

Experimental assessment of the impacts of ocean acidification and urchin grazing on benthic kelp forest assemblages

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ABSTRACT

Ocean acidification (OA) is likely to differentially affect the biology and physiology of calcifying and non-calcifying taxa, thereby potentially altering key ecological interactions (e.g., facilitation, competition, predation) in ways that are difficult to predict from single-species experiments. We used a two-factor experimental design to investigate how multispecies benthic assemblages in southern California kelp forests respond to OA and grazing by the purple sea urchin, *Strongylocentrotus purpuratus*. Settlement tiles accrued natural mixed assemblages of algae and invertebrates in a kelp forest off San Diego, CA for one year before being exposed to OA and grazing in a laboratory experiment for two months. Space occupying organisms were identified and pooled into six functional groups: calcified invertebrates, non-calcified invertebrates, calcified algae, fleshy algae, sediment, and bare space for subsequent analyses of community structure. Interestingly, communities that developed on separate tile racks were unique, despite being deployed close in space, and further changes in community structure in response to OA and grazing depended on this initial community state. On Rack 1, we found significant effects of both $p\text{CO}_2$ and grazing with elevated $p\text{CO}_2$ increasing cover of fleshy algae, but sea urchin grazers decreasing cover of fleshy algae. On Rack 2, we found a ~ 35% higher percent cover of sediment on tiles reared in ambient $p\text{CO}_2$ but observed ~27% higher cover of bare space in the high $p\text{CO}_2$ conditions. On Rack 3, we found an average of 45% lower percent cover of calcified sessile invertebrates at ambient $p\text{CO}_2$ than in high $p\text{CO}_2$ treatments on Rack 3. Net community calcification was 137% lower in elevated $p\text{CO}_2$ treatments. Kelp sporophyte densities on tiles without urchins were 74% higher than on tiles with urchins and kelp densities were highest in the elevated $p\text{CO}_2$ treatment. Urchin growth and grazing rates were 49% and 126% higher under ambient than high $p\text{CO}_2$ conditions. This study highlights consistent negative impacts of OA on community processes such as calcification and grazing rates, even though impacts on community structure were highly context-dependent.

1. Introduction

Ocean acidification (OA) is likely to impact marine organisms globally, resulting in significant changes to marine ecosystems. Despite our understanding of how OA impacts single species (Kroeker et al., 2010; Harley et al., 2012), scaling up impacts from organism to ecosystem is challenging due to numerous interactions (e.g.

competition, predation, etc.) that occur both within and between species. In order to more accurately predict the consequences of OA on ecosystems, it is therefore important to explore the effects of OA on intact species assemblages.

Environmental drivers such as seawater pH, temperature, and nutrient concentrations can influence the structure and diversity of benthic communities (Schiel et al., 2004; Connell and Russell, 2010;

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Hale et al., 2011; Kroeker et al., 2011; Sunday et al., 2016; Teixidó et al., 2018). Shifts from communities dominated by calcified taxa to communities dominated by fleshy taxa have been documented under OA-like conditions (Hoegh-Guldberg et al., 2007; Hall-Spencer et al., 2008; Russell et al., 2009; Kroeker et al., 2012; Fabricius et al., 2015; Agostini et al., 2018) and even across natural present-day gradients in pH (Price et al., 2012). These studies suggest that changes in ocean chemistry can have dramatic impacts on the structure and function of benthic communities.

Biotic interactions, such as herbivory, play an important role in structuring marine communities (Paine and Vadas, 1969; Breitburg, 1984; Harrold and Reed, 1985; Watanabe and Harrold, 1991; Byrnes et al., 2013). For instance, overgrazing by sea urchins can cause phase shifts from kelp forests to urchin barrens (Steneck et al., 2002). On Caribbean coral reefs, the mass die-off of black sea urchins, *Diadema antillarum*, coupled with historical overfishing of herbivorous fish led to a shift from tropical reefs dominated by corals to reefs dominated by macroalgae (Hughes, 1994). Therefore, changes in the magnitude of grazing by sea urchins, whether positive or negative, could have major consequences for marine ecosystems. Furthermore, Provost et al. (2016) found that within a large scale mesocosm experiment, changes to the strengths of multiple trophic (e.g. grazing, predation) and competitive interactions in response to ocean warming and OA can reduce kelp forest integrity and have the potential to negatively impact kelp forest resilience. Similarly, a combination of intense warming and the loss of sea urchin predators, such as the sunflower star (*Pycnododia helianthoides*) due to sea star wasting disease, resulted in a > 90% reduction in the kelp forest canopy and shifts to urchin barrens along the northern coast of California (Rogers-Bennett and Catton, 2019). However, it is still not clear how OA will combine with biotic interactions, such as grazing, to alter diverse species assemblages.

The combined effects of OA and grazing are likely to be complex. Many grazers build protective shells or skeletons by precipitating calcium carbonate and are therefore likely to be directly impacted by OA. Current research suggests that growth and calcification of numerous grazing taxa (e.g. Echinodermata, Gastropoda) are often reduced under acidified conditions (Dupont et al., 2010; Kroeker et al., 2010; Gazeau et al., 2013). The direct impacts of OA on grazing rates, however, appear to be more complicated. Studies assessing the impacts of OA on grazing rates have returned variable results. For instance, some studies have found that OA decreases consumption rates of seaweeds, either directly (Russell et al., 2013) or indirectly through changes in the palatability of resources (Poore et al., 2013). Conversely, other studies have shown increases in consumption under OA, potentially as a consequence of decreased nutritional content of algal resources (Falkenberg et al., 2013a, 2013b) or through compensation as primary production concurrently increases under OA (Ghedini et al., 2015). Thus, the responses of grazers to OA are likely nuanced and may even be system specific.

Kelp forests that dominate subtidal rocky habitats within the California Current System (CCS) have received relatively little attention with regard to their response to future environmental change (but there is some individual species work e.g. Brown et al., 2014). These ecosystems, however, are some of the most diverse and productive on the planet (Steneck et al., 2002). OA is predicted to progress rapidly within the CCS (Chan et al., 2017) due to the decreased ability to buffer the chemical changes induced by additional anthropogenic CO₂ in already acidic seawater (Frankignoulle, 1994; Gruber et al., 2012). Therefore, kelp forest ecosystems within the CCS may be at increased risk from the threats of OA.

The goals of this study were to elucidate the impacts of future OA and grazing on kelp forest communities in southern California by addressing the following questions: 1) How do benthic rocky reef community assemblages respond to experimentally induced OA in the presence and absence of sea urchin grazers? and, 2) How will OA alter sea urchin grazer growth and consumption? We hypothesized that the cover and

biomass of calcified taxa would decrease following exposure to high pCO₂, but increase in the presence of grazers. In addition, we hypothesized that cover and biomass of non-calcified taxa within these communities would increase in high pCO₂, but decrease in the presence of grazers. We also hypothesized that sea urchin growth and grazing rates would be reduced in high pCO₂ conditions and these altered grazing rates would explain differences in fleshy macroalgal cover between low and high pCO₂ treatments in the presence of urchins. A better understanding of the combined effects of OA and grazing on intact species assemblages will improve our ability to predict the emergent effects of OA on kelp forest ecosystems.

