

1 **Title:** Experimental assessment of the impacts of ocean acidification and urchin
2 grazing on benthic kelp forest assemblages

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4 **Running page head:** OA impacts on kelp communities

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Abstract

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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

43 $p\text{CO}_2$ than in high $p\text{CO}_2$ treatments on Rack 3. Net community calcification was
44 137% lower in elevated $p\text{CO}_2$ treatments. Kelp sporophyte densities on tiles
45 without urchins were 74% higher than on tiles with urchins and kelp densities
46 were highest in the elevated $p\text{CO}_2$ treatment. Urchin growth and grazing rates
47 were 49% and 126% higher under ambient than high $p\text{CO}_2$ conditions. This
48 study highlights consistent negative impacts of OA on community processes
49 such as calcification and grazing rates, even though impacts on community
50 structure were highly context-dependent.

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52 *Keywords:* acidification; community structure; kelp forest; sea urchins; grazing

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54 *Authors' contributions:*

55 **Emily Donham:** Conceptualization, Methodology, Resources, Investigation,

56 Formal analysis, Investigation, Writing – Original Draft. **Scott Hamilton:**

57 Conceptualization, Methodology Writing- Original Draft. **Nichole Price:**

58 Conceptualization, Methodology, Writing – Review & Editing. **Susan Kram:**

59 Resources, Investigation, Writing- Reviewing and Editing. **Emily Kelly:**

60 Resources, Investigation, Writing- Reviewing and Editing. **Maggie**

61 **Johnson:** Methodology, Writing- Reviewing and Editing. **Alexander Neu:**

62 Resources, Investigation, Writing- Reviewing and Editing, **Jennifer**

63 **Smith:** Conceptualization, Methodology, Writing- Reviewing and Editing.

64 All authors approved the final version.

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Introduction

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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.

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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, 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.

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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,

87 overgrazing by sea urchins can cause phase shifts from kelp forests to urchin
88 barrens (Steneck et al., 2002). On Caribbean coral reefs, the mass die-off of
89 black sea urchins, *Diadema antillarum*, coupled with historical overfishing of
90 herbivorous fish led to a shift from tropical reefs dominated by corals to reefs
91 dominated by macroalgae (Hughes 1994). Therefore, changes in the magnitude
92 of grazing by sea urchins, whether positive or negative, could have major
93 consequences for marine ecosystems. Furthermore, Provost et al. (2016) found
94 that within a large scale mesocosm experiment, changes to the strengths of
95 multiple trophic (e.g. grazing, predation) and competitive interactions in response
96 to ocean warming and OA can reduce kelp forest integrity and have the potential
97 to negatively impact kelp forest resilience. Similarly, a combination of intense
98 warming and the loss of sea urchin predators, such as the sunflower star
99 (*Pycnododia helianthoides*) due to sea star wasting disease, resulted in a >90%
100 reduction in the kelp forest canopy and shifts to urchin barrens along the northern
101 coast of California (Rogers-Bennett and Catton 2019). However, it is still not
102 clear how OA will combine with biotic interactions, such as grazing, to alter
103 diverse species assemblages.

104 The combined effects of OA and grazing are likely to be complex. Many
105 grazers build protective shells or skeletons by precipitating calcium carbonate
106 and are therefore likely to be directly impacted by OA. Current research
107 suggests that growth and calcification of numerous grazing taxa (e.g.
108 Echinodermata, Gastropoda) are often reduced under acidified conditions

109 (Dupont et al., 2010; Kroeker et al., 2010; Gazeau et al., 2013). The direct
110 impacts of OA on grazing rates, however, appear to be more complicated.
111 Studies assessing the impacts of OA on grazing rates have returned variable
112 results. For instance, some studies have found that OA decreases consumption
113 rates of seaweeds, either directly (Russell et al., 2013) or indirectly through
114 changes in the palatability of resources (Poore et al., 2013). Conversely, other
115 studies have shown increases in consumption under OA, potentially as a
116 consequence of decreased nutritional content of algal resources (Falkenberg et
117 al., 2013a,b) or through compensation as primary production concurrently
118 increases under OA (Ghedini et al., 2015). Thus, the responses of grazers to OA
119 are likely nuanced and may even be system specific.

120 Kelp forests that dominate subtidal rocky habitats within the California
121 Current System (CCS) have received relatively little attention with regard to their
122 response to future environmental change (but there is some individual species
123 work e.g. Brown et al., 2014). These ecosystems, however, are some of the
124 most diverse and productive on the planet (Steneck et al., 2002). OA is
125 predicted to progress rapidly within the CCS (Chan et al., 2017) due to the
126 decreased ability to buffer the chemical changes induced by additional
127 anthropogenic CO₂ in already acidic seawater (Frankignoulle 1994, Gruber et al.,
128 2012). Therefore, kelp forest ecosystems within the CCS may be at increased
129 risk from the threats of OA.

130 The goals of this study were to elucidate the impacts of future OA and
131 grazing on kelp forest communities in southern California by addressing the
132 following questions: 1) How do benthic rocky reef community assemblages
133 respond to experimentally induced OA in the presence and absence of sea
134 urchin grazers? and, 2) How will OA alter sea urchin grazer growth and
135 consumption? We hypothesized that the cover and biomass of calcified taxa
136 would decrease following exposure to high $p\text{CO}_2$, but increase in the presence of
137 grazers. In addition, we hypothesized that cover and biomass of non-calcified
138 taxa within these communities would increase in high $p\text{CO}_2$, but decrease in the
139 presence of grazers. We also hypothesized that sea urchin growth and grazing
140 rates would be reduced in high $p\text{CO}_2$ conditions and these altered grazing rates
141 would explain differences in fleshy macroalgal cover between low and high $p\text{CO}_2$
142 treatments in the presence of urchins. A better understanding of the combined
143 effects of OA and grazing on intact species assemblages will improve our ability
144 to predict the emergent effects of OA on kelp forest ecosystems.

