Chasing the Future

How Will Ocean Change Affect Marine Life?

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The benthic respiration system (BRS) floats at the surface of the Gulf of California on a free vehicle "elevator" used to ferry gear to the bottom or back in support of remotely operated vehicle dives.

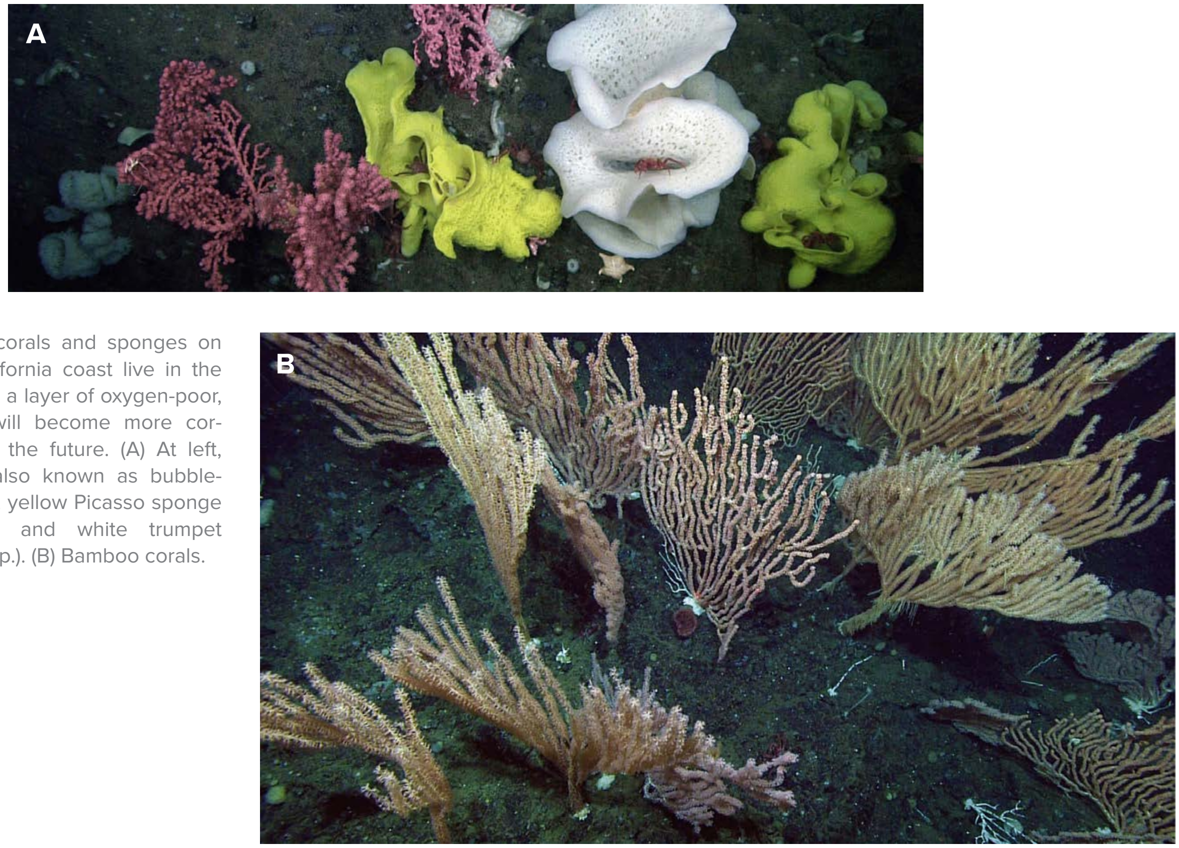

FIGURE 1. Deep-sea corals and sponges on Sur Ridge off the California coast live in the oxygen minimum zone, a layer of oxygen-poor, CO_2-rich water that will become more corrosive and hypoxic in the future. (A) At left, *Paragorgia arborea*, also known as bubblegum coral, and at right, yellow Picasso sponge (*Staurocalyptous* sp.), and white trumpet sponge (*Chonelasma* sp.). (B) Bamboo corals.

ABSTRACT. Understanding the potential consequences of rising ocean carbon levels and related ocean changes for marine life and ecosystems is a high priority for the ocean research community and marine resource management. In the mid-1990s, two geoengineering proposals to mitigate global warming by carbon sequestration in the deep sea led to research measuring the effects of increased deep ocean carbon dioxide levels on marine animals. A few years later, ocean acidification and its effects on marine life became an international research priority. Here we provide an overview of several technical developments by scientists and engineers at the Monterey Bay Aquarium Research Institute (MBARI) that have enabled and enhanced deep-sea exploration and experiments to assess the effects of changing ocean conditions on benthic marine animals. Improvements in remotely operated vehicles (ROVs) have increased the efficiency of dive operations and enabled more complex measurements and experiments at great ocean depths. In situ respirometers and Free Ocean CO_2 Enrichment (FOCE) mesocosms have allowed measurement of physiological and behavioral responses of deep-sea animals to environmental change. A laboratory-based, gas-controlled aquarium system that regulates oxygen and pH in chilled waters was engineered to measure the physiological responses of deep-sea animals and biological communities to expected future environmental conditions. Recently, MBARI engineers and scientists developed an Upwelling Simulator, a lab-based aquarium control system that mimics ocean conditions during coastal upwelling. This system is programmable, allowing independent control of pH, oxygen, and temperature to enable experiments that examine the effects of present-day upwelling conditions, expected future conditions, or other changes in these environmental conditions. In sum, collaboration between the marine operations group, engineers, and scientists at MBARI has advanced methods to explore the ocean and understand the consequences of ocean change for marine organisms and ecosystems.

INTRODUCTION

Centuries-old deep-sea corals (Figure 1) growing in currents that swirl past seamounts off central California are somewhat isolated from changes in surface ocean conditions, but like their shallow-water counterparts, they will eventually have to contend with reduced pH in the ocean environment (called ocean acidification) and other changes in ocean conditions linked to fossil fuel emissions. Life is already tough for deep-sea animals that are sensitive to high-CO_2 levels. Long before humans began to burn fossil fuels, deep waters in the northeastern Pacific were already rich in carbon dioxide, corrosive, and low in oxygen due to accumulating effects of respiration over the timescale of ocean thermohaline circulation (~1,000 years; Broecker, 1991). The ongoing rise in CO_2 emissions is driving warming and ocean acidification as well as deoxygenation in deep waters (Bopp et al., 2013) that will persist for millennia, making life in the deep Pacific even more challenging for corals and other species.

