

# What Changes in the Carbonate System, Oxygen, and Temperature Portend for the Northeastern Pacific Ocean: A Physiological Perspective

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*The northeastern Pacific Ocean is undergoing changes in temperature, carbonate chemistry, and dissolved oxygen concentration in concert with global change. Each of these stressors has wide-ranging effects on physiological systems, which may differ among species and life-history stages. Simultaneous exposure to multiple stressors may lead to even stronger impacts on organisms, but interacting effects remain poorly understood. Here, we examine how single- and multiple-stressor effects on physiology may drive changes in the behavior, biogeography, and ecosystem structure in coastal marine ecosystems, with emphasis on the California Current Large Marine Ecosystem. By analyzing the effects of stressors on physiological processes common to many marine taxa, we may be able to develop broadly applicable understandings of the effects of global change. This mechanistic foundation may contribute to the development of models and other decision-support tools to assist resource managers and policymakers in anticipating and addressing global change-driven alterations in marine populations and ecosystems.*

**Keywords:** acidification, California Current Large Marine Ecosystem, dissolved oxygen, global change, hypoxia

**I**n many regions of the world ocean, global change is leading to alterations in a suite of abiotic factors that have wide-ranging effects on the physiologies of marine organisms (Gattuso and Hansson 2011, Gattuso et al. 2015). Understanding how environmentally driven changes in physiological systems lead to alterations at higher levels of biological organization, including ecosystem function and biogeographical patterning, is central to a mechanistic and predictive understanding of global change's effects on the future state of marine ecosystems. Here, we examine how stresses that result from the exposure of marine organisms to changes in temperature, dissolved oxygen (DO), and ocean acidification (OA)—including increased partial pressure of carbon dioxide ( $p\text{CO}_2$ ) and reduced pH and carbonate saturation state ( $\Omega$ ); see supplemental text 1 for definitions)—three classes of stressors that, singly or in combination, may have significant effects at all levels of biological organization. Note that the terms *stress* and *stressor* are often used rather loosely in the literature. Here, we offer the following definitions: A *stressor* is any physical or chemical factor that can perturb the physiological and biochemical systems of organisms and, above some threshold value of intensity, lead to suboptimal values for these traits. Therefore, a change in the intensity of a

stressor does not necessarily mean that the organism experiences *stress*, which we define as the displacement of traits from their optimal range of values (see Hochachka and Somero 2002). Our analysis is not a comprehensive review of the rapidly growing literature on the known or potential physiological consequences of global change but rather is focused on selected studies that, we believe, provide an especially sound basis for building a framework for elucidating how physiological stress can lead to broader-scale effects. An important focus of our analysis is on the effects of multiple stressors, a topic that has not been adequately examined to date in experimentation on the effects of global change on marine organisms. We show that such integrated analyses can provide new insights into the causes of already observed range shifts and changes in ecosystem status and, importantly, can contribute to the foundation needed for projecting the future effects of global change. The predictive capacity afforded by incorporating physiology into model development may benefit the efforts of resource managers and policymakers tasked with managing marine resources (see the “Scaling up to management and policy” box). Finally, our analysis points to ways in which physiological studies should be designed in order to fill gaps in our knowledge and provide the most

**Box 1. Scaling up to management and policy: How can physiological data contribute?**

Managers of living marine resources and those tasked with environmental decisionmaking are challenged to develop strategies that minimize the negative consequences of ocean acidification, hypoxia, and other stressors for species and systems of interest. Such strategies need to be developed in what is now a relatively data-poor context, because despite rapid growth in the number of laboratory studies performed, studies at the population and ecosystem scales of most concern to managers are relatively few. Given this situation, how can physiological studies contribute to the information needs of managers?

First, physiological studies can improve the mechanistic understanding of organismal response. Marine organisms are exposed to changes in a number of variables (temperature, dissolved oxygen concentration, carbon system variables, etc.) that have the potential to affect their performance in terms of survival, growth, reproduction, and behavior. The conservation and management of living marine resources under changing environmental conditions require that the effects of individual variables can be linked mechanistically to organismal response. The clear attribution of specific responses to changes in one or more environmental variables is an essential first step in the development of any management strategy.

Second, physiological studies can increase confidence in projections and scenario planning. Decisionmakers increasingly rely on scenario planning to discriminate between alternative futures (e.g., Gattuso et al. 2015 and references therein). For example, scenarios used to construct alternative futures under climate change include estimates of organismal functional response to incremental changes in temperature, oxygen, and carbon-system variables. Experimental studies that accurately estimate these individual-based responses can increase confidence in projections.

Third, physiological studies can contribute to the determination of threshold values and the development of indicators. Resource managers will benefit from the determination of appropriate threshold values for environmental variables that could be used to trigger regulatory response. Moreover, physiology-based indicators of population status and trends under conditions of environmental change can be used to inform environmental assessments and guide management responses. Studies to determine physiological thresholds and those that provide an explanatory link between environmental conditions and population-level responses can be used to develop measures that meet the needs of regulators.

biologically realistic analysis of the effects of single and multiple stressors.

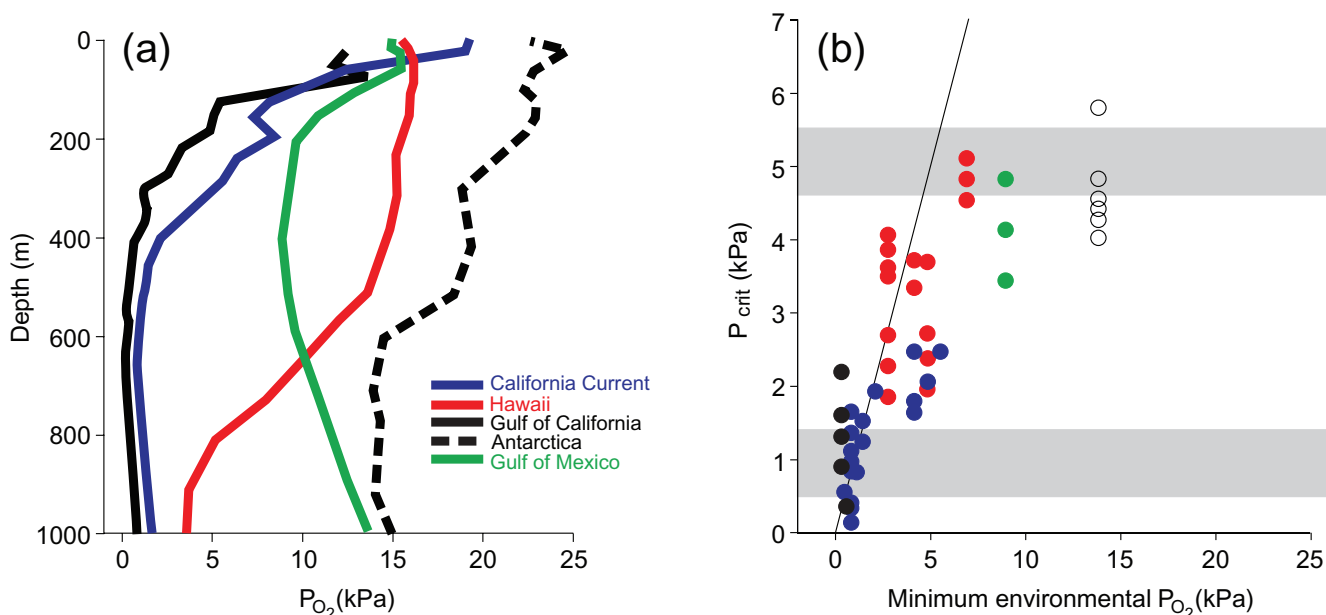
**Changes in the coastal waters of the northeastern Pacific Ocean**

The coastal and near-coastal waters of the northeastern Pacific Ocean, the California Current System (CCS), provide an especially opportune study system for improving our abilities to understand and forecast the intersection between organismal physiology and the key elements of global change. The Large Marine Ecosystem within the CCS is one of the most thoroughly studied coastal ecosystems, where long-term observations have highlighted the sensitivity of ecosystem-scale temperature, DO, and carbonate-chemistry changes to basin- and global-scale modes of climate variability such as the Pacific Decadal Oscillation (PDO) and the El Niño Southern Oscillation (ENSO; Chavez et al. 2003, Deutsch et al. 2011, Feely et al. 2012). In addition, anthropogenic carbon dioxide (CO<sub>2</sub>) emissions have directly and demonstrably altered carbonate chemistry in the CCS (Feely et al. 2008), and climate change is projected to affect DO concentration and temperature through changes in ocean circulation and stratification. Variations in carbonate chemistry, DO, and temperature have already been shown to affect organismal performance in the CCS (Keller et al. 2010, Kroeker et al. 2013, Bednaršek et al. 2014a, Ekstrom et al. 2015). Assuming unabated release of massive amounts of CO<sub>2</sub> from fossil fuel consumption, the changes observed to date in the CCS portend a future that will confront marine

ecosystems with increasingly severe challenges (Gattuso et al. 2015).