2. Materials and methods

2.1. Study sites

We employed a manipulative factorial experiment assessing the effects of OA and herbivory on benthic community structure from natural assemblages that developed on settlement tiles for one year in situ. To achieve natural communities, we installed three racks of settlement tiles elevated slightly off the seafloor, each with 18 individual 100 cm² sanded PVC tiles. Tile racks were installed at 14 m depth and separated by 5–10 m within the kelp forest, at Mia's Reef in San Diego County (N 32° 51' 14.8", W 117° 16' 52.4") on August 2, 2012 (Fig. S1). Tiles were uncaged in order to allow access to the natural community of predators and herbivores on the reef. Mia's Reef, similar to other southern California kelp forests, is characterized by limestone that is fragmented into large boulders (Dayton, 1985). The main habitat-forming species is *Macrocystis pyrifera* with an understory community dominated by low lying fleshy red and brown macroalgae, calcified red algae, bryozoans and sponges. Purple sea urchins, *Strongylocentrotus purpuratus*, bat stars, *Patiria miniata*, and various benthic fish species (e.g. *Hypsypops rubicundus*, *Semicossyphus pulcher*, *Oxyjulis californica*) were commonly seen at the study site. A large terrace provided continuous bathymetry in which to deploy settlement tile arrays under the kelp canopy and among the understory community.

2.2. Experimental system

On September 20, 2013, we retrieved tiles ($n = 40$; 14 tiles were left on the reef for a separate study) and transported them in seawater to the laboratory at Scripps Institution of Oceanography (SIO). We cleaned tiles of all mobile invertebrates (e.g. small crabs, limpets) using forceps and then randomly placed tiles into individual square 1.5 L glass containers (mesocosms) within a water table. We supplied tiles with ambient flow-through seawater and light ($\sim 30 \mu\text{mol m}^{-2} \text{s}^{-1}$) for one week to acclimate to laboratory conditions. We collected juvenile purple sea urchin grazers, *Strongylocentrotus purpuratus*, (test diameter ~ 1.5 cm) from the Point Loma kelp forest during the week of September 23, 2013. Prior to their use as experimental grazers, we held sea urchins in ambient flow-through aquaria and fed them fronds of *M. pyrifera* ad libitum.

We conducted all laboratory experiments in a flow-through seawater system at SIO (for description see Kram et al., 2015). We bubbled an air or CO₂-gas mixture into individual mesocosms continuously supplied with flow-through seawater; temperature was allowed to vary naturally in this system. Ambient pH conditions in our experiment (pH = 8.00, Table 1) were similar to mean pH conditions within the La Jolla kelp forest (pH ~ 7.95 , Takeshita et al., 2015). We manipulated high pCO₂ (low pH) conditions by bubbling an air/CO₂ blend at a rate that lowered the seawater pH by 0.4 (± 0.05 SE) units below ambient (Table 1). This treatment pH was chosen based on the IPCC's Representative Concentration Pathway (RCP) 8.5 projected conditions for the year 2100 (IPCC, 2014). We created ambient pCO₂ conditions by bubbling air at the same rate as the high pCO₂ treatment mesocosms. We used a hand-held pH meter (HACH HQ40d Portable pH meter) with a glass electrode pH

Table 1

Mean environmental conditions within experimental mesocosms for the duration of the 56-day experiment (± 1 SE). Discrete samples from mesocosms both with and without tiles were pooled within treatments.

Trt	N	Temp ($^{\circ}$ C)	pCO ₂ (μ atm)	pH _{SW}	[CO ₂] (μ mol kg ⁻¹ SW)	[HCO ₃ ⁻] (μ mol kg ⁻¹ SW)	[CO ₃ ²⁻] (μ mol kg ⁻¹ SW)	Ω_{Calcite}	$\Omega_{\text{Aragonite}}$	Irradiance (μ mol m ⁻² s ⁻¹)
Ambient pCO ₂	12	13.15 \pm 0.08	443.65 \pm 8.92	8.00 \pm 0.01	17.67 \pm 0.37	1917.10 \pm 4.87	129.21 \pm 2.09	3.11 \pm 0.05	1.98 \pm 0.03	30.60 \pm 3.12
High pCO ₂	14	13.03 \pm 0.03	1569.38 \pm 230.05	7.53 \pm 0.04	62.71 \pm 9.17	2113.63 \pm 8.65	50.27 \pm 3.488	1.21 \pm 0.084	0.77 \pm 0.053	30.47 \pm 2.80

probe (HACH, PCH201) to measure pH and temperature daily at midday (12:00–13:00) in each of the experimental mesocosms and calibrated the glass electrode pH probe daily with certified Tris buffer from the Dickson laboratory at SIO. Two distinct pCO₂ conditions were thus maintained for the entirety of the experiment (ambient pCO₂ = 443 μ atm and high pCO₂ = 1569 μ atm; Table 1) from October 7, 2013 until December 3, 2013. Light levels were monitored within all mesocosms using a hand-held PAR sensor (QSL-2200, Biospherical Instruments) once a week at midday (12:00–13:00; Table 1). Mesocosm location within the water table was rotated every two weeks for the duration of the experiment to minimize the effects of minor differences in light and flow across the water table.

We added juvenile *S. purpuratus* individuals ($n = 1$ per tile) to half of the mesocosms as grazers to yield a total of four factorial treatments (ambient pCO₂ - grazer, ambient pCO₂ + grazer, high pCO₂ - grazer, high pCO₂ + grazer). Although sea urchin densities are generally lower in nature than those used in this experiment (here 1 urchin/ 0.01m²), they do approach similar densities during the formation and persistence of urchin barrens (Byrnes et al., 2013). In addition, we used small juvenile urchins, which have a much lower per capita grazing impact than larger adult urchins (Sala and Graham, 2002). Three or four tiles were randomly selected from each of the 3 racks ($n = 10$ tiles per treatment) and these were reared for ~two months in each of the four factorial treatment conditions. Four control mesocosms were maintained without tiles or grazers at both ambient and high pCO₂ to evaluate the effects of biological processes by the organisms on the pCO₂ levels in each of the treatments.

We collected discrete water samples in 500 mL Corning brand sample bottles from four or five randomly selected mesocosms per pH treatment at the beginning, after 1 month, and at the end of the experiment. After collection, we immediately spiked discrete samples with 240 L of HgCl₂ solution. We measured pH with a spectrophotometer (Shimadzu, UV-1800) and total alkalinity using open-cell titration on triplicate samples (Metrohm, 905 Titrando) following standard protocols (Dickson et al., 2007). We calculated salinity from density using a densimeter (Mettler Toledo, DX45). We calculated carbonate chemistry parameters (Table 1) based on measured pH, total alkalinity, salinity, in situ temperature and pressure using CO2SYS (Pierrot et al., 2006) with stoichiometric dissociation constants defined by Mehrbach et al. (1973) and refit by Dickson and Millero (1987).