In the 1990s, growing concern about global warming promoted interest in various ideas for avoiding atmospheric emissions by sequestering carbon in the deep sea, including the direct injection of liquid carbon dioxide into deep waters (Marchetti, 1977) and fertilization of surface waters with iron to accelerate the biological pump (Gribbin, 1988). If implemented on a climate-relevant scale, both geoengineering approaches could result in a large and rapid rise in deep ocean carbon dioxide levels that would reduce the pH of deep-sea waters. Models of a deep-sea CO_2 injection program estimated changes in excess of –0.5 pH units over vast regions of the deep ocean, depending on the amount of CO_2 injected (Caldeira and Wickett, 2005). Increasing our understanding of the potential consequences of deep-sea carbon storage for fauna living there shifted the focus of the Monterey Bay Aquarium Research Institute's (MBARI's) benthic biology program and led to laboratory and field experiments designed to measure responses of deep-sea animals to elevated CO_2 levels. Just a few years later, seminal papers by Kleypas et al. (1999) and others (see Brewer, 2013) detailing the implications of changing ocean carbonate chemistry for coral reefs began to catalyze the emergence of ocean acidification as a key research theme in ocean science (e.g., Caldeira and Wickett, 2003). Determining how organisms living in habitats that range from shallow waters to the deep sea will cope with changing ocean chemistry has required new methods and tools to perform experiments in situ, and new aquarium control systems to support laboratory studies of species' responses to expected future ocean conditions.

Here, we review several key developments at MBARI that have enabled exploration and research to understand how animals might cope with the challenges of life in a high-CO_2 ocean. These developments include advances in remotely operated (ROV) technology, in situ experimental systems, and advanced laboratory aquaria.

MBARI'S OCEAN ACCESS AND ROVS

Situated in Monterey Bay with ready access to deep Pacific waters, MBARI's ships and ROVs (Figure 2) have helped explore, discover, and study the diversity of deep-sea fauna and their sensitivity to natural and anthropogenic change. Within hours of Moss Landing Harbor, MBARI's ships and ROVs can range waters from the continental shelf to the abyssal plain and visit submarine canyons, ridges, and seamounts.

ROVs *Ventana* and *Doc Ricketts* represent the continuing evolution of deep-sea submersible science. *Ventana*, first obtained in the late 1980s from the oil industry, has evolved through the years to meet the needs of MBARI's submersible science program, and *Doc Ricketts* brought new capabilities in 2009. As

FIGURE 2. MBARI ships and remotely operated vehicles. (A) R/V *Western Flyer*. (B) ROV *Doc Ricketts*. (C) R/V *Rachel Carson*. (D) ROV *Ventana*.

deep-sea studies have escalated technical support requirements, these ROVs have been upgraded to accommodate increased payload (the amount of gear that the ROVs can haul up or down from the surface) and to include state-of-the-art imaging, high-resolution subsea navigation, a broad suite of sensors, various sample collection devices, and robotic arms and systems for deploying and manipulating experimental gear, enabling sophisticated subsea operations to depths of 4 km.

Each enhancement has improved the ROVs' support for science and engineering, but advances in imaging and navigation, and the ability to carry increased payload, have been game changing. Imagine being dropped on an unfamiliar hillside on a dark, moonless night with only a small flashlight, searching for a small marker you placed months before, knowing your location only within the footprint of a few football fields. In the late 1990s, navigation errors of up to several hundred meters made it difficult to find previous study sites. Improvements in acoustic tracking have reduced navigation errors to as little as a few meters, and ROV location can now be plotted on high-resolution (1 m scale) maps made possible with MBARI-developed mapping systems. Today, scientists can "see" the seascape in great detail and know precisely where the ROV is at any time. Now, little dive time is wasted wandering in the dark deep waters, and the efficiency of ROV dives is very high. High-definition cameras have also changed the nature of ROV dives. In the early days, staring at the video screen in the control room was very different from viewing the seabed through the window of a manned submersible. Now, high-definition cameras make viewing a video screen akin to looking through the window of an aquarium (and, if you'd like a rest, you can go get a cup of coffee—not so easy in a manned sub). The future is even more exciting, as 4K (or higher resolution) cameras, as well as low-light cameras capable of imaging bioluminescence in the deep sea, are becoming available for use on ROVs. The ability to carry a larger payload has also been key to success. Think of the ROV as a pickup truck that allows a scientist to deliver and recover experimental gear to the bottom. Earlier vehicles had very limited payload capacity, but ROVs *Doc Ricketts* and *Ventana* can carry upwards of 272 kg, and they also have variable buoyancy tanks to balance the vehicles as they lift or release heavy gear.

FIGURE 3. Pool of about 60 liters of liquid carbon dioxide at 3,600 m depth off Monterey, California, during a deep-sea carbon-dioxide injection experiment to evaluate the effects of the CO_2-rich dissolution plume on animals held in cages or inhabiting sediments nearby.

IN SITU TECHNOLOGY DEVELOPMENT FOR HIGH-CO_2 STUDIES

Deep-Sea CO_2 Injection Studies

Through a series of deep-sea CO_2 release experiments on the abyssal seafloor off California, our initial "future ocean" studies asked the question: how will deep-sea animals respond to pH changes caused by a deep-sea CO_2 sequestration program? Brewer et al. (1999) developed a system to transport and release CO_2 at depth from MBARI's ROVs. Using this system, we performed experiments that released 20–100 liters of liquid CO_2 into PVC rings (Figure 3) at depths of over 3,000 m (Barry et al., 2005). Great depth is required owing to the compressibility of liquid CO_2, which is lighter than seawater at depths less than ~2,600 m, above which it would have simply floated away. Over days to weeks, liquid CO_2 pools would dissolve into bottom waters and be carried down-current as a CO_2-rich, low-pH dissolution plume. These experiments required complex manipulations and measurements, from delivering and positioning gear and carbon dioxide to close-up imaging. Perturbations in bottom water pH were measured along with the condition or survival of species inhabiting the sediment (crustaceans and polychaetes) or held in cages (holothurians, echinoids, gastropods, fishes) at distances to 100 m from CO_2 pools (Barry et al., 2004, 2005, 2013; Carman et al., 2004; Thistle et al. 2005; Barry and Drazen, 2007; Thistle et al., 2007; Bernhard et al., 2009; Ricketts et al., 2009). The strength of these experiments was their realism—simulating exactly the changes in seawater chemistry that accompany CO_2 dissolution at depth. Their weakness was the difficulty in determining thresholds for impacts on deep-sea animals. Even at these great depths, tidal variation in the direction of bottom currents led to high variability in pH near CO_2 pools, alternately exposing animals to a dissolution plume or ambient seawater, depending on their positions in relation to the CO_2 plumes.

