

# **Kelp Forests as a Refugium: A Chemical and Spatial Survey of a Palos Verdes Restoration Area**

Project Report



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## 1. Abstract

Ocean acidification, caused by increasing atmospheric carbon dioxide (CO<sub>2</sub>) concentrations, has pervasive impacts on marine organisms that are critical to ecosystem stability, ocean health, and fishery productivity. As marine photosynthesizers, kelp have been shown to modify local seawater chemistry by removing dissolved CO<sub>2</sub>, and thus are hypothesized to provide a pH refuge for local marine organisms. However, the extent to which restored kelp forests can truly serve as a pH refuge has yet to be fully explored. To address this, we collected water samples at 4 distinct depths throughout multiple days to analyze seawater chemistry outside and within a restored kelp forest off the coast of the Palos Verdes Peninsula. Using a combination of boat-mounted flow through sensors and drone technology, we also mapped surface dissolved carbon dioxide concentrations on a broader spatial scale. We found evidence to suggest that kelp forests can modify local water chemistry to create more favorable water conditions, both with depth and over a large spatial scale. Though the extent to which kelp forest species may benefit from this refuge is less clear, given kelp forests' ecological importance for many species of invertebrates, fish, and mammals, and their potential for modifying seawater chemistry, it is important to determine whether kelp forests can function as a pH refuge in increasingly acidic oceans.

## 2. Introduction

Atmospheric CO<sub>2</sub> levels have been steadily rising since the start of the Industrial Revolution, reaching levels beyond what the Earth has experienced in the last 800,000 years (Lüthi et al. 2008). As a natural carbon sink, the ocean has absorbed more than 30% of anthropogenic carbon dioxide emissions, an amount that is projected to increase in the coming decades (IPCC 2014). However, many factors other than atmospheric CO<sub>2</sub> concentrations (Revelle and Seuss, 1957), such as coastal upwelling, internal tides, and biological activity determine the carbon fluxes within the ocean and complicate studies of the system.

In terms of the physical chemistry of the ocean, dissolution of CO<sub>2</sub> into seawater follows the chemical equilibrium  $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{HCO}_3^- + \text{H}^+ \rightarrow \text{CO}_3^{2-} + 2\text{H}^+$ . Increased concentrations of CO<sub>2</sub> in the atmosphere have led to increased concentrations of certain carbon species in the ocean, such as hydrogen ions (Takahashi et al. 2006). Changes in these chemical concentrations have had wide-ranging effects on ocean chemistry and thermodynamics, including declines in ocean pH (Haugan and Drange 1996) and decreases in carbonate saturation state (Feely et al. 2004). These effects on ocean chemistry and on the carbonate system may be even more pronounced in the coast of California, where such events are becoming less frequent, stronger, and longer in duration due to climate change (Iles et al. 2011). However, coastal environments regularly experience fluctuating pH, temperature, and nutrient levels, which may make them more resilient to the products of ocean acidification (Capone and Hutchins 2013).

These chemical changes have pervasive impacts on various marine organisms that are

critical to ecosystem stability, ocean health, and fishery productivity (Andersson et al. 2015). Moreover, upwelling zones in California, due to their high nutrient concentrations, support an ecologically diverse multilevel trophic system (Bakun et al. 2015), with one organism forming large forests and supporting this intricate food web - Giant Kelp (*Macrocystis pyrifera*). Kelp forests are integral features of the California coast because of their high ecological importance and economic value (Steneck et al. 2002; Du Brow 2013). These highly productive ecosystems bring in approximately \$40 billion in revenue to the state of California each year through both recreational and commercial fishing (Kildow and Colgan 2005). Unfortunately, the kelp canopy cover in key California ecosystems, such as the Santa Monica Bay, has fallen 75% over the last 100 years as a result of human expansion, pollution, overfishing, and changes in climate such as temperature rise (Pondella et al. 2013). Though, with recent restoration efforts and legislation, kelp forest cover is gradually increasing in Southern California, allowing for the recruitment of previously disturbed kelp forests and their inhabitants, such as the Kelp Bass *Paralabrax clathratus* (Burdick et al. 2015).

The recovery of local kelp forests is also important because they may modify local seawater chemistry. Several studies have shown that marine photosynthesizers may respond positively to increases in oceanic carbon dioxide concentrations, due to the “carbon fertilization” of photosynthesis (Delille et al. 2000, 2009; Ries et al. 2009; Krause-Jensen et al. 2015). The removal of the carbon species from the surrounding seawater by photosynthesizers may alter the carbonate chemistry, reducing its acidity. This positive response may lead to local changes in seawater chemistry, providing a localized area of higher pH.

While changes in the chemistry of the ocean have debated impacts on biological systems, the physical chemistry behind these changes has been widely agreed upon for several decades. Three main inorganic carbon compounds control the carbonate chemistry of the ocean: carbon dioxide (CO<sub>2</sub>), bicarbonate (HCO<sub>3</sub><sup>-</sup>), and carbonate (CO<sub>3</sub><sup>2-</sup>) (Doney et al. 2009). These compounds are connected by a series of reversible reactions wherein atmospheric CO<sub>2</sub> dissolves in seawater to form carbonic acid (H<sub>2</sub>CO<sub>3</sub>), which dissociates into bicarbonate and carbonate:



An increase in atmospheric CO<sub>2</sub> shifts the equilibrium to the right, increasing the concentration of hydrogen ions (H<sup>+</sup>), and decreasing concentrations of carbonate ions (CO<sub>3</sub><sup>2-</sup>). Oceanic pH is strictly tied to hydrogen ion concentrations (pH = -Log<sub>10</sub> [H<sup>+</sup>]), so this increase in H<sup>+</sup> leads to a decrease in pH, resulting in acidification. Using this relationship and modeled projections, Haugan and Drange (1996) found that in the past 200 years, surface ocean pH has already seen a reduction of 0.1 pH units and in the next 100 years, it is predicted to drop an additional 0.3 – 0.4 pH units. This decline corresponds to a near 150% increase in H<sup>+</sup> and 50% decrease in CO<sub>3</sub><sup>2-</sup> (Orr et al. 2005; Doney et al. 2009), which will have consequences beyond just a change in pH.

Carbonate chemistry is also influenced by the relationship between carbonate and

calcium species in seawater according to the following equation:



Many calcifying organisms found in kelp forests, such as mollusks, crustaceans, and plankton rely on this reaction to precipitate their calcareous shells. Though the impacts of acidification on particular species are unsure (e.g., Ries et al. 2009), generally when there is a net precipitation of  $\text{CaCO}_3$ , organisms can easily build up shells and body structures. However, when there is net  $\text{CaCO}_3$  dissolution,  $\text{CaCO}_3$  structures begin to dissolve and organisms face difficulty building and maintaining their shells (Orr et al. 2005; Fabry et al. 2008).

To determine if restored kelp forests in Santa Monica Bay can create this type of pH refuge for organisms from ocean acidification, we addressed changes in water chemistry over depth, time of day, and over a large spatial scale along the coast. We also investigated whether phytoplankton as other marine photosynthesizers impacted these possible changes in water chemistry alongside the kelp. Lastly, we performed a systematic literature review of several hundred species native to kelp forests to identify the range of pH sensitivities that may influence the definition of a pH refuge.

### 3. Methods

#### 3.1 Water Chemistry

To determine whether restored kelp forests can serve as an ocean acidification refuge from a chemical perspective, we collected water samples from two sites in Santa Monica Bay near the coast of the Palos Verdes Peninsula four times between 31 January and 24 May 2017. Samples were collected off of the Palos Verdes Peninsula within a restored kelp forest (33.75715°N, -118.41866°W) and at a control site ~100m offshore (33.75707°N, -118.42085°W) (Figure 1). The protocols for sample collection were made at four discrete depths at each location to sample from the surface to the benthos, and were identical for each site. The maximum depth was 14m at the kelp site and 18m at the offshore site, thus the four collection depths at each site were approximately {0m, 4m, 8m, 14m} and {0m, 6m, 12m, 18m}, respectively. Given that kelp photosynthesis is limited by sunlight, we believed it to be important to compare the suitability of different depths as possible refugia for kelp forest organisms between both sites.

We expected to see little variation in the offshore site throughout the day, while water chemistry at the kelp site was expected to be more variable on shorter time scales. To address this, measurements at the offshore site were collected at the beginning and end of each field day, and measurements at the kelp site were collected every hour for 5-6 hours throughout the day in order to capture temporal changes in water chemistry.



### 3.2 Phytoplankton Impacts

Given that Giant Kelp are not the only species photosynthesizing in kelp forests, we also collected water samples to characterize the abundance and composition of microorganisms inside and outside of the restored kelp forest. These samples were collected from the same batch of water obtained for seawater chemistry analysis at all four depths and both locations. Samples were not collected on the 1/31/2017 field day and are thus not included in this analysis.

During field sampling days, water samples of approximately 20mL from each Niskin bottle collection were set aside for phytoplankton analysis. Samples were stored in sterile, 50 mL bottle containers coated with pre-measured 3 $\mu$ L of 0.5% glutaraldehyde and refrigerated to preserve the oceanic phytoplankton and prevent further photosynthesis and/or predation events prior to flow cytometric analysis at UCLA. Although we gathered multiple samples throughout the field days, we chose to use samples from the last kelp site measurements and the final offshore site measurements, which were at ~3:00 - 4:30 PM on each date.

We utilized flow cytometry to analyze and characterize phytoplankton abundance. Using a fixed volume micropipette, a lab technician at UCLA's Flow Cytometry Core Laboratory transferred 50 $\mu$ L of CountBright Absolute Counting Beads and 1 mL of seawater into sample tubes. The concentration of the counting beads was 50,000 beads/50 $\mu$ L. A control sample was set up using 0.2 $\mu$ m filtered seawater and the counting beads in order to determine the exact concentration of counting beads. These counting beads were utilized in order to determine the absolute count of photosynthetic phytoplankton, instead of relative counts.

