 77.5\ M^{-0.55}$, 10 °C normalized) are significant, the size range and sample size are insufficient for confidence in the slopes of those curves (see "Methods" section). Relationships with size were insignificant for *N. difficilis* and *N. gracilis*. Mean mass- and temperature-corrected rates for all species in this study fall below the cross-species regression ($MO_2 = 36.50\ M^{-0.25}$) presented by Ikeda (2013) for euphausiids (Fig. 1A). This may be a consequence of different experimental protocols between studies. The present study used longer acclimation and measurement periods relative to most euphausiid studies (a few hours only), resulting in less organismal stress and less influence of feeding prior to capture.

MO_2 measured under hypoxia (Table 1) was significantly lower (58–69%; $P < 0.005$, *t*-test; Fig. 1B) than MO_2 measured in air-saturated conditions for all species, although hypoxic oxygen consumption rates are not reported for *N. difficilis* from the California Current due to the more than 80% mortality under hypoxic conditions (Table 2).

Mortality

No significant mortality occurred in hypoxia at 10 °C for the ETP species with up to 12 h exposure (the longest exposure tested). Similarly, mortality of *E. diomedeae* was low in hypoxia at 22 °C (Table 2). *Nematoscelis difficilis*, in contrast, had 80% mortality after 3 h exposure to $PO_2 = 2.4$ kPa and 100% mortality after 3 h exposure to $PO_2 = 0.8$ kPa. Because of this high mortality, hypoxic oxygen consumption, lactate, and total ATP consumption are not reported for *N. difficilis*.

Lactate

Under hypoxic treatments, all species accumulated lactate, but at relatively modest rates (*N. gracilis*, 2.12 ± 1.34, $n = 9$; *E. eximia*, 2.51 ± 0.93, $n = 5$; *E. diomedeae*, 1.28 ± 0.66, $n = 4$, μmol lactate $g^{-1}h^{-1}$; Table 1, Fig. 2), indicating little reliance on anaerobic ATP production to counter the oxygen deficit. The lactate levels (as opposed to accumulation rates) were near or below the limits of detection (<2.0 mmol lactate g^{-1}) for most individuals in air-saturated conditions and these control levels were subtracted as background in determination of lactate accumulation rates.

Lactate accumulated to significantly higher levels ($P = 0.043$) in the euphausiids tested immediately upon capture in the core of the OMZ during the day (47.21 ± 12.41 mmol lactate g^{-1}, $n = 5$) compared to those collected at night in oxygenated surface waters (10.97 ± 1.14 μmol lactate $g^{-1}h^{-1}$, $n = 5$, Fig. 2). These high levels suggest that stress from capture results in high activity levels that generate lactate and that anaerobic metabolism supports these activity levels to an even greater extent when oxygen is limiting in the nighttime trawls. The much lower control lactate values (below detection limits in most cases) following shipboard acclimation indicate that specimens had recovered from the stress of capture prior to respiration measurements.

Table 1 Oxygen consumption rates (MO_2, μmol O_2 $g^{-1}h^{-1}$) and lactate accumulation rates (Lac, μmol $g^{-1}h^{-1}$) in four species of euphausiids

	N	M, mg	MO_2	*N*	M, mg	Lac
E. eximia (ETP)						
Normoxic	10	29.3 (8.8)	7.51 (2.81)	–	–	
Hypoxic (0.8 kPa)	13	40.6 (13.6)	3.16 (1.65)	5	37.2 (6.98)	2.51 (0.93)
N. gracilis (ETP)						
Normoxic	13	23.3 (8.7)	5.40 (0.89)	–		–
Hypoxic (0.8 kPa)	9	34.3 (6.9)	1.68 (0.75)	9	34.3 (6.85)	2.12 (1.34)
E. diomedeae (RS, 22 °C)						
Normoxic	4	30.9 (25.9)	34.52 (15.01)	–		–
Hypoxia (1.6 kPa)	4	26.8 (6.4)	12.11 (3.27)	4	26.8 (6.4)	1.28 (0.66)
N. difficilis (CC)						
Normoxic	14	68.8 (12.8)	10.34 (2.59)	–		–
Hypoxic (2.4 kPa)	No	survivorship				

The wet mass (M) of the measured specimens are presented in microgram and physiological rates are presented per gram wet mass, corrected to a common body mass (20 mg) assuming a scaling coefficients of − 0.25 (Fig. 1A). Standard deviations are presented parenthetically. Sample sizes (*n*) are provided for each species and measurement. Acclimation and measurement were at 10 °C except for *E. diomedeae*, which was measured at 22 °C. These temperatures are consistent with the temperature at their daytime habitat depth. Levels of hypoxia exposure were also consistent with daytime habitat depth for each species and are compared to air-saturated controls (normoxia). Capture locations are presented with each species name (ETP, Eastern Tropical Pacific; RS, Red Sea; CC, California Current).

