THE ABILITY OF FRAGMENTED KELP FORESTS TO MITIGATE OCEAN ACIDIFICATION AND THE EFFECTS OF SEASONAL UPWELLING ON KELP-PURPLE SEA URCHIN INTERACTIONS

By

Kindall Ann Murie

A Thesis Presented to

The Faculty of Humboldt State University

In Partial Fulfillment of the Requirements for the Degree

Master of Science in Biology

Committee Membership

Dr. Paul E. Bourdeau, Committee Chair

Dr. Jeffery Abell, Committee Member

Dr. Eric P. Bjorkstedt, Committee Member

Dr. Brian N. Tissot, Committee Member

Dr. Joseph A. Tyburczy, Committee Member

Dr. Paul E. Bourdeau, Graduate Coordinator

May 2020

ABSTRACT

THE ABILITY OF FRAGMENTED KELP FORESTS TO MITIGATE OCEAN ACIDIFICATION AND THE EFFECTS OF SEASONAL UPWELLING ON KELP-PURPLE SEA URCHIN INTERACTIONS

Kindall Murie

Bull kelp (*Nereocystis leutkeana*) forests along the coast for northern California have decreased dramatically as a result of a 'perfect storm' of multiple environmental stressors. The disappearance of a predatory sea star and subsequent increase in purple sea urchins (*Strongylocentrotus purpuratus*) and the recurrence of marine heat waves have caused these once diverse ecosystems to be rapidly converted into relative species-depauperate urchin barrens. By examining the interactive effects of both a rapidly changing abiotic environment and the increase in urchin grazing pressure that is affecting this vital ecosystem, we can better understand its ultimate fate and make better-informed decisions to manage and protect it. As once large and persistent kelp forests are converted into fragmented landscapes of small kelp patches, kelp's ability to take up dissolved inorganic carbon and reduce nearby acidity and increase both dissolved oxygen and bio-available calcium carbonate may be reduced, preventing it from serving as an environmental stress-free 'oasis' of reduced environmental stresses for local marine organisms and affecting ecosystem dynamics. In my first chapter, I examined whether small, fragmented kelp patches are able to retain their ability to alter local seawater chemistry to the same extent a large persistent kelp forests that have been studied

previously. I found that in the canopies of small kelp patches, multiple parameters of carbonate chemistry fluctuated more than in the kelp benthos and in adjacent urchin barrens, consistent with metabolic activity by the kelp. Further, kelp fragments increased pH and aragonite saturation and decreased pCO_2 during the day to a similar degree as large, intact kelp forests. These results suggest that small kelp patches could mitigate OA stress during the day and serve as spatial and temporal refugia for canopy-dwelling organisms. I also found that the benthic environment in kelp forests and adjacent urchin barrens is subject to unbuffered decreases in temperature, dissolved oxygen and pH. Thus, in chapter two, I assessed how current-day and future-predicted fluctuations in the duration and magnitude of these upwelling-associated stressors would impact the grazing, growth, and survivorship of purple urchins from kelp forest and urchin barren habitats. With upwelling predicted to increase in both intensity and duration with global climate change, understanding whether urchins from different habitats are differentially affected by upwelling-related stressors will give insight into how current and future stressors may be able to help 'tip the scales' and convert the increasing number of urchin barrens back into healthy productive kelp forests. I found condition-dependent susceptibility in urchins to increased magnitude and duration upwelling-related stressors. Grazing and gonadal development in kelp forest urchins was most negatively affected by distant future upwelling conditions, whereas in urchin barren urchins, grazing and survival were sensitive to exposure to upwelling in general, and also to increase in magnitudes of acidity, hypoxia, and temperature across both upwelling and non-upwelling events in the future. These results have important implications for population dynamics of urchins and

their interactions with bull kelp, which could strongly affect ecosystem dynamics and transitions between kelp forests and urchin barrens. Taken together, the two chapters my thesis provide valuable insight into the potential resilience of bull kelp, a critical foundation species in northeastern Pacific coastal habitats, in the face of a rapidly changing multi-stressor environment.

ACKNOWLEDGEMENTS

I would like to thank my funding sources: the United States National Science Foundation Graduate Research Fellowship Program (NSF GRFP), The Phycological Society of America Grant-in-aid of Research, California Sea Grant though the Humboldt Marine and Coastal Sciences Institute (MCSI), the bank of Kri Kri, and a California State University Council on Ocean Affairs, Science & Technology (CSU-COAST) Grant Development Program Award to my advisor.

I would first like to thank all of my committee members for their support and insightful feedback on my thesis. Big thanks to Dr. Tyburczy for helping me with processing water samples at the Burke-o-lator and figuring out almost all of the frustrating nuances of carbonate chemistry for me. Dr. Abell and his amazing team (Ben Freiberger and his daughter Elenor) for providing standards for the Burkolator and an extra pair of hands in collecting data. A big thanks to Dr. Bjorkstedt for his amazing wizardly R-coding skills and paving the way with the dreadful Loligo system. Finally, a big thanks to Dr. Tissot for loaning me field equipment, lab space, and valuable advice.

I would like to thank the Hog Island Oyster Company and Juan Avellaneda for allowing me to take over the Burkolator and stay late; it was a huge help finishing up the water samples. I also like to thank CENCOOS for providing data on local environmental conditions.

I would like to thank the faculty and staff at HSU for the continued logistical support. Rich Alvarez (DSO) and Steve Monk (BSO) for providing the means and the

training to scuba dive and the use of boats for this project. I greatly appreciate the level of dedication they both show toward their students. HSU's programs in marine sciences are very lucky to have them and their amazing dive and boating program. I would also like to thank Grant Eberle for not only the last two years but the many before it, he has had to put up with me since I was a junior. I appreciate his patience and help in and around the wet lab. Roxanne Robertson for helping me with chlorophyll analysis. A huge thanks to Kyle Weis for making everything run, from the remodeling of the OA system to making sure my HOBO sensors were not taken by the ocean. I could have not completed these two chapters without him. My mom also thanks Kyle because thanks to his help the odds of me chopping off one of my bodily limbs in the shop or catching the whole wet lab and myself on fire were significantly decreased ($p<0.0001$). A huge thanks to the ladies in front office, Stephanie Steffen, Liz Weaver, and Yvonne Kugies for making my life as a graduate student so much easier. And finally, a special thanks to Yvonne for all the emotional support and thoughtful memories (Potato Feast to name one), I appreciate you always being in my corner.

