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Lethal and functional thresholds of hypoxia in two key benthic grazers

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ABSTRACT: Climate drivers can impact ecosystem structure and processes by changing the composition of organisms through lethal impacts, or by altering the ecological roles of organisms through sublethal physiological and behavioral impacts. We examined potential effects of upwelling-driven hypoxia on California Current kelp forests by assessing hypoxia impacts on 2 species having important functional roles in these ecosystems. We quantified dissolved oxygen (DO) thresholds for lethal and functional-role impacts in purple and red sea urchins (*Strongylocentrotus purpuratus* and *Mesocentrotus franciscanus*). For both species in 2 size classes, we measured median lethal exposure time to severe hypoxia of 1.0 mg l^{-1} of DO, and quantified grazing at 7.5 (ambient), 6.5, 5.5, and 4.5 mg l^{-1} DO. Sea urchins of both species and size classes were tolerant of severe hypoxia, but showed 39 to 47% declines in grazing at 5.5 mg l^{-1} DO. We compared these thresholds to a decade-long dataset of DO in a Monterey Bay kelp forest and found that sea urchins are unlikely to experience direct mortality under current or near-future exposures to upwelling-driven hypoxia. In contrast, they are likely to experience sublethal effects of low oxygen and decreased grazing rates for substantial periods of time under current conditions. These results represent the first documentation of hypoxia-modulated kelp grazing in sea urchins. They indicate that upwelling-driven hypoxia can have important impacts on these ecologically important benthic grazers and possibly on whole ecosystems through altered trophic interactions, highlighting that sublethal, functional-role impacts of climate drivers can be as or more important than lethal impacts.

KEY WORDS: Hypoxia · Thresholds · Sublethal · Functional roles · Sea urchins · Kelp forests

INTRODUCTION

Global climate change is already altering the physical and physiological drivers that marine species, communities, and ecosystems experience. For example, rising temperatures, ocean acidification, deoxygenation, sea level rise, and increasing storm activity are all occurring and are expected to intensify (Harley et al. 2006, Doney et al. 2012). Because climate-driven impacts on ecosystems have major implications for the ecosystem services that people depend on, it is important and urgent that we increase our understanding of how species respond to climate drivers, and how those responses can scale up to effects on ecosystem processes and structure.

Climate drivers can impact ecosystems by changing the abundance and composition of species, such as through differential mortality. Syntheses of experimental and field studies have highlighted winners and losers in the face of climate-driven stressors such as warming and ocean acidification (Somero 2010, Kroeker et al. 2013, Cavole et al. 2016). Because species often perform different functional roles within their ecosystems, changes in the relative abundances of species can alter ecosystem structure, processes, and functions (Micheli & Halpern 2005, Cardinale et al. 2006, Mouillot et al. 2013). For example, ocean

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acidification can bring about shifts in the relative abundance of different habitat-forming taxa in ecosystems like coral reefs and intertidal mussel beds, based on differences in their tolerances to low pH conditions. These changes in species composition can then lead to changes in the ecological functions performed, such as habitat provision (Sunday et al. 2017).

However, climate impacts on ecosystem processes can also arise through direct changes in species' ecological roles. Physical and physiological drivers can alter species' behavior or activity levels, thus changing their functional contribution to key ecosystem processes. For example, physical drivers such as hydrodynamic forces can limit the timing and spatial extent of foraging (Duggins 1981, Siddon & Witman 2003), and temperature fluctuations can impose metabolic constraints on feeding rates (e.g. Sanford 1999, O'Connor 2009). Such changes in species' functional roles may impact ecosystem processes and functioning even in the absence of significant changes in population abundances, species composition, and community structure. These effects often occur under less extreme conditions than do the lethal impacts that alter species' relative abundances. Therefore, they are likely to occur more frequently, and for longer periods of time. At the same time, such functional-role impacts may be much harder to detect in the field because they involve changes in process rates, rather than the more visible changes in species numbers.