In the analysis that follows, we first provide a brief overview of the changes that characterize the waters of the CCS and many other regions of the world ocean and discuss how changes in these stressors affect physiological processes. We follow this with specific examples from the physiological literature that provide a basis for broader ecological, biogeographic, and policy analyses.

**Dissolved oxygen.** Changing levels of DO reflect a complex interplay between physical and biological drivers. Warmer surface waters hold less oxygen, and the stratification that results from surface warming can strengthen the thermocline and reduce the overturning circulation that is essential in ocean-ventilation processes (Keeling et al. 2010). In productive eastern boundary-current systems, changes in the timing and intensity of upwelling are occurring (Garcia-Reyes and Largier 2010, Wang et al. 2015) that can further amplify global deoxygenation trends (Bakun et al. 2010). The upward expansion (shoaling) of hypoxic waters in the CCS has been observed, and the vertical range and horizontal breadth of the oxygen minimum zone (OMZ) may be increasing as well (Bograd et al. 2008, Rosa and Seibel 2008, Booth et al. 2012). Hypoxia, although sublethal in most cases, is already affecting coastal marine ecosystems. Keller and colleagues (2010, 2015) reported that biomass, species diversity, and condition factors for demersal fishes and invertebrates were significantly correlated with DO



**Figure 1. Oxygen-binding abilities reflect ambient dissolved oxygen (DO).** Frame A: The oxygen profiles in waters columns in the Eastern Pacific California Current (blue), Gulf of California (black, solid), Hawaiian Pacific (red), Gulf of Mexico (green), and Southern Ocean (Antarctica; black, dashed). Frame B: The critical oxygen partial pressure ( $P_{crit}$ ) values of pelagic fishes and invertebrates adapted to different minimal oxygen contents. The color-coding reflects the information in frame A (Antarctic species are open circles). In hypoxic regions, there is a close correlation between minimal oxygen level and  $P_{crit}$  (line of unity). Species with  $P_{crit}$  values falling to the left of the line of unity experience oxygen levels that require partial reliance on anaerobic metabolism (or metabolic suppression at low oxygen levels). Species falling on the line of unity live near their evolved limits for oxygen extraction. The gray bars portray two different types of oxygen thresholds. The plateau in  $P_{crit}$  noted above approximately 5 kilopascals (kPa) indicates that further increases in oxygen concentration provide no additional benefit to the organism. The lower gray band represents oxygen concentrations that appear not to have selected for lower  $P_{crit}$  values. Figure from Seibel (2011) with permission from the Company of Biologists. Abbreviations: m, meters;  $P_{O_2}$ , partial pressure oxygen.

levels in an offshore hypoxic zone along the US West Coast. The occurrence of episodes of anoxia in nearshore waters in the northeastern Pacific has been observed and marked by large-scale mortalities of animals that lack the ability to move away from anoxic regions (Grantham et al. 2004, Chan et al. 2008). Animals vary greatly in their reliance on aerobically powered activity, their abilities to extract oxygen from seawater and transport it to respiring tissues, and their capacities for sustaining metabolism using anaerobic pathways of ATP generation when hypoxia occurs, either from low DO (environmental hypoxia) or elevated metabolic rates (physiological hypoxia; see supplemental text 2 for definitions of oxygen parameters; Hochachka and Somero 2002). Species with the capacities to rely on anaerobic metabolic pathways during bouts of hypoxia or anoxia (facultative anaerobes) may fare better during periods of low oxygen than species that are obligate aerobes (most fishes fall into this category, despite the fact that species vary widely in hypoxia tolerance).

From these facts, it follows that no single value of oxygen concentration can be used as a common “threshold” level for the onset of physiological stress. Criteria used to define hypoxia need careful re-evaluation. The commonly

used definition of hypoxia as a DO concentration less than or equal to 2 milligrams (mg) oxygen ( $O_2$ ) per liter (see supplemental text 2 for the relationships among the different formats for expressing DO values) is not descriptive of the oxygen relationships of many and perhaps most marine fishes and invertebrates (Vaquer-Sunyer and Duarte 2008). Seibel (2011) emphasizes that the crucial  $O_2$  thresholds for aquatic organisms must be based on the criteria of organismal performance and survival, not on some single oxygen concentration. One commonly used performance criterion is based on the response of oxygen-consumption rate (aerobic respiration) to decreasing DO. This variable is the *critical oxygen partial pressure* ( $P_{crit}$ ), defined as the partial pressure of oxygen at which the rate of oxygen consumption first begins to decrease with further decreases in DO. Values of  $P_{crit}$  vary among animals in relation to their activity levels—highly active animals generally have higher  $P_{crit}$  values—and the levels of DO that characterize their habitat conditions. Organisms from low DO habitats tend to have correspondingly low  $P_{crit}$  values (figure 1; Seibel 2011). Over a span of oxygen partial pressures between approximately 2 and 7 kilopascals (kPa),  $P_{crit}$  values for many pelagic marine animals are proportional to the ambient DO levels typical of

their habitats. At oxygen levels above approximately 7 kPa, further increases in oxygen content appear to provide no physiological advantage to organisms, and  $P_{crit}$  exhibits a plateau. Below 2 kPa, further decreases in  $P_{crit}$  may not be possible, physiologically (see Seibel 2011). Treating  $O_2$  levels independently of effects of other stressors can, of course, lead to incorrect conclusions about metabolic relationships of ectotherms. As we discuss below, ambient-temperature and carbonate-system variables such as  $pCO_2$  can affect the ability of an animal to maintain an aerobic poise to its metabolism.

**Carbonate chemistry (pH,  $pCO_2$  and saturation state).** Ocean acidification refers to long-term changes in seawater chemistry such as reductions in pH and carbonate mineral saturation state caused primarily by the uptake of carbon dioxide from the atmosphere. Such carbonate chemistry changes have widespread effects on the physiological processes of many taxa of marine organisms (Kroeker et al. 2013). It is important to realize that different taxa as well as different physiological processes and developmental stages of a given species may respond to different aspects of changes in the carbonate system (see supplemental text 1 for definitions of carbonate-system variables). Calcifiers may be most sensitive to carbonate saturation state in the context of shell deposition (Waldbusser et al. 2014), but changes in  $pCO_2$  or pH may also affect a species' rate of energy metabolism and other pH-sensitive cellular processes such as ion channel activities (Waldbusser et al. 2015). pH regulatory mechanisms and their efficacy in controlling extra- and intracellular pH differ among species and, in many cases, between life stages of a single species. Therefore, OA may have differential effects on preadult and adult life stages. As in the case of DO, there is no single value of pH (or any other carbonate-system variable) that can be used as a ubiquitous "threshold" for biologically significant effects. The challenges from OA differ among oceanic regions. OA effects may be particularly strong in regions such as the CCS, where anthropogenic  $CO_2$  entering at the ocean surface adds to  $CO_2$  produced through respiration in subsurface waters, substantially enriching the total dissolved inorganic carbon (DIC) of coastal waters (Feely et al. 2008, Harris et al. 2013).

**Temperature.** Global change is affecting the temperature of the CCS in complex ways. Surface waters are warming, but increased episodic upwelling of cold deep water may confront organisms with rapid decreases in temperature of several degrees Celsius ( $^{\circ}C$ ), which occur in conjunction with falling pH and DO (Feely et al. 2008, Booth et al. 2012). Alterations in temperature have direct effects on virtually all structures and processes of organisms (Hochachka and Somero 2002). These effects include alterations in rates of biochemical processes, changes in pH values of extra- and intracellular fluids (pH in both compartments falls by approximately 0.017 pH unit per degree Celsius increase in body temperature), altered stabilities of macromolecules

and membranes, changes in gene expression, and shifts in the equilibria of chemical processes involved in building structures, notably those composed of calcium carbonates. Thermal optima and breadth of thermal-tolerance range vary greatly among species; species evolved in stable thermal habitats generally have narrow thermal-tolerance ranges (stenothermy) relative to species that have evolved in highly variable thermal conditions (eurythermy). Acclimation capacity, which contributes importantly to the definition of the breadth of the thermal-tolerance range, differs substantially among species as well.

The temperature dependence of physiological processes (indexed by the temperature coefficient  $Q_{10}$ , which describes the effect of a  $10^{\circ}C$  change in temperature on reaction rate and usually lies in the range of 1.5–2.5) leads to a 5%–10% change in rate with each  $1^{\circ}C$  change in temperature (Hochachka and Somero 2002). The effects of temperature change on metabolic rates present challenges that differ among species based on their intrinsic metabolic rates and tolerance of variation in DO (Verberk et al. 2011). Because the increased demand for oxygen at higher temperatures due to  $Q_{10}$  effects on respiration occurs in conjunction with a reduction in oxygen solubility, challenges to sustaining *metabolic scope*, defined as the ability to increase aerobic respiration when heightened activity is needed (e.g., in predator–prey interactions) will arise (Deutsch et al. 2015).