A total of 9 samples were run for each appointment at the lab facility. The fluorophores analyzed were Chlorophyll PerCP-A, APC-A, Phycoerythrin (PE-A), Divinyl Chlorophyll A to determine fluorescence events. The phytoplankton size and complexity were analyzed using forward scatter and side scatter parameters. For the first round of data processing, the analysis was concluded once the number of counting bead events had reached around 10,200 to 10,300 events. For the second and third round of analyses, the analyses were concluded once the number of counting bead events had reached exactly 10,000 events. We characterized events to be of high fluorescence by gating strategies based on Haynes et. al (2015)., who conducted an analysis of marine plankton using flow cytometry (Haynes et. al. 2015). The overall phytoplankton abundance count was determined by gating the P1 channel. Events outside of the P1 gate were determined to be bacteria, viruses, or events too small to count as significant.

We utilized the following equation to determine the absolute counts of events and multiplied the final number by 1000 in order to get a final unit of events (as cells)/L, see supplementary Data Folder for spreadsheets with final calculations:

Calculation of cell concentration:

$$\frac{A}{B} \times \frac{C}{D} = \text{concentration of sample as cells}/\mu\text{L}$$

Where:

A = number of cell events

B = number of bead events

C = assigned bead count of the lot (beads/50  $\mu\text{L}$ )

D = volume of sample ( $\mu\text{L}$ )

All samples were processed at UCLA's Flow Cytometry Core Laboratory, under the guidance of Ingrid Schmid, the lab manager, with the lab's LSRII Digital Analyzer.

### 3.3 Ecological Survey

To better understand how to define a "pH refuge", we performed a comprehensive literature review of the effects of increasing ocean acidity for 228 species native to Southern California's kelp forests. This search was based off a list provided by The Bay Foundation, and generated from the Vantuna Research Group (Occidental College) Monitor for Southern California Kelp Forests, for species identified as ecologically and commercially significant to the kelp forests of Santa Monica Bay. Each species was searched in Google Scholar using the full species name with "ocean acidification," "pH response," or "CO<sub>2</sub>" appended and literature was surveyed from the first 5 pages of results. We excluded literature that was missing information such as the duration of the test or the concentration of CO<sub>2</sub> at which the organism was tested. Literature was also excluded that lacked quantitative data on the sensitivity of OA response. Search engine results showing related species or species of the same genus were likewise excluded to ensure that our results reflected information on the specific kelp species of importance.

### 3.4 Spatial Survey

In order to capture water chemistry variation in our study area over a larger spatial scale, we employed a combination of flow-through sensing of surface dissolved carbon dioxide concentration and drone image mapping. Using a CO<sub>2</sub>-Pro instrument (Pro-Oceanus Systems, Inc., Nova Scotia, Canada), we mapped dissolved CO<sub>2</sub> levels along a 1980 m stretch of surface water along the coast of the Palos Verdes Peninsula on May 24, 2017. In order to determine if this technology could detect changes in kelp canopy density, the area surveyed included regions of light kelp cover, no kelp at the surface, and dense kelp at the surface. Eight discrete locations were chosen to ensure navigation of the boat through these areas: 4 in the open ocean, and 4 in kelp canopy areas. These two transects were parallel to each other, and the 4 points in each transect were approximately 300m apart. The research vessel moved at 3 knots/hr through the transects while moving from point to point. Once each GPS coordinate was reached, engines were turned off and the vessel hovered around the same area for 10 minutes, allowing the CO<sub>2</sub>-

Pro Instrument to calibrate.

We also attempted to create a mosaic image of the peninsula and study site from high resolution Phantom 3 drone imagery collected on June 6, 2017. The 8 chosen coordinate points were uploaded into a phone database and calibrated with the drone's GPS coordinate system. The drone was flown on autopilot where it took approximately 200 images of the specific site. After the images were collected, a geographic information system (ArcGIS), was utilized to create a mosaic of the images. The CO<sub>2</sub>-Pro data map was then overlaid on top of the drone imagery to determine if dissolved CO<sub>2</sub> gradients aligned with varying kelp cover between the offshore region and the kelp forest locations.

## 4. Results

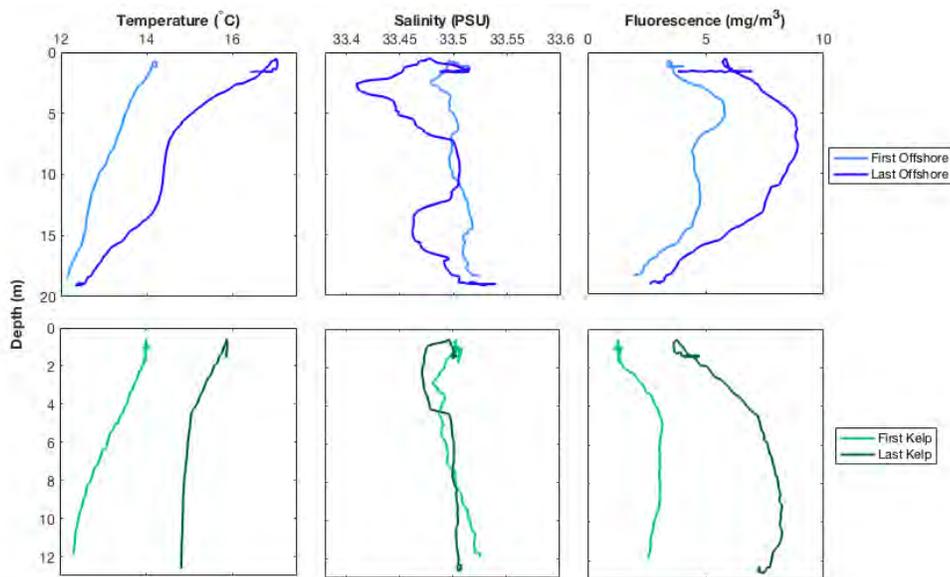
### 4.1 Water Chemistry

Both physical and chemical seawater parameters varied by depth, time of day, sampling date, and site location (Table 1, Figure 2, 3). In addition, the kelp plants at our kelp site grew in slowly over the course of our field campaign, allowing us to compare these parameters over a range of kelp presence between field days (very little kelp on 1/31/2017 to stands ~1-2m tall on 5/24/2017 (pers. comm., Kristen Elsmore)). Unfortunately, the CTD data collected on 1/31/2017 was not usable and is thus not included in this analysis.

Sampling Date	Site	T range (°C)	S range (PSU)	F range (mg/m <sup>3</sup> )	pH				TA (mean ± SE μmol/kg)
					min	max	avg	range	
1/31/2017	Offshore	N/A	N/A	N/A	7.8108	7.8859	7.8402	0.0751	2259.12 ± 7.16
	Kelp	N/A	N/A	N/A	7.8187	7.8561	7.8397	0.0374	2264.45 ± 2.25
3/15/2017	Offshore	2.0157	0.2839	2.9373	7.8031	7.9389	7.8913	0.1358	2231.99 ± 1.25
	Kelp	1.5197	0.4401	5.2606	7.8125	7.9824	7.9258	0.1700	2234.90 ± 0.49
5/2/2017	Offshore	5.2225	0.5609	9.5117	7.6714	8.1251	7.8469	0.1755	2229.57 ± 1.18
	Kelp	3.9271	0.3787	8.6473	7.6898	8.1117	7.9848	0.4220	2234.78 ± 0.46
5/24/2017	Offshore	0.2800	0.6299	3.6240	7.6039*	7.9899*	7.8336*	0.3861*	N/A

**Table 1:** Physical and chemical seawater parameters for the offshore and kelp sites from each sampling date including the temperature range (T range), salinity range (S Range), fluorescence range (F range), pH (min, max, average, range), and total alkalinity (TA) mean  $\pm$  SE. Missing values denoted N/A. \*pH data from 5/24/2017 was incomplete at the time of analysis, thus values were computed using the last three kelp and only the last offshore water samples from the day.

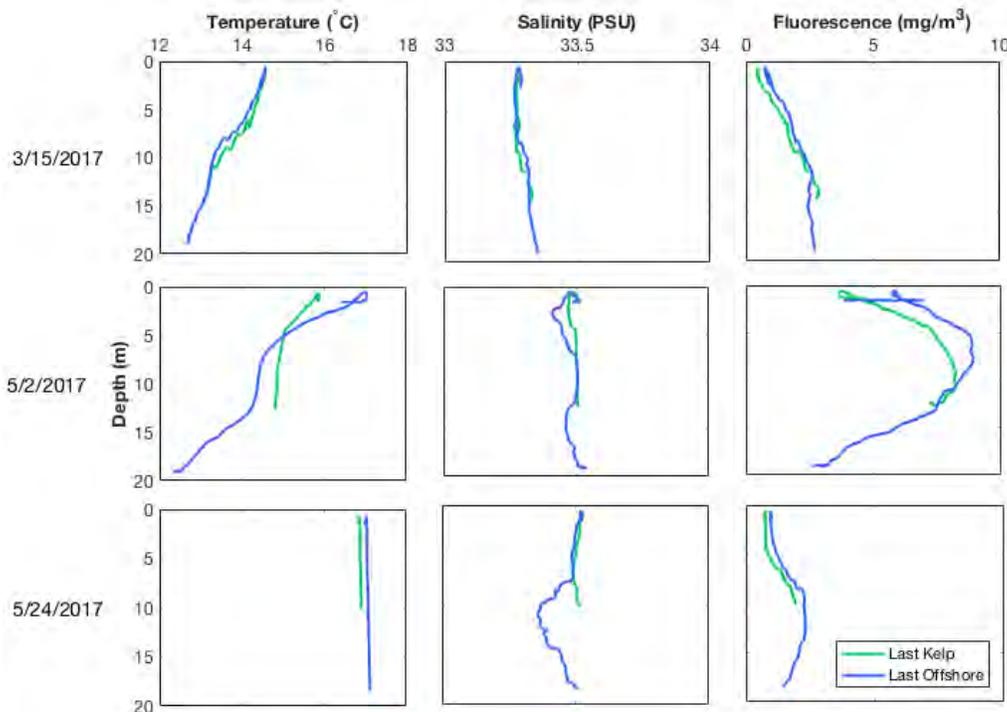
At the offshore site, temperature ranged from 12.08-17.30°C and salinity ranged from 32.96-33.70 PSU over the course of our field campaign. Temperature at the kelp site ranged from 12.29-21.67°C, while salinity ranged from 32.91-33.65 PSU over this same period. In terms of changes throughout the day, temperature and fluorescence generally increased at all depths, while salinity remained nearly constant (for an example from 5/2/2017, see Figure 2). Temperature appeared to experience more change with depth at the offshore site than at the kelp site, perhaps due to location and maximum depth of the site (Figure 2). Peak fluorescence values occurred a few meters below the surface at both sites, though this trend was clearer at the offshore site (Figure 2).