These functional-role impacts can be especially important when they occur in species that play key roles in their ecosystems. For example, small temperature fluctuations mediate keystone predation rates in the intertidal seastar *Pisaster ochraceus*, potentially altering the seastar's keystone role (Paine 1966, 1969) in maintaining benthic diversity in the rocky intertidal zone (Sanford 1999, 2002). Functionally important species can thus act as leverage points through which small changes in physical and physiological drivers may effect larger impacts on the entire ecosystem (Sanford 2002, Harley et al. 2006). Moreover, between-species and size-dependent differences in vulnerability to functional-role impacts can also lead to changes in ecosystem processes and functions (Taylor & Eggleston 2000, Vanderploeg et al. 2009).

We explored the potential consequences of a novel climate stressor, upwelling-driven hypoxia (Stramma et al. 2008, Keeling et al. 2010), on the diverse, productive kelp forest ecosystems of the California Current System by investigating thresholds for impacts on mortality and on the functional role of 2 key kelp consumers and potential 'leverage species' (Harley et al. 2006) within these ecosystems. Upwelling-driven coastal hypoxia has been recorded on the continental shelf within the California Current System since the early 2000s (Grantham et al. 2004, Chan et al. 2008, Booth et al. 2012), and it is expected to increase in severity as climate change intensifies upwelling processes (Bakun et al. 2010, Sydeman et al. 2014) and ocean deoxygenation (Keeling et al. 2010). While extreme hypoxia events have caused some mass mortality events in California Current ecosystems (Grantham et al. 2004, Chan et al. 2008, Micheli et al. 2012), knowledge is limited about how exposures to hypoxia may impact the highly productive and diverse nearshore kelp forest ecosystems within the California Current System. Most work on nearshore hypoxia has been done in estuarine and semi-enclosed coastal systems, where hypoxia is largely respiration-driven (Rabalais & Turner 2001). Species from upwelling systems are poorly represented in syntheses of taxa-specific hypoxia thresholds (Vaquer-Sunyer & Duarte 2008, 2011), so these studies may not be particularly informative for understanding hypoxia impact in kelp forests, though some broad-scale taxa specific differences, such as high hypoxia vulnerability in fish and crustaceans, and greater tolerance in echinoderms and molluscs, may hold across systems (Vaquer-Sunyer & Duarte 2008, Eerkes-Medrano et al. 2013).

We focused on purple and red sea urchins (*Strongylocentrotus purpuratus* and *Mesocentrotus franciscanus*) as 2 functionally important species that co-occur within California Current kelp forest ecosystems. Understanding hypoxia impacts on the survival and/or functional roles of sea urchins can shed light on how kelp forest ecosystems may be affected. Sea urchin grazing is a major contributor to kelp loss, and overpopulation and overgrazing by these species have led to kelp deforestation and ecosystem state shifts into urchin barrens in multiple California Current kelp forests (Harrold & Reed 1985, Watanabe & Harrold 1991, Estes & Duggins 1995, Steneck et al. 2002, Beas-Luna & Ladah 2014). Therefore, hypoxia impacts on sea urchin survival or functional roles could influence kelp forest dynamics, potentially moving these 'tipping points' for ecosystem state shifts. Purple and red sea urchins are also prey for a diverse group of predators such as sea otters, predatory fish, and crustaceans, and can serve as an important link to transfer energy up the food web from primary producers to predators (Beas-Luna et al. 2014). Therefore, sea urchin responses to hypoxia may modulate energy transfer through the food web, possibly increasing the importance of the detrital food web.

Potential differences in hypoxia tolerances may exist between the 2 sympatric sea urchin species, and between different size classes. Unlike the subtidal red sea urchins, purple sea urchins have a depth range that extends into the intertidal zone and may be adapted to nighttime hypoxic conditions in tidepools (Stephenson & Eyre 1934). Body size influences hypoxia tolerance in other organisms (Nilsson & Östlund-Nilsson 2008), and it correlates positively with decreased surface area to volume ratios in sea urchins, which are diffusion-limited in their respiratory capacity (Giese et al. 1966, Giese 1967). Changes in sea urchin species composition and size structure due to such different tolerances could have additional ecosystem consequences. Red sea urchins play additional roles in the ecosystem — their spines act as predator and hydrodynamic refuges for juvenile sea urchins, abalone, and other organisms (Tegner & Levin 1983, Rogers-Bennett & Pearse 2001, Nishizaki & Ackerman 2007), and they are preferred over purple sea urchins by some predators (Nishizaki & Ackerman 2007). Red, but not purple sea urchins are harvested in commercial fisheries throughout the nearshore kelp forests of the California Current (Rogers-Bennett 2013). These fisheries are size-selective in their harvest, and thus alter both the relative abundance and size structure of red sea urchins. Therefore, any species- and size-specific differences in lethal and functional-role oxygen thresholds might lead to interactions between upwelling-driven hypoxia and sea urchin fisheries, and could also have broader consequences for ecosystem function.