Water temperature has complex effects on marine organisms' abilities to build and maintain structures fabricated from calcium carbonates (Orr et al. 2005, Kroeker et al. 2014). Reduced temperatures will lower metabolic rates and could thereby slow down the biochemical reactions supporting calcification and therefore ontogeny and growth. Moreover, and possibly of greater significance, the stabilities of calcite and aragonite are reduced at low temperatures, resulting in a generally decreased saturation state with high latitude. Certain high-latitude waters may already be exposing calcifiers to conditions that hinder the building of skeletal materials (Orr et al. 2005, Bednaršek et al. 2014a). The waters of the CCS have high background DIC because of large-scale patterns in ocean circulation and may be approximately 30% less saturated than those of warmer low-latitude waters (Feely et al. 2008); therefore, the CCS may be relatively vulnerable to further acidification. Temperature–pH–carbonate system relationships therefore are complex and merit multistressor analysis (Kroeker et al. 2014).

**The importance of temporal patterning.** The temporal patterning of alterations in environmental variables is another important factor that can govern the overall stress resulting from changes in individual or multiple stressors. The rate of change in pH and other carbonate-system variables, DO, and temperature and the duration of exposure to stressful levels of these variables will determine the severity of stress on marine organisms (Cheng et al. 2015, Reum et al. 2015). Few field or laboratory studies have addressed the time courses of acclimatization to changes in either individual



stressors or concurrent changes in multiple stressors. We also have limited information on how marine species differ in capacities for acclimatization. For example, the natural variation of carbonate-system variables, DO, and temperature due to semidiurnal fluctuations associated with the tidal cycle or seasonal upwelling events may predispose intertidal and shallow subtidal species to cope relatively well with these stressors (Frieder et al. 2014). In contrast, species evolved in deeper and more stable environments may have lesser abilities to acclimatize and therefore will be in greater jeopardy from environmental change.

**Multistressor effects: Challenges to marine species and to marine scientists.** Exposure to multiple abiotic stressors may lead to effects that are *additive* (*net stress* is the sum of effects of individual stressors), *antagonistic* (stressors have counteracting, or offsetting, effects), or *synergistic* (stressors amplify the influences of other stressors; Gobler et al. 2014, Kroeker et al. 2014). Therefore, single-stressor experiments, although often a necessary first step in analysis, may lead to overly simplified conclusions about the actual impacts of global environmental change. The net effect of multiple stressors may depend on the temporal pattern of exposure to the stressors; the effects arising from sequential exposure to two or more abiotic changes may differ from those caused by concurrent exposures to these same factors. It will be important to design experiments that factorially test multiple stressors and then assess the independent effects of each stressor and their potential interaction terms (see Waldbusser et al. 2014).

### **Stressor effects on metabolism, calcified structures, and behavior: What lies in store for coastal marine ecosystems?**

Below, we examine how changes in DO, the carbonate system, and temperature are affecting three significant aspects of the biology of coastal marine animals: their metabolism, behavior, and abilities to build calcium carbonate structures. We believe that this categorization of the biological effects of key stressors provides a good foundation for mechanistic analyses of how physiological stress leads to changes in biogeographic distribution and ecosystem structure and function. It is important to recognize, of course, that these three aspects of the biology of marine animals are often tightly interlinked for determining fitness.

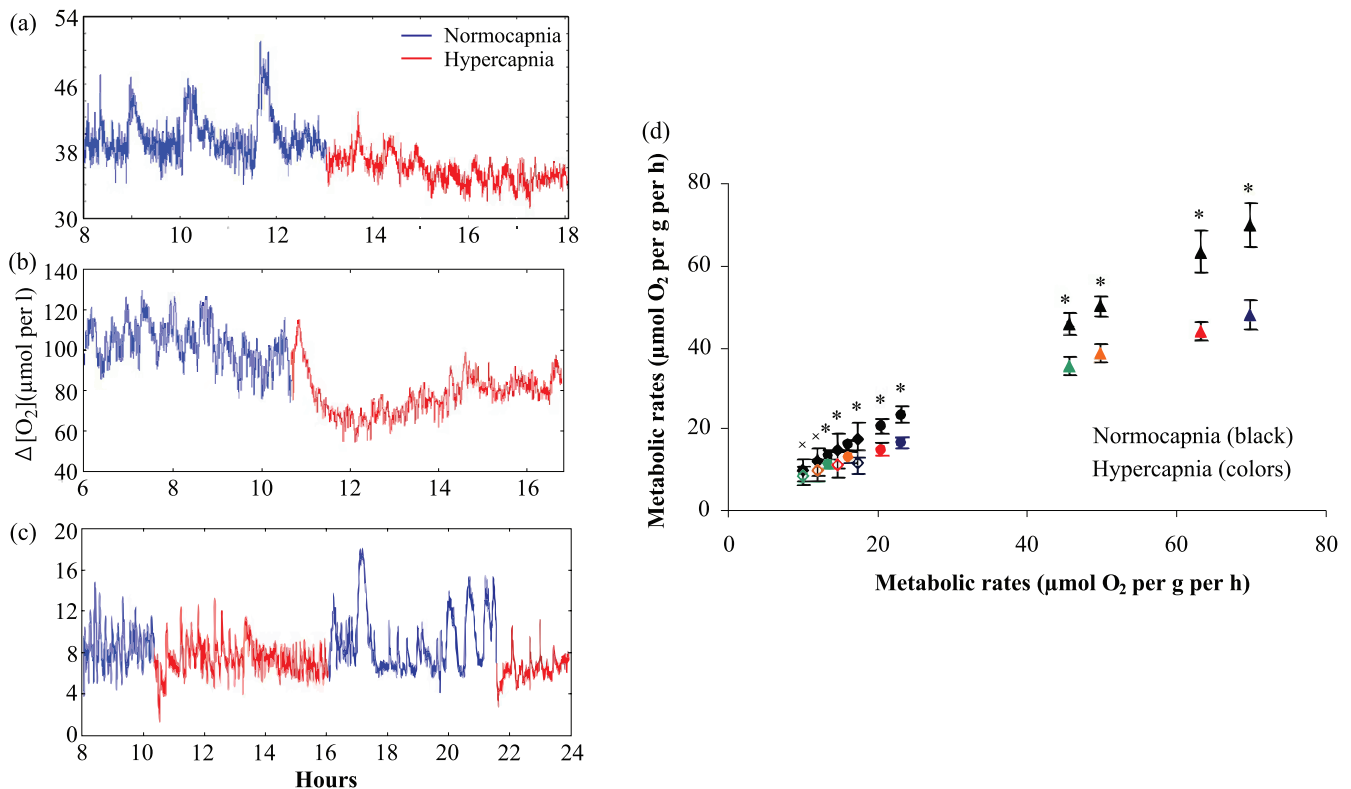
**Metabolism.** There is a large and growing literature on the importance of oxygen–temperature interactions on the physiological states and biogeographic patterning of marine fishes and invertebrates (Deutsch et al. 2015, Haigh et al. 2015). A critical focus of these analyses is on the ability of ectotherms to elevate their rates of oxygen consumption above resting levels during such crucial activities as predation, avoidance of predators, and long-distance migration. The factorial increase in oxygen consumption above resting levels is commonly referred to as *aerobic* (or *metabolic*)

*scope*. More recently, the concept of a *metabolic index* ( $\phi$ ) has been developed, which expresses the ratio of O<sub>2</sub> supply to an organism's resting demand for O<sub>2</sub> (Deutsch et al. 2015). At values of  $\phi$  greater than 1.0, metabolic rates can increase by a factor of  $\phi$  above resting levels. In their analyses of fish and invertebrates from different latitudes and depths, Deutsch and colleagues (2015) discovered that values of  $\phi$  in the range 2–5 were characteristic of most species. Therefore, marine habitats permissive of aerobic animal life appear to allow animals to increase their metabolic rates two to five times above resting rates.

When we view these oxygen requirements in the context of global change through the duration of this century, the combined effects of rising temperature on oxygen solubility and respiration rates (Q<sub>10</sub> effects) portend major biogeographic shifts in distributions of marine ectotherms. There will be a global reduction in  $\phi$  throughout the upper part of the marine water column (0 to approximately 400 meters), with model-dependent estimates ranging between 17% and 25% (average of approximately 21%; Deutsch et al. 2015). The larger fraction (approximately 2/3) of the decrease in  $\phi$  will result from Q<sub>10</sub> effects on respiration rates. Decreases in  $\phi$  will be especially large in the Pacific Ocean, notably in the CCS, where reductions in DO will play a greater role than in most regions of the ocean. Throughout the ocean, these effects on  $\phi$  will drive distributions of many, but not all, species in a poleward direction or toward greater depths in the water column. The complex effects of these species-specific latitudinal and depth-related shifts in distribution on marine ecosystems remain essentially unknown (see Gaylord et al. 2015).