**Figure 2:** Depth profiles of temperature (°C), salinity (PSU), and fluorescence (mg/m³) for the 5/2/2017 sampling date showing the first and last offshore casts collected during the sampling day (morning and late afternoon) [top row, light to dark blue] and the first and last kelp casts collected during the sampling day (morning and late afternoon) [bottom row, light to dark green]. Data are presented using a 50-value moving average.

There were also noticeable changes in physical and biological water parameters between sampling dates (Figure 3). Temperatures at both the kelp and offshore sites increased as the months progressed, consistent with a transition from winter to spring temperatures (Figure 3, left

column). Salinity remained relatively constant between sites, but increased slightly between the sampling date in March and early May (Figure 3, middle column). Fluorescence profiles for both sites were similar in March and late May, but were much different on the sampling date in early May (Figure 3, right column). The low temperatures at depth, visible thermocline, slightly higher salinity, and elevated fluorescence levels on 5/2/2017 suggest that we may have also captured an upwelling event, or strong water column stratification (Figure 3, middle row). Changes in the ranges of these values are reported in Table 1.

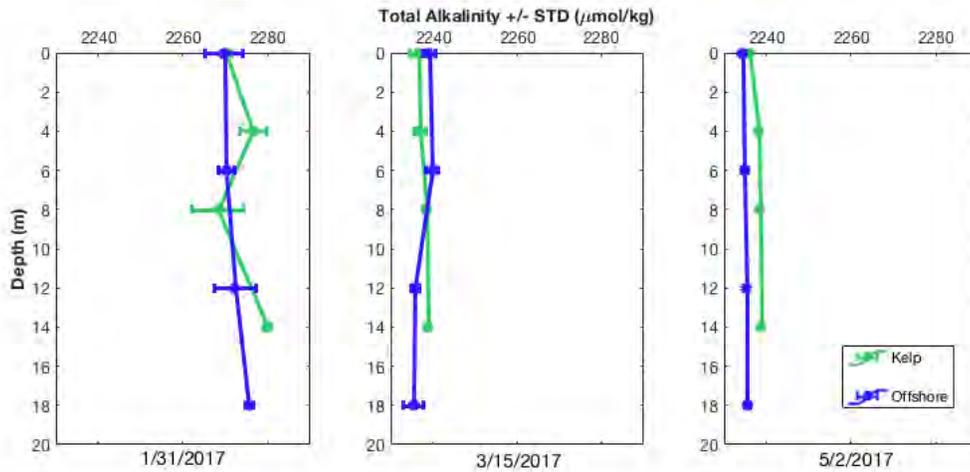


**Figure 3:** Depth profiles of temperature ( $^{\circ}\text{C}$ ), salinity (PSU), and fluorescence ( $\text{mg}/\text{m}^3$ ) (columns left to right) for each field day (rows from top to bottom) for the last kelp and last offshore CTD casts performed each day  $\sim$ 3:00-4:30PM (green and blue lines, respectively). Data are presented using a 50-value moving average.

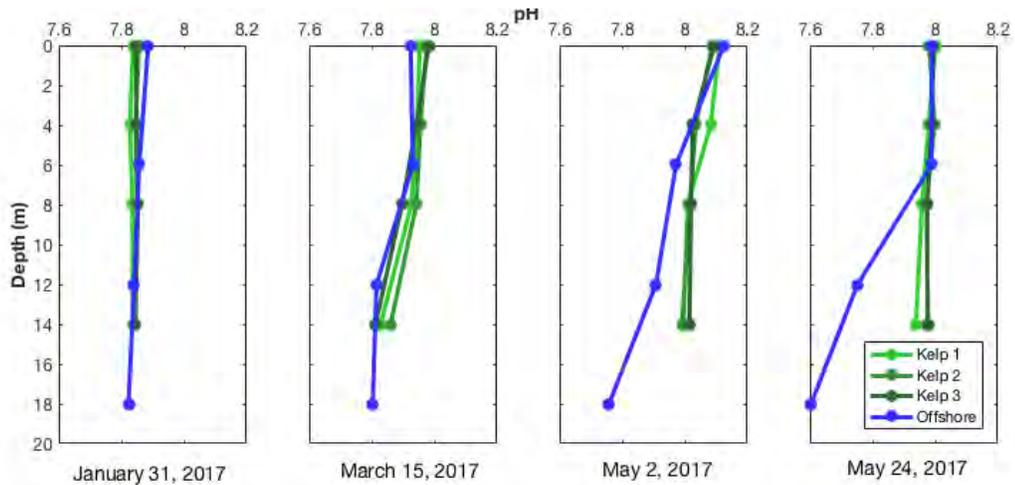
Total alkalinity at the offshore site ranged from  $2221.38 \pm 0.81$  to  $2278.03 \pm 0.43 \mu\text{mol}/\text{kg}$  and from  $2225.76 \pm 0.87$  to  $2279 \pm 0.80 \mu\text{mol}/\text{kg}$  at the kelp site over the sampling period. TA was relatively constant across depths and between sites for all individual sampling dates (Figure 4). However, TA did vary across sampling dates, with lower values in March and early May, compared to higher values in January (Figure 4, Table 1). Mean TA at the kelp site was consistently higher than at the offshore site (Table 1). While mean TA at the offshore site consistently decreased across sampling dates, mean TA at the kelp site decreased and then remained nearly constant between March and early May (Table 1).

Over the course of our field campaign, pH values at the offshore site ranged from 7.6714

to 8.1251, and 7.6898 to 8.1117 at the kelp site (Table 1). On the first field day, pH values did not appear to vary much with depth, between sites, or over the course of the day (Figure 5, first panel). However, pH values between the kelp and offshore sites became more differentiated over the course of the last three field days (Figure 5, second through fourth panels) and the average pH of the kelp site became increasingly more basic (higher pH) than the offshore site (Table 1). Over time, pH at the offshore site steadily decreased with depth, whereas the kelp site pH was relatively stable with depth, even across the span of several hours (Figure 5). On the field days in May (5/2 and 5/24), pH was higher at the kelp site compared to the offshore site, particularly near the benthos.



**Figure 4:** Total alkalinity (TA) depth profiles from each field day (panels from left to right: 1/31/2017, 3/15/2017, 5/2/2017, 5/24/2017). TA values are plotted for the last kelp and offshore water samples collected from each day (green and blue, respectively) at each depth collected  $\pm$  STD in  $\mu\text{mol/kg}$ .



**Figure 5:** pH depth profiles from each field day (panels from left to right: 1/31/2017, 3/15/2017, 5/2/2017,

5/24/2017) showing the growth of a potential pH refuge over time. pH values are plotted for the last three kelp water samples of the day (light to dark green) and the last offshore water sample (blue) at each depth collected.

After all the available data were synthesized, the software CO<sub>2</sub>sys was used to calculate the pCO<sub>2</sub> of the samples from March 15 and May 2. From the calculated results, there was a clear trend between decreased pH and higher values of pCO<sub>2</sub> (Table 2).

Sampling Date	Site	Depth (m)	pH	TA (mean ± SE μmol/kg)	pCO <sub>2</sub> (μatm)
3/15/2017	Offshore	0	7.9824	2231.99 ± 1.25	234.2
		6	7.9427		267.7
		12	7.8968		309.1
		18	7.8125		396.2
	Kelp	0	7.9243	2234.90 ± 0.49	272
		4	7.9318		278.3
		8	7.8155		391.7
		13	7.8031		412.7
5/2/2017	Offshore	0	8.1251	2229.57 ± 1.18	140.9
		6	7.9722		244.1
		12	7.9084		292.5
		18	7.7580		467.9
	Kelp	0	8.0878	2234.78 ± 0.46	162.3
		4	8.0355		201.7
		8	8.0158		214.3
		13	7.9911		229.7

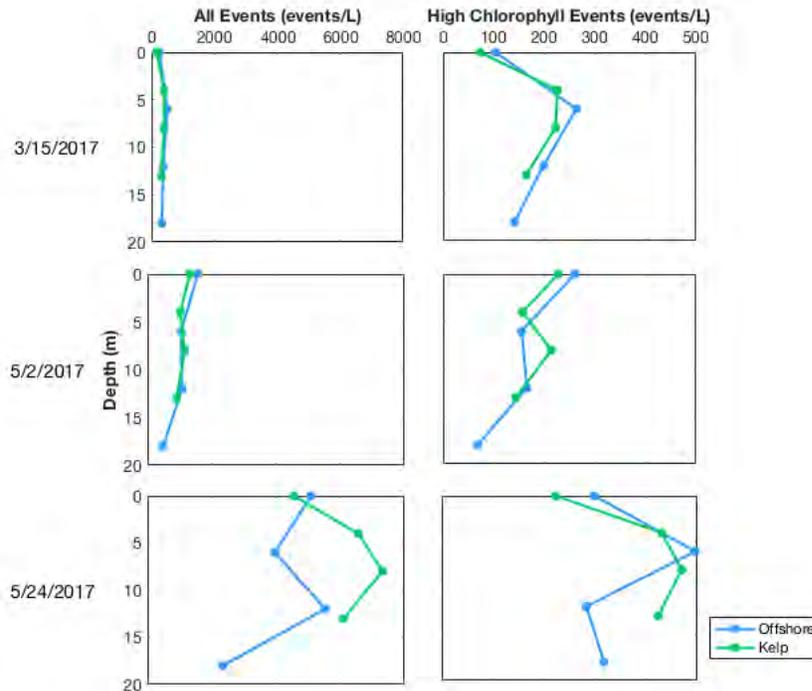
**Table 2:** CO<sub>2</sub>sys analysis results. pCO<sub>2</sub> was calculated at different depths with the corresponding Total Alkalinity data from different sites, and pH for multiple depths.