We examined dissolved oxygen (DO) thresholds for impacts on sea urchin survival and for their potential functional roles as kelp grazers in a California kelp forest ecosystem. Specifically, we quantified and compared the effects of DO concentrations on 2 size classes of 2 kelp forest sea urchin species to ask the questions (1) what are the DO thresholds for impacts on sea urchin survival, (2) what are the DO thresholds for impacts on sea urchin kelp grazing, (3) are there differences in these thresholds between sea urchin species and size classes, and (4) how do these thresholds compare to current and expected DO conditions in California Current kelp forests? We addressed the final question by placing sea urchin DO thresholds within the context of a decade-long data set of DO concentrations from Monterey Bay, in central California.

MATERIALS AND METHODS

Collection and maintenance

We collected red and purple sea urchins (*Mesocentrotus franciscanus* and *Strongylocentrotus purpuratus*) in 2 size classes, ‘small’ (2 to 3 cm test diameter) and ‘large’ (6 to 7 cm test diameter), from 9 to 12 m depths at Van Damme State Park, California, in August 2015. This collection site was selected for ease of access to a broad size range of sea urchins from both species, and pilot trials showed no difference in hypoxia responses between small sea urchins collected from this site and those collected from Monterey Bay. Sea urchins were immediately transported to flow-through seawater tanks in a sheltered outdoor aquaria facility at the Hopkins Marine Station in Pacific Grove, California, and maintained under ambient conditions in running seawater tanks. Sea urchins were fed giant kelp, *Macrocystis pyrifera*, ad libitum and were allowed to acclimate for at least 10 wk before experiments.

DO manipulations

Experiments were run in a sheltered outdoor aquaria facility at the Hopkins Marine Station. For all experiments, we manipulated DO concentrations within 189 l insulated aquarium tanks supplied by flow-through seawater. To establish and maintain treatment levels of DO, we bubbled nitrogen gas into aquaria seawater through 60 mm fine-pore air diffusers. The flow of nitrogen gas was controlled by solenoid valves connected to an Arduino microcontroller system that continuously monitored DO concentrations in the aquaria using an optical DO probe (Vernier Software and Technology) and initiated feedback loops to maintain DO concentrations at treatment levels (N. H. N. Low et al. unpubl.). DO levels in the tanks were monitored and verified daily using a handheld data logger with a DO probe (YSI Pro Plus; Xylem). Other seawater parameters reflected the ambient, incoming seawater supply from Monterey Bay and were consistent across aquaria. Water temperatures stayed between 14.1 and 14.9°C (mean ± SD = 14.5 ± 0.18°C) and seawater pH remained between 7.88 and 7.97 (mean = 7.93 ± 0.028) throughout all experiments. Sea urchins were exposed to ambient light cycles during all experiments (approximately 12 h light:12 h dark).

Lethal thresholds of hypoxia exposure

To investigate the effects of coastal hypoxia on the survival of adult sea urchins, we exposed small and large red and purple sea urchins to 1.0 mg l^{-1} DO for different lengths of time in November 2015. This DO value represents a more extreme level of hypoxia than has ever been recorded in the Monterey Bay kelp forest since 2000 (Booth et al. 2012), but it has been recorded for short durations in other nearshore locations within the California Current (Micheli et al. 2012, Boch et al. 2018).