I also would have not been able to finish my thesis without the help of my amazing volunteers and divers. Jeremy McFarland, Lily McIntire, Halle Shauer, and Gavin Zirkel were the best dive crew I could have asked for. They put up with long car trips, endless number of stairs in full scuba gear, and my attempts to work 15-hour days with no food. Thank you all for reminding me we have to eat, helping me get the job done in the water, and creating countless memories. A huge thank you to my army of volunteers: Ryan Starbuck, Jana Lott, Clarissa Honeker, Connor Sharp, Eden Lolley,

Brittney Mitchell, Garrett Hoffman, Jake Vargas, Juliet Kallen, Morgan Franken, Sally Gammie, Melanie Dominguez, Crystal Hofer, Torre Flagor, Helen Acosta, Ethan Reibsome, Megan Heckman, and Rose Harman. You all are amazing, and I am so glad I got to know each of you.

A huge thanks to my lab mates for all the advice, shared agony over grad school, PEB lab things, and of course all the great lab meetings at the Clam Beach Tavern and the drives to Petrolia. Lily McIntire, Ande Fieber, Wes Hull, Angela Jones, Johnny Roche, Tim McClure, Tharadet Man, Jess Gravelle, Torre Flagor, Taylor Tharaldson, Melanie Dominguez, and Crystal Hofer; you all made my time at HSU so special, so thank you! Also, a special thanks to Lily, she basically kept me alive during this whole process!

I would also like to give the biggest thank you to my advisor Paul. You have definitely been my peanut butter sandwich though this whole process. Without your continued encouragement, enthusiasm, and stubbornness I would not been able to be as successful as I was during my time at HSU. Thank you so much for taking my crazy ideas and making them crazier, for always saying yes when everyone else defiantly wish you said no, and for the ungodly amount of edits and patience it took to get me out this door and on to a Ph.D. I am forever grateful for your insight into how to navigate being a marine ecologist and I can't wait to collaborate and come up with crazing things to tackle in the future.

Lastly, I would like to dedicate this thesis to my mom! With her constant support and pushing/letting me follow my own goals and aspirations, none of this would have

been possible. Thank you for answering all my late-night calls and keeping me grounded. You are the best mom, and I love you MORE!

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CHAPTER 1: BETWEEN TWO FRONDS: FRAGMENTED KELP FOREST CANOPIES MAY PROVIDE REFUGIA FROM OCEAN ACIDIFICATION

INTRODUCTION

Ocean Acidification (OA), and specifically the absorption of CO_2 by our world's oceans is well known to have negative impacts on marine organisms, particularly those that construct their skeletons and shells out of calcium carbonate ($CaCO_3$) (Doney et al. 2009, Gazeau et al. 2013, Hall-Spencer, J. M., & Harvey, B. P 2019). However, photosynthetic macrophytes (e.g., kelps and seagrasses) have the potential to modify local seawater chemistry in a way that could buffer calcifying organisms from OA stress (Raven, J.A et al. 2008, Cornwall, C.E. et al. 2012, Duarte, C.M. 2017). Forest-, and canopy-forming kelps are particularly good candidates for modifying local sea water chemistry because of their ability to uptake both bicarbonate and CO_2 as a carbon sources for photosynthesis, thus acting as carbon sinks, reducing nearby ocean acidity, and increasing both dissolved oxygen (DO) and bio-available calcium carbonate for nearby calcifying organisms. Because kelps serve as foundational species (Dayton, P.K. 1975, Castorani, M.C., Reed, D. C., & Miller, R. J. 2018) supporting some of the world's most productive ecosystems (Steneck, R. S. et al. 2002, Graham, M. H., Dayton, P. K., & Erlandson, J. M. 2003) it is essential to understand their role as abiotic ecosystem engineers.

To date, evidence for the ability of forest-forming kelps to alter local seawater chemistry comes from historically large (i.e. 9.9-103.3ha canopy area) and persistent kelp

forests in the northeastern Pacific, which are mainly dominated by giant kelp (*Macrocystis pyrifera*). For example, in southern California *M. pyrifera* forests, daily fluctuations in pH and DO have been shown to be as large as 0.36 pH units and 4.93 mg · L^{-1}, respectively (Frieder, C. A. et al. 2012). In central California, the yearly fluctuations in pH can range from as low as 7.70 up to 8.33, coupled with fluctuations in pCO_2 from 172-952 µatm (Koweek, D.A. et al 2017). Further north, large kelp forests in the Strait of Juan de Fuca, Washington comprised of both *M. pyrifera* and *Nereocystis luetkeana,* exhibit similar daily pH fluctuations with seawater inside the kelp being a maximum change of 0.35 pH units higher, 167 µatm lower in pCO_2, and higher by 0.2 units in aragonite saturation higher by 0.2 units inside the kelp versus outside the kelp (Pfister, C. A., Altabet, M. A., & Weigel, B. L. 2019). Because kelp forests can reduce seawater dissolved inorganic carbon (DIC) and increase pH and oxygen, they play an additional important role as a foundational species in temperate coastal ecosystems, and the need to maintain these services in the future may be critical.