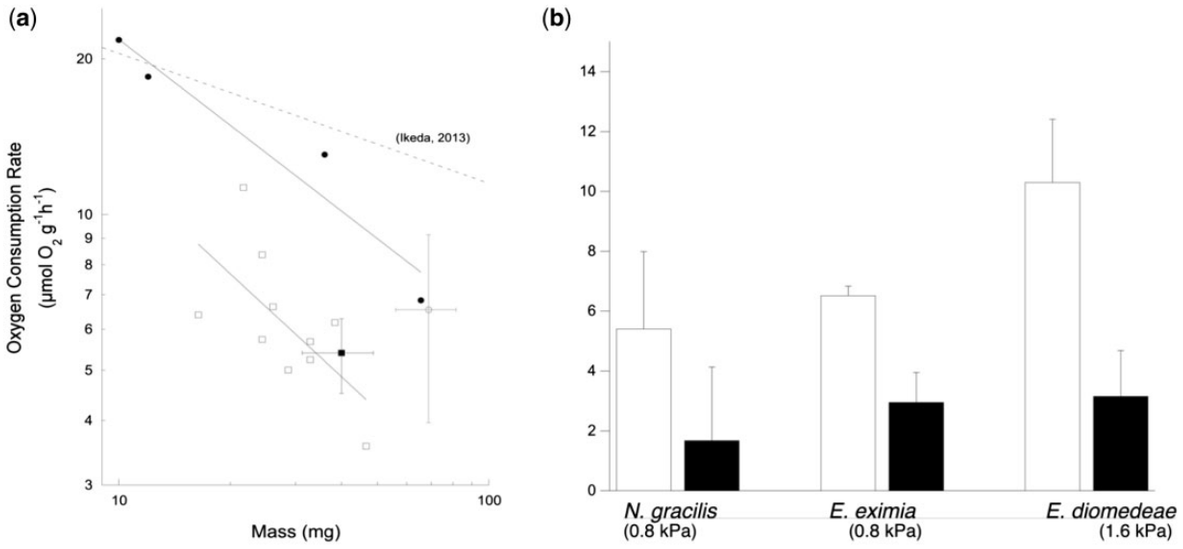

Fig. 1 Oxygen consumption rates (MO_2) of euphausiids living in regions of varying oxygen content. (**A**) Normoxic MO_2 as a function of body mass (M) according to $MO_2 = aM^b$, where *a* is a normalization constant and *b* is a scaling coefficient describing the slope of the power curve. For *Euphausia eximia*, $MO_2 = 55.8M^{-0.66}$ (open squares); For *E. diomedeae*, $MO_2 = 77.5M^{-0.55}$ (filled circles). The size range was not sufficient to provide reliable scaling curves for *N. gracilis* (filled square) and *N. difficilis* (open circle). Uncorrected means ± SD are presented for the latter species. The rates measured here are compared to a relationship ($MO_2 = 36.5M^{-0.25}$, dotted line) from a recent synthesis study on euphausiid metabolism (Ikeda 2013). The measurement temperature was held at 10 °C except for *E. diomedeae* (22 °C) which was corrected to 10 °C assuming a Q_{10} of 2.5 (Ikeda 2013). (**B**) Mean mass-normalized MO_2 (± SD) of the three vertically migrating species from pronounced OMZs in normoxic and hypoxic conditions that mimic the night- (white bar, $PO_2 = 21$ kPa) and daytime (black bar, $PO_2 = 0.8$ kPa in the ETP species and 1.6 kPa in the Red Sea) conditions, respectively. Hypoxic rates for *N. difficilis* are not presented as this species suffered high mortality at all tested PO_2 levels < 2.4 kPa.

Table 2 Mortality following exposure to hypoxia

Species	Region	T°C	Time (h)	PO_2 (kPa)	Mortality (%)
N. gracilis	ETP	10	6	0.8	10
			6	2.4	0
			6	21.0	10
E. eximia	ETP	10	6	0.8	0
			6	2.4	0
			6	21	0
N. difficilis	CC	10	3	0.8	100
			3	2.4	80
			6	21.0	0
E. diomedeae	RS	22	4	0.8	100
			6	1.6	0
			8	2.4	0

ETP, Eastern Tropical Pacific; RS, Red Sea; CC, California Current.