We randomly divided 80 sea urchins of each species–size class combination among 8 aquarium tanks. All tanks were exposed to identical treatments: DO concentrations were decreased from ambient (7.5 mg l^{-1}) to 1.0 mg l^{-1} at a rate of 3 mg l^{-1} h^{-1}. We defined the time when DO reached 1.0 mg l^{-1} as the start of exposure to extreme hypoxia (0 h). At 0, 12, 24, 36, 48, 60, 72, 84, 96, and 108 h of exposure, we randomly selected 8 individuals from each species–size class combination (one from each tank), removed them from the hypoxic treatment, and allowed them to recover in ambient, normoxic conditions. Because mortality in sea urchins often occurs with a time lag, we monitored all individuals for 2 wk post-exposure to record both immediate and delayed mortality. Mortality in sea urchins was characterized by the complete loss of spines and the shedding of dead tissue from the test. All mortality occurred within the first 48 h post-exposure, and we did not see any further mortality over 2 wk of observation. For each species and size class of sea urchin, we recorded the exposure duration corresponding to a 50% mortality rate (median lethal exposure time, LT_{50}).

Thresholds of hypoxia for grazing

To investigate the effects of coastal hypoxia on the ecological roles of adult sea urchins, we examined kelp grazing rates of red and purple sea urchins, as a measure of their potential contribution to kelp removal, under different levels of DO. We ran 2 rounds of experiments, one with large sea urchins and one with small sea urchins, between October and November 2015.

For both experiments, each of 12 aquarium tanks was assigned to 1 of 4 DO treatments: 7.5 mg l^{-1} (ambient), 6.5, 5.5, and 4.5 mg l^{-1} (sublethal; Vaquer-Sunyer & Duarte 2008). We used 96 experimental chambers consisting of 5.7 l perforated plastic containers, divided by barriers of 8 × 8 mm plastic mesh to create 2 compartments. A single sea urchin was placed into one half of each experimental chamber along with 30 g of fresh, pre-weighed kelp blades. The other half of each chamber was stocked with the same amount of kelp in order to measure autogenic and microbial-mediated changes in kelp biomass. Experimental chambers were distributed among the 12 tanks such that each tank contained 4 red sea urchins and 4 purple sea urchins, for a total of 12 replicate sea urchins of each species in each DO treatment.

Each grazing experiment ran for 3 d. At the end of each experiment, we removed sea urchins and kelp from the experimental chambers. Sea urchins were euthanized, dried at 60°C for 48 h, weighed to obtain dry mass, then incinerated in a muffle furnace at 550°C and re-weighed to obtain ashed mass. We calculated each sea urchin's ash-free dry mass as the difference between dry mass and ashed mass. Kelp from each chamber was spun 10 times in a salad spinner (Bickel & Perrett 2016) and weighed to determine its final wet mass. To calculate total grazing while accounting for autogenic and microbial-mediated changes in kelp mass, we adjusted the initial kelp mass in the urchin chamber using the percentage of kelp mass lost in the kelp-only chamber ($<5\%$ for all replicates), then subtracted final kelp mass in the urchin chamber from this adjusted initial mass. This total grazing value was then divided by the length of the experiment (3 d) to calculate the per-capita grazing rate (g d^{-1}), and then further divided this by the ash-free dry mass of the sea urchin to obtain the mass-specific grazing rate (g g^{-1} d^{-1}) for each sea urchin.

We analyzed per-capita and mass-specific grazing rates using a nested analysis of variance (ANOVA) with DO level and urchin species as fixed factors, and tank as a random factor nested within the DO treatment. Experiments with large and small sea urchins were conducted and analyzed separately. Post hoc Tukey tests were run to distinguish differences in treatment levels after ANOVA. Based on Shapiro-Wilk and Fligner tests, the assumptions of normality and homoscedasticity were met ($p > 0.29$ for all tests). All statistical analyses were run using R (R Core Team 2016).

Field patterns of DO

To assess how the DO thresholds from our laboratory experiments compared with conditions in a California Current kelp forest, we analyzed a decade-long data set of DO data from the nearshore Monterey Bay. Since 2000, the Monterey Bay Aquarium has monitored and recorded the DO concentrations of seawater entering its intake pipes. This seawater is drawn from a depth of 17 m, and is expected to reflect seawater conditions in the surrounding kelp forest. A detailed analysis of the data set was published by Booth et al. (2012), who quantified exposures to critical biological thresholds proposed by Vaquer-Sunyer & Duarte (2008).