A recent 'perfect storm' of multiple stressors including the recurrence of marine hear waves, the disappearance of the predatory sea star *Pycnopodia helianthoides* and the subsequent increase in purple sea urchins (*Strongylocentrotus purpuratus*) (Harvell, C. D. et al. 2019, Di Lorenzo, E., & Mantua, N. 2016), has caused dramatic reductions (> 90% loss in canopy cover) in historically large bull kelp (*N. luetkeana*) forests along the northern California coast (Rogers-Bennett, L., & Catton, C. A. 2019). As a result, these once large and productive kelp forests have been reduced to fragmented landscapes of small kelp patches and urchin barrens. Because kelp forests provide multiple ecosystem

services (Smale, D. A., et al. 2013), this large-scale deforestation has had multiple impactful cascading effects, including the mass decline (80% mortality) of abalone (*Haliotis* spp.) populations, resulting in the closure of an estimated $44M recreational fishery (Reid, J. et al. 2016); and the collapse of the $3M north coast commercial red sea urchin (*Mesocentrotus franciscanus*) fishery (Rogers-Bennett, L., & Catton, C. A. 2019). With climate change-related stressors predicted to intensify and the local extinction of purple sea urchins' natural predator (*P. helianthoides*) in the absent of sea otters, continued fragmentation of northern California kelp forests seems likely.

Fragmentation of forests, whether kelp or otherwise, will lead to proportionally more edge habitat, which often experiences altered growing conditions because of novel microenvironments characterized by different temperatures and availability of resources, such as light and nutrients (Stewart, H. L et al. 2009, Reinmann, A. B., & Hutyra, L. R. 2017). For example, in terrestrial tropical forests, interiors of intact forests retain almost 3x more carbon than edges and fragments (De Paula, M. D., Costa, C. P. A., & Tabarelli, M. 2011). In addition, increases in edge habitat have also been shown to be associated with 10% reductions in carbon density (Chaplin-Kramer, R. et al. 2015). The highlights the importance of considering landscape fragmentation when quantifying forest effects on local carbon chemistry. In kelp forests, fragmentation alters the canopy area and the density of individuals, subsequently increasing edge habitat, which in turn alters the extent to which the forest modifies its surrounding physical environment (Stewart, H. L et al. 2009, Reinmann, A. B., & Hutyra, L. R. 2017). For example, when kelp canopies are well-developed, fronds at the forest's edge have faster elongation and larger blades,

resulting in higher overall growth rates than those in the forest's interior (Stewart, H. L et al. 2009, Reinmann, A. B., & Hutyra, L. R. 2017). Carbon and nitrogen accumulation by edge fronds is also greater at this time, which leads to growth rates of edge fronds that are almost twice that of interior fronds (Stewart, H. L et al. 2009, Reinmann, A. B., & Hutyra, L. R. 2017). Light availability within a kelp bed is also a function of canopy density (Reed, D. C. & M. S. Foster. 1984) and light in the kelp forest's interior may be lowered to the point that nutrient uptake and photosynthesis are limited due to self-shading or lack of flow (Jackson, G. A. 1977, Gerard, V. A. 1982).

With only small remnants of bull kelp forest remaining on the coast of northern California, we wanted to know whether these habitat patches retain their ability to modify local seawater relative to nearby urchin barren habitat and to a similar extent as in previously studied large, intact kelp forests (mostly comprising of giant kelp) in other parts of the northeast Pacific. To do so, we used combination of *in situ* water samples and sensors to quantify spatiotemporal variation in seawater chemistry in the canopy and benthos of the interiors and edges of two northern California kelp forest fragments and adjacent urchin barrens.

MATERIALS AND METHODS

Site Selection and habitat characterization

Our study sites were Trinidad Harbor (TH) in Trinidad, CA (41.0'N, 124.1'W) and Portuguese State Beach (PB) in Mendocino, CA (39.3'N, -132.8'W) (Fig 1a). Each site was selected for the presence of fragmented bull kelp forests and adjacent urchin barrens with little to no kelp present. TH's kelp forest is located on the south side of Trinidad head, a large rocky promontory surrounded by sea stacks, protecting it from prevailing northwest swells. In the harbor, bull kelp is aggregated on substrate primarily comprised of large boulders, creating small kelp patches (Fig 1b). The adjacent urchin barren is located south of TH approximately 1,568m from the kelp forest, and is less protected from the northwest swell. In contrast, the kelp forest off of PB is mostly found on a flat rocky reef that is divided by wide sand channels, allowing kelp stipes to be more evenly spaced (Fig 1c). Similar to the kelp in TH, the kelp forest off PB is protected by the Mendocino Headlands from a northwest swell and open to the southwest. PB's urchin barren is located approximately 100m from the nearest kelp patch.

To characterize kelp forest and urchin barren habitats at each site, we used both aerial and subtidal SCUBA surveys. Aerial surveys were conducted for both TH and PB in 2018, and just PB in 2019 (Fig 1d), due to the lack of kelp present at TH during our sampling period in 2019. We estimated kelp canopy area using digital images captured from an unmanned aerial vehicle (DJI Mavric Pro) and analyzed with ArcGIS Pro

(version 2.6). Radiometric orthomosaic techniques and spatial classifications (principle component analysis, isodata clustering, and maximum likelihood) allowed us to minimize water reflection and select specifically for bull kelp canopy in the images, while deselecting any areas without kelp. To estimate kelp and urchin density within each habitat, we did a series of subtidal transect surveys. The placement of each transect was dictated by the presence of kelp. We placed the first 30m x 2m swath transect one meter inward from the edge of the kelp patch parallel to the shore from the outer edge of the kelp, which had an average depth between 6-7m, depending on the tide. Subsequent transects were placed 5m apart and parallel to the first transect; ranging in depth from 4-5.5m. Using the same depth contour, we placed three transects in each adjacent urchin barren. Surveys were conducted for each site in each habitat for 2018, but only at PB in 2019. Along each transect, divers counted algal stipes taller than 30cm of all present species that together comprised of both understory algae and canopy forming algae. Divers also counted both red and purple urchins whose tests were larger than 2.5cm, using PISCO protocols (http://www.piscoweb.org/).