2.3. Community structure

To examine the emergent effects of OA and grazing on kelp forest communities, we photographed all tiles at the start of the experiment and visually examined tiles using a mini quadrat at the end of the experiment. We imported photographic images into Coral Point Count with Excel extensions (CPe V4.1, National Coral Reef Institute) and overlaid a 10 \times 10 point grid over each photograph. We classified the space-occupying organism underlying the crosshairs at each grid intersection into one of six functional groups: calcified invertebrates (bryozoans, barnacles and serpulid worms), non-calcified invertebrates (sponges, anemones and tunicates), calcified algae, fleshy algae, sediment, and bare space for subsequent statistical analyses. Rapid growth of fleshy algal species during the experiment made it difficult to discern

space-occupying organisms from photographs at the end of the experimental period. Therefore, at the end of the experiment we placed tiles in 4% formalin in seawater to preserve specimens for subsequent analysis of community structure. We visualized fixed tiles under a dissecting scope, placed a 10 \times 10 gridded mini quadrat over each tile, and identified the space-occupying organism underlying the crosshairs at each grid intersection to functional group (i.e. calcified invertebrates (bryozoans, barnacles and serpulid worms), non-calcified invertebrates (sponges, anemones and tunicates), calcified algae, fleshy algae, sediment, and bare space) for subsequent statistical analyses. Due to the methodological differences in calculating community structure initially and at the end of the experiment, we were unable to directly compare the initial and final community states on each tile. Therefore, we did separate analyses to test for differences in initial communities among racks and then final communities in response to the OA and grazing treatments in the lab.

2.4. Kelp sporophyte density

Our analysis of community composition only took into account the space-occupying organism on the tile, yet some macroalgae, such as kelp (Order Laminariales), have small holdfasts compared to their biomass in the water column and therefore may be underrepresented in the point intercept analyses used above. Thus, due to their important role as habitat forming species in these ecosystems, at the end of the experiment, we removed all juvenile kelp sporophytes (primarily *Macrocystis pyrifera*), and counted the individuals per tile to obtain a density.

2.5. Net community calcification

At the beginning and end of the experiment, we weighed all tiles using the buoyant weight technique (Davies, 1989) to quantify net community calcification (NCC) on tiles from each of the four treatments. Before being placed in treatment conditions and again at the end of the experiment, we also buoyant weighed sea urchin grazers and measured test diameter to the nearest 0.01 mm using calipers. We calculated percent change in buoyant weight day⁻¹ of tiles and sea urchins and sea urchin test diameter as, $\frac{W_f - W_i}{W_i} \cdot \frac{1}{d}$, where W_i is the initial weight (or diameter), W_f is the final weight (or diameter) and d is the experimental duration in days.

2.6. Sea urchin growth and grazing

After 56 days in experimental conditions, we measured differences in the grazing rates of sea urchins following exposure to ambient and elevated pCO₂ levels. We starved sea urchins for three days in individual mesocosms supplied with treatment seawater (but without tiles). After the starvation period, we presented sea urchins with a single pre-weighed (wet weight) kelp frond collected from the field the morning of the grazing trial. We also placed kelp fronds in mesocosms ($n = 3$) without urchins to control for any changes in wet weight in the absence of grazing. Urchins were allowed to graze on kelp for 24 h before the kelp was removed and reweighed. We calculated the per capita sea urchin grazing rate as the biomass of kelp removed per day per gram of buoyant weight of sea urchin, corrected for changes in biomass gained in

the controls. We calculated grazing rate of sea urchins as, $\frac{(W_{ki} - W_{kf}) - (W_{ci} - W_{cf})}{W_u}$, where W_{ki} is the initial wet weight of kelp, W_{kf} is the final wet weight of kelp, W_{ci} is the initial wet weight of controls (ambient or elevated $p\text{CO}_2$), W_{cf} is the final wet weight of controls, W_u is the buoyant weight of the urchin.

2.7. Statistical analyses

In order to assess differences in community composition on tiles initially and in response to OA and grazing, we first square-root transformed the initial and final percent cover data for each functional group and calculated Bray-Curtis resemblance matrices from transformed community data, before conducting additional multivariate statistical tests. When functional groups in which two or more samples contained a value of zero, thus precluding the calculation of Bray-Curtis dissimilarities, we added a dummy variable = 1 (Clarke et al., 2006). To visualize the similarities and differences between treatments in multidimensional space, we constructed non-metric multidimensional scaling (nMDS) plots from resemblance matrices. To test for differences among the tile racks in the initial community composition, we ran Permutational Multivariate Analysis of Variance (PERMANOVA) on initial resemblance matrices using 9999 permutations with the fixed factor of rack.

To test for differences in the final community composition in response to OA and grazing, we ran a two-factor PERMANOVA on final resemblance matrices using 9999 permutations with $p\text{CO}_2$ treatment and sea urchin presence as fixed factors and "rack" ($n = 3$) as a random factor. We used a factor of rack to control for any differences in the initial communities due to microhabitat and settlement variability in the kelp forest. Where we found a significant rack effect in the PERMANOVA results ($P_{\text{perm}} < 0.05$), we ran two-way PERMANOVA's separately on community structure data from each rack with fixed factors of $p\text{CO}_2$ and sea urchin grazing. We chose not to adjust for multiple comparisons as recommended in Moran (2003) given the difficulties in finding significance within highly variable and diverse communities with low samples size. We removed highly non-significant interactions ($P > 0.25$) from statistical models. All permutation-based analyses were conducted in R using *adonis* within the *vegan* package (Oksanen et al., 2020).

To test for differences in the density of kelp sporophytes on tiles as a function of the treatments, we conducted a generalized linear mixed-effects model with log link function and Poisson distribution using *glmer* in the *lme4* package in R (Bates et al., 2015) with $p\text{CO}_2$ and grazer presence as fixed effects and rack as a random factor. To test for differences in NCC, we conducted linear mixed effects models with $p\text{CO}_2$ and grazer presence as fixed effects and rack as a random factor. We used *t*-tests to compare differences between initial mean sea urchin buoyant weight and sea urchin test diameter between ambient and high $p\text{CO}_2$ grazing assays. We compared sea urchin grazing, calcification, and growth between urchins exposed to high $p\text{CO}_2$ or ambient $p\text{CO}_2$ using *t*-tests. All *t*-tests were run in R (R Development Core Team, version 1.2.5033).

3. Results

3.1. Experimental conditions

Two distinct $p\text{CO}_2$ treatments were maintained for the duration of the 56-day experiment with no effect of the tiles on chemistry within the mesocosms (Table 1; Table S1). High $p\text{CO}_2$ treatments were $\sim 1125 \mu\text{atm}$ higher (~ 0.47 pH units lower) than ambient $p\text{CO}_2$ treatments. Ambient seawater temperatures in all mesocosms were approximately 13°C for the duration of the experiment. Mid-day or peak (12:00–13:00) irradiance was $\sim 30.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ across all mesocosms, which was similar to light levels experienced in the field (Fig. S2).