The broad significance of aerobic metabolism notwithstanding, remember that marine ectotherms differ widely in their intrinsic rates of oxygen consumption and in their abilities to employ anaerobic pathways of ATP generation when DO levels are low. Marine fishes and invertebrates with different life histories and habitat preferences exhibit a broad suite of adaptations to oxygen availability. Animals adapted to OMZs generally display low rates of oxygen consumption, low P<sub>crit</sub> values, high blood oxygen affinities that allow the effective uptake of oxygen in the face of low ambient DO, and biochemical adaptations that facilitate shifts to the anaerobic pathways of ATP production when DO levels are limiting for aerobic catabolic processes (Rosa and Seibel 2008, Seibel 2011, Torres et al. 2012). Fish that have evolved anaerobic ATP-generating pathways that lead to ethanol, which does not perturb pH and can be released into the environment, may be especially tolerant of hypoxia and anoxia. This type of metabolism in fishes has recently been shown to be common in vertically migrating fishes, such as the abundant myctophids that periodically enter the OMZ (Torres et al. 2012). The ongoing vertical and horizontal expansion of OMZs may allow for an increase in the distribution ranges of these hypoxia-tolerant species.

Ocean acidification may also affect metabolism and thereby lead to ecological and biogeographic changes. Decreasing pH and rising pCO<sub>2</sub> have been shown to cause reductions in



**Figure 2.** The effects of temperature and partial pressure of carbon dioxide ( $p\text{CO}_2$ ) on oxygen consumption by the jumbo squid (*Dosidicus gigas*). The control values (normocapnia) are from respiration experiments run at a  $p\text{CO}_2$  of approximately 330 parts per million (ppm); the hypercapnia experiments employed  $\text{CO}_2$  at approximately 1000 ppm. Left panels: The effects of temperature ((a), 25 degrees Celsius [ $^{\circ}\text{C}$ ], (b) 20 $^{\circ}\text{C}$ , (c) 10 $^{\circ}\text{C}$ ) and  $p\text{CO}_2$  on the respiration rates and activity of specimens of *D. gigas* of different mass ((a) 8.03 grams [g], (b) 21.93 g, (c) 2.23 g). The blue segments of the tracings denote normocapnia; the red segments denote hypercapnia. The tracings illustrate the activity-dependent effects on metabolism (measured by recording the rates of mantle contraction), with spikes in oxygen consumption coinciding with bouts of mantle contraction. Right panel: A plot showing how metabolic rate at different activity levels is affected by exposure to different temperatures and elevated  $p\text{CO}_2$ . Oxygen-consumption rates of control individuals with different levels of activity are plotted against themselves and against the rates of the same individuals exposed to elevated  $\text{CO}_2$ . Different activity levels (metabolic rates) are indicated by colors: inactive (green), routine (orange), active (red), and maximal (blue). Open diamonds, 10 $^{\circ}\text{C}$ ; solid circles, 20 $^{\circ}\text{C}$ ; solid triangles, 25 $^{\circ}\text{C}$ . The largest effects of hypercapnia are found at 25 $^{\circ}\text{C}$  for animals with the highest activity levels. Source: Figure adapted from Rosa and Seibel (2008). Abbreviations: h, hour; l, liter;  $\text{O}_2$ , oxygen;  $\mu\text{mol}$ , micromoles.

metabolic rates in many animals, but the potential interactions among OA, falling DO, and rising temperatures have received minimal study to date. Perhaps the best illustration of the nature of the potential interactions among these three stressors is provided by studies of the Humboldt squid (*Dosidicus gigas*; Rosa and Seibel 2008). This species is a top predator in the northeastern Pacific and is one of the most commercially valuable animals in the Gulf of California fishery. The Humboldt squid undergoes daily vertical migration between shallow waters that are relatively rich in oxygen and deeper waters of the OMZ, which are cooler and  $\text{CO}_2$  rich. This vertical movement exposes the animal to wide variations in temperature, DO, and  $p\text{CO}_2$ , making it an excellent experimental subject for multistressor analyses.

Rosa and Seibel (2008) investigated how changes in temperature, DO, and  $p\text{CO}_2$  affected oxygen-consumption rates, aerobic scope, and locomotory activity levels of *D. gigas* (figure 2). Elevated  $p\text{CO}_2$  (hypercapnia) led to significant decreases in locomotory activity and aerobic scope at temperatures of 20 $^{\circ}\text{C}$  and 25 $^{\circ}\text{C}$  but not at 10 $^{\circ}\text{C}$ . The challenges to sustaining aerobic scope at the high temperatures that arise from  $Q_{10}$  effects and reduced DO likely are compounded by a reduction in the binding of oxygen to the squid's respiratory protein (hemocyanin) at high  $p\text{CO}_2$ . Exposure to low DO led to a significant suppression of metabolism, a response that is adaptive for an animal that spends considerable time in the OMZ.

Rosa and Seibel (2008) discuss the implications of these multistressor effects in the context of *D. gigas*' vertical and latitudinal distribution ranges. An important feature of the vertical migration behavior is the repayment of the oxygen debt that is incurred during the time spent in the OMZ, when metabolism is shifted toward anaerobic pathways of ATP production. Returning to shallower, more oxygen-replete waters is therefore needed to restore the animal's ability for vigorous swimming, as is needed for prey capture and predator avoidance. Warming of the upper region of the water column therefore can be seen as a challenge to this aspect of the Humboldt squid's biology because of falling DO and  $Q_{10}$  effects on respiration rates. Taken together, these physiological challenges may lead to alterations in the vertical distribution of the squid. A shoaling OMZ with decreased pH and warming surface waters with reduced DO may lead to a compression in vertical distribution range, especially at lower latitudes where surface temperatures are already near the species' upper thermal-tolerance limits (Rosa and Seibel 2008). Any horizontal or latitudinal expansion of the OMZ, however, may lead to an expansion of the species' biogeographic range because of the role that access to OMZ waters plays in its normal diurnal activities. In fact, the biogeographic distribution of *D. gigas* recently has extended northward to Canadian and Alaskan waters (Zeidberg and Robison 2007). The range expansion of this large squid into the waters of the northeastern Pacific may have significant effects on fisheries for bony fish because of the voracious predatory habits of *D. gigas* (Zeidberg and Robison 2007).

The metabolic effects of changes in DO, carbonate-system components, and temperature on marine fishes share similarities with some of the effects noted for the Humboldt squid, notably the effects on aerobic scope (or metabolic index) arising from  $Q_{10}$  relationships and reductions in DO in warming waters. The acid–base regulatory strategies of fishes also play into metabolic-cost relationships related to OA. In bony fishes, the regulation of pH entails ATP-driven exchanges of chloride ( $\text{Cl}^-$ ) and bicarbonate ( $\text{HCO}_3^-$ ) ions. Because  $\text{CO}_2$  readily moves between seawater and body fluids, rising  $p\text{CO}_2$  will increase the cost of acid–base regulation, potentially reducing the amount of energy available for functions such as locomotion and growth. Studies have shown large differences among species of fish in the effects of increased  $p\text{CO}_2$  on respiration rates (Munday et al. 2012, Couturier et al. 2013). In experiments with three species of coral reef fish, Couturier and colleagues (2013) found that resting metabolism did not change with exposure to elevated  $p\text{CO}_2$ , leading the authors to conjecture that near-future increases in  $p\text{CO}_2$  are not likely to alter fishes' metabolism under conditions of low metabolic output. However, these findings do not disprove the occurrence of significant effects of elevated  $p\text{CO}_2$  on energy budgets under low levels of metabolism, because trade-offs in energy allocation may occur within the confines of an unchanged total metabolic rate (Melnzer et al. 2011). Studies such as these, performed

on adult fishes, need to be complemented by work with early life stages, at which the acid–base regulatory mechanisms found in adult fish have not yet developed. Preadult stages of fish that lack the adult gill and intestinal acid–base regulatory systems rely on epidermal cells (ionocytes) for ionic and pH regulation; the capacities of these cells to cope with elevated  $p\text{CO}_2$  have received little examination. One recent study suggests that increased  $p\text{CO}_2$  negatively affects the metabolism of fish embryos and reduces the somatic growth of larvae, which may result in overall lower survival potential due to decreased feeding success and increased predation mortality (Franke and Clemmesen 2011).