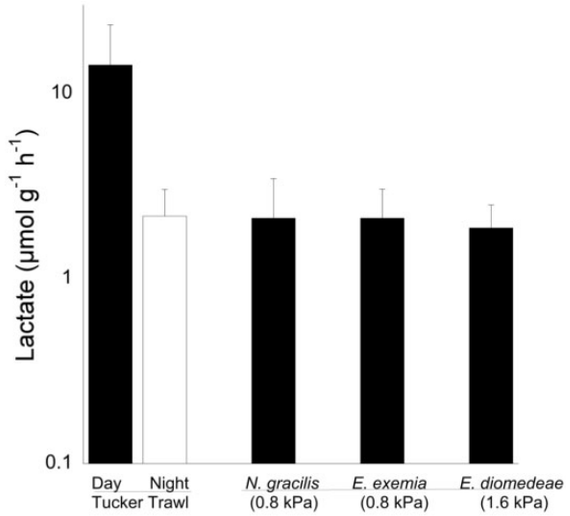

Fig. 2 Lactate accumulation rates (µmol $g^{-1}h^{-1}$, gray bars) during 6 h exposure to continuous hypoxia (PO_2 = 0.8 kPa for ETP euphausiid species and 1.6 kPa for *E. diomedeae* in th Red Sea). The mean lactate levels measured following incubation in control conditions (PO_2 = 21 kPa) were near or below the limit of detection and were subtracted from the hypoxic levels as background. Lactate accumulation in *N. gracilis* captured during day- (300 m, black bar) and nighttime (50 m, white bar) trawls at the approximate depths of the peak scattering layer. The higher lactate levels from trawl caught, relative to laboratory incubated, specimens likely indicate stress during capture. Conversely, the lower values in laboratory incubated specimens indicate that the acclimation period was adequate for recovery from trawl capture.

Total metabolism

The combined aerobic and anaerobic contributions to total metabolism (µmol ATP $g^{-1}h^{-1}$) were

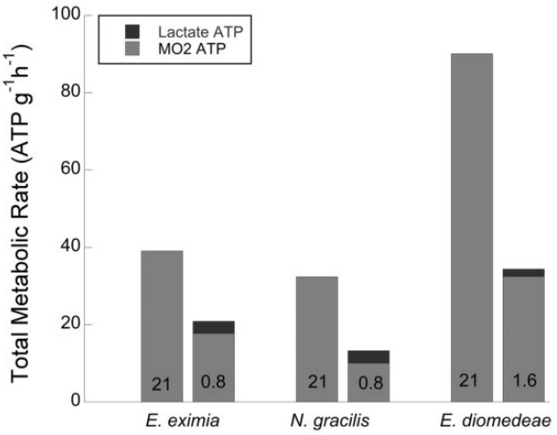

Fig. 3 Total metabolism (aerobic and anaerobic energy equivalents) measured at, or normalized to, 10 °C as indicated by combined oxygen consumption (6 mol ATP mol O_2^{-1}) and lactate accumulation rates (1.5 mol ATP mol $lactate^{-1}$). The numbers on the bars indicate the PO_2 (kPa) in the treatment incubation. *Nematoscelis difficilis* from the California Current did not survive low oxygen treatments so metabolic suppression could not be calculated.

suppressed by 49–64% in *E. eximia*, *N. gracilis*, and *E. diomedeae* during hypoxic exposure, relative to air-saturated controls (Fig. 3).

Critical oxygen partial pressure (P_{crit})

Continuous oxygen consumption measurements for two *E. eximia* produced nearly identical trials with a P_{crit} of 2.1 kPa (28 µM at 10 °C; Fig. 4B). Two additional P_{crit} trials were discarded because the trials were too short to distinguish the rates during acclimation, routine, and oxygen-limited states. In the separate BOD experiment with *E. eximia*, lactate began to increase only after oxygen values in the respiration chambers fell below ~1.1 kPa (15 µM at 10 °C), somewhat below the P_{crit} determined by oxygen consumption (both lactate and oxygen consumption curves are shown in Fig. 4B).

A P_{crit} of 1.0 kPa (at 5 °C) is estimated for the ETP and Red Sea species based on the lower adaptation threshold illustrated in Fig. 4A (redrawn from data at 5 °C, Seibel 2011). Assuming a Q_{10} of 2.5 (Ikeda 2013), 1.0 kPa at 5 °C (Fig. 4B) is equivalent to a P_{crit} of 1.58 kPa at 10 °C. A P_{crit} of 2.4 kPa (at 5 °C) is estimated for *N. difficilis* based on measurements for euphausiid species living in similar oxygen environments (*E. pacifica* in the California Current, Childress 1975 and *E. gibboides* in the Eastern Tropical North Atlantic, Kiko et al. 2016).