3.2. Community structure

Initially, tile communities were covered with calcified and non-calcified invertebrates, calcified and fleshy algae, sediment, and areas of bare space suggesting they were at an earlier stage of development (Fig. 1; Fig. S3). We found that racks 1 and 2, and 2 and 3 differed significantly in initial community structure (Table S2). Racks 1 and 3 had higher abundances of calcified and non-calcified invertebrates. Rack 2 had higher abundances of sediment and bare space (Table S2; Fig. S3).

Following two months of exposure to the experimental treatments, tile communities were still covered with calcified and non-calcified invertebrates, calcified and fleshy algae, and still maintained areas of sediment and bare space (Fig. 2). We found a marginally non-significant effect of $p\text{CO}_2$ on community structure ($P = 0.091$) and no effect of sea urchin grazing or the interaction of $p\text{CO}_2 \times$ grazing (Table 2; Fig. 3D). However, there was also a significant effect of tile rack on community structure ($P = 0.001$), indicating that the final community structure depended on the structure of the initial assemblage.

Due to the significant tile rack effect and marginally non-significant $p\text{CO}_2$ effect, separate PERMANOVAs were run on all three racks. In this analysis, we found significant main effects of $p\text{CO}_2$ and sea urchin grazing on community structure on Rack 1 (Table 2; Fig. 3A), after dropping the non-significant interaction term from the model. The species assemblages on Rack 1 tended to cluster together in multivariate space as a function of the individual treatment. On Rack 2, there was a significant effect of $p\text{CO}_2$ on community structure, but no effects of urchin grazing (Table 2; Fig. 3B). Tiles from the ambient $p\text{CO}_2$ treatments clustered together in multivariate space, while tiles from the high $p\text{CO}_2$ treatments grouped separately. There were no significant differences in community structure from Rack 3 when the interaction of $p\text{CO}_2 \times$ grazer was included in our model. Without the non-significant interaction, we detected significant $p\text{CO}_2$ effects on community structure (Table 2; Fig. 3C), such that tiles from the ambient $p\text{CO}_2$ treatments clustered separately from the tiles in the high $p\text{CO}_2$ treatments.

Further analyses conducted on the responses of individual functional groups elucidated which groups contributed to differences in community structure observed across treatments. On Rack 1, we found significant effects of both $p\text{CO}_2$ and grazing (Table S4; Fig. 4A). At ambient $p\text{CO}_2$, grazing reduced fleshy algal cover by 35%; however, at high $p\text{CO}_2$, fleshy algal cover was only reduced by 26% in the presence of grazers. On Rack 2, we found a $\sim 35\%$ higher percent cover of sediment on tiles reared in ambient $p\text{CO}_2$ but observed $\sim 27\%$ higher cover of bare space in the high $p\text{CO}_2$ conditions (Table S5; Fig. 4B). On average we found a 45% lower cover of calcified sessile invertebrates at ambient $p\text{CO}_2$ than in high $p\text{CO}_2$ treatments on Rack 3 (Table S6; Fig. 4C).

3.3. Kelp sporophyte density

No juvenile kelp sporophytes were visible on tiles at the outset of the experiment. However, juvenile kelp sporophytes were present on 22 of 40 tiles (across all racks) at the end of the experiment. There was a significant effect of $p\text{CO}_2$ on kelp density, with fewer kelp sporophytes present on tiles from the ambient $p\text{CO}_2$ treatments, but a marginally non-significant effect of sea urchin grazers on kelp density overall (Table 3). Instead, we found a significant interaction between $p\text{CO}_2$ and grazing, in which the grazing effect (i.e., difference in kelp density between +grazing and -grazing) was much stronger in the high $p\text{CO}_2$ treatment. In other words, $p\text{CO}_2$ increases kelp density, except in the presence of grazers (Fig. 5).

3.4. Net community calcification

Final buoyant weights of tiles from Mia's Reef, after 56 days in treatment conditions, were almost always lower than initial buoyant weights (i.e., a net loss of calcium carbonate) across all treatments over the duration of the experiment (Fig. 6). However, we observed a



Fig. 1. Photographic examples of settlement tiles from each rack array at the beginning of the experiment. Tiles are 100 cm².

significant reduction and a greater decline in the buoyant weight of organisms living on the tiles in high $p\text{CO}_2$ treatments relative to the ambient treatments (LMM, $p\text{CO}_2$: $t_{34} = -4.06$, $P < 0.0001$, urchin: $t_{34} = -1.26$, $P = 0.22$, $p\text{CO}_2 \times$ urchin: $t_{34} = -0.67$, $P = 0.51$). We did not detect a statistically significant effect of grazer presence on the change in buoyant weight, although tiles with grazers tended to have a greater mean reduction in buoyant weight relative to the non-grazer treatment from a given $p\text{CO}_2$ treatment (Fig. 6). Under ambient $p\text{CO}_2$, the presence of urchins decreased net community calcification by 24%. High $p\text{CO}_2$ led to a reduction in net community calcification relative to the control (ambient $p\text{CO}_2$, -urchin) by 58% in the absence of grazers and 68% in the presence of grazers. There was no significant interaction between $p\text{CO}_2$ and sea urchin grazing on buoyant weight.

3.5. Sea urchin growth and grazing

Initial mean sea urchin buoyant weight and sea urchin test diameter did not differ between treatments (t -test buoyant weight, $t_{18} = 0.26$, $P = 0.79$; t -test test diameter, $t_{18} = 0.17$, $P = 0.87$). However, after two months in treatment conditions, sea urchin growth measured as buoyant weight was 60% higher in ambient $p\text{CO}_2$ than at high $p\text{CO}_2$, while there was no detectable difference in the change in test size between treatments, suggesting that sea urchin tests became thinner under high $p\text{CO}_2$ conditions (t -test percent change in buoyant weight, $t_{17} = -2.20$, $P = 0.042$; Fig. 7a; t -test percent change in test diameter, $t_{17} = -1.03$, $P = 0.32$).

Results from the grazing trial showed significantly higher grazing rates in sea urchins exposed to ambient $p\text{CO}_2$ than high $p\text{CO}_2$ treatments (t -test, $t_{17} = 3.65$, $P = 0.002$; Fig. 7b). Kelp in the ambient CO_2 treatments lost mass as a result of sea urchin grazing, whereas kelp in the high CO_2 treatments gained mass, even in the presence of grazers.

4. Discussion

Ocean acidification is expected to have widespread impacts on marine ecosystems (Gaylord et al., 2015). Here, we show that kelp forest assemblages from southern California were negatively affected by OA. Acidification reduced net community calcification rates in naturally assembled kelp forest understory communities. OA and grazing by *S. purpuratus* altered community structure within the kelp forest assemblages, yet changes to the underlying functional groups were dependent on initial community composition. Grazing by sea urchins reduced the density of kelp sporophytes within our assemblages, but negative impacts of OA on growth and grazing rates of sea urchins suggests that grazing pressure may also shift in future ocean conditions.

The differences seen here highlight the importance of studying the effects of environmental change on intact species assemblages where the emergent effects of species interactions (e.g. competition, predation, facilitation) create naturally heterogeneous landscapes that have the potential to alter the outcomes of OA.