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Materials and Methods

Study sites

148 We employed a manipulative factorial experiment assessing the effects of
149 OA and herbivory on benthic community structure from natural assemblages that
150 developed on settlement tiles for one year *in situ*. To achieve natural

151 communities, we installed three racks of settlement tiles elevated slightly off the
152 seafloor, each with 18 individual 100 cm² sanded PVC tiles. Tile racks were
153 installed at 14 m depth and separated by 5-10 m within the kelp forest, at Mia's
154 Reef in San Diego County (N 32° 51' 14.8", W 117° 16' 52.4") on August 2, 2012
155 (Fig. S1). Tiles were uncaged in order to allow access to the natural community
156 of predators and herbivores on the reef. Mia's Reef, similar to other southern
157 California kelp forests, is characterized by limestone that is fragmented into large
158 boulders (Dayton et al., 1985). The main habitat-forming species is *Macrocystis*
159 *pyrifera* with an understory community dominated by low lying fleshy red and
160 brown macroalgae, calcified red algae, bryozoans and sponges. Purple sea
161 urchins, *Strongylocentrotus purpuratus*, bat stars, *Patiria miniata*, and various
162 benthic fish species (e.g. *Hypsypops rubicundus*, *Semicossyphus pulcher*,
163 *Oxyjulis californica*) were commonly seen at the study site. A large terrace
164 provided continuous bathymetry in which to deploy settlement tile arrays under
165 the kelp canopy and amongst the understory community.

166

167 *Experimental system*

168 On September 20, 2013, we retrieved tiles ($n=40$; 14 tiles were left on the
169 reef for a separate study) and transported them in seawater to the laboratory at
170 Scripps Institution of Oceanography (SIO). We cleaned tiles of all mobile
171 invertebrates (e.g. small crabs, limpets) using forceps and then randomly placed
172 tiles into individual square 1.5 L glass containers (mesocosms) within a water

173 table. We supplied tiles with ambient flow-through seawater and light ($\sim 30 \mu\text{mol}$
174 $\text{m}^{-2} \text{sec}^{-1}$) for one week to acclimate to laboratory conditions. We collected
175 juvenile purple sea urchin grazers, *Strongylocentrotus purpuratus*, (test diameter
176 $\sim 1.5 \text{ cm}$) from the Point Loma kelp forest during the week of September 23,
177 2013. Prior to their use as experimental grazers, we held sea urchins in ambient
178 flow-through aquaria and fed them fronds of *M. pyrifera ad libitum*.

179 We conducted all laboratory experiments in a flow-through seawater
180 system at SIO (for description see Kram et al., 2015). We bubbled an air or CO_2 -
181 gas mixture into individual mesocosms continuously supplied with flow-through
182 seawater; temperature was allowed to vary naturally in this system. Ambient pH
183 conditions in our experiment (pH = 8.00, Table 1) were similar to mean pH
184 conditions within the La Jolla kelp forest (pH ~ 7.95 , Takeshita et al. 2015). We
185 manipulated high $p\text{CO}_2$ (low pH) conditions by bubbling an air/ CO_2 blend at a
186 rate that lowered the seawater pH by 0.4 ($\pm 0.05 \text{ SE}$) units below ambient (Table
187 1). This treatment pH was chosen based on the IPCC's Representative
188 Concentration Pathway (RCP) 8.5 projected conditions for the year 2100 (IPCC
189 2014). We created ambient $p\text{CO}_2$ conditions by bubbling air at the same rate as
190 the high $p\text{CO}_2$ treatment mesocosms. We used a hand-held pH meter (HACH
191 HQ40d Portable pH meter) with a glass electrode pH probe (HACH, PCH201) to
192 measure pH and temperature daily at mid-day (12:00-13:00) in each of the
193 experimental mesocosms and calibrated the glass electrode pH probe daily with
194 certified Tris buffer from the Dickson laboratory at SIO. Two distinct $p\text{CO}_2$

195 conditions were thus maintained for the entirety of the experiment (ambient $p\text{CO}_2$
196 = 443 μatm and high $p\text{CO}_2$ = 1569 μatm ; Table 1) from October 7, 2013 until
197 December 3, 2013. Light levels were monitored within all mesocosms using a
198 hand-held PAR sensor (QSL-2200, Biospherical Instruments) once a week at
199 midday (12:00-13:00; Table 1). Mesocosm location within the water table was
200 rotated every two weeks for the duration of the experiment to minimize the
201 effects of minor differences in light and flow across the water table.

202 We added juvenile *S. purpuratus* individuals ($n=1$ per tile) to half of the
203 mesocosms as grazers to yield a total of four factorial treatments (ambient $p\text{CO}_2$
204 -grazer, ambient $p\text{CO}_2$ +grazer, high $p\text{CO}_2$ -grazer, high $p\text{CO}_2$ +grazer).
205 Although sea urchin densities are generally lower in nature than those used in
206 this experiment (here 1 urchin/ 0.01 m^2), they do approach similar densities during
207 the formation and persistence of urchin barrens (Byrnes et al., 2013). In addition,
208 we used small juvenile urchins, which have a much lower per capita grazing
209 impact than larger adult urchins (Sala and Graham 2002). Three or four tiles
210 were randomly selected from each of the 3 racks ($n=10$ tiles per treatment) and
211 these were reared for ~two months in each of the four factorial treatment
212 conditions. Four control mesocosms were maintained without tiles or grazers at
213 both ambient and high $p\text{CO}_2$ to evaluate the effects of biological processes by
214 the organisms on the $p\text{CO}_2$ levels in each of the treatments.