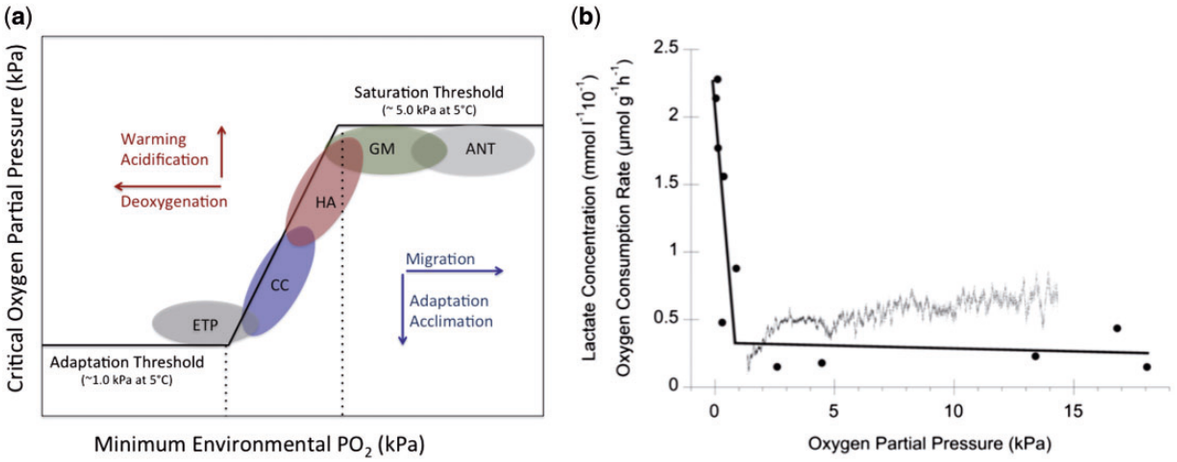

Fig. 4 (**A**) A schematic (redrawn from Seibel 2011) showing the variation in critical oxygen partial pressures (P_{crit}) as a function of the minimum environmental PO_2 to which a species is exposed. The colored ovals represent the range of P_{crits} for dozens of species measured in those regions (labeled ETP, eastern tropical pacific; CC, California current; HA, Hawaii; GM, Gulf of Mexico; and ANT, Antarctica). The measurements supporting this figure were made at, or normalized to, 5 °C (Seibel 2011). An upper saturation threshold is identified at about 5 kPa beyond which further specific adaptation to low oxygen is not required. Below this threshold, the P_{crits} of all species closely match the minimum environmental PO_2 to which they are exposed, indicating specific physiological adaptation in support of aerobic metabolism. A lower adaptation threshold is identified at ~1.0 kPa below which further adaptation for oxygen extraction appears constrained. The arrows represent the potential displacement of species as P_{crits} increase due to warming and acidification and their possible recovery via adaptation and acclimation. (**B**) *Euphausia eximia* critical oxygen partial pressures (P_{crit}) determined via two methods. Oxygen consumption rate (continuous curve) and accumulated lactate (drawn lines) plotted against the experimental oxygen partial pressure. The point at which the rate of oxygen consumption is no longer regulated independently of the PO_2 is defined as the P_{crit}. Two specimens exhibited identical regulated rates of oxygen consumption with discernable critical oxygen levels (breakpoint in the continuous curve). Only one is shown (P_{crit} = 2.1 kPa, 10 °C). Lactate (mmol g^{-1}; circles, solid lines, divided by 10 for presentation with oxygen consumption data) began to accumulate during exposure to increasing hypoxia at ~1.1 kPa.

Environmental profiles

Representative oxygen and temperature profiles for each region are shown in Fig. 5. The OMZ was more pronounced in the ETP than at the other sites and was characterized by a relatively shallow mixed layer where the oxygen approached air saturation. The thermocline below the mixed layer in this tropical region was abrupt, with a temperature change from 25°C to 28 °C in the mixed layer to 15°C to 18 °C at the top of the upper oxycline (only 20–40 m depth) and 10–12 °C at its base (250–350 m). The thermocline was coincident with a steep oxycline, more so in the Tehuantepec Bowl than in the Costa Rica Dome. Oxygen reached a minimum of 1.8 µM (0.1 kPa) at 40 m in the Tehuantepec Bowl (Wishner et al. 2013 and Maas et al. 2014 for representative oxygen and temperature profiles). The California Current was characterized by more moderate temperatures (15 °C at the surface) and a gradual oxycline that reached a minimum of ~12 µM O_2 (0.8 kPa at 5 °C) near 700 m depth. The oxygen content in the Red Sea was relatively high, reaching a minimum of ~60 µM (4 kPa) at 300 m depth. However, the temperature was stable at 22 °C throughout the depth range.