We used this dataset (spanning 2000 to 2010) to quantify the frequency and duration of exposure to the levels of DO used in our laboratory experiments. We calculated the fraction of time spent below these DO levels over the 10 yr period. Because we expected seasonal variation in the duration and frequency of exposure to low DO, we also calculated these exposure times monthly.

RESULTS

Lethal thresholds of hypoxia exposure

Red and purple sea urchins exhibited visible signs of stress, such as decreased mobility and partially collapsed tube feet, after 12 h of exposure to severe hypoxia (1.0 mg l^{-1}). However, all sea urchins in the experiments were able to recover from exposures of up to 36 h. No mortality was observed in any of the sea urchins exposed to 12, 24, or 36 h of severe hypoxia. LT_{50} varied between 48 and 60 h (Fig. 1). The LT_{50} value in red sea urchins was greater than that of purple sea urchins across both size classes, but no differences were observed for size class.

Thresholds of hypoxia for kelp grazing

Kelp grazing rates varied significantly between DO treatments for both large ($F_{3,8}$ = 13.6, p = 0.0017) and small ($F_{3,8}$ = 22.1, p < 0.001) sea urchins (Fig. 2). There were significant differences in per-capita grazing rates between species, with purple sea urchins generally consuming more kelp than red sea urchins (large urchins: $F_{1,80}$ = 36.2, p < 0.001; small urchins: $F_{1,80}$ = 4.41, p = 0.04). However, responses to DO were similar between the 2 species (DO level × species interaction for large urchins: $F_{3,80}$ = 1.84, p = 0.15; for small urchins: $F_{3,80}$ = 2.33, p = 0.08).

Post hoc Tukey tests showed that sea urchins of both species and sizes consumed significantly less kelp in the 5.5 and 4.5 mg l^{-1} DO treatments relative

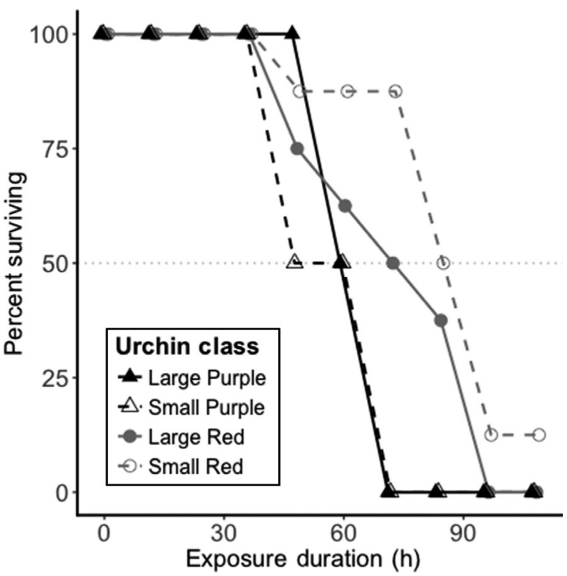

Fig. 1. Percent survivorship of large individuals (solid points and lines) and small individuals (open points and dashed lines) of purple sea urchins *Strongylocentrotus purpuratus* (triangles) and red sea urchins *Mesocentrotus franciscanus* (circles) following different exposure durations to 1.0 mg l^{-1} of dissolved oxygen. Horizontal dotted line: 50% survivorship, corresponding to the median lethal exposure time (LT_{50})