In the design of experiments focused on the effects of OA on physiological systems, it is crucial to simulate as closely as possible the values of carbonate-system variables and the temporal patterns of exposure to these variables that are likely to confront the study organisms (supplemental text 3). The literature on OA effects includes studies done with widely different values of  $p\text{CO}_2$  exposure—in some cases, levels that are substantially higher than those predicted for centuries—and varied durations of exposure, which are at times mismatched with the duration of exposure to changes in pH found in natural systems (see critique in Reum et al. 2015). For example, the relatively rapid changes in  $p\text{CO}_2$  and pH that accompany upwelling events may not allow time for the types of acclimatization that may occur over days to weeks of laboratory exposure. Further studies of a variety of fishes that employ realistic levels of near-future  $p\text{CO}_2$  and do so over time frames that simulate natural exposures (e.g., those accompanying upwelling) are needed to help clarify the challenges posed by OA. To make these studies even more biologically realistic, the effects of oxygen content and temperature (Munday et al. 2009) must be examined as well.

**Behavior.** The behavior of marine ectotherms is influenced in many ways by changes in DO, carbonate-system variables, and temperature. Animals can sense changes in each of these variables, which may afford them the potential to move away from areas where the stressor(s) in question lead to physiological stress. The changes in latitudinal and depth distributions discussed earlier under the heading “Metabolism” illustrate this aspect of adaptive behavioral responses to changes in DO and temperature. However, in other cases, a change in one or more of these stressors may lead to anomalous or maladaptive behavior, which can have significant and even lethal effects. As in the case of DO, OA, and temperature effects on metabolism, there are relatively few studies in which all three stressors have been examined in the context of behavior. Therefore, the following analysis is, of necessity, based largely on single-stressor experiments.

**Oxygen.** Fish and many motile invertebrates respond to falling DO by moving away from hypoxic areas. This behavior may be initiated when oxygen concentrations approach 50% saturation, levels that are twice those conventionally regarded as hypoxic (supplemental text 2). Oxygen concentrations that

initiate escape behavior are closer to those at which processes such as growth and locomotory capacity are impeded and are well above DO levels that are lethal to fish (Deutsch et al. 2015). Wide differences exist in threshold values of DO in which behavioral and physiological changes are induced and in capacities of different species and life stages to avoid areas of low DO. Sluggish demersal species may be less able to move away from areas of low DO than pelagic species with stronger swimming abilities. Different life stages of fish encounter different challenges from low DO. Fish eggs, larvae, and juveniles may have higher mass-specific oxygen requirements than adults and generally lack or have limited abilities to avoid hypoxic water. Therefore, early life stages may be especially vulnerable to decreased DO.

Decreasing DO concentrations can affect the horizontal and vertical movements of ectotherms independently of changes in temperature. Projected declines in DO in certain regions of the northeastern Pacific suggest a reduction in area with adequate levels of DO for many demersal and pelagic fish. Decreasing DO is likely to compress the vertical habitat available for certain species and alter the range and extent of suitable habitats along the northeastern Pacific coast (Gilly et al. 2013). In the Southern California Bight, McClatchie and colleagues (2010) predicted that the shoaling of low oxygen waters over the next two decades could lead to a loss of approximately 18% of available vertical water column habitat for rockfish and the occurrence of hypoxic conditions in 55% of their total habitat. The periodic intrusion of strongly hypoxic or anoxic waters may lead to at least short-term reductions in suitable fish habitat for both predators and prey (Grantham et al. 2004, Koslow et al. 2011). Studies of flatfish provide insight into the potential changes in distribution of demersal fish in relation to regional changes in DO. In a known hypoxic area along the Oregon Coast, the biomass of multiple species of sole was significantly positively associated with bottom DO concentration, with DO explaining nearly 70% of the variation in biomass for some species (Keller et al. 2010). However, the distribution of Dover sole, which regularly inhabit deep waters near or within the OMZ and may be adapted to hypoxic conditions, showed no association with DO.

Challenges posed by reduced DO may vary across life stages for certain fishes. Changes in DO concentrations in shallow estuaries, where the early life stages of some fishes occur, could affect the reproduction and recruitment of these species (Breitburg et al. 2009). Vertical distributions may be life-stage specific, further complicating predictions of the effects of low DO. The vertical distributions of hypoxia-tolerant species of fish and invertebrates could shoal and expand horizontally in concert with enlargement of the OMZ (Bograd et al. 2008). Therefore, replacement of hypoxia-intolerant species by others that are adapted to low oxygen may be a future consequence of falling DO concentrations.

Recent advances in telemetry, such as electronic tags that can record fish movements and behavior along with *in situ*

environmental conditions over long periods, will likely provide new insights into the role of physiology in the behavior of fishes in response to decreasing DO. Rankin and colleagues (2013) determined that hypoxic conditions reduced the home range of copper rockfish by one-third, although quillback rockfish behavior was not significantly affected. They propose that along with DO, water temperature, and chemistry, species-specific behavioral tendencies and thresholds all must be considered.

**Ocean acidification.** The effects of OA on behavior have been observed in a number of marine fishes and invertebrates (Brieffa et al. 2012). Much attention is currently being given to the behavioral effects of OA that involve olfaction, predator-prey interactions, locomotory function, and habitat choice (Munday et al. 2012). The exposures of tropical-reef fish to CO<sub>2</sub> levels predicted for the end of this century caused alterations in olfactory-mediated behaviors, notably homing abilities and the abilities for distinguishing kin from predators; auditory capacities, such as the ability to detect sounds emanating from native habitats; and learning abilities (Munday et al. 2012). Recent studies of a northeastern Pacific rockfish (*Sebastes diploproa*) exposed to pH values currently observed during episodic upwelling of low pH waters revealed significant increases in anxiety levels, as indexed by swimming behavior (Hamilton et al. 2014). Heightened anxiety persisted for a week after exposure to low pH water, illustrating how episodic exposure to low pH water can have prolonged effects on behavior.

One mechanistic basis of these behavioral effects in fishes involves the perturbation of ion gradients across nerve cell membranes (Nilsson et al. 2012). Under conditions of OA, the increases in blood HCO<sub>3</sub><sup>-</sup> concentrations that occur through pH regulatory processes are accompanied by reductions in plasma chloride ion concentrations in order to maintain charge balance (Hamilton et al. 2014). When ion channels that transport Cl<sup>-</sup> are opened as a result of the binding of the neurotransmitter gamma-amino-butyric acid-A (GABA-A), Cl<sup>-</sup> would normally enter the neuron and lead to a reduction of neural activity. However, under OA, there is an outflow of Cl<sup>-</sup> from the neuron, which leads to an increased level of activity in the neural pathway and a disruption of normal behavioral patterns (Hamilton et al. 2014).

Another recently discovered mechanism important in establishing the behavioral responses of fish to OA involves superficial sensory neurons that detect seawater pH in the fish's immediate vicinity (Caprio et al. 2014). A marine catfish that relies on the detection of small changes in pH to identify sites where prey occurs lost its pH-sensing ability rapidly under conditions of OA (Caprio et al. 2014). The sensing of small (less than 0.1 unit) differences in pH that result from the respiratory activity (CO<sub>2</sub> generation) of its prey was maximal at pH of 8.1–8.2. Below pH 8.0, this sensing capacity rapidly decreased. If the set points of pH-sensing systems are adapted for the pH values of “normal” seawater (near pH 8.1–8.2), OA may render these systems dysfunctional.



The binding of signaling molecules (semiochemicals) such as pheromones to receptors can be pH sensitive because of the direct pH effects on charge states of the semiochemicals themselves and the effects on the chemicals' interactions with the receptors' ligand binding sites (Reisert and Restrepo 2009). pH effects on semiochemical-receptor interactions have been observed for a diversity of small molecules, including peptides, nucleosides, thiols, and organic acids (Hardege et al. 2011). The range of behaviors likely to be affected by OA includes sperm-egg interactions (fertilization) and larval settling (see references in Gattuso and Hansson 2011). This largely unexplored aspect of OA merits additional study because of the breadth of possible OA-triggered effects.

**Temperature.** The abilities of aquatic ectotherms to sense changes in temperature and, if the environment allows, to move to regions where temperatures are optimal for function are well characterized for many species. The shifts in latitudinal and vertical distributions of marine ectotherms already observed and likely to occur with the continued warming and deoxygenation of the ocean have been discussed above (see Deutsch et al. 2015). It is not yet known how OA may affect the sensory abilities linked to temperature detection (or the detection of DO concentrations). If this sensory capacity is impaired by OA, then the abilities of motile ectotherms to move to waters with preferred temperatures may be impeded.