Discussion

As indicated by published data on critical oxygen partial pressures (P_{crit}, the majority of which were carried out at 5 °C), a low oxygen threshold of ~1.0 kPa exists below which further adaptations promoting oxygen extraction appear constrained (Seibel 2011; Fig. 4B). In other words, mesozooplankton and micronekton species can adjust their capacities for the uptake and transport of oxygen from hypoxic waters to match the lowest oxygen partial pressures encountered in their natural range down to about 1.0 kPa at 5 °C. Critical oxygen partial pressures lower than this are rare among midwater animals (Childress and Seibel 1998), yet many vertically migrating species spend the daytime at a much lower PO_2, which limits their routine metabolic rates.

Seibel (2011) hypothesized that many species suppress metabolism to survive diel forays into sub-critical oxygen levels. In support of this hypothesis, three of the four species of euphausiid studied here exhibit

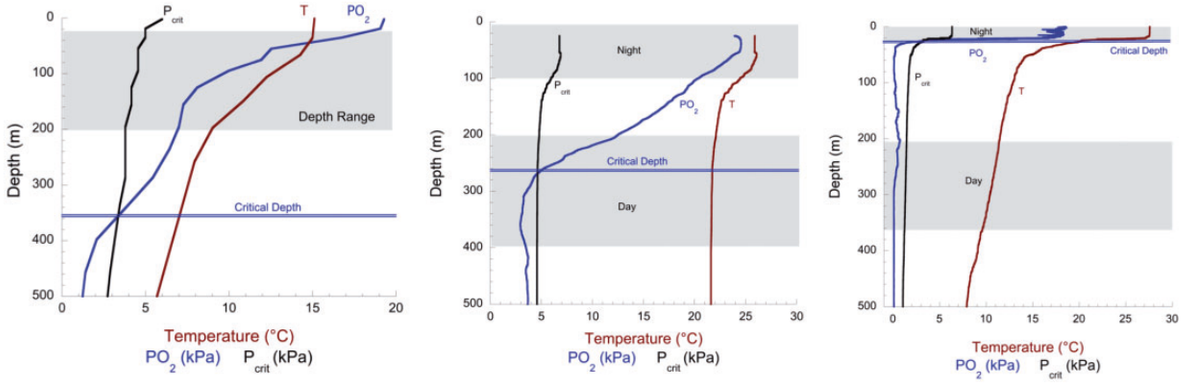

Fig. 5 Critical oxygen partial pressures (P_{crit}; black) change as a function of temperature (T, red) and thus, depth. At each depth, P_{crit} was normalized for temperature using a Q_{10} of 2.5 (Ikeda 2013). A baseline P_{crit} of 1.0 kPa at 5 °C is estimated for ETP species based on the adaptation threshold proposed by Seibel (2011; Fig. 4A) and is consistent with mortality data for all ETP species and with the lactate and oxygen consumption data presented here for *E. eximia* (Fig. 4B). We have estimated a P_{crit} of 2.4 kPa for *N. difficilis* in the California Current based on mortality data and on measurements of related species in similar oxygen environments (Childress, 1975; Kiko et al., 2016). The depth at which the environmental oxygen partial pressure (PO_2, blue) equals the P_{crit} is indicated as a critical depth (D_{crit}) for A) *N. difficilis* in the California Current, (**B**) *E. diomedea* in the Red Sea and (**C**) *N. gracilis* and *Euphausia eximia* in the ETP. Because of the high temperature (~22 °C) across the depth range in the Red Sea, the P_{crit} is near 4–5 kPa, which is reached at a depth of ~270 m. In the ETP the temperature, and thus, P_{crit} is much lower but the oxygen minimum zone is much more pronounced leading to a critical depth of only ~30 m. The California Current has still lower temperatures and intermediate PO_2s leading to a deeper D_{crit} despite the higher P_{crit} of the resident species. The shaded regions indicate the day- and nighttime distributions of the studied species.

a pronounced suppression of total metabolism (49–64%) under hypoxic, relative to air-saturated, conditions at the same temperature (Fig. 3). Oxygen consumption was reduced by 58–69% (Fig. 1B) and the ATP derived from anaerobic glycolysis, as indicated by the accumulation of lactate (Fig. 2), accounted for only a small fraction of the energy deficit under low oxygen (Fig. 3). *Nematoscelis difficilis* from the California Current do not naturally experience PO_2s below their P_{crit} and did not survive more than 3 h at a PO_2 ~2.4 kPa.