## 4.2 Phytoplankton Impacts

From our flow cytometry analyses, we created depth profiles of the total phytoplankton abundance and high chlorophyll events from three field days (3/15/17, 5/2/17, and 5/24/17) to compare phytoplankton abundance over time and depth. Our results showed that the overall phytoplankton abundance generally increased over time from March to May (Figure 6). In March, the highest absolute count of phytoplankton abundance at the offshore site was 504.1 events/L at 6m and at the kelp site, the highest was 403.8 events/L at 4m. This increased to the maximum absolute count of 5531.3 events/L at 12m for the offshore site and 7318.8 events/L at 8m for the kelp site.

For the offshore site, there was a significant increase in overall phytoplankton abundance.

Between the March and May (5/2/2017) field days, the overall phytoplankton abundance at the offshore and kelp sites remained relatively the same. However, the results from the 5/24/2017 field day showed a significant increase in phytoplankton abundance at the kelp site to the maximum absolute count, particularly at depths of 4 - 8m (Figure 6). The increase in abundance at the kelp site can possibly be attributed to an upwelling event or the increasing sunlight penetration into the water during the transition into spring months.



**Figure 6:** Depth profiles of all phytoplankton events and high chlorophyll events (columns left to right) from three field days (rows from top to bottom) for the last kelp and last offshore sample collections each day (green and blue lines, respectively) in events/L.

The number of high chlorophyll events steadily increased during the course of our research. At the offshore site, the number of events/L ranged from 68.3 events/L to 495.6 events/L. At the kelp site, our counts ranged from 72.8 events/L to 469.7 events/L. The number of high chlorophyll events in March and May (5/2/2017) showed no significant differences between the kelp and offshore site, except at the kelp site at 8m on 5/2/2017. Our last field day (5/24/2017) showed an increase in high chlorophyll events, particularly below the surface depth at 4m and 8m.

### 4.3 Ecological Survey

Of the 226 species searched, our systematic literature review only revealed ocean acidification research for 14 of these species (Table 3). For this fraction of species for which

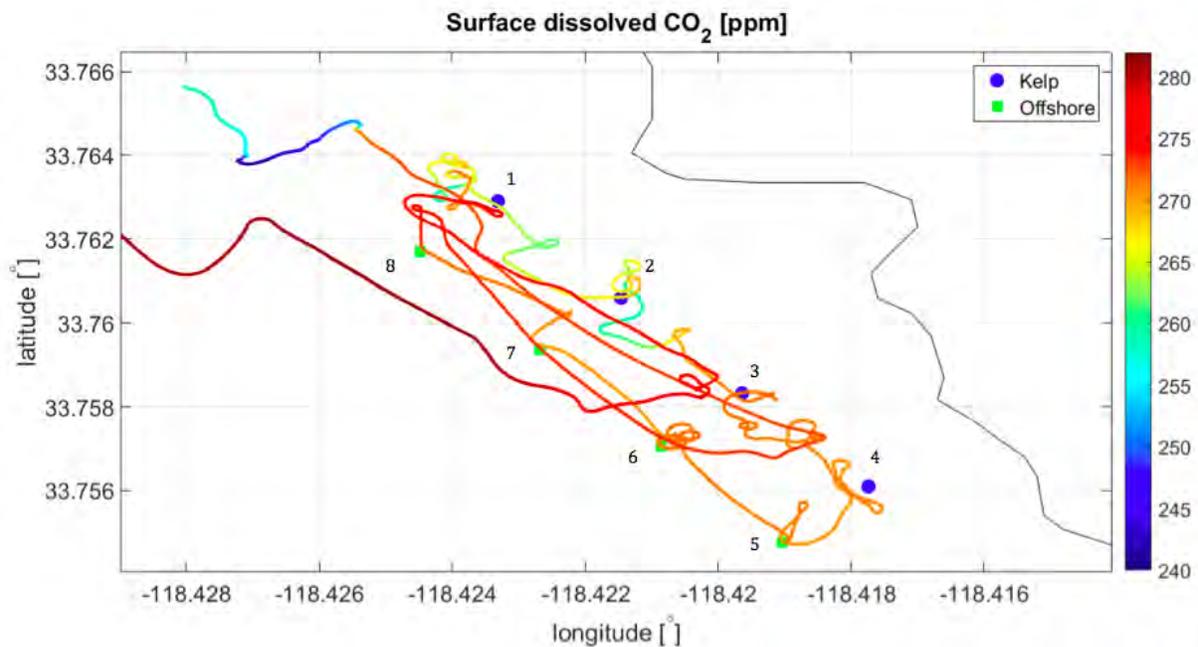
research was published, we found that studies on sensitivity to OA are diverse in duration and levels of pCO<sub>2</sub> exposure, with negative effects seen at as low as 540 ppm CO<sub>2</sub> and as high as 1800 ppm CO<sub>2</sub>. For detailed summaries of the results for each species, organized approximately by phyla or family, see Appendix 2.

Species	Common Name	Ambient Level	Duration Tested	Levels Tested	Response(s) Measured	Sensitivity	Citation
<i>Strongylocentrotus franciscanus</i>	Red Sea Urchin	400 ppm	30s fertilization window, 3hrs development	400, 800, 1800 ppm	Fertilization efficiency (FE), Efficiency of egg to block polyspermy	↓ in FE at both levels, blocking efficiency ↓ at 1800 ppm	<a href="#">Reuter et al. 2010</a>
<i>Strongylocentrotus purpurulus</i>	Purple Sea Urchin	800 ppm	Measured after 14 days of exposure	800, 900 ppm	Body Length, Protein Desposition Efficiency	Protein desposition efficiency ↓ at 900 ppm, ↓ in body size and 50% ↑ in protein synthesis	<a href="#">Pan et al. 2015</a>
<i>Anthropleura elegantissima</i>	Intertidal Anemone	pH 8.1	Measured after 1 week of acclimation and 6 weeks of exposure	pH 8.1, 7.3	Respiration and Photosynthesis	↑ rates of photosynthesis, ↑ mitotic index in acidified conditions	<a href="#">Towanda et al. 2012</a>
<i>Pisaster ochraceus</i>	Purple Sea Star	380 ppm	70 days	380 ppm, 780 ppm	Calcified mass	Calcified mass ↓ relative to control under acidified conditions, though total growth rates ↑ (switched to soft tissue production)	<a href="#">Gooding et al. 2009</a>
<i>Sebastes atrovirens</i> , <i>Sebastes carnatus</i> , <i>Sebastes caurinus</i>	Canopy Rockfish: Kelp, Gopher, Copper	540 ppm	Measured after 3-4 month exposure in 4 levels	540, 820, 2100, 3300 ppm	Behavioral and Physiological Changes	Altered brain function: behavioral lateralization (2 spp.), showed ↑ escape time (brain damage) (3 spp.) and ↓ swimming endurance	<a href="#">Fennie 2015</a>
<i>Sebastes mystinus</i> , <i>Sebastes saxicola</i> , <i>Sebastes serranoides</i>	Benthic Rockfish: Blue, Stripetail, Olive	540 ppm	Measured after 3-4 month exposure in 4 levels	540 ppm, 820 ppm, 2100 ppm, and 3300 ppm	Behavioral and Physiological Changes	↑ escape time in Olive Rockfish, ↓ weight gain in Stripetail Rockfish, ↓ in length of Blue Rockfish in highest ppm condition only	<a href="#">Fennie 2015</a>
<i>Clinocottus analis</i>	Woolly Sculpin	400 μatm	7 day acclimation, 7 or 28 day treatment	400 (ambient) and 1100 (elevated) μatm	Hypoxia Response (routine metabolic rate, P <sub>O2</sub> , Na <sup>+</sup> ,K <sup>+</sup> -ATPase activity)	Acclimation to acidified conditions elevated routine metabolic rates and acid-base regulatory capacity (Na <sup>+</sup> ,K <sup>+</sup> -ATPase activity), which resulted in ↓ hypoxia tolerance (i.e. elevated critical oxygen tension).	<a href="#">Hancock and Place 2016</a>
Crustose coralline algae	Crustose coralline algae	400 μatm	Measured once per week for 7 weeks with some diurnal sampling	400 μatm, 765 μatm	Recruitment Rate and Growth	Both recruitment rate and growth were significantly reduced under acidified conditions.	<a href="#">Kuffner et al. 2008</a>
<i>Macrocystis pyrifera</i>	Giant Kelp	Exp 1: 190 μatm Exp 2: 280 μatm Exp 3: 270 μatm	Exp 1: 5 days Exp 2: 5 days Exp 3: 7 days	Exp 1: 117, 125, 208, 1140, 1250 μatm Exp 2: 133, 152, 280, 1080, 1230, 1320 μatm Exp 3: 270, 830, 1200 μatm	Meiospore germination and sex determination	Lowered pH caused ↓ germination, but added DIC had the opposite effect, indicating that ↑ CO <sub>2</sub> at lower pH ameliorates physiological stress, no sex determination effect, growth ↑ under moderate μatm, and remained positive under high acidification conditions.	<a href="#">Roleda et al. 2011</a>
<i>Nereocystis luetkeana</i>	Bull Kelp	360 ppm	55 days	360, 3000 ppm	Phlorotannin content; biomass; herbivory; decay	↑ phlorotanin content; biomass ↑; no effect on herbivory; ↓ decay in response to higher CO <sub>2</sub>	<a href="#">Swanson and Fox 2007</a>

**Table 3:** Summary of literature review results for each species, indicating important aspects of ocean acidification studies and their impacts on the species.