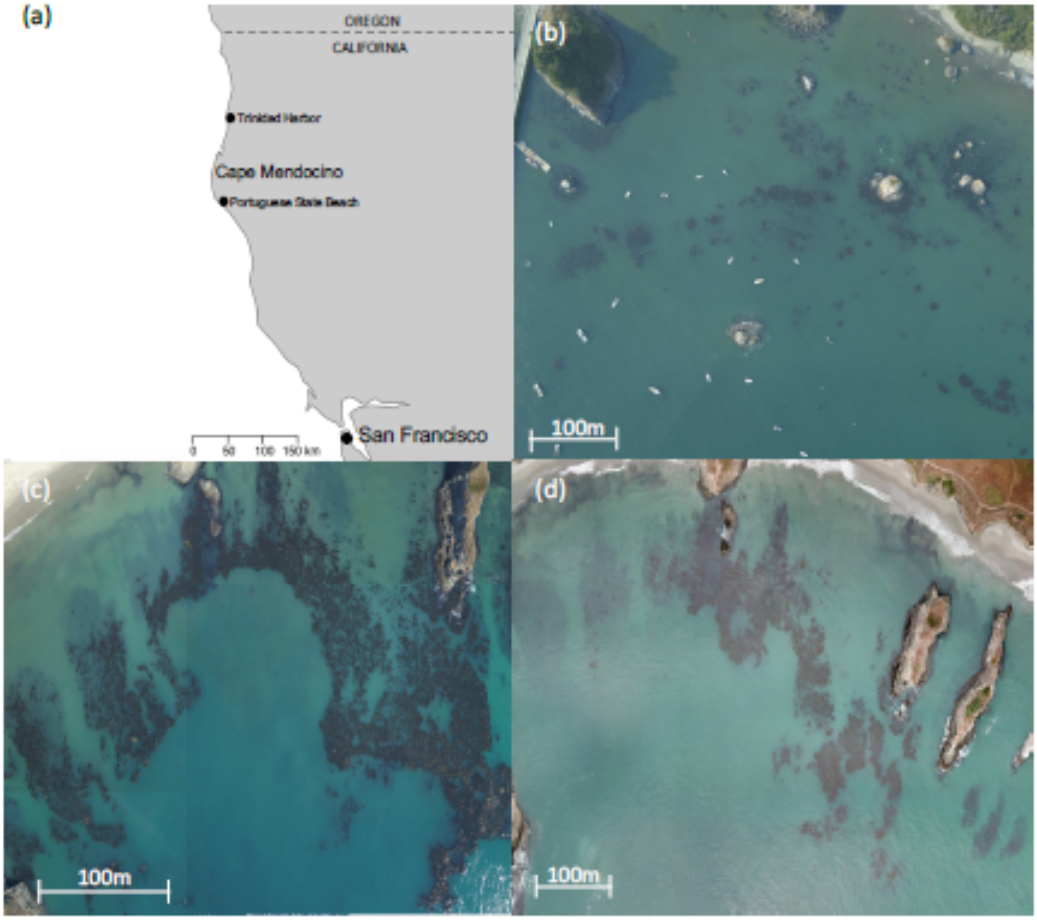

Figure 1. Study locations of two fragmented kelp forests in northern California. (a) Map of northern California with locations of both Trinidad Harbor and Portuguese Beach. (b) Kelp canopy coverage in Trinidad Harbor in 2018. (c-d) Portuguese Beach kelp canopy coverage in both 2018 and 2019, respectively.

Within- and among-habitat variation in seawater chemistry

To measure spatial variation in the total carbonate chemistry of seawater both at the surface and the benthos, and inside and outside of kelp forest fragments, we collected *in situ* water samples (n = 127 at PB, n = 222 at TH) at PB and TH in 2018. Samples were taken every week in TH and every two weeks at PB, weather permitting. We collected water samples one meter below the surface (m.b.s.) and one meter above the bottom (m.a.b.) to quantify bull kelp's capacity to alter local seawater chemistry in the canopy vs. the benthos. This was repeated in urchin barrens, creating four distinct habitats: kelp canopy (KC), kelp bottom (KB), urchin barren surface (US), and urchin barren bottom (UB). Samples were collected by divers by hand in 350 mL amber glass bottles to inhibit light from entering, and then immediately poisoned with 100 µL of saturated mercuric chloride ($HgCl_2$) to halt any biological activity; thus, no organisms in the sample could alter the seawater chemistry via metabolic processes, allowing the sample to reflect the water properties at the time of collection. pCO_2 and DIC measurements were made via gas equilibration and stripping, respectively, followed by infrared detection, after (Bandstra L, Hales B & Takahashi T. 2003), and modified for discrete samples as in (Hales, B., Chipman, D., & Takahashi, T. 2004). To ensure accuracy, gas and liquid standards included a range of values representative of modern-ocean seawater and the instrument was calibrated and monitored throughout run sequences with Certified Reference Materials (CRM) provided by A. Dickson (Scripps Institution of Oceanography). To calculate complete carbonate chemistry (including pH,

total alkalinity [TA], Ω aragonite, and Ω calcite) we used the seacarb package (Lavigne H., Epitalon, J.-M. & Gattuso J.-P. 2011 in R (v3.2.10) (R Core Team R.2013).

Temporal variation in seawater chemistry within and among habitats

To quantify temporal variation in seawater chemistry at the surface and the benthos inside and outside of kelp fragments and to confirm that diel fluctuations in pH and dissolved oxygen (DO) were consistent with diel photosynthetic activity by bull kelp, we deployed sensor arrays in each of the four habitats at PB: at 1 m.b.s and 1 m.a.b inside and outside of the kelp fragments from June to September 2019. Each sensor array consisted of a HOBO™ pH, DO, conductivity, and light loggers (Onset, HOBO™). Loggers also recorded seawater temperatures and were randomly rotated through habitat locations throughout the duration of the study, to remove potential bias from individual sensor errors. Each logger recorded data continuously at 15-minute intervals throughout the duration of the study. We calibrated each pH and DO sensor weekly. pH sensors were calibrated using a 3-point calibration and presented on the total hydrogen ion concentration scale after cross-calibration with Tris buffers (Dickson, A. G., Sabine, C. L., & Christian, J. R. 2007). We also collected weekly discrete water samples directly adjacent to sensors, which allowed us to verify sensor readings, and calculate total carbonate chemistry, as in 2018. DO sensors were calibrated using a one-point calibration at 100% saturation. DO sensors were corrected for temporal variation in salinity using conductivity logger data and had nominal accuracy of up to ±0.5 $mg \cdot L^{-1}$ according factory specification (HOBOwarePro v 3.7.16).