**Calcification.** Because of the importance of calcification processes in the biology of bivalves, considerable attention has been given to the effects of changes in carbonate-system variables on the growth of larval and adult life stages of several species, including commercially valuable mussels and oysters. Challenges from OA to aquaculture operations in the case of the Pacific oyster (*Crassostrea gigas*) have drawn wide concern and public attention (Barton et al. 2012, Ekstrom et al. 2015) and has no doubt helped to spur additional research. There is a large, rapidly growing, and, at times, conflicting literature on bivalve responses to OA. Some disagreements among studies may reflect differences in the exposure conditions used in the experiments. For example, the variations among experiments in how the carbonate system was regulated can affect results, as can the variations among species and life stages in sensitivities to different components of the carbonate system. The process of OA necessarily involves changes in multiple properties of the carbonate-system parameters that covary strongly and predictably in the open ocean. In estuarine systems, in contrast, covariation can break down such that increasing  $p\text{CO}_2$  may be accompanied by widely varying saturation state values for carbonate minerals. Controlled laboratory studies have begun to disentangle the effects of individual carbonate-system parameters on organism physiology. In oysters and mussels, saturation state has emerged as the crucial determinant of calcification (Waldbusser et al.

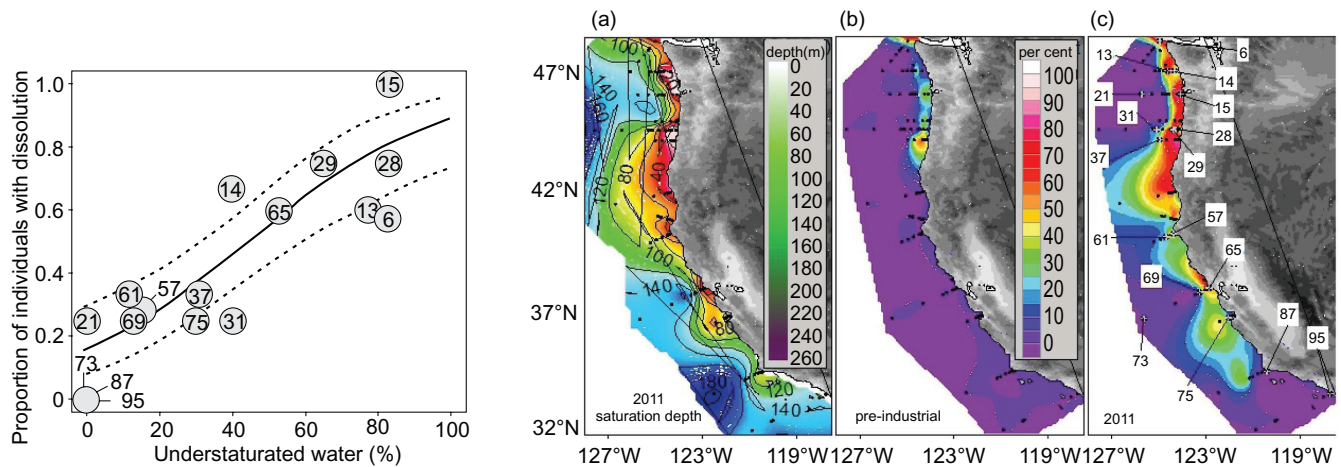
2014), whereas pH has demonstrable control on respiration (Waldbusser et al. 2015).

In other cases, additional variables beyond those of the carbonate system may affect results. OA effects may be influenced by treatment temperature (Kroeker et al. 2014) and dietary conditions (Melzner et al. 2011, Hettinger et al. 2013). For example, interaction between food supply and response to elevated  $p\text{CO}_2$  has been observed in mussels and oysters. Increased  $p\text{CO}_2$  led to the dissolution of the inner surface of the shell of *Mytilus edulis*, and this effect was exacerbated under conditions of reduced food availability (Melzner et al. 2011). Melzner and colleagues (2011) proposed that when mussels are confronted with limitations in food supply, energy is preferentially allocated to processes other than shell repair. Studies of the larvae of the Olympia oyster (*Ostrea lurida*) that examined combinations of  $p\text{CO}_2$  and food level found that elevated  $p\text{CO}_2$  had negative effects on larval growth, metamorphic success, and total dry weight (Hettinger et al. 2013). High food availability was able to partially but not completely offset the effects of elevated  $p\text{CO}_2$ . Therefore, even under conditions of enhanced primary productivity, the impacts of OA may still be felt by some marine calcifiers.

The natural temporal patterning of changes in pH also needs to be considered in the design of experiments intended to shed light on the effects of OA. Frieder and colleagues (2014) showed that natural semidiurnal fluctuations in pH led to insignificant effects on development in *M. californianus* and *M. galloprovincialis*, whereas exposure to a constant low pH (pH 7.6) led to a delayed transition from the trochophore to the veliger stage.

One important consequence of OA's inhibition of calcium carbonate deposition is a reduction in the mechanical integrity of shells. The larvae of *M. californianus* cultured under OA conditions predicted by the year 2100 built shells with reduced mechanical strength (Gaylord et al. 2011), potentially increasing the susceptibility of OA-stressed larvae to predation and desiccation. Along these lines, investigations of Olympia oysters (*Ostrea lurida*) documented smaller individuals cultured under OA conditions that were significantly more vulnerable to predation by drilling snails (Sanford et al. 2014). The success of *Mytilus* mussels in a habitat may also depend on the animal's ability to maintain adequately strong byssal fibers for attachment to the substrate. OA can reduce this capacity (O'Donnell et al. 2014). The growth of *Mytilus trossulus* under elevated  $p\text{CO}_2$  reduced byssus tenacity by approximately 40% because of the weakening of the plaque that attaches the fiber to the substrate. Combinations of OA and increased storm (wave) intensity could therefore operate synergistically in dislodging mussels from the rocky intertidal.

Other bivalves have shown interactive effects of multiple stressors. For example, acidification and hypoxia were found to have additive and negative synergistic effects on growth, survival, and metamorphosis in early-stage bivalves (scallop, *Argopecten irradians*, and clams, *Mercenaria mercenaria*; Gobler et al. 2014). Expanded multistressor studies of OA, hypoxia, and temperature on bivalves are warranted because



**Figure 3.** Left frame: The proportion of pteropods exhibiting severe shell dissolution as a function of the percentage of the water column in the upper 100 meters (m) that is undersaturated with respect to aragonite. The numbers in circles correspond to the station locations shown in the right frame of this figure. The solid line is the logistic regression line fitted to the data; the dashed lines are 95% confidence intervals. Right frame: The carbonate saturation patterns along the Northeastern Pacific coast of the US. (a) The depth of the aragonite saturation horizon. (b) The percentage (%) of the upper 100 m of the water column in the California Current Ecosystem estimated to be undersaturated during (preindustrial time) and (c) in August–September 2011. The pteropod sampling locations are indicated by the numbers within the squares (these correspond to the numbers in the circles in the left frame). Source: Figure adapted from Bednaršek and colleagues (2014a) with permission from the Royal Society.

of the important commercial role of these species in the northeastern Pacific and elsewhere (Ekstrom et al. 2015).

Shelled (thecosome) pteropods are small, pelagic snails that provide an especially good window for viewing the consequences of OA on marine food webs. In the California Current Large Marine Ecosystem, pteropods play significant roles by serving as major dietary items for several commercially important fishes, including pink, chum, and sockeye salmon and pollock. Pteropods can explain 30% of the variation in salmon survival in economically important fishing areas (Doubleday and Hopcroft 2014). They also are prey for higher-trophic level species such as seabirds and whales. Thecosome pteropods have a thin and delicate aragonite shell that can dissolve under conditions of high  $p\text{CO}_2$  and low aragonite saturation state (Bednaršek et al. 2014a). Low temperatures can also present challenges to aragonite deposition by pteropods (Orr et al. 2005).

A recent study of the pteropod *Limacina helicina* documented that in certain regions of the northeastern Pacific, pteropods are already exposed to seawater pH and aragonite saturation states that are low enough to impair biological structure and function (Bednaršek et al. 2014a). A survey of the waters along the coastline from Washington to California, where the species is abundant, revealed that pteropods displaying substantial damage to their shells were associated with upwelled waters, common from late spring to early autumn (figure 3). In the top 100 m of the water column, shell dissolution was strongly correlated with the percentage of water undersaturated with respect to aragonite (figure 3), especially in the near-coastal environment.

In 2011, on average, 53% of onshore pteropods and 24% of offshore specimens showed severe damage from dissolution. In samples in which more than 80% of the water column was undersaturated with respect to aragonite, up to 100% of pteropods showed shell dissolution. The percent of the upper 100 m of the water column estimated to have been undersaturated with respect to aragonite in the preindustrial period is contrasted with the measured extent of undersaturation in August–September of 2011 and shows a more than sixfold increase in the extent of the undersaturated waters along the West Coast (figure 3). At the shelf break, anthropogenic DIC is estimated to have increased the frequency of undersaturated conditions by about 20% since the preindustrial era (Harris et al. 2013).