The disparity between day and nighttime rates of metabolism is even more pronounced when differences in habitat temperature and activity levels are considered. With temperature coeffihcients (Q_{10} = factorial change in rate with a 10 °C temperature change) ranging from 1.9 to 2.8 (Torres and Childress 1983; Werner et al. 2012; Ikeda 2013), euphausiids may exhibit a normoxic routine metabolic rate approaching four times higher at 25 °C, typical of near-surface waters in the ETP, compared to rates in air-saturated water at 10 °C. Moreover, Klevjer and Kaartvedt (2011) showed that swimming activity in euphausiids is substantially reduced (~50%) at depth during the daytime and is further reduced in low oxygen. A doubling of the activity level must be supported by a similar increase in metabolism (Torres and Childress 1983). Thus, the shallow, nighttime metabolic activity of *N. gracilis* and *E. eximia* may be as much as 10–20 times higher than that exhibited at hypoxic depths during the day.

Vertical migration and hypoxia tolerance

Euphausiids have diverse diel migration patterns, and ascend and descend to species-specific depths. However, off-shelf species migrate to a daytime depth between 200 and 500 m (peak abundance near 300 m; Brinton 1979; Sameoto et al. 1987; Escribano et al. 2009; Maas et al. 2014). Antezana (2009) specifically showed that *N. gracilis* and *E. eximia* both descend into the core of the OMZ below 300 m each night. *Euphausia diomedeae* is a strong diel migrator in the Red Sea as well with a daytime peak at 200–400 m (Wiebe et al. 2016). *Nematoscelis difficilis* is reportedly a non-migrator and lives above the more modest OMZ of the California Current (Tremblay et al. 2012), which may explain why this species survived less than 3 h at PO_2 = 2.4 kPa and 10 °C (Table 2). The other three species studied here all demonstrated metabolic suppression and survived the entire hypoxic exposure duration (Table 2; Fig. 3).

Both *E. eximia* and *N. gracilis* survived 6 h or more at PO_2 = 0.8 kPa (10 °C). A PO_2 of 0.8 was fatal to both of the other species, although *E. diomedeae* survived 1.6 kPa at 22 °C. High temperature will elevate oxygen demand and the critical oxygen partial pressure (P_{crit}, Deutsch et al. 2015), with a Q_{10} of

~2.5 (Ikeda 2013). At 22 °C, 1.6 kPa is equivalent to 0.7 kPa at 10 °C. This indicates that the combination of high temperature and moderate hypoxia is more physiologically challenging than a much lower PO_2 at low temperature. We postulate that *E. diomedeae* in the Red Sea has, like species from the ETP, reached the limits of adaptation (the proposed lower adaptation threshold, Seibel 2011) despite the higher oxygen content in the Red Sea.

Euphausia mucronata is a common OMZ species for which there is conflicting evidence for regarding hypoxia tolerance. Teal and Carey (1967) showed that *E. mucronata* from the ETP could maintain its routine oxygen consumption rate to a PO_2 of ~1.7 kPa (at 20 °C). Kiko et al. (2016) recently reported a P_{crit} of ~0.6 kPa (at 10 °C) for *E. mucronata* from the OMZ in the Eastern Tropical South Atlantic. Below the P_{crit}, oxygen consumption rates declined, reaching rates about 50% of the normoxic rate. Moreover, those animals tested continued to swim (albeit at a slower pace) until a PO_2 of <0.3 kPa. Antezana (2002) found no difference in oxygen consumption, swimming, or feeding activity during exposure of *E. mucronata* to oxygen concentrations found just above and below the OMZ core. He further demonstrated that many OMZ-associated species, including *N. gracilis* and *E. eximia*, have enhanced gill surface areas relative to species living in more oxygenated regions, suggesting enhanced O_2 extraction capabilities in these species. Increased antioxidant capacity may also play an important role in enhanced survival of OMZ species. Such capacity may relieve the stress associated with reoxygenation each night as the euphausiids ascend (Tremblay et al. 2010).

Euphausiids in the present study accumulated much more lactate in a 30-min trawl than they did in 6 h of hypoxic exposure (Fig. 2), suggesting that escape reactions, dependent on anaerobic energy production, are intensified during net capture. Higher lactate levels in euphausiids from the daytime trawl at 300 m, compared to the nighttime trawl at 50 m, suggest that hypoxia reduces the anaerobic threshold (exercise intensity at which lactate begins to accumulate). Similar results were found for the euphausiid, *Meganyctiphanes norvegica* (Spicer et al. 1999). Predator–prey interactions are probably minimal in the core of the OMZ during the daytime and activity levels were obviously elevated during net capture and retrieval.