#### 4.4 Spatial Analyses

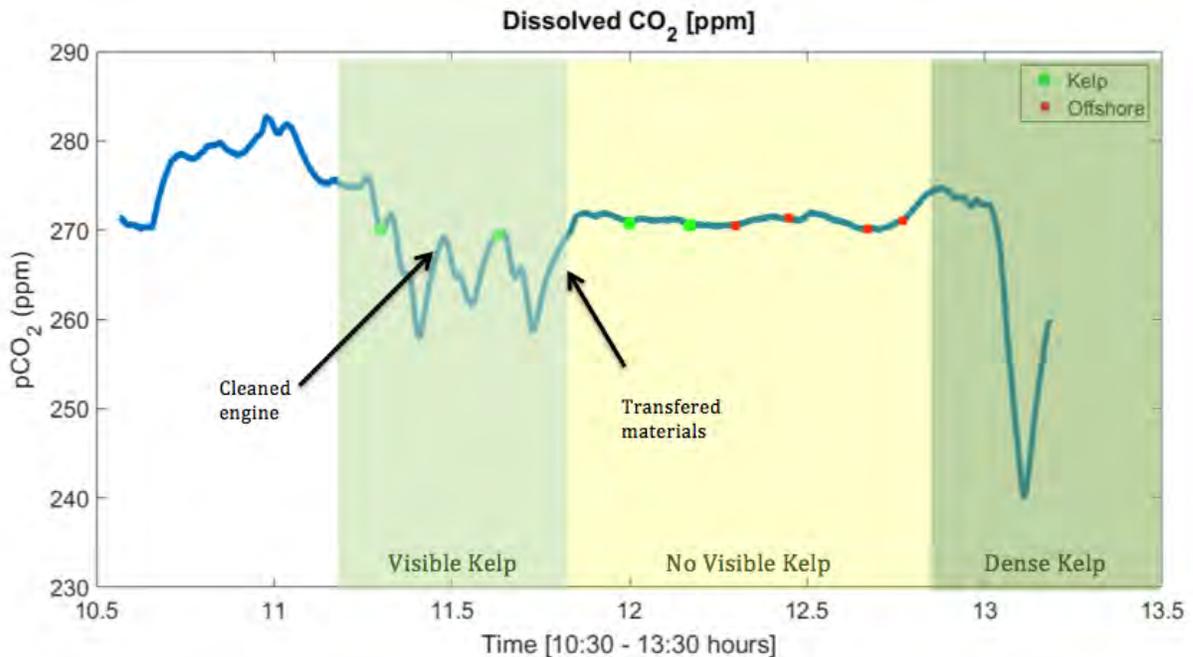
Using the CO<sub>2</sub>-Pro instrument, we were able to map surface CO<sub>2</sub> concentrations along the path of the research vessel through our 8 pre-determined coordinates (Figure 7, Appendix 3). We discovered only our first two locations (33.762902°N, -118.423319°W and 33.760601°N, -118.421461°W) had kelp plants reaching the surface of the water, which allowed us to compare surface CO<sub>2</sub> concentrations over areas of kelp with varying kelp density. The boat began the transect through the kelp forest from the northwest, traveled southeast parallel to the coast, and returned back heading northwest parallel to the route but further offshore. Figure 7 indicates each of the stops in sequential order. At each of the 8 points, we allowed the boat to hover for approximately 8-10 minutes to allow the CO<sub>2</sub>-Pro instrument to equilibrate with the surrounding water to ensure accurate measurements at each point, which explains the spirals in the plot along the transect (Figure 7). An additional stop occurred in order to transfer materials to The Bay Foundation's vessel, indicated by the crossing of the transect in the middle of the plot (Figures 7, 8.)



**Figure 7.** Map of UCLA Zodiac path on May 24, 2017 along the coast of Palos Verdes with varying concentration of dissolved carbon dioxide within surface water, where warm colors correspond to higher values, and cool colors lower values.

We observed an approximate 40 ppm variation of CO<sub>2</sub> over the entire region (Figure 7, 8). Surface CO<sub>2</sub> concentrations appeared to relate inversely to kelp cover along the transect (Figure 8). In the first part of the day, we traveled from Marina Del Rey to our site (non-highlighted region) along the coast. We then reached our first two points, where the fluctuating levels of CO<sub>2</sub> corresponded to the patchiness of kelp in the region. Between our recordings at

these locations, it was necessary to clean one of the engines because it was collecting kelp (indicated in Figure 8). Next, we traveled to two locations within the area of our water sampling as well as four locations further offshore. At these points, there was no kelp visible at the surface. Here, a higher and more stable level of CO<sub>2</sub> was recorded. Finally, we traversed through an extremely dense region of kelp where we observed a sharp decline in CO<sub>2</sub> levels. The portion (labeled 'Dense Kelp', Figure 8) corresponds to the upper section of the map with lower CO<sub>2</sub> levels, indicated in dark blue.



**Figure 8.** Surface CO<sub>2</sub> (ppm) over time, with qualitative observations of kelp visibility and procedure.

The available drone imagery of our spatial survey reveals the kelp canopy to be consistent with our qualitative observations. Due to weather and technical complications, the quality of the second portion of drone imagery was too low to be utilized for our analysis. The first portion of imagery accurately shows the kelp canopy, however, it only corresponds to a small fragment of our CO<sub>2</sub>-Pro data. Using ArcGIS, we were able to georeference the CO<sub>2</sub>-Pro data map onto the drone imagery. In order to ensure visibility of the kelp canopy, we created a shapefile of the data and overlaid the shapefile on the imagery (Figure 9). This section of the route was the first location on the trip (33.762902°N, -118.423319°W).



**Figure 9.** Overlay of UCLA Zodiac’s path (May 24, 2017) with drone imagery (captured June 6, 2017) corresponding to time points 11.3 through 11.7 in Figure 8 (times are approximate).

## 5. Discussion

From a combination of sampling methods over several dimensions of space and time, we were able to collect substantial evidence to suggest that kelp forests may indeed be able to serve as a pH refuge for organisms in the face of ocean acidification. We also confirmed that the presence of this apparent refuge was largely not influenced by phytoplankton communities present in the water column, further strengthening the hypothesis that kelp itself is modifying surrounding water chemistry. Additionally, we confirmed our hypothesis that kelp forest organisms may respond negatively to ocean acidification at differing levels, suggesting that defining a pH refuge will have to take place on a species-by-species basis.

Using the data from the various CTD casts collected throughout each sampling day, we were able to observe changes in the physical state of the seawater at both sites with depth and through time. Throughout the day, temperature increased at both sites, corresponding to increased solar heating from morning to afternoon (Figure 2). Fluorescence signals also increased throughout the day, perhaps corresponding to increasing light levels or more favorable water temperatures for maximized photosynthesis (Figure 2). Nearly constant salinity throughout the day, with depth, across the sampling period, and between sites suggests that there was little mixing of different water masses at either site (Figure 2). The CTD data also captured some larger changes in water parameters between sampling days, notably on the first sampling date in May (5/2/2017). The presence of a clear thermocline and slightly higher salinity, coupled with the much higher fluorescence values indicate that there may have been an upwelling event occurring on this day, where cold, nutrient-rich water from the benthos further offshore was brought toward the coast (Figure 3). This would not be surprising, considering California’s ocean

has a rich upwelling system, which often extends into Santa Monica Bay (Iles et al. 2011). Given that the magnitude of this change was slightly higher at the offshore site, it is likely that the effects of this upwelling may have been diluted or mixed with surrounding water before reaching the kelp site.

Between sites and by depth, total alkalinity was relatively constant for each sampling day (Figure 4, Table 1). However, we are unsure what the implications of the large change in TA between 1/31/2017 and 3/15/2017 are, as they do not appear to be explained by the other data collected. Over the sampling period, TA at the kelp site increasingly diverged from the TA values at the offshore site, which may be one mechanism by which kelp can modify the seawater.

Perhaps most central to this study was our assessment of whether a restored kelp forest may be able to serve as a pH refuge. The fact that the kelp at our site grew in late this year was a unforeseen factor in the current study, but it added an interesting dimension to the study design, allowing us to observe the development of a potential refuge over time. On the first sampling date (1/31/2017), which corresponded to little to no kelp cover (pers. comm., Kristen Elsmore), there was little difference in pH between depths, sites, or over the course of the day (Figure 5). By the second sampling day (3/15/2017), with still very little kelp (pers. comm., Kristen Elsmore), there appeared to be small changes in pH with depth, with pH decreasing slightly below 6-8m depth, though both sites followed the same trend (Figure 5). However, by the third sampling day (5/2/2017), which corresponded to ~0.5-1m kelp cover (pers. comm., Kristen Elsmore) and potentially an upwelling event, pH at the offshore site was much lower than the pH at the kelp site at depth (Figure 5). Given that the kelp grows in from the seafloor to the surface, it is expected that the effect of kelp presence on seawater chemistry would begin at depth and propagate upward as it grows. Thus, while surface pH values were similar between the offshore and kelp sites through the day, they diverged at depth, suggesting that kelp may be modifying the water chemistry throughout the day at the kelp site. By the final sampling date (5/24/2017), which corresponded to kelp ~1-2m in height (pers. comm., Kristen Elsmore), this divergence between decreasing pH at the offshore site and higher, constant pH at the kelp site with depth was even clearer (Figure 5). As the kelp was still well below the surface, the pH values in the upper 8 m were similar between sites. However, it appears that the elevated pH at the kelp site remained relatively stable with depth and throughout the day, showing that kelp may indeed be able to raise local water pH at depth and throughout the day.