Several ecological consequences of OA-induced damage to pteropod shells are likely. Changes in the abundance and distribution of pteropods that result from OA effects are likely to cause changes in prey availability for higher-trophic level species, especially in high-latitude waters and in continental shelf regions where productivity is high. Impaired shell production will reduce the strength of the biological carbon pump because less carbonate ballast will be produced in the upper regions of the water column (Bednaršek et al. 2014b). Pteropods are responsible for an estimated 20%–42% of total carbonate production in the ocean (Bednaršek et al. 2012), so carbon pump effects may be large. At saturation states projected to occur in the northeastern Pacific by 2050 at a depth of 100 m, the flux of biological removal may be halved (Bednaršek et al. 2014b). This reduction in the carbon pump could potentially create a positive feedback

that reduces the rate of the biological removal of carbon from the upper ocean under conditions of intensifying carbon enrichment from anthropogenic sources.

The occurrence of aragonite in otoliths of teleost fishes suggests that OA-induced changes in pH and  $p\text{CO}_2$  in the intra- or extracellular fluids could influence the growth of these sensory structures. The eggs and larvae of several species of fish have been used in studies of the OA effects on otolith growth and structure. In a majority of cases, elevated  $p\text{CO}_2$  and decreased pH led to larger otolith mass in fish of similar size. The elevation in blood bicarbonate ion concentrations, which is part of the fish's pH regulatory processes, could have facilitated the deposition of calcium carbonate in otoliths. Checkley and colleagues (2009) found that otolith masses of young white sea bass (*Atractoscion nobilis*), were 10%–14% and 24%–26% larger in fish cultured at 993 and 2558 microatmospheres ( $\mu\text{atm}$ )  $\text{CO}_2$ , respectively, compared with the control (380  $\mu\text{atm}$   $\text{CO}_2$ ) fish. In view of the roles of otoliths in the maintenance of balance and in the detection of sound and acceleration, these OA-induced changes in otolith mass could impair behavior and might compound the behavioral effects of OA resulting from changes in chloride ion gradients across neural membranes (Hamilton et al. 2014).

## Conclusions

The consequences of global change–driven alterations in ocean temperature, oxygen concentration, and carbonate-system components pose particularly important challenges for the coastal marine ecosystems of the northeastern Pacific Ocean. Physiological analysis can provide important insights into the mechanistic factors that underlie the already observed changes in the biogeographic and vertical distribution patterning, food web productivity, and other characteristics of these ecosystems. More importantly, perhaps, the insights gained from diverse types of appropriately designed physiological studies (supplemental text 3) can be used to predict what the future holds for the CCS and, by extension, other coastal and near-shore marine ecosystems. Physiological analyses of diverse marine ectotherms have helped to define the species- and life-stage-specific threshold levels at which changes in a stressor lead to physiological stress. The threshold effects for one stressor may be dependent on changes occurring in another stressor, as was suggested by the strong interactions that occur among alterations in temperature, DO, and  $p\text{CO}_2$ . Physiological insights may help to provide a foundation for understanding the effects at the ecosystem level and serve as useful input for the development of policies and management strategies that foster societal and economic adaptation to changing ecosystems and the resources they provide (see the “Scaling up to management and policy” box).

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## Supplemental material

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## References cited

- Bakun A, Field DB, Redondo-Rodriguez ANA, Weeks SJ. 2010. Greenhouse gas, upwelling: Favorable winds and the future of coastal ocean upwelling ecosystems. *Global Change Biology* 16: 1213–1228.
- Barton A, Hales B, Waldbusser GG, Langdon C, Feely RA. 2012. The Pacific oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels: Implications for near-term ocean acidification effects. *Limnology and Oceanography* 57: 698–710.
- Bednaršek N, Možina J, Vogt M, O'Brien C, Tarling GA. 2012. The global distribution of pteropods and their contribution to carbonate and carbon biomass in the modern ocean. *Earth Systems Science Data* 4: 167–186.
- Bednaršek N, Feely RA, Reum JCP, Peterson B, Menkel J, Alin SR, Hales B. 2014a. *Limacina helicina* shell dissolution as an indicator of declining habitat suitability owing to ocean acidification in the California Current Ecosystem. *Proceedings of the Royal Society B*. 281: 2014–2023.
- Bednaršek N, Tarling GA, Bakker DCE, Fielding S, Feely RA. 2014b. Dissolution dominating calcification process in polar pteropods close to the point of aragonite undersaturation. *PLOS ONE* 9 (art. e109183).
- Bograd SJ, Castro CG, Di Lorenzo E, Palacios DM, Bailey H, Gilly W, Chavez FP. 2008. Oxygen declines and the shoaling of the hypoxic boundary in the California Current. *Geophysical Research Letters* 35 (art. L12607). doi:10.1029/2008GL034185.
- Booth JAT, McPhee-Shaw EE, Chua P, Kingsley E, Denny M, Phillips R, Bograd SJ, Zeidberg LD, Gilly WF. 2012. Natural intrusions of hypoxic, low pH water into nearshore marine environments on the California coast. *Continental Shelf Research* 45: 108–115.
- Breitbart DL, Hondorp DW, Davies L, Diaz RJ. 2009. Hypoxia, nitrogen, and fisheries: Integrating effects across local and global landscapes. *Annual Review of Marine Science* 1: 329–349.
- Briffa M, de la Haye K, Munday P. 2012. High  $\text{CO}_2$  and marine animal behaviour: Potential mechanisms and ecological consequences. *Marine Pollution Bulletin* 64: 1519–1528.
- Caprio J, Shimohara M, Marui T, Harada S, Kiyohara S. 2014. Marine teleost locates live prey through pH sensing. *Science* 344: 1154–1156.
- Chan F, Barth JA, Lubchenco J, Kirincich A, Weeks H, Peterson WT, Menge BA. 2008. Emergences of anoxia in the California Current Large Marine Ecosystem. *Science* 319: 920.
- Chavez FP, Ryan J, Lluch-Cota SE, Niqun CM. 2003. From anchovies to sardines and back: Multidecadal change in the Pacific Ocean. *Science* 299: 217–221.
- Checkley DM Jr, Dickson AG, Takahashi M, Radich JA, Eisenkolb N, Asch R. 2009. Elevated  $\text{CO}_2$  enhances otolith growth in young fish. *Science* 324: 1683.