4.1. Community structure

Numerous studies have shown shifts from reefs dominated by calcifiers to reefs dominated by fleshy algal species under OA-like scenarios (Jokiel et al., 2008; Fabricius et al., 2011; Kroeker et al., 2012). The majority of these studies have focused, however, on coral reef communities where the dominant habitat forming species are calcifiers (i.e. scleractinian corals). We found that the $p\text{CO}_2$ and grazing effects on community structure were variable and dependent on the initial community composition. Since functional group taxa responded differently across racks, overall effects were masked when racks were pooled together. Notably, although there were no detectable differences in initial community composition on tiles from racks 1 and 3, the responses of functional group taxa to acidification and grazing varied between these two racks. Tiles from rack 1 exhibited significant effects of $p\text{CO}_2$ and grazing on fleshy macroalgae; the presence of herbivorous grazers reduced the percent cover of non-kelp fleshy macroalgae on tiles, while acidification increased percent cover. Both of these findings are consistent with the predicted outcomes of grazing or OA on fleshy seaweed species (Harley et al., 2012; Poore et al., 2012). Conversely, OA decreased cover of calcified invertebrates on rack 3 yet had no impacts on any other taxa, suggesting that percent cover alone may not determine the emergent effects of OA and grazing on kelp forest ecosystems. Instead, the biotic interactions among species may play a critical role in determining community dynamics in response to environmental change. On rack 2, decreases in sediment were compensated by increases in bare space on tiles reared under OA conditions. It is possible that the sediments on tiles were made of carbonates and were therefore more readily dissolved under acidic conditions. Interestingly, similar results were not seen on other tile assemblages, which suggests that differences in the percent cover of other functional group taxa may interact with sediment removal/dissolution.

All tile assemblages were obtained from the same reef outcrop (spaced approximately five meters away from each other and differing in depth by less than one meter) and experienced similar environmental conditions (e.g., light, temperature, pH, grazing pressure) before being exposed to ocean acidification and grazing in the laboratory. The importance of small-scale heterogeneity on reefs has been unstudied in the context of OA, yet it may play an important role in the persistence of

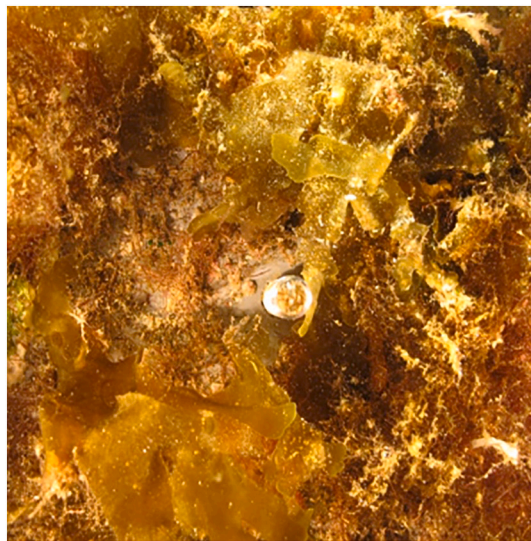
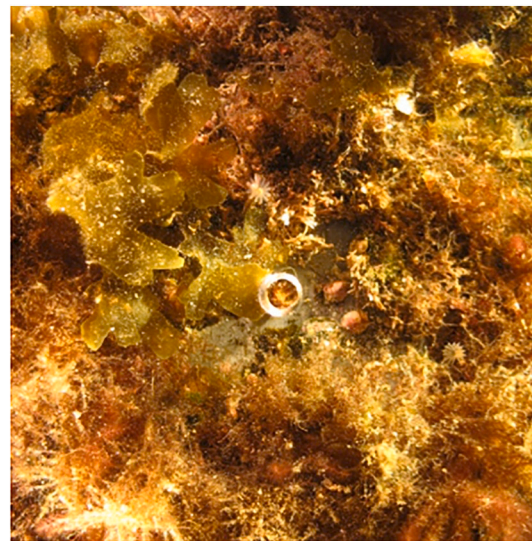
Ambient $p\text{CO}_2$, -grazerAmbient $p\text{CO}_2$, +grazerHigh $p\text{CO}_2$, -grazerHigh $p\text{CO}_2$, +grazer

Fig. 2. Photographic examples of settlement tiles with representative mixed algal and invertebrate communities in each of the four treatment conditions after 56 days. Tiles are 100 cm².

marine ecosystems. Initial differences in community composition were likely affected by microhabitat variation in environmental conditions, settlement, competition and growth of benthic species (Ferguson et al., 2013) and those differences in initial communities influenced the final response of the communities to OA and grazing. Cornwall et al. (2014) found that diffusive boundary layers (DBL) around algal assemblages could alter the effects of OA by decreasing flow and allowing pH to increase within the algal assemblage. DBL around an individual also has the potential to increase calcification due to pH buffering within the calcifying fluid, though benefits are species-specific (Comeau et al., 2019). Therefore, variability in not just the abundance, but also the species identity and spatial arrangement of organisms could change the response of the community to acidification. The potential for local buffering through photosynthesis has been shown at a much larger scale with highly productive photoautotrophic species capable of increasing the pH within their local environment (Anthony et al., 2013; Koweeck et al., 2018; Manzello et al., 2012; Nielsen et al., 2018). Even minor

differences in flow and light levels within the kelp forest could alter assembly processes (Edwards, 1998; Wernberg and Goldberg, 2008) and therefore lead to different species arrangements and subsequent responses to both OA and grazing.

Interest in the consequences of environmental change for kelp forest ecosystems has increased since recent studies have shown that early life history stages of kelp may be particularly vulnerable to high $p\text{CO}_2$ and temperature (Gaitán-Espitia et al., 2014). Early life history stages are likely to become increasingly important to the recovery of kelp forests in future oceans as disturbances that act to remove kelp plants become more frequent and intense (Byrnes et al., 2011). When first brought into the lab, no kelp thalli were visible to the naked eye on tile surfaces, suggesting that they were likely present at some microscopic stage (gametophyte or sporophyte) upon removal from the reef. OA increased the density of juvenile kelp sporophytes on tiles, but only in the absence of grazers. Shukla and Edwards (2017) found that sporophyte recruitment and microscopic growth were greater under elevated $p\text{CO}_2$

Table 2

PERMANOVA results of the effects of $p\text{CO}_2$ (ambient and high), sea urchins (presence and absence), and rack (1, 2 and 3) on community composition on settlement tiles, significant differences are in bold.