In our efforts to expand the spatial scale of our survey, we were also able to collect evidence suggesting that kelp forests can modify surface dissolved CO<sub>2</sub> concentrations along a larger spatial scale. By traversing a transect that included areas of no kelp cover, sparse kelp, and very dense kelp at the surface along the coast of the Palos Verdes Peninsula, we were able to show that kelp cover appeared to scale inversely with surface CO<sub>2</sub> concentrations (FigureS 7,8). Fluctuating levels of CO<sub>2</sub> corresponded to the patchiness of kelp in the first region, higher and more stable levels of dissolved CO<sub>2</sub> corresponded to the lack of kelp in the second region, and a sharp decline in CO<sub>2</sub> concentrations corresponded to an extremely dense region of kelp near the

end of the field day (Figure 8). Fully mature kelp tends to have a dense canopy and direct access to the sun, therefore performing more photosynthesis, and removing more dissolved CO<sub>2</sub> from the water. Under the assumption that low concentrations of dissolved CO<sub>2</sub> correspond to higher pH values (which was confirmed with our CO<sub>2</sub>sys calculations, Table 2), this data complements the pH data collected at depth from our field site, further providing evidence that kelp can modify surrounding carbonate chemistry and provide a refuge, both at depth and at the surface.

From these results, we conclude that kelp is able to influence water chemistry parameters, particularly pH and dissolved CO<sub>2</sub> concentrations over the course of a day, with depth, and at the surface across a large spatial scale. The ability of kelp to modify local seawater is also supported in the existing literature. Though our conclusions are limited in that we were not able to collect water chemistry data at depth and throughout the day in a fully-grown kelp forest, a similar study of pH at a kelp forest off the coast of La Jolla, CA using moored buoys found that the kelp forest increased pH ~0.1 units following an upwelling event (Frieder et al. 2012). Our data suggests that kelp may be able to modify pH up to ~0.3 pH units with depth, showing that we were still able to capture important variation in pH over our sampling campaign. In addition, although we did not collect surface CO<sub>2</sub> concentration data over a large temporal scale, Frieder et al. (2012) found that pH changed more with depth than alongshore. Delille et al. (2009) also studied the potential of kelp forests to serve as a refuge, though in the Southern Ocean. They found that kelp drastically draws down dissolved inorganic carbon (DIC), though this was strongly tied to a seasonal cycle due to the limited light for photosynthesis in the winter, and was influenced significantly by microplankton (Delille et al. 2009). Although not addressed in the current study, future studies of the potential for kelp forests to serve as a pH refuge should consider sampling over the course of an entire 24-hour period (as in Delille et al. 2009; Frieder et al. 2012). As photosynthesizers like Giant Kelp cannot perform photosynthesis overnight, it will be useful to examine the dynamics of the refuge over semidiurnal, diurnal, and event time scales (Delille et al. 2009; Frieder et al. 2012).

Using flow cytometric analyses of the the water samples, we were also able to show that changes in water chemistry at the kelp site were likely not strongly influenced by phytoplankton presence. Since there were no significant differences between the overall phytoplankton abundances at the offshore and kelp sites during the 3/15/2017 and 5/2/2017 field data (Figure 6), this suggested that any changes in the water chemistry were not strongly correlated with phytoplankton photosynthesis activity. The significant rise in counts of phytoplankton at the kelp site at the next field day (5/24/2017) may be attributed to the effects of the suggested, prior upwelling event on 5/2/2017 or because the climate has changed. When CTD fluorescence data was compared with the flow cytometry data, our flow cytometry results did not clearly indicate an upwelling event on 5/2/2017 that matched the CTD fluorescence data, perhaps suggesting that although there was an increase in fluorescence events, these events were not high chlorophyll fluorescence events. However, the effects of the suggested 5/2/2017 upwelling event may be confirmed by the increase in overall phytoplankton counts at the kelp site on the last field day

(5/24/2017) (Figure 6). A comparison of the high chlorophyll event profile to the CTD fluorescence profile showed similarities in which the counts of chlorophyll events were consistently greater at depth ranging from 4-8m. Overall, the flow cytometry analyses confirmed that while phytoplankton abundance does change over time, it may not have a strong influence on the seawater chemistry. Further research and a more temporal survey of phytoplankton abundance in the future would allow us to see the any additional changes in phytoplankton abundance as the weather progresses from the spring to summer months.

While we have shown that kelp forests may be able to modify local water chemistry, it is important to consider whether the magnitude of this change will be enough to protect a variety of kelp forest species under future ocean acidification. We found varied ocean acidification (OA) sensitivities between different species of the same genus, such as *Sebastes* rockfish (Fennie 2015) and *Strongylocentrotus* urchins (Reuter et al. 2010; Pan et al. 2015), and between various species present in the kelp forest (Table 3). This shows that defining whether a kelp forest can serve as a refuge may depend on the species in question, complicating predictions of future ecological stability.

Our findings were also limited by the dearth of consistent and widespread studies of organismal responses to increasingly acidic conditions. Among the OA research on kelp species, most studies tested organismal responses only to one, or possibly a few, projected pCO<sub>2</sub> levels. For example, Gaylord et al. (2011) found that the mussel *Mytilus californianus* showed adverse responses at 540 ppm compared to mussels exposed to the baseline of 380 ppm. However, whether or not mussels exposed to 381-539 ppm pCO<sub>2</sub> would still suffer OA-related impairments is unknown. The elevated pCO<sub>2</sub> conditions tested were all at least 160 ppm pCO<sub>2</sub> higher than the respective pCO<sub>2</sub> control levels for each experiment, though we saw at most a 40 ppm pCO<sub>2</sub> difference in our measurements between water inside the kelp forest and in an adjacent site (Figure 8). There is a need to study how kelp organisms respond to smaller pCO<sub>2</sub> elevations, e.g. 40 ppm-100 ppm higher than the baseline control ppm, over longer periods of time. A comprehensive study exposing multiple kelp species to finer increments of pCO<sub>2</sub> over longer periods of time, while observing seasonal and diurnal pCO<sub>2</sub> variability would better account for the dynamics of possible OA adaptation by both kelp forests and its inhabitants.

It is also important to note that kelp itself is not completely immune to the effects of ocean acidification. We found that pH levels within dense kelp forests are indeed higher than pH levels where there was no kelp (Figure 7,8), an effect that may very well be protecting the kelp itself, given that kelp spores have been shown to be vulnerable in high pCO<sub>2</sub> conditions (Gaitán-Espitia et al. 2014). As future pCO<sub>2</sub> levels become more extreme, kelp spores may become dependent on dense, mature kelp forests to maintain pH levels that allow for germination. In addition, developing kelp may also have to compete with other non-calcifying photosynthesizers that benefit from rising CO<sub>2</sub> concentrations, such as algal turf (Connell and Russell 2010). In a high pCO<sub>2</sub> future, kelp populations may be at risk if a climate-related event devastates kelp forests, or if urchin populations continue to decimate kelp cover. The lack of developed kelp, and

thus, lack of a pH modulator after such events, may prevent the germination of kelp spores needed to replace a decimated population. This, in turn, may also threaten kelp forest species that not only rely on kelp to provide a physical habitat, but also as a pH refuge from changing ocean acidity. However, studies show that while decreased pH impacts kelp spores, a coupled increase in dissolved inorganic carbon may ameliorate these negative effects (Roleda et al. 2011), though this may be complicated by changes in temperature or other factors (Gaitán-Espitia et al. 2014; Fernández et al. 2015). Thus, future studies of pH refugia in kelp forests must look at change all aspects of the carbonate system, not just pH, as well as other physical seawater parameters.

## 6. Conclusion

By analyzing water chemistry over several spatial and temporal scales, we were able to show that kelp may indeed modify water chemistry to serve as a pH refuge for some organisms. However, the extent to which this refuge might benefit kelp forest species may depend on the specific species. We have shown that a variety of kelp forest species exhibit various behavioral and physiological responses to more acidic conditions, however the ability to predict which species may benefit the most or least is complicated by a lack of relevant literature and inconsistencies in study design. This study serves as a foundation for future research on the ability of California's kelp forests to serve as a pH refuge, as more research is needed to fully understand the dynamics of these possible refugia over both space and time.