In Situ Respirometers

Hyperbaric Fish Trap Respirometer

Measurement of oxygen consumption is a basic metric of metabolic rates and energy consumption in animals, and rates vary widely among marine taxa (Seibel and Drazen, 2007). For deep-sea species, measurements of oxygen consumption under laboratory conditions may be impossible due to the animals' intolerance to or stress resulting from depressurization. Using in situ methods for taking measurements is thus preferable. This is particularly true for fishes with physoclistous swim bladders, such as deep-sea grenadiers in the family Macrouridae, a common group of benthopelagic scavengers at bathyal to abyssal depths worldwide (Priede and Bagley, 2000). What are the natural metabolic rates of active deep-sea fishes, and how will they respond to changing CO_2 and O_2 levels? To address this question, MBARI scientists and engineers fabricated a hyperbaric fish trap (Figure 4) capable of capturing deep-sea fishes (to 4,000 m) and returning them to the laboratory for physiological studies under the pressure, oxygen, and temperature conditions of their capture depth (Drazen et al., 2005).

The trap is deployed over the side of the ship as a free vehicle with a descent weight and an acoustic release. A baited hook is positioned outside the trap door where, once hooked, a fish is pulled into the chamber by a spring-loaded reel mechanism that also closes the door tightly. To retrieve the trap, a ballast weight is released acoustically using a surface command; the trap then floats to the surface for recovery, retaining the pressure of capture. Upon return to the laboratory, the trap is connected to a high-pressure pump and water recirculation system. An oxygen optode, a camera with LED lighting, and a recirculation pump are contained in the trap to allow continuous measurements of oxygen consumption and observations of fish condition. Using this system, three macrourids (*Coryphaenoides acrolepis*) were captured over the first seven deployments in Monterey Bay at depths near 1,400 m, with a respiration rate of 79 µmol O_2 kg^{-1} h^{-1}.

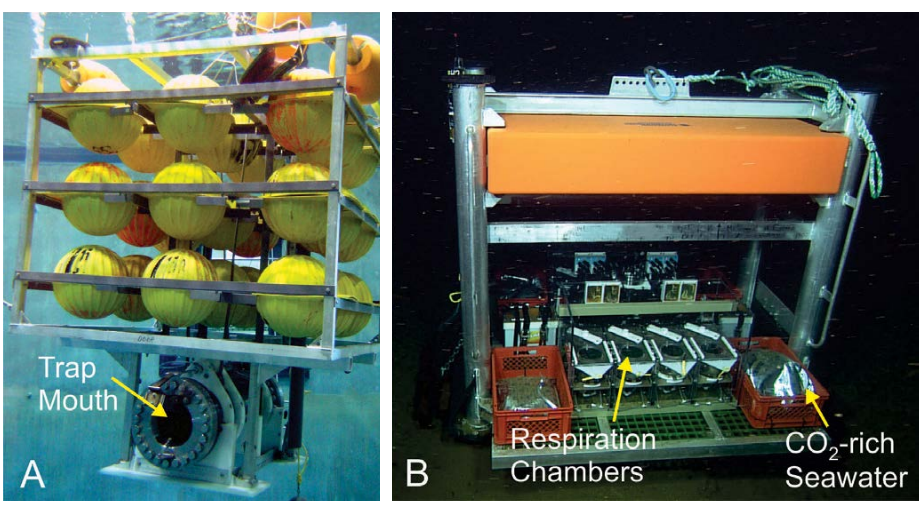

FIGURE 4. (A) Hyperbaric fish-trap respirometer (Drazen et al., 2005). The stainless steel fish trap is mounted below a frame holding 27 floats with sufficient buoyancy (~670 kg) to float the trap system to the surface following release of its ballast at the end of a deep-sea deployment. (B) Benthic respiration system used for in situ measurement of oxygen consumption by deep-sea animals under ambient, hypoxic, or acidified conditions.

Benthic and Midwater Respiration System

Although the hyperbaric fish trap was useful, it was not suitable for most deep-sea taxa. Therefore, an autonomous, in situ respiration system was developed that was capable of measuring oxygen consumption of a wider array of deep-sea animals under ambient or experimental conditions. Two models of the autonomous respiration system are based upon the same control system, and are used to study benthic or midwater species (Figure 4).

The benthic respirometer has eight metabolic chambers, allowing oxygen consumption rates of multiple individuals of benthic macroinvertebrates and fishes to be measured during a single deployment. Each metabolic chamber is equipped with an oxygen optode, a stirring pump to ensure chamber waters are well mixed, a flushing pump to periodically replenish chamber waters, and sample pumps used to inject or withdraw fluids from each chamber. These sample pumps allow the injection of modified (e.g., oxygen-rich) seawater into chambers for experiments to measure any change in oxygen consumption under experimentally altered O_2 or pH or both.

The benthic respiration system is deployed with a ballast weight as a free vehicle and sinks to the seafloor. An ROV then dives to the respirometer and captures individuals of targeted species using a suction sampler, then releases them into a metabolic chamber, and closes the chamber lid. A control system operates according to a preprogrammed schedule that defines the duration of the incubation cycle, the stirring cycle, O_2 measurement frequency, and any sample injection or withdrawal events. All pumping events and oxygen data from each chamber (and from an O_2 sensor mounted on the respirometer frame) are logged internally. Incubation periods are typically two to four hours, after which the flushing pump is activated to replenish waters in the chamber to avoid asphyxiation of contained animals.

A typical schedule for a deployment would begin with 10 cycles of two to four hours, each under "control" conditions (i.e., no injection of altered seawater), followed by several cycles in which conditions in one or more chambers are perturbed by injecting hypoxic, hyperoxic, or low-pH waters to examine changes in metabolic rates in response to environmental changes. After several "perturbation" cycles, ambient conditions are restored and numerous control cycles are repeated to determine if metabolic rates return to their pre-perturbation levels. Upon completion of the deployment, an acoustic release command is sent from the surface to release the ballast, and the system floats to the surface for shipboard recovery where animals from each chamber are then removed and processed.