215 We collected discrete water samples in 500 mL Corning brand sample
216 bottles from four or five randomly selected mesocosms per pH treatment at the

217 beginning, after 1 month, and at the end of the experiment. After collection, we
218 immediately spiked discrete samples with 240 L of HgCl₂ solution. We measured
219 pH with a spectrophotometer (Shizmadzu, UV-1800) and total alkalinity using
220 open-cell titration on triplicate samples (Metrohm, 905 Titrando) following
221 standard protocols (Dickson et al., 2007). We calculated salinity from density
222 using a densimeter (Mettler Toledo, DX45). We calculated carbonate chemistry
223 parameters (Table 1) based on measured pH, total alkalinity, salinity, *in situ*
224 temperature and pressure using CO2SYS (Pierrot et al., 2006) with
225 stoichiometric dissociation constants defined by Mehrbach et al. (1973) and refit
226 by Dickson and Millero (1987).

227

228 *Community structure*

229 To examine the emergent effects of OA and grazing on kelp forest
230 communities, we photographed all tiles at the start of the experiment and visually
231 examined tiles using a mini quadrat at the end of the experiment. We imported
232 photographic images into Coral Point Count with Excel extensions (CPCe V4.1,
233 National Coral Reef Institute) and overlaid a 10 x 10 point grid over each
234 photograph. We classified the space-occupying organism underlying the
235 crosshairs at each grid intersection into one of six functional groups: calcified
236 invertebrates (bryozoans, barnacles and serpulid worms), non-calcified
237 invertebrates (sponges, anemones and tunicates), calcified algae, fleshy algae,
238 sediment, and bare space for subsequent statistical analyses. Rapid growth of

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

254

255 *Kelp sporophyte density*

256 Our analysis of community composition only took into account the space-
257 occupying organism on the tile, yet some macroalgae, such as kelp (Order
258 Laminariales), have small holdfasts compared to their biomass in the water
259 column and therefore may be underrepresented in the point intercept analyses
260 used above. Thus, due to their important role as habitat forming species in these

261 ecosystems, at the end of the experiment, we removed all juvenile kelp
262 sporophytes (primarily *Macrocystis pyrifera*), and counted the individuals per tile
263 to obtain a density.

264

265 *Net community calcification*

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

275

276 *Sea urchin growth and grazing*

277 After 56 days in experimental conditions, we measured differences in the
278 grazing rates of sea urchins following exposure to ambient and elevated $p\text{CO}_2$
279 levels. We starved sea urchins for three days in individual mesocosms supplied
280 with treatment seawater (but without tiles). After the starvation period, we
281 presented sea urchins with a single pre-weighed (wet weight) kelp frond collected
282 from the field the morning of the grazing trial. We also placed kelp fronds in

283 mesocosms ($n=3$) without urchins to control for any changes in wet weight in the
284 absence of grazing. Urchins were allowed to graze on kelp for 24 hours before
285 the kelp was removed and reweighed. We calculated the per capita sea urchin
286 grazing rate as the biomass of kelp removed per day per gram of buoyant weight
287 of sea urchin, corrected for changes in biomass gained in the controls. We
288 calculated grazing rate of sea urchins as, $\frac{(W_{ki}-W_{kf})-(W_{ci}-W_{cf})}{W_u}$, where W_{ki} is the
289 initial wet weight of kelp, W_{kf} is the final wet weight of kelp, W_{ci} is the initial wet
290 weight of controls (ambient or elevated $p\text{CO}_2$), W_{cf} is the final wet weight of
291 controls, W_u is the buoyant weight of the urchin.

292

293 *Statistical analyses*

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

305 (PERMANOVA) on initial resemblance matrices using 9999 permutations with
306 the fixed factor of rack.

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

321 To test for differences in the density of kelp sporophytes on tiles as a
322 function of the treatments, we conducted a generalized linear mixed-effects
323 model with log link function and Poisson distribution using `glmer` in the `lme4`
324 package in R (Bates et al., 2015) with $p\text{CO}_2$ and grazer presence as fixed effects
325 and rack as a random factor. To test for differences in NCC, we conducted linear
326 mixed effects models with $p\text{CO}_2$ and grazer presence as fixed effects and rack as

327 a random factor. We used t-tests to compare differences between initial mean
328 sea urchin buoyant weight and sea urchin test diameter between ambient and
329 high $p\text{CO}_2$ grazing assays. We compared sea urchin grazing, calcification, and
330 growth between urchins exposed to high $p\text{CO}_2$ or ambient $p\text{CO}_2$ using t-tests. All
331 t-tests were run in R (R Development Core Team, version 1.2.5033).

332

333

Results

334

Experimental conditions

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

343

Community structure

345 Initially, tile communities were covered with calcified and non-calcified
346 invertebrates, calcified and fleshy algae, sediment, and areas of bare space
347 suggesting they were at an earlier stage of development (Fig. 1; Fig. S3). We
348 found that racks 1 and 2, and 2 and 3 differed significantly in initial community

349 structure (Table S2). Racks 1 and 3 had higher abundances of calcified and non-
350 calcified invertebrates. Rack 2 had higher abundances of sediment and bare
351 space (Table S2; Fig. S3).

352 Following two months of exposure to the experimental treatments, tile
353 communities were still covered with calcified and non-calcified invertebrates,
354 calcified and fleshy algae, and still maintained areas of sediment and bare space
355 (Fig. 2). We found a marginally non-significant effect of $p\text{CO}_2$ on community
356 structure ($P = 0.091$) and no effect of sea urchin grazing or the interaction of
357 $p\text{CO}_2 \times$ grazing (Table 2; Fig. 3D). However, there was also a significant effect of
358 tile rack on community structure ($P=0.001$), indicating that the final community
359 structure depended on the structure of the initial assemblage.