Metabolic suppression and the biological pump

The most complete study of metabolic suppression for a vertical migrator is that for *Dosidicus gigas*, a large squid endemic to the eastern Pacific. It shuts down ATP demand by as much as 60% by suppressing expensive cellular processes such as translation and transcription (Seibel et al. 2014). The remaining energy demand is met via a combination of enhanced oxygen extraction (Seibel 2013; Trueblood and Seibel 2013), diminished ATP and phosphagen stores and anaerobic metabolism via both glycolytic and mitochondrial pathways (Seibel et al. 2014; Rosa and Seibel 2010). Reductions in *aerobic* metabolism at daytime habitat depths, due to low oxygen and independent of temperature, have now been demonstrated for squids (Seibel et al. 2014), pteropods (Maas et al. 2012), copepods (Childress 1977; Svetlichny et al. 2000; Auel et al. 2005; Kiko et al. 2015), pelagic crabs (Kiko et al. 2015), amphipods (Elder and Seibel 2015), and krill (Kiko et al. 2016 and present study). Additional studies (reviewed in Seibel 2011) suggest this strategy is common for all vertically migrating zooplankton, including fishes. That total metabolism, including anaerobic contributions, is suppressed has been demonstrated only for *D. gigas*, mentioned above, the amphipod, *Phronima sedentaria* (Elder and Seibel 2015) and the krill studied here. Additional work in preparation finds similar results for species ranging from copepods to crabs and other squids (Seibel, unpublished data).

The possibility that most vertical migrators suppress total metabolism during daily excursions into pronounced oxygen minimum zones has important implications for estimates of their contribution to active carbon flux yet has been considered only superficially to date. The present analysis suggests that, at least in pronounced OMZs, krill suppress metabolism, which is typically accomplished via reductions in energetically expensive cellular processes such as protein synthesis, ion transport, transcription, and translation (Hand 1998; Storey and Storey 2004; Seibel et al. 2014). Such shutdown of cellular function must preclude, or at least constrain feeding, locomotion, excretion and other whole-animal functions while at depth.

Reductions in aerobic metabolism due to reduced oxygen vary from ~35% to 80% in the species referenced above. Temperature will of course further reduce metabolism in most regions during deep migrations. The carbon dioxide excreted is proportional to the oxygen consumed with a ratio (respiratory quotient) of 0.7–1.0 depending on the fuel (e.g., lipid, carbohydrate, or protein) being metabolized. Atomic nitrogen excretion to oxygen consumption ratios (O:N) vary more broadly in the range of 3–16 for pure protein catabolism, to 50 or 60 for a mixed diet of lipid and protein (Mayzaud and

Conover 1988). A reduction in CO_2 excretion equal to that in oxygen consumption is a reasonable first approximation but this will vary with diet and other factors. Nitrogen excretion is much more variable and should be measured directly. Unlike aerobic metabolism, anaerobic metabolism is not directly coupled to excretion. However, some pathways are still linked. For example, myctophid fishes have been suggested, based on measurement of alcohol dehydrogenase activities, to produce ethanol as an anaerobic end-product in low oxygen (Torres et al. 2012). This pathway results in excretion of both CO_2 and ethanol, but direct measurements of these end products have not yet been made.

Habitat compression and expanding oxygen minimum zones

Recent evidence reveals that ocean oxygen concentrations are decreasing and oxygen minimum zones are expanding in most regions due to natural and anthropogenic forcing (Stramma et al. 2008; Bograd et al. 2008; Keeling et al. 2010; Deutsch et al. 2011). The biological implications of ocean deoxygenation are largely unexplored but habitat compression is a major concern for oceanic populations (Prince and Goodyear 2006; Stramma et al. 2011; Seibel 2011; Gilly et al. 2013; Wishner et al. 2013). Mesopelagic fish and zooplankton populations fluctuate on complex, and species-specific time-scales in response to climate forcing in the California Current (Di Lorenzo and Ohman 2013), but recent declines are believed to result from the shoaling oxygen partial pressure, which forces individuals into shallower, better illuminated waters where they are more susceptible to predation (Koslow et al. 2011, 2014). A recent study links the range expansion of a hypoxia-tolerant predator, *D. gigas*, to the expanding OMZ and changes in the distribution of its myctophid (fish) prey (Gilly et al. 2013). This is consistent with the prediction of Seibel (2011) that, in the California Current system, small changes in oxygen will result in major shifts in ecosystem structure, from one dominated by permanent mesopelagic inhabitants to one more typical of pronounced OMZs dominated by strong diel migrators. In a comparison of regions with differing oxygen profiles in the ETP, Wishner et al. (2013) showed that the deeper daytime depth inhabited by vertical migrators did not change with oxygen. However, the depth to which species must return at night to metabolism accumulated anaerobic end-products (i.e., repay their oxygen debt) is compressed into a much shallower, narrower, and more concentrated layer. This renders resident species more susceptible to predators at night.