Variation in seawater chemistry between the kelp interior and kelp edge

To quantify diel fluctuations in pH and DO both in the interior and edge of the kelp canopy, we also deployed a sensor array 1 m.b.s and 1 m.a.b at the edge of the forest fragment at PB in summer 2019. Due to the expansion of the kelp forest perimeter at the end of July, and the senescence of kelp in the interior at the end of August, we only used data spanning from July 13th-29th to ensure that we were only comparing differences between the kelp forest edge and the healthy kelp interior.

Among-habitat variation in chlorophyll, flow, and light

To analyze chlorophyll, we collected weekly seawater samples in 250 mL brown plastic bottles from the kelp canopy interior, kelp edge, and the urchin barren surface. Samples were taken 5 meters apart while remaining in each habitat. Samples were then vacuum filtered them through 47mm GF/F filters for chlorophyll analysis. The filters were placed in borosilicate tubes along with 4ml of 90% acetone and stored in a spark-free freezer for 18-24 hours prior to fluorometric analyses. Fluorometric measurements before and after the addition of 10% HCl were compared to a solid standard to calculate chlorophyll *a* concentration.

We categorized flow in the different habitats based on the dissolution rate of plaster clod cards [40]. Clod cards were fastened to our sensor arrays in each habitat (kelp interior, kelp edge, and urchin barren) at three depths (1 m.b.s, 1 m.a.b., and the midpoint of the water column). After approximately 48 h, blocks were collected, dried at 65C, and

final weights recorded. Dissolution rates were quantified as proportion of initial mass lost.

Two light loggers (HOBO™Pendant 64 K—UA-002-64) were deployed on each sensor array in each habitat, one 1 m.b.s, and one 1 m.a.b. Although the HOBO sensors recorded light intensity in units of Lux, we were only interested in relative differences in light intensity among habitats and so we did not convert these data to PAR values.

Statistical analysis

We constructed 95% confidence intervals around mean daily differences in pH, pCO_2, Ω aragonite, Ω calcite, DIC, and TA between habitats (i.e., kelp canopy minus kelp bottom, urchin barren surface minus urchin barren benthos, kelp canopy minus urchin barren surface, and kelp benthos minus urchin barren benthos) to assess statistical significance. 95% CI are more informative than traditional significance tests, as they allow hypothesis testing while providing a range of parameter values to reflect the degree of uncertainty in the difference estimate. If the 95% CI did not overlap zero, the daily difference for a given carbonate parameter was deemed significant at $\alpha = 0.05$. Temporal pH and DO data from each habitat were converted to daily day and night means and differences between these means were analyzed with paired t-tests. We assessed the relationships between seawater temperature and pH and seawater temperature and DO in the different habitats using simple ordinary least squares regression. We used paired t-tests to analyze between-habitat differences in chlorophyll, and ANOVA to analyzed differences in low among habitats. I used Bonferroni-corrected p-values to adjust for

multiple hypothesis tests. All analyses were done using R (v3.6.1) (R Core Team R. 2013)

RESULTS

Kelp canopy area and habitat characterization

In 2018, the remnant kelp forest canopies in Trinidad Harbor (TH) and Portuguese Beach (PB) were approximately 0.32 and 1.05ha, respectively. In 2019, we observed a reduction in kelp canopy at Portuguese Beach to 0.85ha. Bull kelp density in remnant forests in 2018 was 3.78 and 3.28 stipes · m^{-2} at TH and PB, respectively. In 2019, despite the overall reduction in canopy cover, PB's bull kelp density increased to 4.44 stipes · m^{-2}. Stipe density for both bull kelp and understory flora (e.g., *Pterygophora californica, and Laminaria spp.)* was zero in adjacent urchin barrens.

Within-habitat spatial variation in seawater chemistry

Seawater carbonate chemistry varied within kelp and urchin barren habitats in a manner consistent with a kelp canopy effect. pH was 0.11 and 0.07 pH units higher significantly in the kelp canopy (KC) than the kelp benthos (KB) at PB and TH, respectively (Fig 2a). Further, pCO_2 was significantly 130.87 µatm lower in the kelp canopy than in the kelp benthos at PB and 80.85 µatm lower at TH, representing a 23 and 12% difference in pCO_2 (Fig 2b). Aragonite saturation state was significantly larger by 0.44 (PB) and 0.28 (TH) units in the KC than KB, and similarly, calcite saturation was significantly 0.69 (PB) and 0.44 (TH) units higher in the KC than in the KB (Fig 2c, d).

In contrast, we saw no significant difference in both dissolved inorganic carbon (DIC) and total alkalinity (TA) at either PB or TH when comparing KC and KB (Fig 2e, f).

Differences in seawater carbonate chemistry between the urchin barren surface (US) and urchin barren benthos (UB) varied between sites. In the PB urchin barren, carbonate chemistry differences between the surface and benthos were in a similar direction to those in the kelp forest, but the differences were roughly half the magnitude; with pH 0.05 units higher in the US than the UB at PB (Fig 2a). Further, pCO_2 at PB was 79.92 μatm lower in the US than in the UB, resulting in a 12% difference in pCO_2 (Fig 2b). Aragonite and calcite saturation states were larger by 0.26 and 0.31 units in the US than in the UB (Fig 2c, d). Similar to the comparison in the kelp, we saw no difference in both DIC and TA at PB when comparing US and UB (Fig 2e, f). In the TH urchin barren, there were no significant differences in carbonate chemistry between the surface and the benthos (Fig 2a-f).