360 Due to the significant tile rack effect and marginally non-significant $p\text{CO}_2$
361 effect, separate PERMANOVAs were run on all three racks. In this analysis, we
362 found significant main effects of $p\text{CO}_2$ and sea urchin grazing on community
363 structure on Rack 1 (Table 2; Fig. 3A), after dropping the non-significant
364 interaction term from the model. The species assemblages on Rack 1 tended to
365 cluster together in multivariate space as a function of the individual treatment.
366 On Rack 2, there was a significant effect of $p\text{CO}_2$ on community structure, but no
367 effects of urchin grazing (Table 2; Fig. 3B). Tiles from the ambient $p\text{CO}_2$
368 treatments clustered together in multivariate space, while tiles from the high
369 $p\text{CO}_2$ treatments grouped separately. There were no significant differences in
370 community structure from Rack 3 when the interaction of $p\text{CO}_2 \times$ grazer was

371 included in our model. Without the non-significant interaction, we detected
372 significant $p\text{CO}_2$ effects on community structure (Table 2; Fig. 3C), such that tiles
373 from the ambient $p\text{CO}_2$ treatments clustered separately from the tiles in the high
374 $p\text{CO}_2$ treatments.

375 Further analyses conducted on the responses of individual functional
376 groups elucidated which groups contributed to differences in community structure
377 observed across treatments. On Rack 1, we found significant effects of both
378 $p\text{CO}_2$ and grazing (Table S4; Fig. 4A). At ambient $p\text{CO}_2$, grazing reduced fleshy
379 algal cover by 35%; however, at high $p\text{CO}_2$, fleshy algal cover was only reduced
380 by 26% in the presence of grazers. On Rack 2, we found a ~35% higher percent
381 cover of sediment on tiles reared in ambient $p\text{CO}_2$ but observed ~27% higher
382 cover of bare space in the high $p\text{CO}_2$ conditions (Table S5; Fig. 4B). On average
383 we found a 45% lower cover of calcified sessile invertebrates at ambient $p\text{CO}_2$
384 than in high $p\text{CO}_2$ treatments on Rack 3 (Table S6; Fig. 4C).

385

386 *Kelp sporophyte density*

387 No juvenile kelp sporophytes were visible on tiles at the outset of the
388 experiment. However, juvenile kelp sporophytes were present on 22 of 40 tiles
389 (across all racks) at the end of the experiment. There was a significant effect of
390 $p\text{CO}_2$ on kelp density, with fewer kelp sporophytes present on tiles from the
391 ambient $p\text{CO}_2$ treatments, but a marginally non-significant effect of sea urchin
392 grazers on kelp density overall (Table 3). Instead, we found a significant

393 interaction between $p\text{CO}_2$ and grazing, in which the grazing effect (i.e., difference
394 in kelp density between +grazing and -grazing) was much stronger in the high
395 $p\text{CO}_2$ treatment. In other words, $p\text{CO}_2$ increases kelp density, except in the
396 presence of grazers (Fig. 5).

397

398 *Net community calcification*

399 Final buoyant weights of tiles from Mia's Reef, after 56 days in treatment
400 conditions, were almost always lower than initial buoyant weights (i.e., a net loss
401 of calcium carbonate) across all treatments over the duration of the experiment
402 (Fig. 6). However, we observed a significant reduction and a greater decline in
403 the buoyant weight of organisms living on the tiles in high $p\text{CO}_2$ treatments
404 relative to the ambient treatments (LMM, $p\text{CO}_2$: $t_{34} = -4.06$, $P < 0.0001$, urchin:
405 $t_{34} = -1.26$, $P = 0.22$, $p\text{CO}_2 \times$ urchin: $t_{34} = -.67$, $P = 0.51$). We did not detect a
406 statistically significant effect of grazer presence on the change in buoyant weight,
407 although tiles with grazers tended to have a greater mean reduction in buoyant
408 weight relative to the non-grazer treatment from a given $p\text{CO}_2$ treatment (Fig. 6).
409 Under ambient $p\text{CO}_2$, the presence of urchins decreased net community
410 calcification by 24%. High $p\text{CO}_2$ led to a reduction in net community calcification
411 relative to the control (ambient $p\text{CO}_2$, -urchin) by 58% in the absence of grazers
412 and 68% in the presence of grazers. There was no significant interaction
413 between $p\text{CO}_2$ and sea urchin grazing on buoyant weight.

414

415 *Sea urchin growth and grazing*

416 Initial mean sea urchin buoyant weight and sea urchin test diameter did
417 not differ between treatments (t-test buoyant weight, $t_{18} = 0.26$, $P = 0.79$; t-test
418 test diameter, $t_{18} = 0.17$, $P = 0.87$). However, after two months in treatment
419 conditions, sea urchin growth measured as buoyant weight was 60% higher in
420 ambient $p\text{CO}_2$ than at high $p\text{CO}_2$, while there was no detectable difference in the
421 change in test size between treatments, suggesting that sea urchin tests became
422 thinner under high $p\text{CO}_2$ conditions (t-test percent change in buoyant weight, t_{17}
423 $= -2.20$, $P = 0.042$; Fig. 7a; t-test percent change in test diameter, $t_{17} = -1.03$, $P =$
424 0.32).

425 Results from the grazing trial showed significantly higher grazing rates in
426 sea urchins exposed to ambient $p\text{CO}_2$ than high $p\text{CO}_2$ treatments (t-test, $t_{17} =$
427 3.65 , $P = 0.002$; Fig. 7b). Kelp in the ambient CO_2 treatments lost mass as a
428 result of sea urchin grazing, whereas kelp in the high CO_2 treatments gained
429 mass, even in the presence of grazers.