Free Ocean CO_2 Enrichment

As interest and support grew for ocean acidification studies, the literature proliferated quickly, most commonly through studies that exposed organisms to high CO_2 levels in laboratory aquaria (e.g., Browman, 2016). Although such studies have been essential, adding more realism in ocean acidification research by measuring responses of species assemblages and natural communities over longer periods where direct and indirect effects can emerge was recognized as a stronger approach. In support of this goal, Brewer et al. (2005) initiated a program to develop a Free Ocean CO_2 Enrichment (FOCE) system to emulate the Free Air CO_2 Enrichment (FACE) technology (McLeod and Long, 1999) used to measure CO_2 effects in terrestrial communities.

A FOCE system consists of one or more partially open mesocosms placed on the seabed in a natural ocean habitat and connected to a flume that directs the flow of ambient (control) or CO_2-enriched (experimental) seawater through the mesocosm(s) (Gattuso et al., 2014). For experimental treatments, CO_2-enriched seawater (produced by various methods) is injected into the flume upstream of the mesocosm location to permit equilibration of seawater carbonate chemistry prior to its entry into the mesocosm(s) (Kirkwood et al., 2015). Other than the experimental change in seawater pH, organisms or species assemblages enclosed by a mesocosm are exposed to conditions as near as possible to natural (i.e., normal flow, water quality, light, plankton, species interactions, etc.).

A control system (typically shore-based) coupled to sensors (temperature, oxygen, salinity, pH) mounted inside and outside mesocosms regulates the pH within the mesocosms. A feedback loop regulates the release of CO_2-enriched seawater to maintain either a prescribed pH or an offset in pH from nearby ambient levels.

MBARI engineers and scientists developed a FOCE system for deep-sea studies (Figure 5) and were involved in FOCE development at several global locations (Kline et al., 2012; Barry et al., 2014; Gattuso et al., 2014; Kirkwood et al., 2015). A FOCE system for near-shore, subtidal use in Monterey Bay is under development.

LABORATORY AQUARIUM SYSTEMS FOR OCEAN CHANGE STUDIES

How will the physiology, development, growth, and other metrics of marine animal performance respond under future ocean conditions? Laboratory studies, while lacking the realism of field studies, allow for tight control of experimental conditions and a range of measurements typically not possible in the field. However, the control of multiple environmental variables relevant for ocean change studies has not been widely available. To improve experiments evaluating species' responses to ocean change, MBARI focused on developing aquarium systems that would allow control of oxygen, temperature, and pH over the range of temporal variation typical in ocean habitats.

Gas-Controlled Aquarium System

Are deep-sea animals more sensitive to environmental change than related taxa from shallow waters? For more detailed studies of physiological responses of deep-sea organisms to the expansion and intensification of oxygen minimum zones as well as reduced ocean pH, a gas-controlled aquarium (GCA) system was built to simulate changes in future pH and oxygen levels in deep-sea waters (Barry et al., 2008). The GCA controls pH and dissolved oxygen (DO) through the regulation of gas (N_2, air, CO_2) flowing to membrane contactors (gas exchange modules) connected to four 80-gallon (303-liter) control tanks of chilled seawater. DO and pH sensors in each control tank are linked to mass flow controllers that alter gas flow

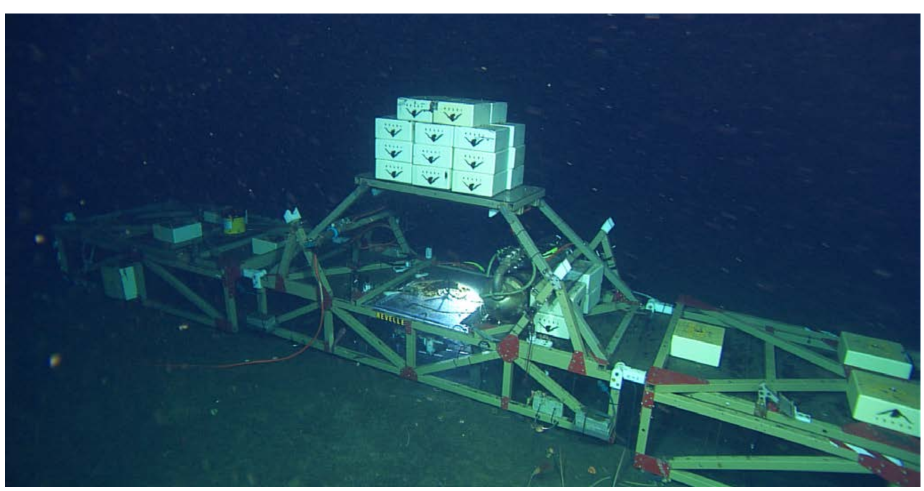

FIGURE 5. The deep-sea Free Ocean CO_2 Enrichment (FOCE) mesocosm is shown during deployment at 900 m depth in Monterey Bay.

rates according to pH and DO set points. The GCA has supported several studies evaluating the response of a variety of deep-sea animals to future deep-sea conditions (e.g., Pane and Barry, 2007; Pane et al., 2008; Kim et al., 2013a,b; Taylor et al., 2014; Hamilton et al., 2017).

Upwelling Simulator

As ocean change research matured, interest in and methods for investigating the influence of changes in multiple potential stressors over the timescales of variation typical in ocean ecosystems have increased (Breitburg et al., 2015). While the GCA is ideal for maintaining very stable changes in pH and DO expected for deep-sea environments, it could not simulate rapid changes in conditions that are typical in shallower waters. To allow experiments that more realistically emulate the timescales of variation and the range of conditions expected in coastal upwelling systems, we developed an Upwelling Simulator (UpSys) aquarium system. This advanced aquarium control system allows regulation of pH, oxygen, and temperature according to a user-defined schedule, making it possible to simulate current and future upwelling, as well as conditions (e.g., a low-temperature, low-oxygen, but high-pH event) that can help us to better understand the relative importance of individual potential stressors in multistressor experiments.