Source of variation	df	Mean squares	Pseudo-F	Pr(>F)
All				
$p\text{CO}_2$	1	0.027	2.127	0.091
Grazer	1	0.012	0.957	0.411
Rack	2	0.106	8.330	>0.001
Residuals	35	0.013		
Rack 1				
$p\text{CO}_2$	1	0.035	2.818	0.041
Grazer	1	0.034	2.702	0.046
Residuals	10	0.012		
Rack 2				
$p\text{CO}_2$	1	0.028	2.971	0.038
Grazer	1	0.003	0.271	0.884
Residuals	11	0.009		
Rack 3				
$p\text{CO}_2$	1	0.029	2.318	0.046
Grazer	1	0.006	0.526	0.779
Residuals	10	0.012		

compared to ambient, which may explain the elevated kelp densities in high $p\text{CO}_2$ conditions in our experiments. Other studies have shown either no effect of $p\text{CO}_2$ on microscopic early life history stages of *M. pyrifera* (Roleda et al., 2011) or decreased fitness in response to OA

(Gaitán-Espitia et al., 2014), which would be expected to result in either no differences or decreases in macroscopic sporophyte density. Importantly, recent work by Hollarsmith et al. (2020) showed significant variation in the performance of early life history stages of kelp in response to OA and temperature across populations, suggesting that local adaptation/acclimation could alter the outcome of environmental change. Furthermore, since the initial life history stage(s) and density of gametophytes and/or sporophytes in our study were unknown, it will be important to directly assess how impacts at various microscopic stages (i.e., settlement, fertilization, germination, sporophyte production) manifest at macroscopic stages to alter the landscape scale patterns of kelp forest recovery dynamics.

4.2. Community processes

Our experiment found that acidification significantly reduced net community calcification. Past studies on both coral reef and temperate algal communities have shown lower accretion rates of corals and coralline red algae under elevated $p\text{CO}_2$ (Hoegh-Guldberg et al., 2007; Jokiel et al., 2008; Hofmann et al., 2012; Albright et al., 2018). Decreases in calcification rates are likely due to a reduction in the availability of CO_3^{2-} , a necessary building block of biogenic carbonates. It is possible that the $p\text{CO}_2$ effect seen here is due to both reduced calcification, via decreased growth and thinner calcium carbonate skeletons, as well as increased dissolution of exposed CaCO_3 . The calcified taxa

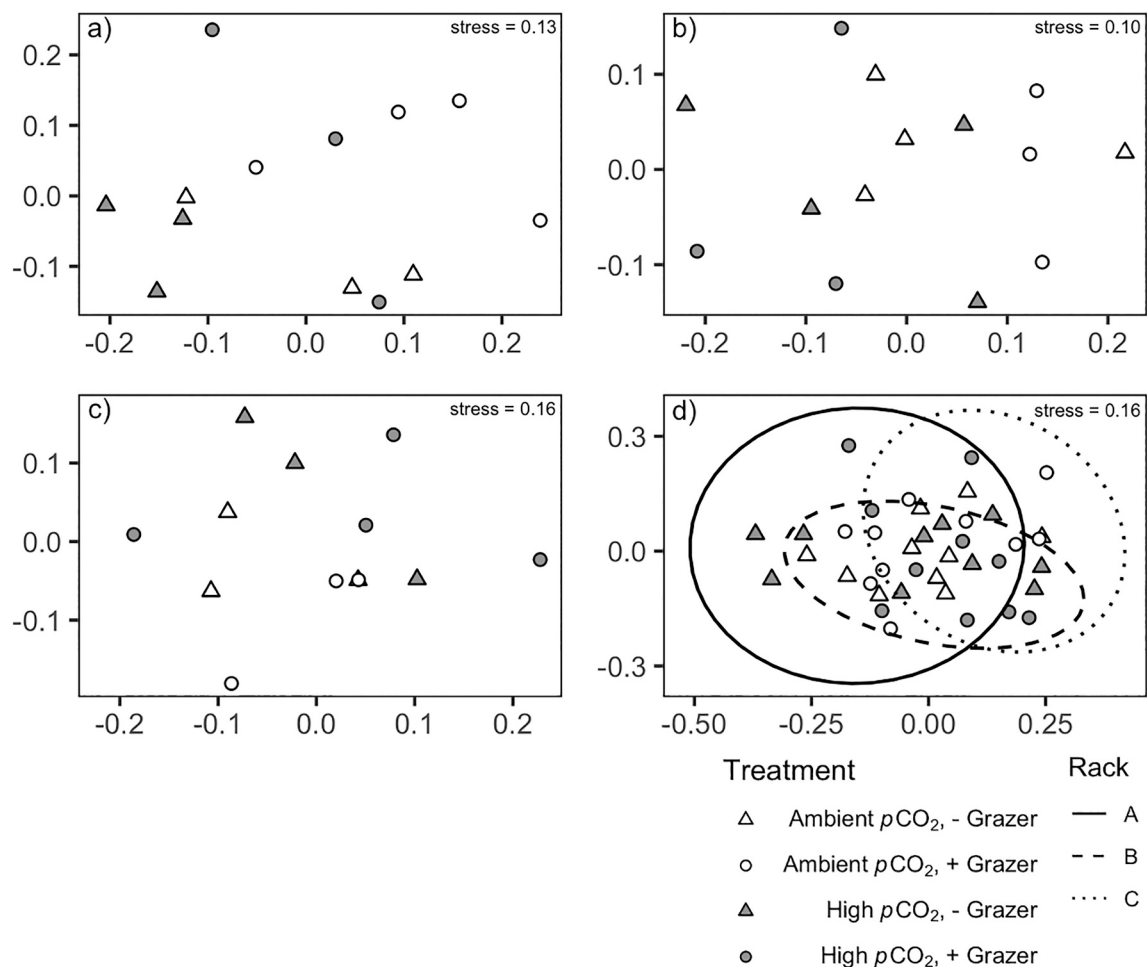


Fig. 3. nMDS plots showing similarities in community composition between settlement tile benthic assemblages reared in experimental $p\text{CO}_2$ (gray = high $p\text{CO}_2$, white = ambient $p\text{CO}_2$) and urchin grazing (triangles = +urchins, circles = -urchins) treatments. Points (tiles) closer together indicate communities more similar than points further apart. Panels a), b) and c) show nMDS plots of community data separated by racks 1–3 respectively, while panel d) shows all community data together on the same plot with 95% confidence ellipses around each rack.