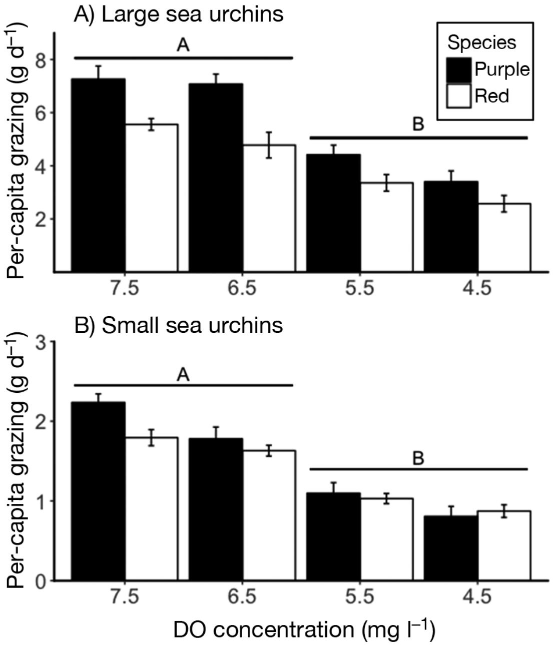

Fig. 2. Per-capita kelp grazing (mean ± SE g d^{-1}, n = 12 ind.) for (A) large and (B) small individuals of purple sea urchins *Strongylocentrotus purpuratus* (filled bars) and red sea urchins *Mesocentrotus franciscanus* (open bars) under 4 different dissolved oxygen (DO) concentrations, from ambient (7.5 mg l^{-1}) to sub-lethal (4.5 mg l^{-1}). Letters indicate differences in kelp grazing between DO treatments, based on Tukey's HSD tests

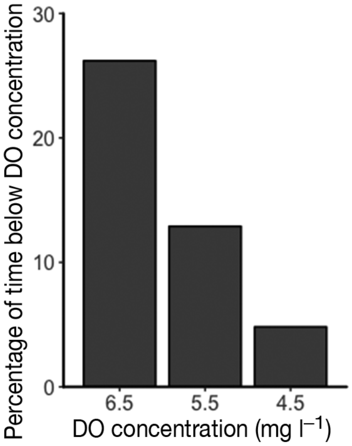

Fig. 3. Percentage of time spent below different levels of dissolved oxygen (DO) over 10 yr (2000–2010) in the nearshore Monterey Bay

to the ambient treatment (Fig. 2). On average, large sea urchins reduced their kelp grazing by 39% and small sea urchins reduced their kelp grazing by 47% when exposed to 5.5 mg l^{-1} of DO. Mass-specific grazing rates showed the same trends over the different DO treatments (large urchins $F_{3,8}$ = 11.9, p = 0.0025; small urchins: $F_{3,8}$ = 13.7, p = 0.0016).

Field patterns of DO

Between 2000 and 2011, DO concentrations at the Monterey Bay Aquarium intake pipe dropped below 6.5 mg l^{-1} for 26% of the time (Fig. 3). DO concentrations below 5.5 mg l^{-1} occurred 13% of the time, and concentrations below 4.5 mg l^{-1} occurred 5% of the time. Severe hypoxia conditions of 1.0 mg l^{-1} or less were never recorded.

There was substantial seasonal variation in exposure to low DO (Fig. 4). During the upwelling months from April to September, an average of 18 to 20% of the time was spent at DO levels low enough to reduce sea urchin grazing (<5.5 mg l^{-1}). In contrast, during the months of October to March, only 1 to 8% of the time was spent below 5.5 mg l^{-1} of DO.

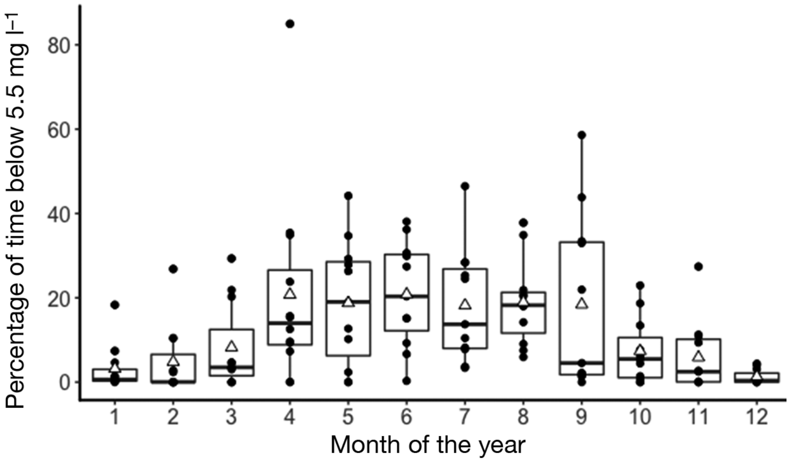

Fig. 4. Percentage of time spent at dissolved oxygen levels low enough to reduce sea urchin grazing (<5.5 mg l^{-1}), by month of the year, over 10 yr (2000–2010) in the nearshore Monterey Bay. Filled circles: percentage values from each individual year; open triangles: mean percentage values. Bar: median; box: interquartile range (IQR); whiskers: data range within ±1.5×IQR above/below IQR