430

431

Discussion

432

433 Ocean acidification is expected to have widespread impacts on marine
434 ecosystems (Gaylord et al., 2015). Here, we show that kelp forest assemblages
435 from southern California were negatively affected by OA. Acidification reduced
436 net community calcification rates in naturally assembled kelp forest understory

437 communities. OA and grazing by *S. purpuratus* altered community structure
438 within the kelp forest assemblages, yet changes to the underlying functional
439 groups were dependent on initial community composition. Grazing by sea
440 urchins reduced the density of kelp sporophytes within our assemblages, but
441 negative impacts of OA on growth and grazing rates of sea urchins suggests that
442 grazing pressure may also shift in future ocean conditions. The differences seen
443 here highlight the importance of studying the effects of environmental change on
444 intact species assemblages where the emergent effects of species interactions
445 (e.g. competition, predation, facilitation) create naturally heterogeneous
446 landscapes that have the potential to alter the outcomes of OA.

447

448 *Community structure*

449 Numerous studies have shown shifts from reefs dominated by calcifiers to
450 reefs dominated by fleshy algal species under OA-like scenarios (Jokiel et al.,
451 2008, Fabricius et al., 2011, Kroeker et al., 2012). The majority of these studies
452 have focused, however, on coral reef communities where the dominant habitat
453 forming species are calcifiers (i.e. scleractinian corals). We found that the $p\text{CO}_2$
454 and grazing effects on community structure were variable and dependent on the
455 initial community composition. Since functional group taxa responded differently
456 across racks, overall effects were masked when racks were pooled together.
457 Notably, although there were no detectable differences in initial community
458 composition on tiles from racks 1 and 3, the responses of functional group taxa to

459 acidification and grazing varied between these two racks. Tiles from rack 1
460 exhibited significant effects of $p\text{CO}_2$ and grazing on fleshy macroalgae; the
461 presence of herbivorous grazers reduced the percent cover of non-kelp fleshy
462 macroalgae on tiles, while acidification increased percent cover. Both of these
463 findings are consistent with the predicted outcomes of grazing or OA on fleshy
464 seaweed species (Harley et al., 2012, Poore et al., 2012). Conversely, OA
465 decreased cover of calcified invertebrates on rack 3 yet had no impacts on any
466 other taxa, suggesting that percent cover alone may not determine the emergent
467 effects of OA and grazing on kelp forest ecosystems. Instead, the biotic
468 interactions among species may play a critical role in determining community
469 dynamics in response to environmental change. On rack 2, decreases in
470 sediment were compensated by increases in bare space on tiles reared under
471 OA conditions. It is possible that the sediments on tiles were made of
472 carbonates and were therefore more readily dissolved under acidic conditions.
473 Interestingly, similar results were not seen on other tile assemblages, which
474 suggests that differences in the percent cover of other functional group taxa may
475 interact with sediment removal/dissolution.

476 All tile assemblages were obtained from the same reef outcrop (spaced
477 approximately five meters away from each other and differing in depth by less
478 than one meter) and experienced similar environmental conditions (e.g., light,
479 temperature, pH, grazing pressure) before being exposed to ocean acidification
480 and grazing in the laboratory. The importance of small-scale heterogeneity on

481 reefs has been unstudied in the context of OA, yet it may play an important role
482 in the persistence of marine ecosystems. Initial differences in community
483 composition were likely affected by microhabitat variation in environmental
484 conditions, settlement, competition and growth of benthic species (Ferguson et
485 al., 2013) and those differences in initial communities influenced the final
486 response of the communities to OA and grazing. Cornwall et al. (2014) found
487 that diffusive boundary layers (DBL) around algal assemblages could alter the
488 effects of OA by decreasing flow and allowing pH to increase within the algal
489 assemblage. DBL around an individual also has the potential to increase
490 calcification due to pH buffering within the calcifying fluid, though benefits are
491 species-specific (Comeau et al., 2019). Therefore, variability in not just the
492 abundance, but also the species identity and spatial arrangement of organisms
493 could change the response of the community to acidification. The potential for
494 local buffering through photosynthesis has been shown at a much larger scale
495 with highly productive photoautotrophic species capable of increasing the pH
496 within their local environment (Anthony et al., 2008; Koweek et al., 2018;
497 Manzello et al., 2012; Nielsen et al., 2018). Even minor differences in flow and
498 light levels within the kelp forest could alter assembly processes (Edwards 1998,
499 Wernberg and Goldberg 2008) and therefore lead to different species
500 arrangements and subsequent responses to both OA and grazing.

501 Interest in the consequences of environmental change for kelp forest
502 ecosystems has increased since recent studies have shown that early life history

503 stages of kelp may be particularly vulnerable to high $p\text{CO}_2$ and temperature
504 (Gaitán-Espitia et al., 2014). Early life history stages are likely to become
505 increasingly important to the recovery of kelp forests in future oceans as
506 disturbances that act to remove kelp plants become more frequent and intense
507 (Byrnes et al., 2011). When first brought into the lab, no kelp thalli were visible to
508 the naked eye on tile surfaces, suggesting that they were likely present at some
509 microscopic stage (gametophyte or sporophyte) upon removal from the reef. OA
510 increased the density of juvenile kelp sporophytes on tiles, but only in the
511 absence of grazers. Shukla and Edwards (2017) found that sporophyte
512 recruitment and microscopic growth were greater under elevated $p\text{CO}_2$ compared
513 to ambient, which may explain the elevated kelp densities in high $p\text{CO}_2$
514 conditions in our experiments. Other studies have shown either no effect of
515 $p\text{CO}_2$ on microscopic early life history stages of *M. pyrifera* (Roleda et al., 2011)
516 or decreased fitness in response to OA (Gaitán-Espitia et al., 2014), which would
517 be expected to result in either no differences or decreases in macroscopic
518 sporophyte density. Importantly, recent work by Hollarsmith et al. (2020) showed
519 significant variation in the performance of early life history stages of kelp in
520 response to OA and temperature across populations, suggesting that local
521 adaptation/acclimation could alter the outcome of environmental change.
522 Furthermore, since the initial life history stage(s) and density of gametophytes
523 and/or sporophytes in our study were unknown, it will be important to directly
524 assess how impacts at various microscopic stages (i.e., settlement, fertilization,

525 germination, sporophyte production) manifest at macroscopic stages to alter the
526 landscape scale patterns of kelp forest recovery dynamics.