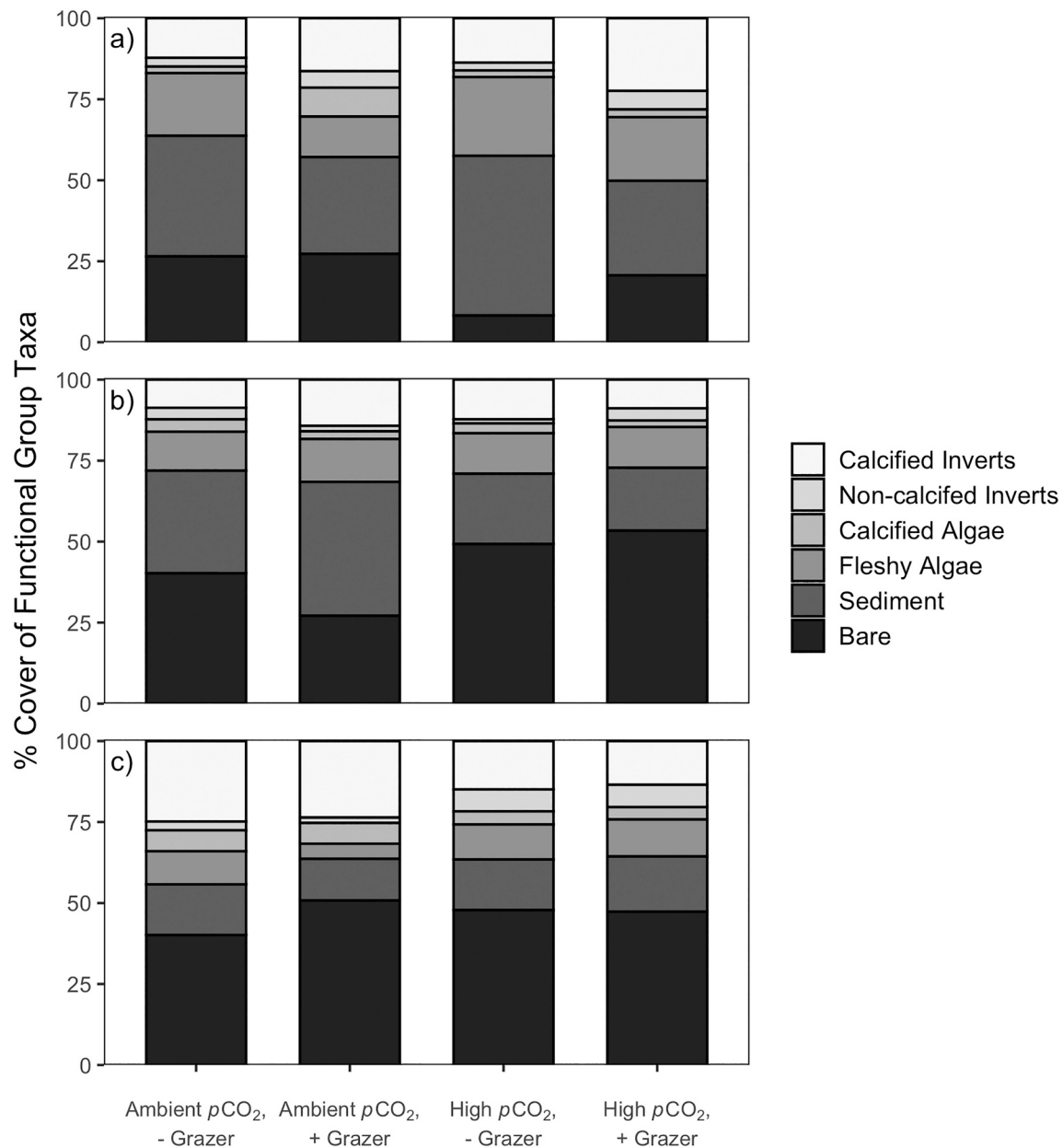


Fig. 4. Percent cover of functional group taxa on tiles by rack 1 (a), rack 2 (b), and rack 3 (c) after 56 days in experimental conditions.

Table 3

Results of generalized linear mixed model testing the effects of $p\text{CO}_2$ (ambient and high), sea urchins (presence and absence), and rack (1, 2 and 3) on the density of juvenile kelp on settlement tiles, significant differences are in bold.

Source of variation	n	z-value	P-value
Fixed effects			
$p\text{CO}_2$	40	3.354	0.001
Urchin	40	6.276	0.053
$p\text{CO}_2 \times \text{Urchin}$	40	2.337	0.028
	n	Variance	std dev
Random effect			
Rack	40	0.008	0.092

within our assemblages were primarily coralline red algae and bryozoans. Coralline algae and bryozoans are often negatively affected by acidification because they precipitate a more soluble form of calcium carbonate, magnesium calcite (Andersson et al., 2008; Taylor et al., 2014; McCoy and Kamenos, 2015). We did observe weight loss on tiles

across all treatments. This may be due to the lower irradiances in experimental conditions compared to in situ measurements shortly before the outset of the experiment (Fig. S2) or could be due to seasonal differences in calcification rates of organisms on the tiles. Regardless, despite the overall decrease in calcified biomass in all treatments, we found a significantly greater reduction on tiles reared under high $p\text{CO}_2$ conditions.

We found that sea urchin growth and grazing rates were significantly depressed after two months in high $p\text{CO}_2$ conditions. Russell et al. (2013) also found that the herbivorous gastropod, *Littorina littorea*, showed decreased consumption rates in high $p\text{CO}_2$ conditions. Brown et al. (2014) found an initial (after one month) decrease in consumption rates of *S. purpuratus* reared at low pH, yet no difference in grazing rates between *S. purpuratus* reared at ambient pH and low pH after two months. This result is particularly interesting since we did find decreased grazing rates after two months of exposure to OA conditions. Urchins from our study were collected from the same kelp forest as urchins in the Brown et al. (2014) study yet were approximately one third

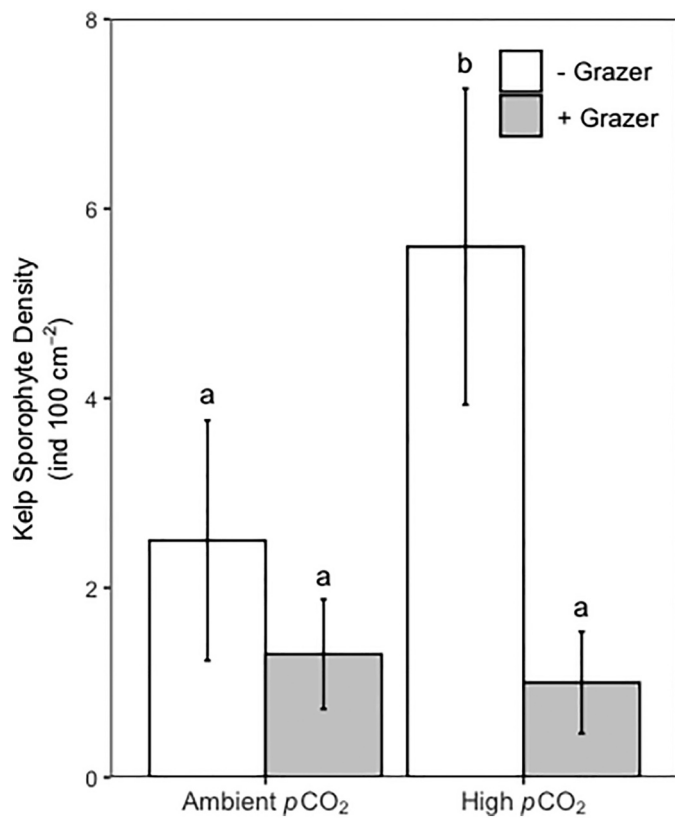


Fig. 5. Density of juvenile kelp on experimental tiles at the end of 56 days in different pCO₂ and urchin grazing treatments. Shared letters above error bars indicate mean density did not differ between treatments. Error bars denote ±1 SE.