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## 9. Appendices

### Appendix 1.

#### **Kelp Forest Water Sampling**

Protocol by Priya Shukla

19-Oct-2016

Updated by Kristen Elsmore

11-Jan-2017

#### **Samples Collected**

- CTD profile (Conductivity, Temperature, Depth, oxygen, and PAR)
- Total alkalinity via Niskin water samples
- Spec pH via Niskin water samples
- Chlorophyll via filtration apparatus
- Phytoplankton via Niskin water samples

#### **Materials**

- 250 mL Glass Bottles for Surface and Depth Samples of pH for each site/sample
- 250 mL Plastic Nalgene Bottles for Alkalinity at each site/sample
- Weatherproof labels for each bottle
- 2 spare Glass Bottles
- 2 spare Plastic Nalgene Bottles
- 2 buckets for alkalinity samples (to keep separated from poisoned samples)
- Large cooler for pH samples
- Gloves (S, M, & L)
- Parafilm strips
- Poison Kit: 100  $\mu$ L & 30  $\mu$ L Pipettes, 50 mL bottle of HgCl<sub>2</sub>, Napkins, Jar of Drierite
- Bag for HgCl<sub>2</sub> Waste
- Small cooler for chlorophyll filter samples
- Dry Ice for chlorophyll samples
- 4 opaque Plastic Nalgene Bottles for temporary holding of chlorophyll samples (1 per depth)
- Chlorophyll filtering apparatus (filtration set up, plastic flask, hand pump)
- Bag of pre-cut foil with weatherproof labels
- Glass Fiber Filters
- Forceps
- 2 squirt bottles of DI water
- Niskin bottles (2)
- Niskin tube (blue)
- Line w/ weight for Niskin samples
- CTD
- Line w/ weight for CTD casts
- CTD cleaner fluid

- Datasheet for water samples and CTD casts
- HgCl<sub>2</sub> safety sheet

## **Methods**

*CTD Sampling* \*Do cast right before each water sampling series

1. CTD can either be connected to a Davit Arm and lowered into the water or be deployed by a person over the side of the boat
2. Remove caps from the oxygen and PAR sensors (one small black and one larger white)
3. Turn CTD on (check for lights on fluorometer)
4. Lower into water about 1m below the surface and allow bubbles to leave tubing. Leave submerged for 2 minutes – sensor is designed to turn on after 45 secs – 1 min of submersion
5. Lift CTD up to the surface until the ring on the top of the cage is at the surface
6. Lower to bottom slowly, at an even speed: hand over hand should be fine.
7. Once you feel the CTD gently hit the bottom, bring it back up to the surface (we use the data collected on the downcast, so speed on the way up is not as important)
8. Retrieve CTD and turn off
9. Note CTD cast order and cast time on datasheet

*Niskin Water Chemistry Sampling*

1. Ensure air vent (knob) is closed (doesn't need to be air-tight) and that spigot/nipple is pulled out (rotate spigot/nipple quarter-turn away from metal pin to avoid accidentally pushing spigot/nipple in)
2. Prepare Niskin by opening each door and threading the plastic of one door through the plastic loop of another (do not loop past white, plastic ball!).
3. Hook non-threaded loop through the metal trigger (triggered by messenger to close doors)
4. Drop Niskin to desired depth.
  - a. For bottom samples, you will *feel* when Niskin hits bottom (line will go slack), raise Niskin slightly from bottom and use line to move Niskin up and down to “clean”.
  - b. For surface samples, if close enough to water, lean over side of boat and place Niskin below surface and “swish” Niskin side-to-side while maintaining horizontal position.
5. Close Niskin doors to obtain water sample.
  - a. For bottom samples, attach messenger to rope and release into water to trigger Niskin doors.
  - b. For surface samples, close one door, and allow Niskin to fill completely by elevating open door's position in water column without breaching surface and contaminating with air (Niskin should be at an angle). Close second door when Niskin is full – you will know the Niskin is full when gas bubbles stop exiting through the open door.
6. Bring Niskin Aboard

7. Attach blue Niskin tube to nipple/spigot, align metal pin with hole in nipple/spigot, push nipple/spigot in, and open air vent
  - a. Let flow to get rid of air bubbles. To get rid of stubborn air bubbles, pinch Niskin tubing above bubble and slightly raise tubing while air vent is open to push bubbles out.
8. Lower Niskin tube to bottom of each bottle and count as water fills until positive meniscus forms, then count again to ensure 100% replenishment of water
9. Gently remove tube from bottle (maintain positive meniscus)
10. To clean Niskin tubing, whip in air multiple times prior to next bottle sample.

*Poisoning for pH- Glass Bottles (mercuric chloride, HgCl<sub>2</sub>)*

1. Samples should be poisoned within 1 hour of collection.
2. Make sure you are wearing gloves and that no skin is exposed during the poisoning process.
3. Place poisoning kit in a steady location that will not move until poisoning is complete.
4. Unscrew cap from bottle with poison – Tip: keep poison bottle propped up in a corner of the box to reduce its likelihood of being knocked over.
5. Loosely unscrew sample bottle cap without removing it to avoid oxygen exchange with water sample and ambient air.
6. Fill pipette with poison and deposit into sample bottle, tightly screwing on the cap immediately after poisoning. This kit should include two fixed-volume pipettes: 30µL & 100 µL
  - a. For 250 mL water sample, 100 µL of poison is required
  - b. ~~For 100 mL water sample, 60 µL of poison is required~~
7. Once sampling is complete, confirm that all caps are tightly screwed onto their respective sample bottles and seal with parafilm.

*In the event of a poison spill ...*

1. If on a non-human surface, soak up poison with drierite and dispose of in HazMat waste container upon return to lab
2. If poison spills on human skin, remove contaminated clothes and rinse thoroughly with freshwater for at least 15 minutes and contact a physician afterwards.

*Alkalinity- Plastic Nalgene Bottles*

1. Lower Niskin tube to bottom of each bottle and count as water fills until positive meniscus forms, then count again to ensure 100% replenishment of water
2. Gently remove tube from bottle
3. Pour a little water out so the bottle is a little over ¾ full (so it doesn't crack or open up when frozen)
4. Place in bucket with the rest of the samples (just to keep them separate from the poisoned samples)
5. Freeze alkalinity bottle samples within **X** hours

*Chlorophyll Samples*

1. Pour the remaining water from the Niskin into its respective temporary Chlorophyll Nalgene (labeled by depth) and leave in cooler until it can be processed (filtered)
2. Label a pre-cut piece of foil with Date, Time, Depth, and Replicate# (1, 2, or 3) <sup>\*should have 3 filters for each Niskin sample</sup>
3. Prep the filtering apparatus by wetting both pieces with DI water
4. With forceps, place a clean filter in between the two pieces of the filtering apparatus
5. Put the filtering apparatus on the plastic flask and attach the tubing of the hand pump
6. Gently shake the Chlorophyll Nalgene to mix the water sample
7. Pour 50mL of water into the filtering apparatus and pump until the sample is dry
8. Be sure the pump never exceeds 4 on the pressure gauge
9. With forceps, gently remove the filter from the filter setup
10. Fold filter in half, twice (with the algae on the inside), and then wrap up securely in its pre-labeled foil
11. Place sample into the plastic samples bag and store in small cooler with dry ice
12. Repeat until there are 3 replicate filter samples
13. Rinse entire filtration setup and Chlorophyll Nalgene bottles with DI water in between samples
14. Keep on dry ice until samples are brought back to Bodega Marine Lab to be frozen
15. Samples can be frozen for up to 2 months before degradation

*Task Breakdown*

<i>Task</i>	<i>Number of People</i>	<i>Notes</i>
CTD Casting	1-2	Must be done prior to each set of water sample collections
Niskin bottle deployment	1-2	Collect these samples as close together in time as possible
pH and alkalinity bottle filling	1-2	Try to conserve as much sample water as possible
pH sample poisoning	1	Can be done after all 4 samples have been collected <b>*Kristen will do this</b>
Chlorophyll filtering	2	Can be done while other tasks are going on
Phytoplankton sampling	1 (?)	TBD

## Appendix 2.

Summary of papers with ocean acidification effects on ecologically or economically relevant kelp forest species, organized roughly by phyla:

### *Mollusca*

A study by Gaylord et al. (2011) examined the effects of projected pCO<sub>2</sub> levels in 2100 compared to conditions measured in 2011 on the mechanical integrity of shells of *Mytilus californianus*, a kelp inhabiting mussel. Embryos and larvae were exposed to pCO<sub>2</sub> conditions at 380 ppm, 540 ppm, and 970 ppm. Shell integrity was measured in the middle of the larval period and immediately after settlement (5 and 8 days after fertilization, respectively). On day 5 after fertilization, Gaylord et al. (2011) found that shells were 13% weaker in the 540 ppm condition compared to the 380 ppm conditions. Shells exposed to 970 ppm were 20% weaker and 7% smaller. After 8 days, shells in 540 ppm were 12% weaker and 15% thinner, while shells in 970 ppm were 15% weaker, 15% thinner, and 5% smaller. These results show that ocean acidification may reduce mussels' ability to survive their juvenile stage and therefore expose them to high mortality rates.

### *Cnidaria*

Towanda et al. (2012) found that lowered pH had no negative effects on respiration or photosynthesis of the intertidal sea anemone *Anthopleura elegantissima*. Cloned pairs were maintained at pH 8.1 for a week and then put into two groups at different pH conditions: 8.1 and 7.3. After 6 weeks in the lower pH conditions, there were no significant changes seen in the body mass of the anemone, demonstrating that it can survive in hypercapnic conditions (elevated pCO<sub>2</sub> levels). In fact, photosynthesis increased in the elevated pCO<sub>2</sub> treatments. Intertidal organisms are regularly exposed to water with higher pCO<sub>2</sub> levels during low tide when waters are stagnant and evaporate quickly under the sun, which may explain why the *A. elegantissima* may be already adapted to large fluctuations in pH.

### *Echinoderma*

Reuter et al. (2010) found that low pH conditions decreased fertilization success by reducing sperm motility and gamete compatibility in *Strongylocentrotus franciscanus*, or the Red Sea Urchin. These urchins are economically important organisms, due to their prized gonads, a delicacy served in Japanese restaurants. Urchin eggs were exposed to pCO<sub>2</sub> treatments at 400 ppm (control), 800 ppm, and 1800 ppm (pH= 8.04, 7.81, and 7.55 respectively). Fertilization was allowed for thirty seconds, after which potassium chloride (KCl) salt was added to immobilize sperm. They found that increased pCO<sub>2</sub> resulted in decreased fertilization success. Fertilization Efficiency decreased 79% in the 800 ppm treatment and 89% in the 1800 ppm treatment. Efficiency of the egg block to polyspermy significantly decreased in the 1800 pm treatment.