527

528 *Community Processes*

529 Our experiment found that acidification significantly reduced net
530 community calcification. Past studies on both coral reef and temperate algal
531 communities have shown lower accretion rates of corals and coralline red algae
532 under elevated $p\text{CO}_2$ (Hoegh-Guldberg et al., 2007; Jokiel et al., 2008; Hofmann
533 et al., 2012; Albright et al., 2018). Decreases in calcification rates are likely due
534 to a reduction in the availability of CO_3^{2-} , a necessary building block of biogenic
535 carbonates. It is possible that the $p\text{CO}_2$ effect seen here is due to both reduced
536 calcification, via decreased growth and thinner calcium carbonate skeletons, as
537 well as increased dissolution of exposed CaCO_3 . The calcified taxa within our
538 assemblages were primarily coralline red algae and bryozoans. Coralline algae
539 and bryozoans are often negatively affected by acidification because they
540 precipitate a more soluble form of calcium carbonate, magnesium calcite
541 (Andersson et al., 2008; Taylor et al., 2014; McCoy and Kamenos, 2015). We
542 did observe weight loss on tiles across all treatments. This may be due to the
543 lower irradiances in experimental conditions compared to *in situ* measurements
544 shortly before the outset of the experiment (Fig. S2) or could be due to seasonal
545 differences in calcification rates of organisms on the tiles. Regardless, despite

546 the overall decrease in calcified biomass in all treatments, we found a
547 significantly greater reduction on tiles reared under high $p\text{CO}_2$ conditions.

548 We found that sea urchin growth and grazing rates were significantly
549 depressed after two months in high $p\text{CO}_2$ conditions. Russell et al. (2013) also
550 found that the herbivorous gastropod, *Littorina littorea*, showed decreased
551 consumption rates in high $p\text{CO}_2$ conditions. Brown et al. (2014) found an initial
552 (after one month) decrease in consumption rates of *S. purpuratus* reared at low
553 pH, yet no difference in grazing rates between *S. purpuratus* reared at ambient
554 pH and low pH after two months. This result is particularly interesting since we
555 did find decreased grazing rates after two months of exposure to OA conditions.
556 Urchins from our study were collected from the same kelp forest as urchins in the
557 Brown et al. (2014) study yet were approximately one third the size (test
558 diameter). It is possible that age and/or size class may play a role in species
559 tolerance to OA; however, neither study used multiple size classes of urchins and
560 therefore this cannot be tested explicitly. Interestingly, our study used fresh kelp
561 collected the same day as the initiation of the grazing trial, whereas Brown et al.
562 (2014) used kelp that had been reared in the same pH conditions as the urchin
563 grazers, which may have influenced urchin grazing rates differently in the two
564 studies. Falkenberg et al. (2013b) found increased consumption rates in the sea
565 snail, *Austrocochlea concamerata* and Ghedini et al. (2015) found evidence for
566 trophic compensation (increased consumption with increased growth of primary
567 producers) in the gastropod *Turbo undulatus* under high $p\text{CO}_2$ conditions. The

568 changes in consumption seen in these studies may be due to changes in the
569 nutritional quality of the algae used in the assays (Falkenberg et al., 2013a,b). It
570 is possible that kelp also change their nutritional quality in response to
571 acidification, which could further explain the differing results between our study
572 and that of Brown et al. (2014). Given the variability in responses of grazers to
573 OA, future research assessing both direct (i.e. physiological) and indirect (e.g.
574 nutritional quality) effects of acidification on grazer species simultaneously are
575 crucial since changes to the strength of algal-grazer interactions could alter
576 community structure and ecosystem function.

577 Although grazing had few direct effects on community structure in our
578 quadrat analyses, the density of juvenile kelp sporophytes in the presence of sea
579 urchin grazers tended to be lower. Kelp forest grazers, such as *S. purpuratus*,
580 graze upon both microscopic (Sala and Graham 2002) and macroscopic stages
581 of *M. pyrifera* and recent work by Ng. et al. (2020) showed a reduction in the
582 interaction strength of *S. purpuratus* and *M. pyrifera* microscopic stages in future
583 climate change scenarios of reduced oxygen levels. Changes to sporophyte
584 density in response to grazing would likely alter other physical properties of the
585 environment. Alsterberg et al. (2013) found both direct and indirect effects of
586 OA, warming, and grazers on benthic microalgae in a seagrass community. In
587 the presence of grazers, OA and warming did not significantly affect benthic
588 microalgae. In the absence of grazers, the direct effects of acidification and
589 warming positively affected benthic microalgae, which then negatively affected

590 benthic microalgae through the indirect effects of macroalgal shading. It is likely
591 that the indirect effects of macroalgal shading and changes to flow regimes could
592 interact with the acidification response of kelp forest benthic communities over
593 longer timescales.

594

595 *Conclusion*

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

612

613

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614

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628

629

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- 878

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 (°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 sec ⁻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

880 Table 2. PERMANOVA results of the effects of pCO₂ (ambient and high), sea
 881 urchins (presence and absence), and rack (1, 2 and 3) on community
 882 composition on settlement tiles, significant differences are in **bold**.