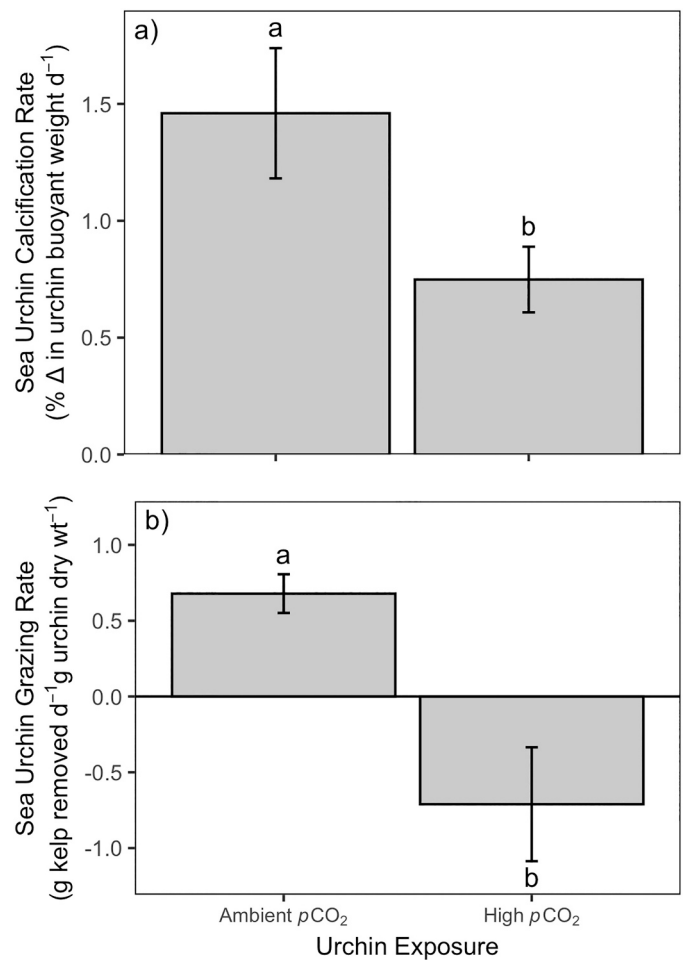


Fig. 7. Calcification rates a) of sea urchins reared on settlement tiles in ambient and high pCO₂ conditions, standardized to initial size; Grazing rates b) of sea urchins on *Macrocystis pyrifera* fronds after 56 days in experimental conditions. Shared letters indicate calcification rate or grazing rate did not differ between treatments. Error bars denote ±1 SE.

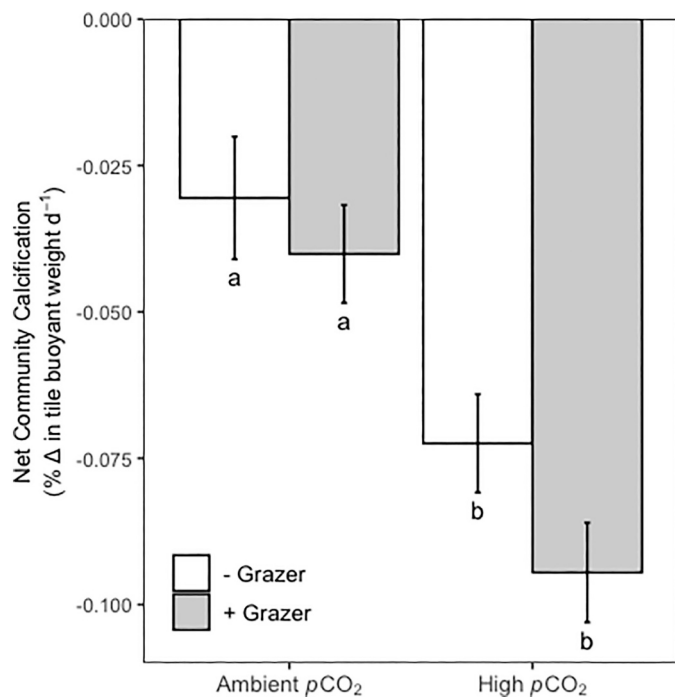


Fig. 6. Net community calcification on settlement tiles. Shared letters below error bars indicate mean % change in buoyant weight did not differ between treatments. Error bars denote ±1 SE.

the size (test diameter). It is possible that age and/or size class may play a role in species tolerance to OA; however, neither study used multiple size classes of urchins and therefore this cannot be tested explicitly. Interestingly, our study used fresh kelp collected the same day as the initiation of the grazing trial, whereas [Brown et al. \(2014\)](#) used kelp that had been reared in the same pH conditions as the urchin grazers, which may have influenced urchin grazing rates differently in the two studies. [Falkenberg et al. \(2013b\)](#) found increased consumption rates in the sea snail, *Austrocochlea concamerata* and [Ghedini et al. \(2015\)](#) found evidence for trophic compensation (increased consumption with increased growth of primary producers) in the gastropod *Turbo undulatus* under high pCO₂ conditions. The changes in consumption seen in these studies may be due to changes in the nutritional quality of the algae used in the assays ([Falkenberg et al., 2013a, 2013b](#)). It is possible that kelp also change their nutritional quality in response to acidification, which could further explain the differing results between our study and that of [Brown et al. \(2014\)](#). Given the variability in responses of grazers to OA, future research assessing both direct (i.e. physiological) and indirect (e.g. nutritional quality) effects of acidification on grazer species simultaneously are crucial since changes to the strength of algal-grazer interactions could alter community structure and ecosystem function.

Although grazing had few direct effects on community structure in our quadrat analyses, the density of juvenile kelp sporophytes in the presence of sea urchin grazers tended to be lower. Kelp forest grazers, such as *S. purpuratus*, graze upon both microscopic ([Sala and Graham,](#)

2002) and macroscopic stages of *M. pyrifera* and recent work by Ng and Micheli (2020) showed a reduction in the interaction strength of *S. purpuratus* and *M. pyrifera* microscopic stages in future climate change scenarios of reduced oxygen levels. Changes to sporophyte density in response to grazing would likely alter other physical properties of the environment. Alsterberg et al. (2013) found both direct and indirect effects of OA, warming, and grazers on benthic microalgae in a seagrass community. In the presence of grazers, OA and warming did not significantly affect benthic microalgae. In the absence of grazers, the direct effects of acidification and warming positively affected benthic microalgae, which then negatively affected benthic microalgae through the indirect effects of macroalgal shading. It is likely that the indirect effects of macroalgal shading and changes to flow regimes could interact with the acidification response of kelp forest benthic communities over longer timescales.

5. Conclusion

The impacts of ocean acidification on kelp forest ecosystems will likely vary at both local and regional scales. Here, we show differences in the susceptibility of kelp forest benthic assemblages to OA and the interactive effects of grazing over small spatial scales (<5 m). These findings suggest that small-scale heterogeneity could play an important role in the resilience of ecosystems in an increasingly acidic ocean. Yet, despite the nuanced effects of OA and grazing on community structure, the impacts on community processes, such as calcification and grazing rates, remain relatively robust. Additionally, recent work by Beas-Luna et al. (2020) suggests that warming (specifically marine heat waves) can have dramatic impacts on kelp forest community structure. In particular, the predictions with increased warming in southern CA are for kelp forests to shift from primary producers to more diverse consumer functional groups, which is likely to interact in complex ways with OA. Future research to assess the most important metrics for determining resilience to OA, as well as how multiple stressors interact (e.g. warming and OA) to affect kelp forest ecosystems, will be important in developing frameworks to predict future change.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jembe.2021.151548>.

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