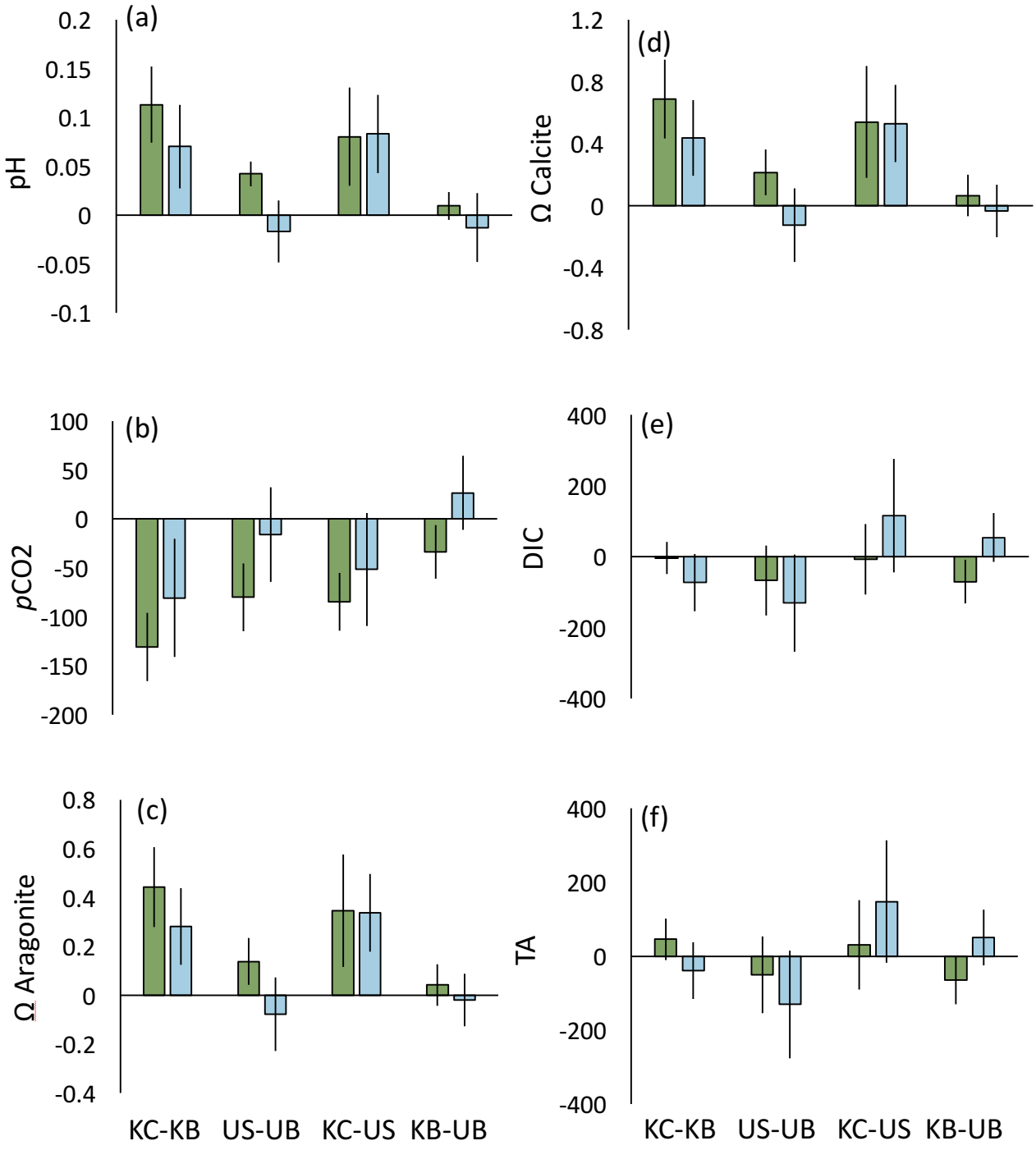
(a)
pH
0.2
0.15
0.1
0.05
0
-0.05
-0.1
(b)
pCO2
100
50
0
-50
-100
-150
-200
(c)
Ω Aragonite
0.8
0.6
0.4
0.2
0
-0.2
-0.4
KC-KB
US-UB
KC-US
KB-UB
(d)
Ω Calcite
1.2
0.8
0.4
0
-0.4
-0.8
(e)
DIC
400
200
0
-200
-400
(f)
TA
400
200
0
-200
-400
KC-KB
US-UB
KC-US
KB-UB

Figure 2. Average paired (per day) differences between habitats for carbonate parameters (a) pH, (b) pCO_2, (c) Ω aragonite, (d) Ω calcite, (e) DIC, and (f) TA, for both Trinidad Harbor (blue) and Portuguese Beach (green). Error bars represent 95% C.I. Abbreviations of habitats are: KC – kelp canopy, KB – kelp benthos, US – urchin barren surface, UB – urchin barren benthos.

Among-habitat spatial variation in seawater chemistry

To reinforce that the paired differences in carbonate chemistry was due to photosynthetic activity by the kelp, we also wanted to make comparisons across habitats between the kelp forest habitats and the adjacent urchin barren habitats. We found that carbonate chemistry differed inside and outside of kelp fragments in a manner consistent with a kelp effect. pH was 0.08 units higher in the KC than the US at both PB and TH (Fig 2a). In addition, pCO_2 at PB was 84.53 µatm lower in the KC than in the US and 51.49 µatm lower at TH, representing a 20 and 11% difference in pCO_2, respectively (Fig 2b). Note that the difference in pCO_2 at TH was only marginally significant. Aragonite and calcite saturation states were higher by 0.35 and 0.54 units in the KC than in the US at both sites (Fig 2c, d). Similar to the comparison in the kelp and urchin barren, we saw no difference in both DIC and TA at PB and TH when comparing KC and US (Fig 2e, f).

Finally, when comparing KB with the UB we found that only pCO_2 and DIC were lower in the KB at PB, while the rest of the carbonate chemistry parameters where not significantly different. At PB pCO_2 and DIC were lower in the KB by 7 and 4%, respectively (Fig 2b, e).