DISCUSSION

Current and near-future exposures to low oxygen conditions in the nearshore kelp forest ecosystems of the California Current (Booth et al. 2012, 2014, this study, C. B. Woodson et al. unpubl.) are unlikely to cause direct mortality of sea urchins. Purple and red sea urchins *Strongylocentrotus purpuratus* and *Mesocentrotus franciscanus* were tolerant of very low DO levels (1.0 mg l^{-1}), with all experimental individuals recovering from up to 36 h of exposure to these conditions. These oxygen levels were never observed in over a decade of field data from Monterey Bay. Severe hypoxia has been documented at other nearshore locations within the California Current (e.g. Isla Natividad, Baja California, Mexico), but for periods not exceeding 24 h and typically much shorter (2 to 4 h; Micheli et al. 2012, Boch et al. 2018). Similarly high hypoxia tolerances have been found in the larval stages of *S. purpuratus* (Eerkes-Medrano et al. 2013), suggesting that direct, lethal impacts of hypoxia on sea urchin abundances are not likely to occur within California Current kelp forests. Our measurements of LT$_{50}$ in *S. purpuratus* and *M. franciscanus* fall within the broad range of LT$_{50}$s (32 to >700 h), but are below the mean (201 h) measured in other echinoderms—primarily asteroids and ophiuroids—under severe (≤2 mg l^{-1}) hypoxia (Vaquer-Sunyer & Duarte 2008). To our knowledge, these represent the first measurements of hypoxia tolerance in nearshore echinoids.

In contrast, sublethal effects on physiology and behavior are likely to already occur under present-day conditions in the nearshore kelp forests of the California Current. Across both species and sizes, sea urchins significantly reduced their grazing rates under 5.5 mg l^{-1} of DO. This represents a DO threshold that is less extreme than the sublethal threshold of 4.6 mg l^{-1} proposed by Vaquer-Sunyer & Duarte (2008), and substantially less extreme than the mean sublethal threshold that they reported for echinoderms (1.22 mg l^{-1}). Our findings support the idea that there is no single value for DO thresholds that holds across all species or even for species within a taxonomic group, especially for species that are found in different habitats (Seibel 2011, Somero et al. 2016). In addition, the 5.5 mg l^{-1} threshold found here represents DO conditions that are currently relatively common in kelp forests of the California Current System. A decade of field oxygen data indicate that DO concentrations fall below 5.5 mg l^{-1} for 18 to 20% of the time during upwelling seasons in Monterey Bay. Repeated patterns of exposure to DO levels below 5.5 mg l^{-1} have also been seen in other nearshore California Current ecosystems, including the Southern California Bight (Booth et al. 2014), northern California (F. Micheli et al. unpubl.) and at sites in Baja California, Mexico, where DO levels can stay below 4.5 mg l^{-1} for 1 to 3 wk consecutively (Micheli et al. 2012, Boch et al. 2018).

Based on a decade of DO data from a Monterey Bay kelp forest (Booth et al. 2012, this study) and measurements from other locations (e.g. Micheli et al. 2012, Booth et al. 2014), we observed that the DO threshold for functional-role impacts in *S. purpuratus* and *M. franciscanus* is much more likely to be crossed than are the thresholds for mortality. Therefore, the functional-role impacts of coastal hypoxia in this ecosystem are likely much more prevalent than hypoxia-driven mortality under both current and near-future climate change scenarios. These findings support the idea that 'physiological rate effects', defined as changes to the rates of key biological processes that occur in an organism's normal range of physiological conditions, can be as or more important than the effects of extreme conditions on ecosystem dynamics (Sanford 2002). While the concept of physiological rate effects was originally used in the context of temperature, our study shows that it is more broadly applicable to other climate drivers, such as DO.