Performance Criteria

As is typical along upwelling-affected shores (Feely et al., 2008; Hofmann et al., 2011; Frieder et al., 2012), the temperature, pH, and dissolved oxygen in nearshore environments in Monterey Bay are highly variable, particularly during upwelling-dominated seasons (Hofmann et al., 2011). Measurements at 17 m depth near Monterey Bay Aquarium (Booth et al., 2012) and at 6 m depth off Hopkins Marine Station (36.6204°N, 121.902°W) during spring 2013 confirmed the rapid variation in conditions related to the advection of upwelled waters across the continental shelf and into nearshore locations (Feely et al., 2008). Temperature, dissolved oxygen, and pH_{total} changed by as much as 3°C, 10 mg O_2 L^{-1}, and 0.5 $pH_{(total)}$ units, respectively, in as little as a one to two hours (Figure 6). Variation in all three parameters was periodic at both locations, with dominant changes associated with the principal semidiurnal (M1; 12.42 h) and diurnal (K1; 23.93 h) lunar tidal constituents, supporting the notion that tidal currents control the advection of upwelled waters in and out of the nearshore. Based on these observations in southern Monterey Bay, UpSys must be capable of altering temperature, oxygen, and pH within experimental chambers by ~3°C, 10 mg O_2 L^{-1}, and 0.5 $pH_{(total)}$ units, respectively, within ~1.5 hours.

A secondary performance objective for UpSys is to enable experiments that disentangle the individual and combined effects of changes in multiple environmental conditions. Upwelling events typically include simultaneous reductions in temperature, dissolved oxygen, and pH that persist for hours to days, with potentially important effects on coastal taxa (e.g., Kim et al., 2013a,b). Owing to changes in several environmental conditions that coincide with upwelling, their effects on marine organisms are typically difficult to define. To determine the singular and interactive effects of upwelling-related changes in temperature, oxygen, and pH on coastal species, UpSys was designed to have independent, programmable control of each parameter. Thus, controlled conditions (T, pH, DO) in multiple experimental aquaria can be used to measure the responses of organisms to various scenarios concerning the intensity, duration, and nature of environmental changes during upwelling events (i.e., changes in any combination of temperature, pH, and dissolved oxygen).

Seawater Control System

UpSys regulates seawater conditions according to a user-specified schedule of set points for temperature, dissolved oxygen, and pH within 15 "conditioning tanks" (CTs). Each CT (7.6-liter RubberMaid Model 1530 water cooler) is equipped with a float valve to regulate seawater input, sensors (T, DO, pH), a gas input line connected to a diffuser stone, a 600-watt heater, and a drain with a distribution manifold that connects delivery

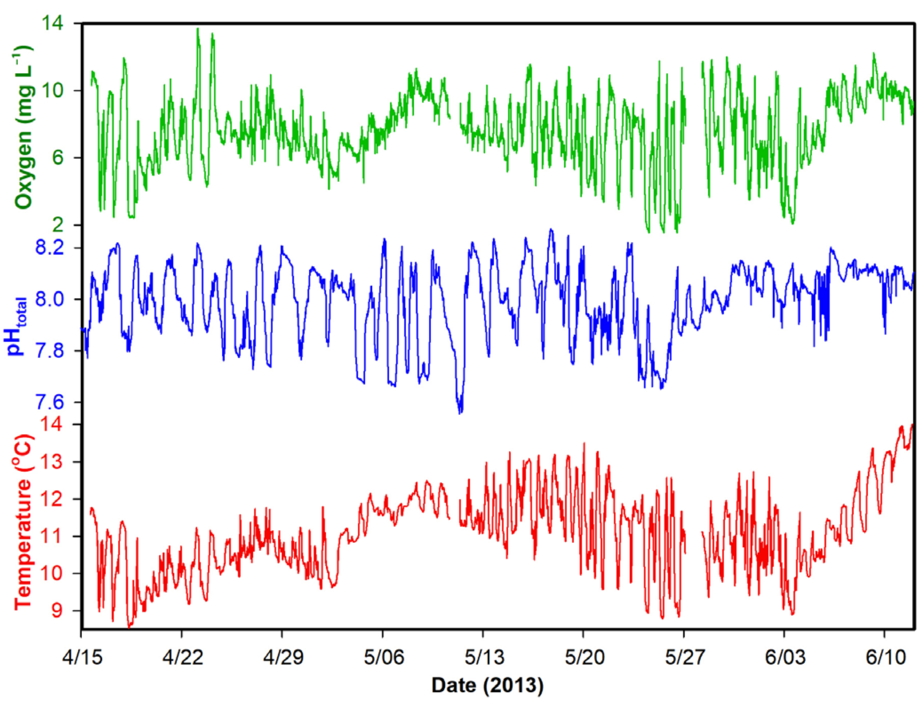

FIGURE 6. Time series of upwelling conditions at 5 m depth off Hopkins Marine Station, with pH measured on the total scale.

lines to experimental chambers (EC; Figure 7). Chilled, ambient seawater from the MBARI seawater system is pumped on demand to each CT, and set points (T, DO, pH) are achieved through heating, as needed, and/or bubbling of gas mixtures through the water to purge or add oxygen and carbon dioxide. The CT's small seawater volume permits rapid regulation of T, DO, and pH as required to simulate their variations in nearshore environments. The flow-through design of the system is capable of controlling seawater conditions for flow rates up to ~1.5–2.0 L min^{-1}. Together, 15 independently controlled CTs allow for production of a broad range of conditions for a single experiment.

Conditioning tanks are mounted on a rack above a seawater table, and the conditioned water flows by gravity to experimental chambers located below (Figure 7). Outflow from each CT is delivered to ECs through 1–10 delivery lines. For each CT, one EC is equipped with pH, DO, and temperature sensors to monitor any drift in parameter values from set points and the lag period between the CT and the EC. Experimental chambers can be of any size, and their suitability depends upon the capacity of organisms contained within them to alter conditions through respiration or excretion, as well as the EC's turnover time (determined by the flow rate). Environmental chambers can be placed in water baths with temperature set points that are also controlled by UpSys.

LabView software communicates with sensors and other UpSys hardware to regulate all functions of the UpSys, through connections to WAGO-I/O-SYSTEM modules. Power, sensors, and valves associated with each CT are connected to WAGO modules and linked by Ethernet to LabView software operating on a desktop computer. A LabView graphic user interface allows users to operate all functions associated with the 15 CTs (Figure 8), from manually entering set points, heater settings, and gas flow rates to full automatic control of conditions according to a set point schedule file. Under automated control, feedback from sensors in each CT is compared with parameter set points to regulate the heater and flow of purge gases to the tank.