Temporal variation in seawater chemistry inside and outside of kelp fragments

Spatial variation was consistent with a kelp canopy effect on local seawater chemistry, but to confirm that photosynthetic activity by the kelp was driving these spatial patterns, we looked at the temporal fluctuations in pH and dissolved oxygen (DO) within all four habitats (KC, KB, US, UB).

On average, pH was 0.11 units higher during the day than at night in the KC (Fig 3a, Table1), and we observed the largest diel fluctuations in pH in the KC (7.47 to 8.32) compared to the kelp benthos; KC pH was 0.16 units higher than KB pH (Fig 3a, Table1). The diel fluctuations in pH within the urchin barrens did not differ between the surface and the benthos (Fig 3b, Table 1). When comparing across habitats, pH was 0.11 units higher in the KC than US during the day (Fig 3c, Table 1), but was not significantly different at night (Fig 3c, Table 1).

Seawater temperature was positively correlated with pH, but the strength of those relationships varied among habitats (Fig 3a-c, insets). Temperature explained only 3% of the variation in pH in the KC but explained 22% of the variation in pH in the KB (Fig 3a inset). In addition, temperature explained 23% of the variation at the US, and 47% in the UB (Fig 3b inset).

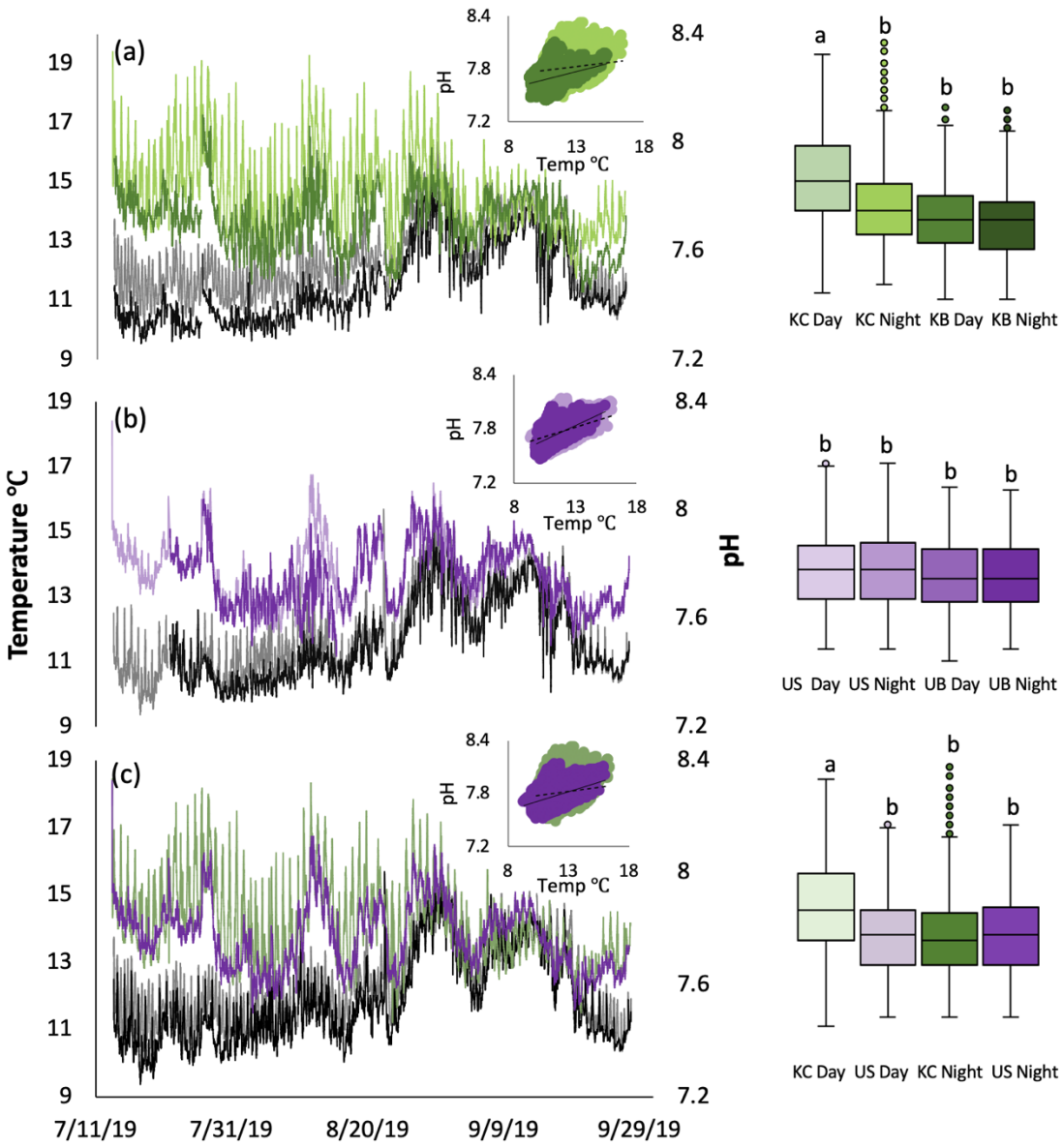

Figure 3. Spatiotemporal variation in pH and temperature in kelp forest and urchin barren habitats. Each panel (a-c) includes fluctuations in pH through time (left), relationship between temperature and pH (inset), and average habitat-specific day and night pH's. (A) Kelp canopy (KC-light green) versus kelp benthos (KB-dark green); temperature of KC is in grey and KB is in Black. (B) is comparing urchin barren surface

(US-light purple) to urchin barren benthos (UB-dark purple); temperature of US is in grey and UB is in black. (C) is comparing kelp canopy (KC-light green) to urchin barren surface (US- light purple); temperature of KC is in grey and KS is in black. Letters denote significant pairwise differences ($P < 0.001$). Inset in graph (a) R^2 for KC (light green) = 0.03 and KB (dark green) = 0.21, (b) R^2 for US (light purple) = 0.23 and UB (dark purple) = 0.47.