- Cheng BS, et al. 2015. Testing local and global stressor impacts on a coastal foundation species using an ecologically realistic framework. *Global Change Biology* 21: 2488–2499. doi: 10.1111/gcb.12895.
- Couturier CS, Stecyk JAW, Rummer JL, Munday PL, Nilsson G. 2013. Species-specific effects of near-future CO<sub>2</sub> on the respiratory performance of two tropical prey fish and their predator. *Comparative Biochemistry and Physiology* 166: 482–489.
- Deutsch C, Holger B, Taka I, Hartmut F, Thompson L. 2011. Climate-forced variability of ocean hypoxia. *Science* 333: 336–339.
- Deutsch C, Ferrel A, Seibel B, Pörtner HA, Huey RB. 2015. Climate change tightens a metabolic constraint on marine habitats. *Science* 348: 1132–1135.
- Doubleday AJ, Hopcroft RR. 2014. Interannual patterns during spring and late summer of larvaceans and pteropods in the coastal Gulf of Alaska, and their relationship to pink salmon survival. *Journal of Plankton Research* 37: 134–150. doi:10.1093/plankt/fbu092.
- Ekstrom JA, et al. 2015. Vulnerability and adaptation of US shellfisheries to ocean acidification. *Nature Climate Change* 5: 207–214. doi:10.1038/nclimate2508
- Feely RA, Sabine CL, Hernandez-Ayon JM, Ianson D, Hales B. 2008. Evidence for upwelling of corrosive “acidified” water onto the continental shelf. *Science* 320: 1490–1492.
- Feely RA, Sabine CL, Byrne RH, Millero FJ, Dickson AG, Wanninkhof R, Murata A, Miller LA, Greeley D. 2012. Decadal changes in the aragonite and calcite saturation state of the Pacific Ocean. *Global Biogeochemical Cycles* 26 (art. GB33301). doi:10.1029/2011 GB004157.
- Franke A, Clemmesen C. 2011. Effect of ocean acidification on early life stages of Atlantic herring (*Clupea harengus* L.). *Biogeosciences* 8: 3697–3707.
- Frieder CA, Gonzalez JP, Bockmon EE, Navarro MO, Levin LL. 2014. Can variable pH and low oxygen moderate ocean acidification outcomes for mussel larvae? *Global Change Biology* 20: 754–764.
- Garcia-Reyes MJ, Largier J. 2010. Observations of increased wind-driven coastal upwelling off central California. *Journal of Geophysical Research* 115 (art. C04011). doi:10.1029/2009JC000556.
- Gattuso JP, Hansson L. 2011. *Ocean Acidification*. Oxford University Press.
- Gattuso JP, et al. 2015. Contrasting futures for ocean and society from different anthropogenic CO<sub>2</sub> emissions scenarios. *Science* 349 (art. aac4722). doi:10.1126/science.aac4722.
- Gaylord B, Hill TM, Sanford E, Lenz EA, Jacobs LA, Sata KN, Russell AD, Hettinger A. 2011. Functional impacts of ocean acidification in an ecologically critical foundation species. *Journal of Experimental Biology* 214: 2586–2594.
- Gaylord B, et al. 2015. Ocean acidification through the lens of ecological theory. *Ecology* 96: 3–15.
- Gilly WF, Beman JM, Litvin SY, Robison BH. 2013. Oceanographic and biological effects of shoaling of the oxygen minimum zone. *Annual Review of Marine Science* 5: 393–420.
- Gobler CJ, DePasquale EL, Griffith AW, Baumann H. 2014. Hypoxia and acidification have additive and synergistic negative effects on the growth, survival, and metamorphosis of early life stage bivalves. *PLOS ONE* (art. e83648).
- Godbold JA, Solan M. 2013. Long-term effects of warming and ocean acidification are modified by seasonal variation in species responses and environmental conditions. *Philosophical Transactions of the Royal Society B* 368 (art. 21031086). (6 November 2015; <http://dx.doi.org/10.1098/rstb.2013.0186>).
- Grantham BA, Chan F, Nielsen FJ, Fox DS, Barth JA, Huyer A, Lubchenko J, Menge BA. 2004. Upwelling-driven nearshore hypoxia signals ecosystem and oceanographic changes in the northeast Pacific. *Nature* 429: 749–754.
- Haigh R, Ianson D, Holt CA, Neate HE, Edward AM. 2015. Effects of ocean acidification on temperate coastal marine ecosystems and fisheries in the northeast Pacific. *PLOS ONE* 10 (art. e0117533). doi:10.1371/journal.pone.0117533.
- Hamilton TJ, Holcombe A, Tresguerres M. 2014. CO<sub>2</sub>-induced ocean acidification increases anxiety in rockfish via alteration of GABAA receptor functioning. *Proceedings of the Royal Society B* 281 (art. 20132509).
- Hardege JD, Rotchell JM, Terschak J, Greenway GM. 2011. Analytical challenges and the development of biomarkers to measure and to monitor the effects of ocean acidification. *Trends in Analytical Chemistry* 30: 1320–1326.
- Harris, K, DeGrandpre MD, Hales B. 2013. Aragonite saturation state dynamics in a coastal upwelling zone. *Geophysical Research Letters* 40: 2720–2725.
- Hettinger A, Sanford E, Hill TM, Russell AD, Sato KNS, Hoey J, Forsch M, Page HN, Gaylord B. 2012. Persistent carry-over effects of planktonic exposure to ocean acidification in the Olympia oyster. *Ecology* 93: 2758–2768.
- Hettinger A, Sanford E, Hill TM, Hosfelt JD, Russell AD, Gaylord B. 2013. The influence of food supply on the response of Olympia oyster larvae to ocean acidification. *Biogeosciences* 10: 6629–6638. doi:10.5194/bg-10-6629-2013.
- Hochachka PW, Somero GN. 2002. *Biochemical Adaptation*. Oxford University Press.
- Keeling RF, Körtzinger A, Gruber N. 2010. Ocean deoxygenation in a warming world. *Annual Review of Marine Science* 9: 199–229.
- Keller AA, Simon V, Chan F, Wakefield WW, Clarke ME, Barth JA, Kamikawa D, Fruh EL. 2010. Demersal fish and invertebrate biomass in relation to an offshore hypoxic zone along the US West Coast. *Fisheries Oceanography* 19: 76–87.
- Keller AA, Ciannelli L, Wakefield WW, Simon V, Barth JA, Pierce SD. 2015. Occurrence of demersal fishes in relation to near-bottom oxygen levels within the California Current large marine ecosystem. *Fisheries Oceanography* 24: 162–176.
- Koslow JA, Goericke T, Lara-Lopez A, Watson W. 2011. Impact of declining intermediate-water oxygen on deepwater fishes in the California Current. *Marine Ecology Progress Series* 436: 207–218.
- Kroeker KJ, Kordas RL, Crim R, Hendriks IE, Ramajo L, Singh GS, Duarte CM, Gattuso J-P. 2013. Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming. *Global Change Biology* 19: 1884–1896.
- Kroeker KJ, Gaylord B, Hill TM, Hosfelt JD, Miller SH, Sanford E. 2014. The role of temperature in determining species’ vulnerability to ocean acidification: A case study using *Mytilus galloprovincialis*. *PLOS ONE* 9 (art. 31000353). doi:10.1371/journal.pone.0100353.
- McClatchie S, Goericke R, Cosgrove R, Auad G, Vetter R. 2010. Oxygen in the Southern California Bight: Multidecadal trends and implications for demersal fisheries. *Geophysical Research Letters* (art. L19602). doi:10.1029/2010GL044497.
- Melzner FP, Strange K, Trubenback J, Thomsen I, Casties U, Panknin S, Gorb N, Gutowska MA. 2011. Food supply and seawater pCO<sub>2</sub> impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. *PLOS ONE* 6 (art. e24223).
- Munday PL, Crawley NE, Nilsson G. 2009. Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral reef fishes. *Marine Ecology Progress Series* 388: 235–242.
- Munday PL, McCormick MI, Nilsson GE. 2012. Impact of global warming and rising CO<sub>2</sub> levels on coral reef fishes: What hope for the future? *Journal of Experimental Biology* 215: 3865–3873.
- Nilsson GE, Östlund-Nilsson S. 2008. Does size matter for hypoxia tolerance in fish? *Biological Reviews* 83: 173–189.
- Nilsson G[E], Dixon DL, Domenici P, McCormick MI, Sorensen C, Watson S-A, Munday PL. 2012. Near-future carbon dioxide levels alter fish behaviour by interfering with neurotransmitter function. *Nature Climate Change* 2: 201–204.
- O’Donnell MJ, George MN, Carrington E. 2014. Mussel byssus attachment weakened by ocean acidification. *Nature Climate Change* 3: 587–590. doi:10.1038/nclimate1846.
- Orr JC, et al. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* 437: 681–686.
- Rankin P, Hannah R, Blume M. 2013. Effect of hypoxia on rockfish movements: Implications for understanding the roles of temperature, toxins, and site fidelity. *Marine Ecology Progress Series* 492: 223–234.



- Reisert J, Restrepo D. 2009. Molecular tuning of odorant receptors and its implication for odor signal processing. *Chemical Senses* 34: 535–545.
- Reum JCP, et al. 2015. Interpretation and design of ocean acidification experiments in upwelling systems in the context of carbonate chemistry co-variation with temperature and oxygen. *ICES Journal of Marine Science* (art. fsu231). doi:10.1093/icesjma/fsu231
- Rosa R, Seibel BA. 2008. Synergistic effects of climate-related variables suggest future physiological impairment in a top ocean predator. *Proceedings of the National Academy of Sciences* 105: 20776–20780.
- Sanford E, Gaylord B, Hettinger A, Lenz EA, Meyer K, Hill TM (2014). Ocean acidification increases the vulnerability of native oysters to predation by invasive snails. *Proceedings of the Royal Society B* 281: 20132681.
- Seibel BA. 2011. Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones. *Journal of Experimental Biology* 214: 326–336.
- Torres J, Grigsby MD, Clarke ME. 2012. Aerobic and anaerobic metabolism in oxygen minimum layer fishes: The role of alcohol dehydrogenase. *Journal of Experimental Biology* 215: 1905–1914.
- Vaquier-Sunyer R, Duarte CM. 2008. Thresholds of hypoxia for marine biodiversity. *Proceedings of the National Academy of Sciences* 105: 15452–15457.
- Verberk WCEP, Bilton DT, Calosi P, Spicer JI 2011. Oxygen supply in aquatic ectotherms: Partial pressure and solubility together explain biodiversity and size patterns. *Ecology* 92: 1565–1572.
- Waldbusser GG, Hales B, Langdon CJ, Haley BA, Schrader P, Brunner EL, Gray MW, Miller CA, Gimenez I 2014. Saturation-state sensitivity of marine bivalve larvae to ocean acidification. *Nature Climate Change* 5: 273–280. doi:10.1038/nclimate2479.
- Waldbusser GG, Hales B, Langdon CJ, Haley BA, Schrader P, Brunner EL, Gray MW, Miller CA, Gimenez I, Hutchinson G. 2015. Ocean acidification has multiple modes of action on bivalve larvae. *PLOS ONE* 10 (art. e0128376).
- Wang D, Gouhier TC, Menge BM, Ganguly AR 2015. Intensification and spatial homogenization of coastal upwelling under climate change. *Nature* 518: 390–394.
- Zeidberg LD, Robison BH. 2007. Invasive range expansions by the Humboldt squid, *Dosidicus gigas*, in the eastern North Pacific. *Proceedings of the National Academy of Sciences* 104: 12948–12950.

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