Heaters can be set to regulate temperature within each CT and also in a water bath that houses a group of experimental

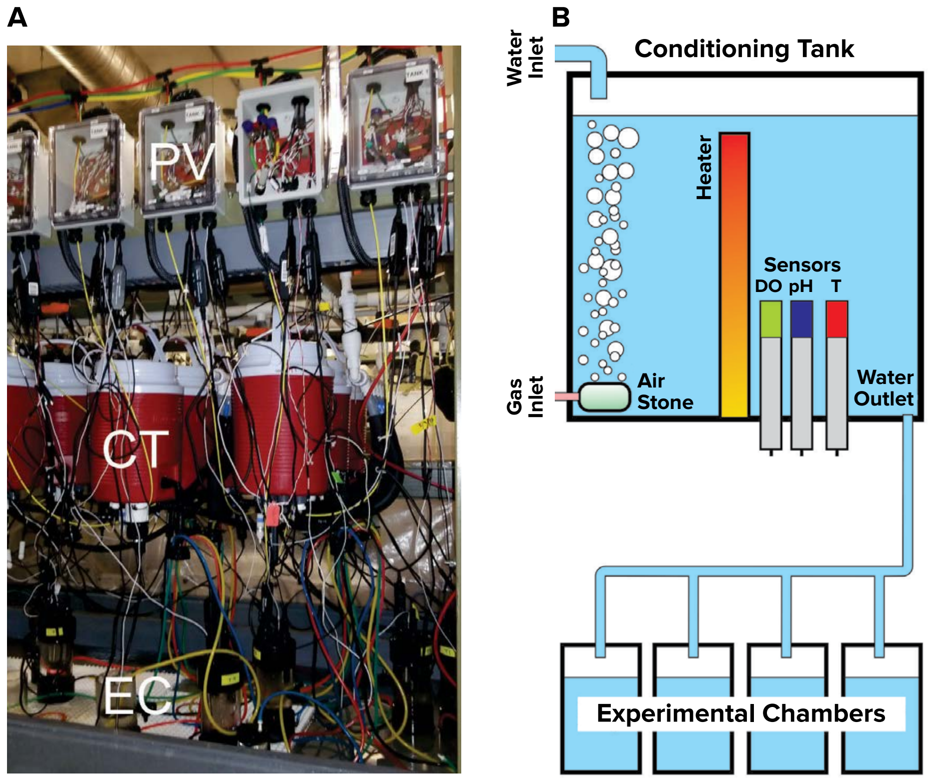

FIGURE 7. Overview of Upwelling Simulator (UpSys) design. Water quality is regulated in the conditioning tank, then distributed by gravity to a number of experimental chambers. (A) Photograph shows UpSys hardware. PV = junction boxes housing gas lines and proportional valves. CT = conditioning tanks. EC = experimental chambers on seawater table. (B) Schematic of conditioning tank. DO = dissolved oxygen. T = temperature.

FIGURE 8. Graphic user interface used with the Upwelling Simulator. Separate tabs are available for each of 15 tanks. Plots indicate the recent (~2 h) history of performance of the tank. A schedule of set points can be stored as a Microsoft Excel spreadsheet.

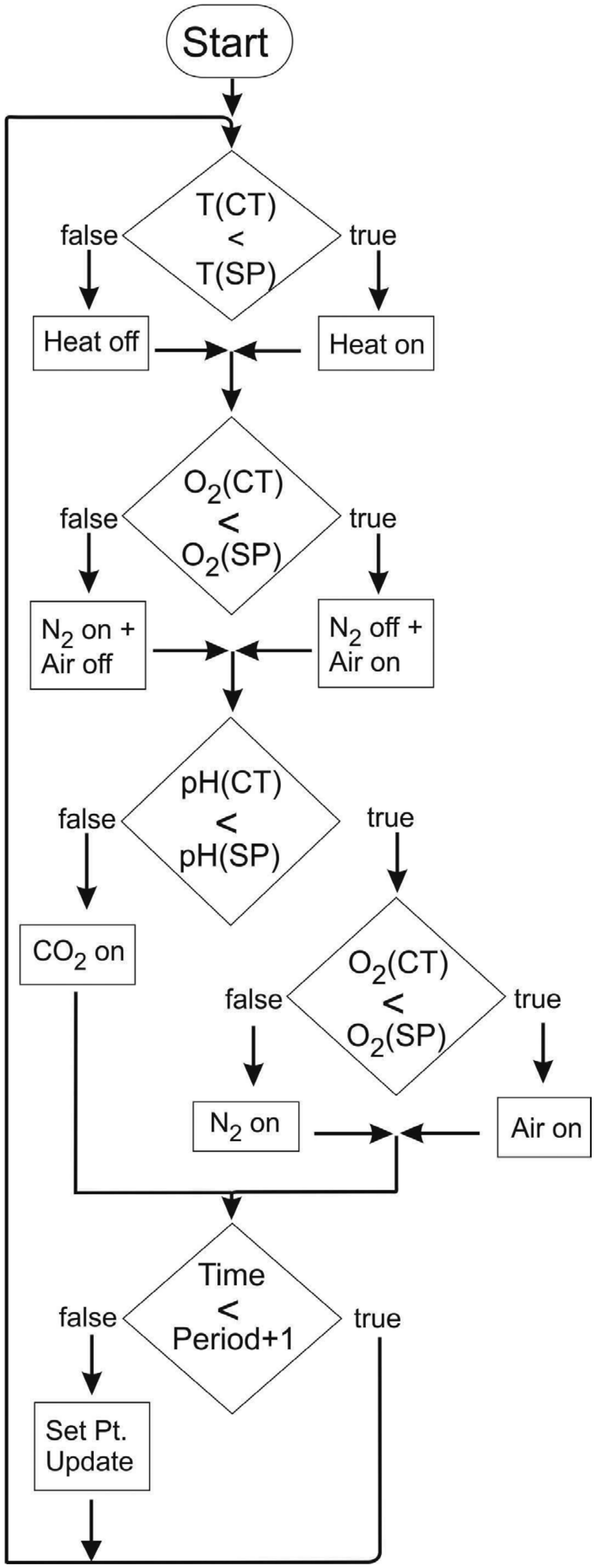

FIGURE 9. Logic diagram for Upwelling Simulator software control for each conditioning tank (CT). T(CT) = Conditioning tank temperature. T(SP) = Temperature set point. Heat on/off indicates control of CT heater. O_2(CT) = Conditioning tank oxygen (mg L^{-1}). O_2(SP) = Oxygen set point. pH(CT) = Conditioning tank pH. pH(SP) = pH set point. N_2, CO_2, Air indicate gas inputs to the bubble stream manifold. Air = CO_2-free air.

chambers. The minimum temperature for the laboratory seawater system is set to a level slightly below the coldest experimental conditions planned for UpSys so that "raw" seawater (chilled, 1 µm-filtered, UV-irradiated seawater) entering each CT will not require cooling for the coldest planned conditions. Depending on its current temperature set point, a 600-watt heater in each CT is automatically activated and can raise the seawater temperature several degrees within five to ten minutes.