The P_{crit} is a useful whole-animal metric that integrates physiological mechanisms for oxygen provision across a range of temperatures (Seibel 2011; Deutsch et al. 2015). As such it may allow prediction of habitat depth changes due to deoxygenation and warming with climate change. Childress and Seibel (1998) showed that P_{crits}, measured at 5 °C for a variety of zooplankton and micronekton from diverse regions, closely match the lowest environmental PO_2 to which they are exposed in their natural habitat, indicating specific physiological adaptation to a species' particular oxygen environment. Animals found in lower oxygen environments have lower P_{crits} that permit aerobic survival there. However, below a PO_2 of ~1.0 kPa there is an apparent limit to the capacity for such adaptation and no further reduction in P_{crit} with declining PO_2 (no further adaptation) is observed (Seibel 2011; Fig. 4B). In other words, P_{crit} and minimum environmental PO_2 become uncoupled below 1.0 kPa. The ETP and Red Sea both contain oxygen levels below this threshold (normalized for appropriate habitat temperature) within the vertical range of the species considered here (Fig. 5).

The P_{crit} has a temperature sensitivity similar to that for metabolism (Deutsch et al. 2015). Assuming each species in the ETP and Red Sea is at the proposed adaptation threshold ($P_{crit} = 1.0$ kPa at 5 °C; Seibel 2011; Fig. 4A), we calculate the P_{crit} across the depth (temperature) range in their respective regions assuming a temperature coefficient (Q_{10}) of 2.5 (Fig. 5), which is a reasonable approximation for metabolism across diverse euphausiid species (Torres and Childress 1983; Werner et al. 2012; Ikeda 2013). The depth at which the environmental PO_2 matches the P_{crit} for a given species can be thought of as a critical depth (Fig. 5), which can be used to predict changing vertical and horizontal distributions of these organisms as a function of oxygen and temperature. Below this depth, aerobic metabolism can no longer be maintained and is suppressed, while anaerobic metabolism (e.g., lactate accumulation) increase.

Because temperature drops sharply with depth in most regions, oxygen availability, relative to demand, is increased at depth in most regions (Deutsch et al. 2015). In the ETP and Red Sea, however, oxygen availability declines faster than does oxygen demand. The P_{crit} is reached at a depth of only 50 m depth in the ETP. In the Red Sea, temperature is high and constant at 22 °C across the depth range but the oxygen minimum zone is deeper and less

pronounced than in the ETP. The P_{crit} for *E. diomedeae* in the Red Sea is, accordingly, reached at a much deeper depth than for the two ETP species (Fig. 5). High mortality at all hypoxic levels tested precludes determination of the P_{crit} for *N. difficilis* in the California Current, but it must be > 2.4 kPa at 10 °C. Childress (1975) reported a P_{crit} of 2.4 kPa for *E. pacifica*, which is a vertical migrator in the California Current. Kiko et al. (2016) estimated a similar P_{crit} of 2.4 kPa for *E. mucronata* in the Eastern Tropical North Atlantic. We suggest that *N. difficilis* does not reach subcritical oxygen within its known vertical distribution (Tremblay et al., 2010), permitting its non-migratory strategy.

In contrast, all three migrating species descend to depths below their estimated P_{crit} during the day (Fig. 5) and must suppress metabolism. Light levels that minimize predation pressure take precedence in determining the daytime habitat depth for these species (Wishner et al. 2013). At night however, krill must remain above their P_{crit}, which is compressed into shallower water in the ETP relative to the Red Sea and the California Current. Thus, P_{crit} acts as a "floor" for the nighttime depth distribution. The interacting effects of warming and deoxygenation may simultaneously raise this "floor" and lower the "ceiling" that constrain species-specific vertical distributions, resulting in habitat compression. The consequences of elevated sea-surface temperatures and expanded oxygen minimum zones for mesopelagic ecology and for the contribution of migrating species to biogeochemical cycles may be substantial.

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