883

Source of variation	<i>df</i>	Mean squares	Pseudo- <i>F</i>	<i>Pr(>F)</i>
<i>All</i>				
pCO 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		
<i>Rack 1</i>				
pCO ₂	1	0.035	2.818	0.041
Grazer	1	0.034	2.702	0.046
Residuals	10	0.012		
<i>Rack 2</i>				
pCO ₂	1	0.028	2.971	0.038
Grazer	1	0.003	0.271	0.884
Residuals	11	0.009		
<i>Rack 3</i>				
pCO ₂	1	0.029	2.318	0.046
Grazer	1	0.006	0.526	0.779
Residuals	10	0.012		

884

885

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

890

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

891

892

893 **Figure Captions**

894 **Figure 1.** Photographic examples of settlement tiles from each rack array at the
895 beginning of the experiment. Tiles are 100 cm².

896

897 **Figure 2.** Photographic examples of settlement tiles with representative mixed
898 algal and invertebrate communities in each of the four treatment conditions after
899 56 days. Tiles are 100 cm².

900

901 **Figure 3.** nMDS plots showing similarities in community composition between
902 settlement tile benthic assemblages reared in experimental $p\text{CO}_2$ (gray = high
903 $p\text{CO}_2$, white = ambient $p\text{CO}_2$) and urchin grazing (triangles = +urchins, circles = -
904 urchins) treatments. Points (tiles) closer together indicate communities more
905 similar than points further apart. Panels a), b) and c) show nMDS plots of
906 community data separated by racks 1-3 respectively, while panel d) shows all
907 community data together on the same plot with 95% confidence ellipses around
908 each rack.

909

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

912

913 **Figure 5.** Density of juvenile kelp on experimental tiles at the end of 56 days in
914 different $p\text{CO}_2$ and urchin grazing treatments. Shared letters above error bars

915 indicate mean density did not differ between treatments. Error bars denote ± 1
916 SE.

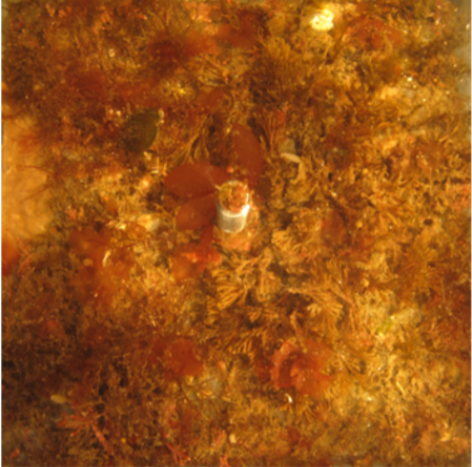
917

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

921

922 **Figure 7.** Calcification rates a) of sea urchins reared on settlement tiles in
923 ambient and high $p\text{CO}_2$ conditions, standardized to initial size; Grazing rates b) of
924 sea urchins on *Macrocystis pyrifera* fronds after 56 days in experimental
925 conditions. Shared letters indicate calcification rate or grazing rate did not differ
926 between treatments. Error bars denote ± 1 SE.