Dissolved oxygen and pH in each CT are regulated by altering the composition and volume of gases in a bubble stream within the CT. Proportional solenoid valves (Clippard EVP Series proportional solenoid valves) connected to N_2 (0–10 L min^{-1}), CO_2-free air (Puregas CO_2 adsorber, model CAS4-11; 0–10 L min^{-1}), and CO_2 (0–1 L min^{-1}) sources are coupled to a diffusor manifold leading to an air stone within each CT. If dissolved oxygen in the CT is below the current set point, the flow of CO_2-free air is increased and N_2 is reduced until oxygen levels rise to meet the DO set point (Figure 9). If DO is above its current set point, the flow of CO_2-free air is reduced and the N_2 is increased to purge oxygen from the CT water until the DO set point is reached. For pH control, carbon dioxide is added or removed by altering the composition and flow of the bubble stream. Carbon dioxide is added if the pH exceeds its set point. If the pH drops below its set point, CO_2 addition ceases and either N_2 or CO_2-free air is added as a purge gas, depending upon the current oxygen status. If DO and pH are both low compared to their set points, then CO_2-free air is used as the purge gas for CO_2 until the pH rises to the set point. If DO is high and pH is low relative to their set points, N_2 is used as a purge gas to reduce the partial pressures of both oxygen and carbon dioxide in the CT (Figure 9). In this way, the bubble stream uses the optimal gas composition to meet the set point requirements for both pH and DO.

Rather than on/off flow of gases, proportional valves for gas metering can be tuned by the PID (Proportional-Integral-Derivative) control algorithms available in LabView to provide fine control of gases, avoiding wide oscillations of parameters around set points. Prescribed flow rates for gases depend upon the output of PID control loops for both oxygen and pH, and in general, flow is proportional to the magnitude of differences between CT sensor values and the set points for oxygen and pH.

Users can create a schedule for each CT as a spreadsheet consisting of a series of periods (rows) defining the duration (minutes) and set points for temperature (°C), dissolved oxygen (mg L^{-1}), and pH_{total} (Table 1). LabView software is used to read set point schedules, monitor sensor feedback from CTs and ECs, activate heaters, and regulate the composition and volume of gas bubbled through each CT to achieve set point conditions for the current period. For each CT, the software regulates conditions for the duration of each period of the schedule, comprising a "cycle" of 1–1,000 periods, and then restarts the cycle at the first period. Alternatively, users can operate the system in manual mode to enter set points. In addition, the control of heaters and the flow of diffusion gases can be automated (i.e., software control loop) or set manually. With this flexibility, users can schedule a wide range of conditions for each CT.

Sensors

Relatively inexpensive Vernier optical DO probes (model ODO-BTA) were used to monitor dissolved oxygen concentrations in conditioning tanks and experimental chambers. These sensors have relatively high stability and accuracy (±0.2 mg L^{-1} O_2) over timescales of weeks, and require little maintenance. Economical Vernier pH electrodes (model PH-BTA) were used to measure pH in experimental chambers, while Accumet pH electrodes (Fisher Model 13-620-108A) were used in the conditioning tanks to obtain accurate pH feedback for the automated UpSys control system. The Accumet pH combination electrodes are stable (± ~0.1 pH units) over weeks, but require regular cleaning and recalibration. To reduce noise, power and signals for each pH sensor were isolated from each other and from the main system electronics.

Upwelling Simulator Performance

UpSys can control seawater temperature, pH, and oxygen levels under a wide range of set point schedules that simulate changes typically observed during coastal upwelling (Figure 10). The ability of UpSys to regulate conditions depends upon several factors, including the rate of seawater flowthrough for the CT, the timescale of variation, and the range of extremes for set points. The flow rate through the CT and into experimental chambers required for an experiment depends upon the respiratory requirements of the animals held in the CT as well as the desired turnover time for water in the EC. For large organisms or those with high metabolic rates, respiration can rapidly alter oxygen and pH conditions within ECs, thereby requiring higher flow rates from the CT to maintain prescribed settings. As flow through the CT is reduced, the mean residence time for water in the CT increases, allowing greater control time to reach set points. Therefore, UpSys is most effective for experiments with relatively low flow requirements (e.g., <1 L min^{-1}). Conversely, as flow-through increases, residence time decreases, with correspondingly lower capacity for parameter regulation within the CT.

Control of seawater parameters is also related closely to the magnitude of changes required; for small changes in temperature (<2°C), dissolved oxygen (<5 mg L^{-1}) or pH (<0.3 units), regulation of conditions by UpSys is typically very effective. For some extreme values, a balance between flow rate and set points is required. For example, for hypoxic set points (<2 mg L^{-1}), flow rates must be relatively low or the residence time of incoming water in the CT is too short to

TABLE 1. Example of upwelling schedule for a conditioning tank.