Pan et al. (2015) studied the effects of ocean acidification on body length and protein synthesis in *Strongylocentrotus purpurulus*, the Purple Sea Urchin. Purple urchins are

ecologically relevant in kelp forests given that their historic overpopulation due to hunting of the main predators has led to major kelp loss along California's coasts (Pondella et al. 2013). Urchins exposed to 900 ppm CO<sub>2</sub> showed a 50% increase of protein synthesis and ion transport and a change in metabolism. This decrease in pH did not alter the types or amounts of protein that urchins normally produce. However, the mechanisms used by urchins to maintain the biochemical molecules needed for growth did show changes. The larvae exposed to 900 ppm needed to channel 84% of their metabolic resources toward protein synthesis and ion transport compared to 55% in the control group. This difference in allocation could limit the urchins' available energy to deal with environmental changes.

*Pisaster ochraceus*, or the purple sea star, has been identified as a keystone species, meaning that it has an ecological role of disproportionate importance. In order to test purple sea stars' reaction to ocean acidification, Gooding et al. (2009) compared responses of purple sea stars exposed to 780 ppm pCO<sub>2</sub> to a control group exposed to 380 ppm. Dry soft tissue, calcified tissue, and water make up the three components of sea star body mass. Overall, growth was not reduced in the high CO<sub>2</sub> treatments. This may be because sea stars, unlike many calcifying organisms, do not have a calciferous outer shell that can be a physical barrier to growth. There were no apparent harmful effects of increased CO<sub>2</sub> on growth or feeding, but it is unknown how lowered levels of calcification may affect sea stars over longer periods of time.

### *Osteichthyes*

Osteichthyes are a superclass of fish that have skeletons made up of bone tissue. Of the research on the behavioral, physiological, and metabolic responses of these 'bony fish,' we found specific studies on the OA responses of White Seabass, Yellowtail Amberjack, and 6 species of rockfish.

In a study by Checkley Jr. et al. (2009), *Atractoscion nobilis* (White Seabass) eggs and larvae were grown under 380, 993, or 2558  $\mu$ atm of CO<sub>2</sub>. After 7 days, otolith (ear bone) size was 7-9% larger under the 993  $\mu$ atm conditions and 15-17% larger under 2558  $\mu$ atm conditions. Although it is unknown whether or not large otolith have harmful effects on fish, asymmetry has been shown to be harmful (Gagliano et al. 2008).

In a study by Abbink et al. (2012), the growth, feed conversion, stress, and metabolic parameters in juvenile *Seriola lalandi*, or Yellowtail Amberjack, were tested at a pH of 6.58, 7.16 and 7.85. Appetites of Yellowtail Amberjacks in the pH 7.16 treatment were suppressed, but not as severely as those in the pH 6.58 treatment. Juvenile fish reared at pH 6.58 had a 92% lower survival rate compared to those from the two higher pH conditions. All fish in the pH 7.16 and pH 7.85 conditions survived. Growth rate, food intake, and food conversion were all found to be significantly lower in fish from the pH 6.58 condition. Fish also experienced physiological homeostasis disruptions and a reduction in blood plasma pH. Because many effects appeared at the extreme pH of 6.58 and many harmful effects were absent in the 7.16 treatment, the Yellowtail Amberjack appears to have a more robust resilience against OA compared to other

organisms.

Fennie et al. (2015) studied behavioral and physiological changes in 6 rockfish species native to California's kelp forests. The kelp, gopher, and copper rockfish (*Sebastes atrovirens*, *Sebastes carnatus*, *Sebastes caurinas*) inhabit the kelp canopy, while blue, stripetail and olive rockfish (*Sebastes mystinus*, *Sebastes saxicola*, *Sebastes serranoides*) are benthic. Over a 3-4 month period, the fish were exposed to 4 levels of CO<sub>2</sub>: 540 ppm, 820 ppm, 2100 ppm, and 3300 ppm. Two canopy species suffered altered brain function, measured by altered behavioral lateralization and all three canopy species had a slower escape time from an escape chamber. The canopy rockfish also showed decreased swimming endurance. Conversely, benthic rockfish showed no significant changes in swimming speed or aerobic performance. This resistance to CO<sub>2</sub> impairments suggests that benthic rockfish may have a blood pH compensation mechanism, allowing them to adapt in elevated pCO<sub>2</sub> conditions. Benthic rockfish inhabit deeper water, and therefore experience higher pCO<sub>2</sub> during development. This early exposure to elevated CO<sub>2</sub> may be the reason for their resilience against CO<sub>2</sub> impairments.

The woolly sculpin, *Clinocottus analis*, is a fish that dwells on the intertidal seabed. Hancock and Place (2016) tested the hypoxia response of these bottom dwelling fish using the parameters of routine metabolic rate, elevated critical oxygen tension PO<sub>2crit</sub>, and Na<sup>+</sup>,K<sup>+</sup>-ATPase activity. After random assignment to either 400 (ambient) or 1100 (elevated) µatm following 7 days of acclimation, responses were measured. 7 days after treatment the woolly sculpin showed no differences in hypoxia tolerance between the high CO<sub>2</sub> group and the control group. After 28 days, however, there was a 33.6% increase in PO<sub>2crit</sub>, evidence that exposure to elevated level of CO<sub>2</sub> caused a decrease in hypoxia tolerance.

#### *Class Florideae*

Kuffner et al. (2008) studied the effects of OA on the recruitment rate and growth of *Corallinaceae Crustose* (coralline algae), a type of red, calcareous algae. In this study, 6 mesocosms of seawater were placed near a coral reef in Hawaii, though these organisms are generalists and are found in most marine ecosystems, including kelp forests. Three were adjusted to have seawater pCO<sub>2</sub> levels of 400 µatm for a control group, while the other half were set at 765 µatm pCO<sub>2</sub>. For 7 weeks, the recruitment rate and growth of coralline algae were measured once a week. In the high pCO<sub>2</sub> mesocosm, coralline algae recruitment rate decreased by 78% and the cover decreased by 92%. The high pCO<sub>2</sub> treatment also had a 52% increase in non-calcifying algae. This indicates that there could be changes in the type of algal communities present in future OA conditions.

#### *Class Phaeophyceae*

*Nereocystis luetkeana*, or Bull Kelp, is the second most common type of kelp off the coast of California. In a study by Swanson and Fox (2007), bull kelp was exposed to 360 ppm (control) and 3000 ppm (experimental group) for 55 days. Changes in phlorotannin content,

biomass, herbivory, and decay were assessed. There was over 3x as much growth in the high pCO<sub>2</sub> treatment, suggesting that bull kelp may be adaptive to elevated pCO<sub>2</sub> levels. There were also increases in phlorotannin content and biomass. There was no effect on herbivory, but there was a decrease in decay due to elevated levels of pCO<sub>2</sub>, which may affect nutrient cycling.

*Macrocystis pyrifera*, or Giant Kelp, makes up much of the kelp forests along southern California's coast. Roleda et al. (2011) studied alterations in meiospore germination and sex determination due to elevated CO<sub>2</sub> levels using three experiments. The first tested giant kelp in a control 190 μatm, and various levels of pCO<sub>2</sub>: 208, 1140, 1250, 117, and 125 μatm in succession. The second experiment used a baseline of 280 μatm and measured responses after exposure to 280, 1080, 1230, 1320, 133, and 152 μatm. In experiments 1 and 2, the number of germinated, non-germinated, and dead spores were counted after 5 days. Kelp was put in 270, 830, 1200 μatm in the third experiment and measured after 7 days. pH was adjusted in each experiment every 3-4 days. The kelp meiospore germination in present day levels of pCO<sub>2</sub> (70-85%) were similar to germination percentages of meiospore exposed to preindustrial pCO<sub>2</sub>. Lowered pH caused decreased germination, but added DIC had the opposite effect, indicating that pCO<sub>2</sub> at lower pH alleviated physiological stress and caused no sex determination effect. Growth remained positive under high acidification conditions. Gametophytes were 32% larger in the moderate 830μatm treatment and 10% larger in the extreme 1200μatm treatment when compared to the baseline at 270μatm. There was a 6-9% reduction in germ cells by adult kelp in extreme pCO<sub>2</sub> levels, indicating that giant kelp are relatively resistant to OA impediments.

Research has also indicated that Giant Kelp spores, reproductive units, are negatively affected by the combination of high temperatures and CO<sub>2</sub> concentrations (Gaitán-Espitia et al. 2014). If there is a continued increase in temperature and acidification, there may not be much kelp that reaches maturity, the stage when it is most capable of carbon storage. Gaitán-Espitia et al. (2014) found a general increase in the number of dead spores at elevated temperatures. Kelp spores can tolerate a temperature up to about 18° C, at which point many fail to germinate. The harmful effects of high temperature on spores were exacerbated by high CO<sub>2</sub>. Low germination was also found at high levels of CO<sub>2</sub> (subsequently high acidity and low pH ~ 7.5). Like many organisms in early life stages, kelp spores are especially vulnerable, possibly because they do not have cell wall. They are less capable of protecting themselves under environmental stressors. Under high temperatures/lower CO<sub>2</sub> levels kelp spores entered a dormant stage, similar to the stage they enter during upwelling events that often occur on the California coast. This is a common response for spores during less than ideal situations which prevents them from germinating until environmental conditions are more preferable. This study shows that there exists a limit to the spore's ability to stay alive in harsh conditions.

### **Appendix 3.**

#### UCLA AOS Zodiac & CO<sub>2</sub>-Pro Sampling Coordinates

##### Nearshore:

1. 33.762902°N, -118.423319°W
2. 33.760601°N, -118.421461°W
3. 33.758322°N, -118.419645°W
4. 33.7560950°N, -118.4177390°W

##### Offshore:

5. 33.761688°N, -118.42448°W
6. 33.759372°N, -118.422666°W
7. 33.75707°N, -118.42085°W (control site)
8. 33.754762°N, -118.419041°W