927



Rack 1



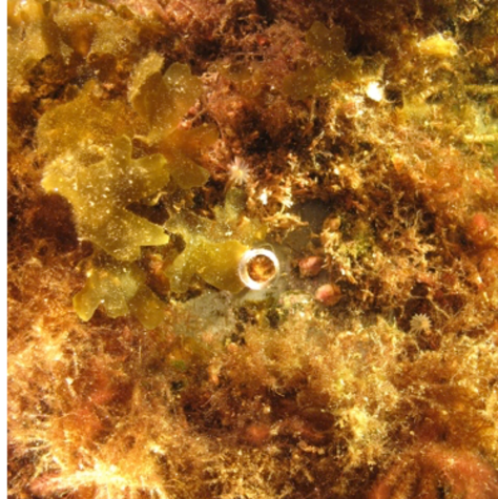
Rack 2



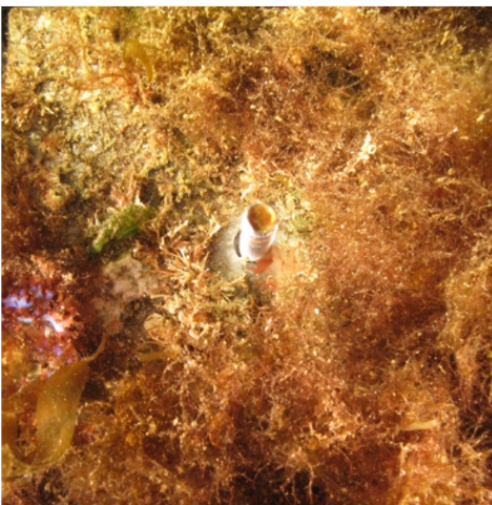
Rack 3



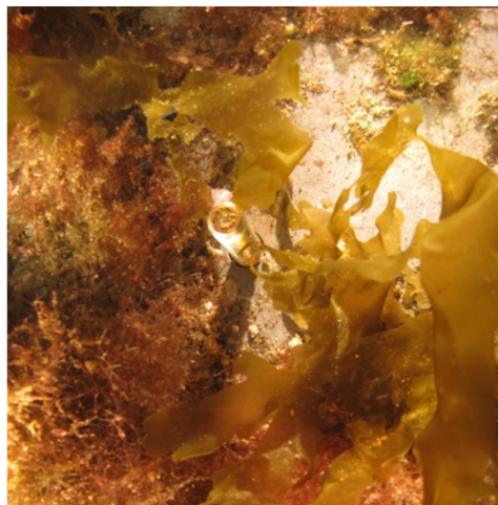
Ambient $p\text{CO}_2$, -grazer



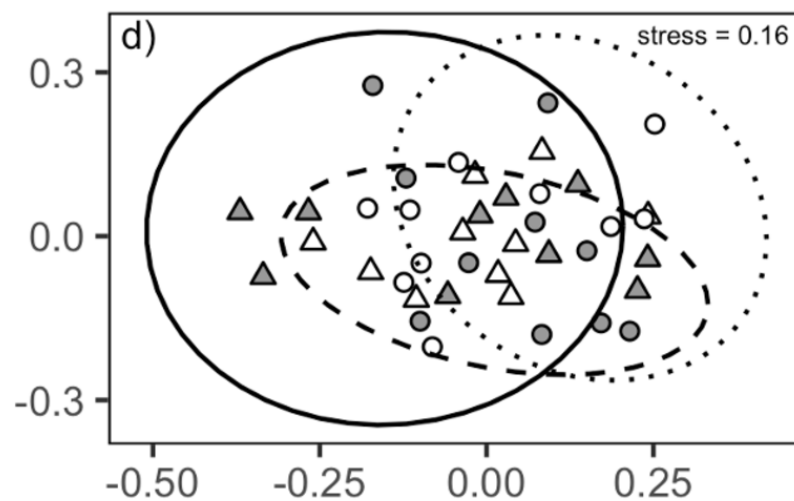
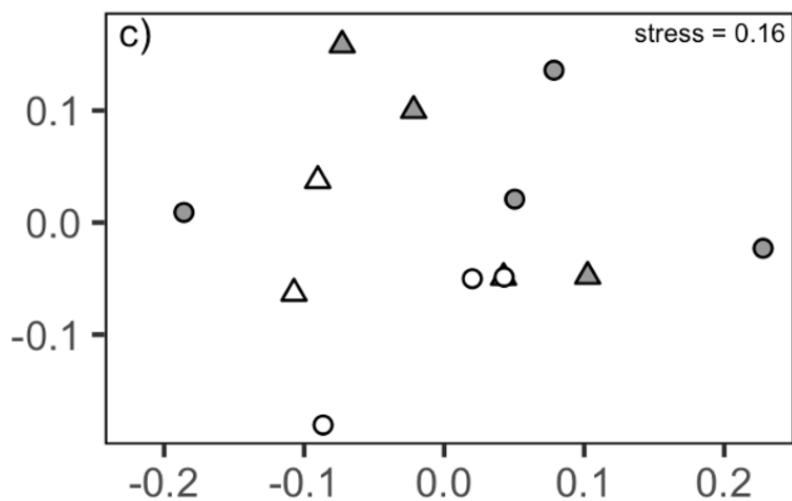
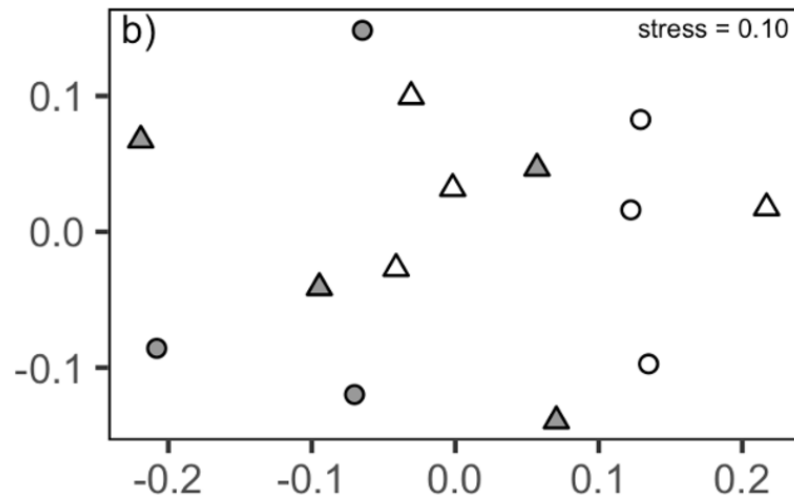
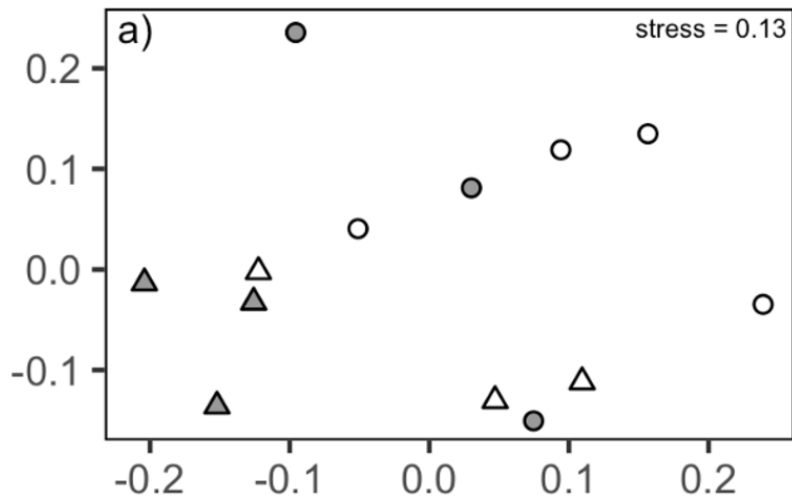
Ambient $p\text{CO}_2$, +grazer



High $p\text{CO}_2$, -grazer



High $p\text{CO}_2$, +grazer



Treatment

△ Ambient $p\text{CO}_2$, - Grazer

○ Ambient $p\text{CO}_2$, + Grazer

▲ High $p\text{CO}_2$, - Grazer

● High $p\text{CO}_2$, + Grazer

Rack

— A

- - B

⋯ C