Our estimates of the potential functional-role impacts of low DO exposures were comparable in magnitude to some non-consumptive predator effects: exposure to 5.5 mg l^{-1} of DO reduced grazing rates in *S. purpuratus* and *M. franciscanus* by an average of 39 to 47%, whereas Matassa (2010) reported a similar 44% decrease in *S. purpuratus* grazing rates in the presence of water-borne cues from spiny lobsters. Therefore, sublethal hypoxia could potentially have a similar magnitude of cascading, community-wide effects as do non-consumptive predator effects (Werner & Peacor 2003, Schmitz et al. 2004), which can be substantially larger than consumptive effects (Trussell et al. 2006). To our knowledge, this is the first documentation of a possible modulation of kelp grazing via hypoxia.

The importance of DO impacts on sea urchin functional roles may also be enhanced by the timing of exposures to low oxygen. There are strong seasonal differences in the prevalence of DO conditions low enough to impact sea urchin grazing rates (<5.5 mg l^{-1}), with low-oxygen conditions being most common during the spring to summer upwelling season (April to September). This upwelling season corresponds to peak recruitment of juvenile kelp (Graham et al. 1997), and the earlier half of the season (April to June) coincides with lower drift kelp production in the Monterey Bay kelp forest (Gerard 1976). Drift kelp shortages are thought to trigger a behavioral shift in sea urchins from sedentary consumers of drift kelp to actively roving benthic grazers that can remove kelp holdfasts and recruits (Dean et al. 1984, Harrold & Reed 1985). By reducing grazing rates at a time when food supply is low and kelp recruitment is occurring, seasonal exposures to low DO conditions may help to maintain kelp forests, especially at high densities of sea urchins.

We found no significant differences between either sea urchin species or size classes in their grazing responses to hypoxia. While there may have been some inter-species differences in lethal responses, our experimental design did not allow this to be tested statistically, and any such differences would likely not be ecologically relevant, given the high hypoxia tolerances we found in both species and size classes. Our findings suggest that hypoxia effects are likely to be similar across sizes of both species of sea urchins, and that their relative abundances and population size structures may not influence the impact of upwelling-driven hypoxia on kelp forest ecosystems.

Our experiments focused on a single physiological driver: DO. However, upwelling-driven low oxygen conditions tend to be correlated with low temperature and/or low pH (Booth et al. 2012, Frieder et al. 2012), and it will be important to consider how sea urchin mortality and kelp consumption are impacted by these multiple simultaneous climate drivers. It is likely that sea urchins will be more tolerant of severe hypoxia under low temperatures (Vaquer-Sunyer & Duarte 2011), whereas the well-documented relationship between temperature and ectotherm metabolic rate (Brown et al. 2004) suggests that urchins' kelp consumption rates may be jointly reduced by low-temperature, low-oxygen upwelling conditions. As a result, the functional-role impacts of upwelling-driven hypoxia are likely to be even more important in a multiple-stressor upwelling context, relative to the impacts on sea urchin mortality. These potential effects need to be studied experimentally, along with the less-predictable effects of low pH.

This study was limited to the short-term impacts of low DO on sea urchins. Although upwelling-driven hypoxia does generally occur as a short-term 'pulse' exposure, organisms are likely to encounter many such short-term exposures within the course of an upwelling season (Booth et al. 2012), and more throughout their lifetime. These repeated exposures to sublethal physiological stress may impose 'cost-of-living' consequences that alter sea urchins' growth and reproductive rates, and ultimately impact their abundances in the long term (Somero 2002). Longer-term experiments are needed to explore these potential impacts, and to determine if organisms may also be able to acclimate to repeated low-oxygen exposures over time.

As the frequency and intensity of upwelling-driven coastal hypoxia is expected to increase with climate change (Bakun et al. 2010, Sydeman et al. 2014), understanding how this driver impacts the structure and functioning of coastal ecosystems is increasingly important. Our study contributes toward that understanding by providing novel insights into the impacts of low DO on an ecologically important species within the kelp forest ecosystem, and more broadly by demonstrating the potential importance of considering the non-lethal impacts of climate drivers on the functional roles of species.

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