Period	Time	pH	O_2	T (°C)
1	12:00	8.0	8	12
2	12:30	7.4	2	9
3	13:00	7.6	5	12

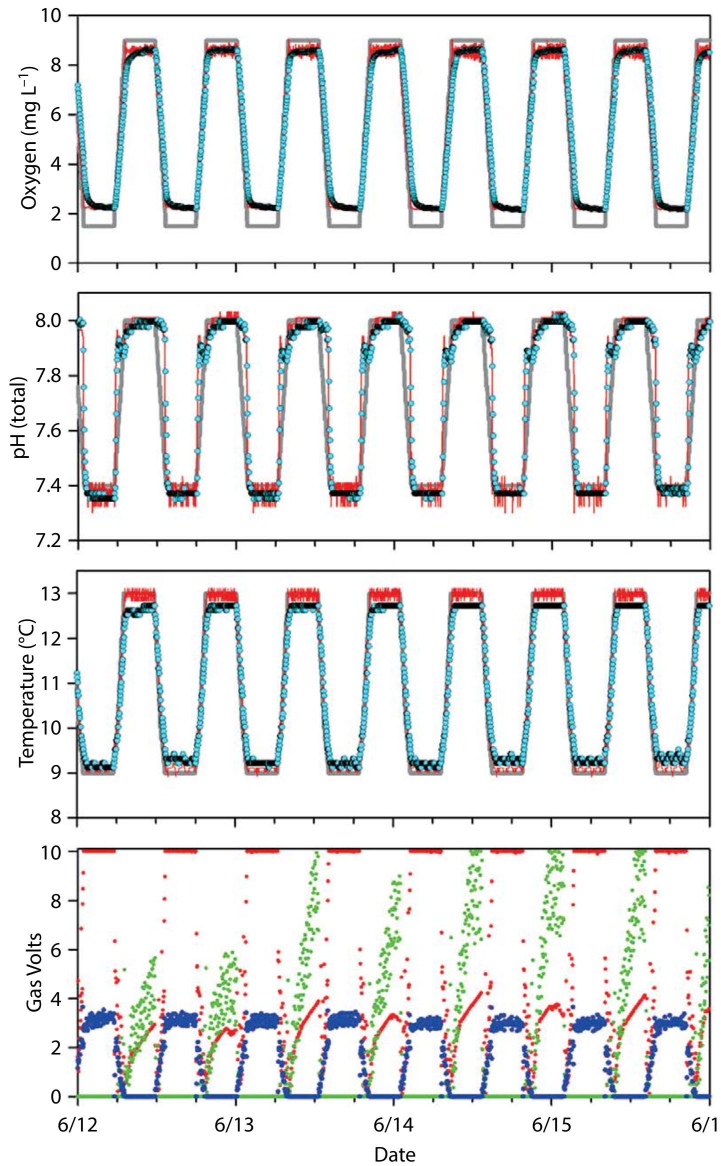

FIGURE 10. Performance of one Upwelling Simulator tank over four days. Time series of oxygen, pH, temperature, and gas flow (volts) shown in four panels. For O_2, pH, and temperature, the gray line (background) indicates the set point, the red line indicates conditioning tank value, and the blue dots indicate the experimental chamber values. Gas voltage output for control (bottom panel) is shown in blue (CO_2), green (CO_2-free air), and red (N_2). Zero to 10 volts correspond to ~0–10 L min^{-1} for N_2 and CO_2-free air, or 0–1.5 L min^{-1} for CO_2.

purge O_2 and reach very low oxygen levels, particularly if the O_2 content of ambient incoming water is high (Figure 10). Similarly, high-pH set points (>8.0) are difficult to accomplish by UpSys under moderate or high flow-through rates.

The Upwelling Simulator enables experiments that measure the effects of climate-related changes in ocean conditions on marine organisms, similar to the Mobile Ocean Acidification Treatment System (MOATS) developed by McElhany (2014), thus advancing our ability to predict the consequences of ocean change for species, communities, and society. The wide range of control and scheduling allows experiments that test the effects of realistic future upwelling scenarios and the creation of conditions for evaluating the effects of changes in various combinations of temperature, oxygen, and/or pH. Initially, UpSys has been used for experiments that examine the sensitivity of various life stages of red abalone (*Haliotis rufescens*) to the range and timing of tidally driven variation in conditions that currently occur in coastal habitats along central California (recent work of author Boch). Performance under current conditions will be compared with performance under potential future conditions, such as prolonged exposure to more hypoxic and lower pH waters during tidal upwelling exposure, along with warmer surface conditions (Figure 11).

SUMMARY

Projected changes in ocean conditions caused by rising atmospheric carbon dioxide levels are larger and more rapid than have occurred for many millions of years (Kerr, 2010), with effects that could persist for tens to hundreds of millennia. Ocean science is now chasing the future—current efforts in research and technology are creating a framework of understanding to predict and hopefully manage the effects of a changing Earth on ocean health and related benefits for society. Methods for advancing our understanding of the effects of ocean change on marine life range widely from studies documenting community patterns along natural gradients in environmental conditions, to manipulative laboratory experiments focusing on specific physiological processes. Integration of these efforts is improving our understanding of the likely consequences of ocean change (Kroeker et al., 2010; Harvey et al., 2013; Przeslawski et al., 2015; Busch and McElhany, 2016).

Research priorities in ocean science have evolved with our understanding of natural and anthropogenic influences on ocean conditions and marine life. MBARI engineers and scientists have contributed to this effort by developing technologies and methods that improve exploration and research in the deep ocean and enable laboratory and field experiments to advance understanding of the effects of ocean change on marine animals and ecosystems. David Packard's vision for MBARI was to bring together engineers and scientists to address key issues in ocean science that are limited by technology. Among many technologies developed by MBARI, several efforts described here have enabled observations and experiments that enhance our understanding of the influence that changes in ocean conditions have on the health of ocean ecosystems.

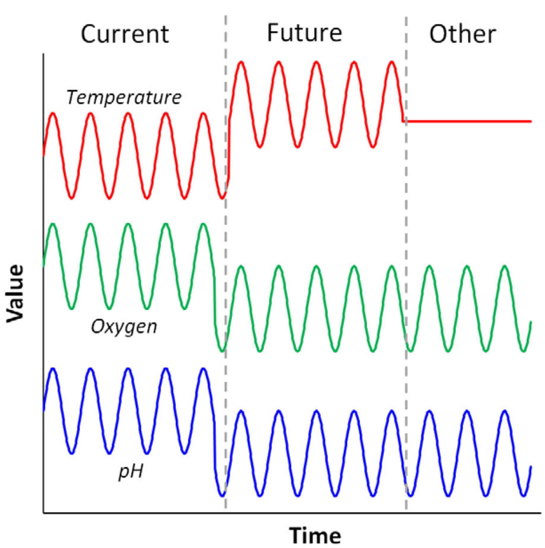

FIGURE 11. Options for Upwelling Simulator experiments. Three periods indicate possible scenarios for evaluating upwelling stress on marine organisms. Current conditions are dominated by semi-diurnal oscillation in temperature, pH, and oxygen. Future scenarios could include shifts in the mean T, pH, and/or O_2, with the same or altered variance. Other scenarios may include holding one or more parameters constant, prolonging high or low values, or various combinations of stability and variability among control parameters.

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