DO was 16% higher during the day than at night in the KC (Fig 4a, Table 1) and exhibited the largest diel fluctuations (6.91-18.10 $mg \cdot L^{-1}$) relative to other habitats (Fig 4a, Table 1). DO did not vary between day and night in the KB, but KC DO was 23% higher than KB DO (Fig 4a, Table 1). DO was not significantly different between the US and UB, regardless of diel period (Fig 4b, Table 1). When comparing across habitats, DO was 16% higher in the KC during the day than US DO during the day, but not different from those habitats at night (Fig 4c; Table 1). Temperature explained only 5% of the

variation in DO in the KC, but 34% in the KB, 17% at the US, and 31% in the UB (Fig 4a-c, insets).

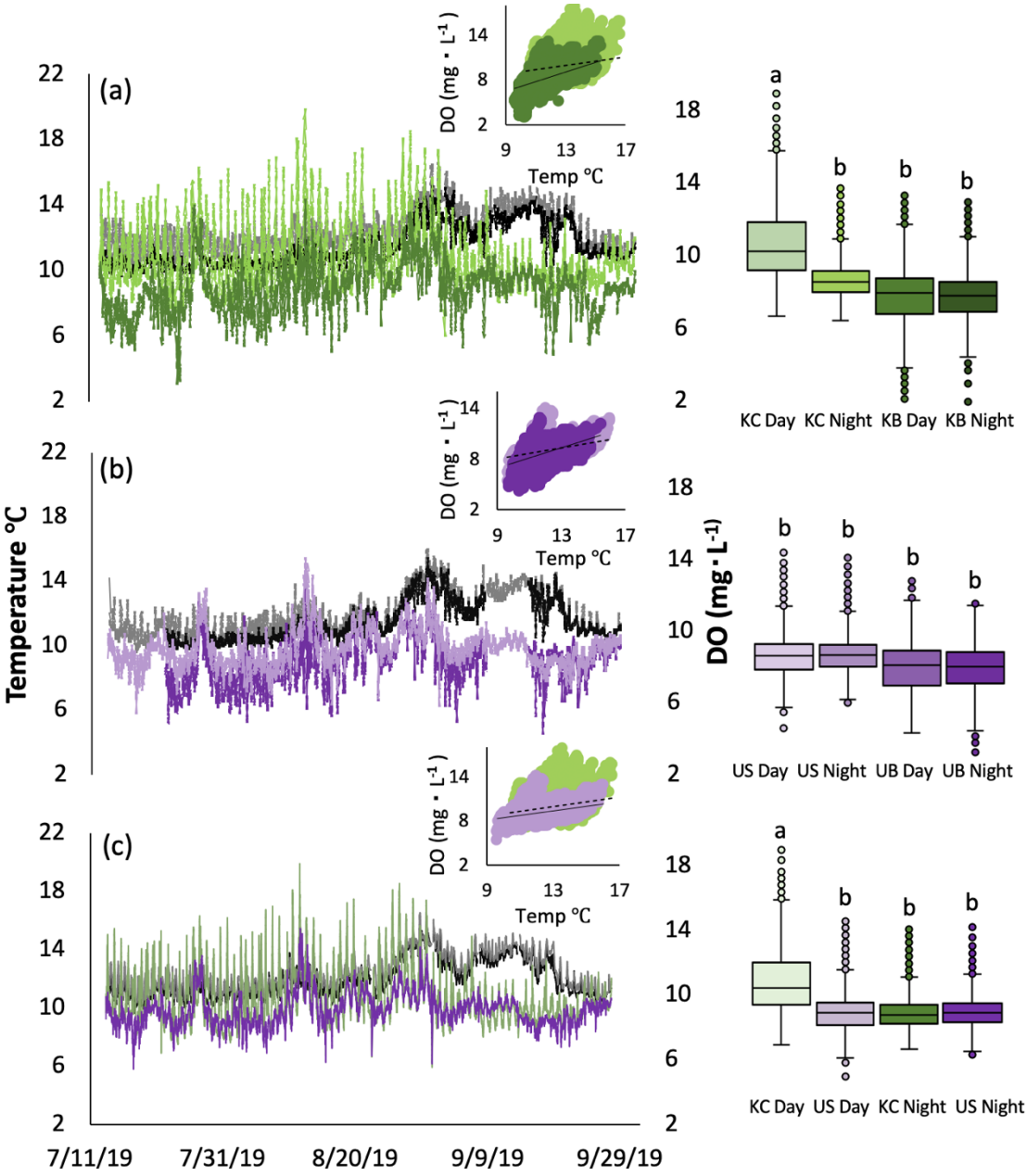

Figure 4. Spatiotemporal variation in dissolved oxygen (DO) and temperature in kelp forest and urchin barren habitats. Each panel (a-c) includes fluctuations in DO through time (left), relationship between temperature and DO (inset), and average habitat-specific

day and night DO's. (a) Kelp canopy (KC- light green) versus kelp benthos (KB- dark green); temperature of KC is in grey and KB is in black. (B) is comparing urchin barren surface (US-light purple) to urchin barren benthos (UB- dark purple); temperature of US is in grey and UB is in black. (C) is comparing kelp canopy (KC- light green) to Urchin surface (US- light purple); temperature of KC is in grey and KS is in black. Letters denote significant pairwise differences ($P < 0.001$). Inset in graph (a) R^2 for KC (light green) = 0.05 and KB (dark green) = 0.34, (b) R^2 for US (light purple) = 0.17 and UB (dark purple) = 0.31.

Table 1. Day and night comparisons of both pH and dissolved oxygen (DO) among kelp canopy (KC), kelp benthos (KB), urchin barren surface (US) and urchin barren benthos (UB). Bolded values denote statistical significance

Comparison	pH *t*	pH *p*	DO *t*	DO *p*
KC_{day} vs KC_{night}	**4.69**	**< 0.001**	**7.57**	**< 0.001**
KB_{day} vs KB_{night}	0.591	0.555	0.404	0.222
US_{day} vs US_{night}	0.079	0.937	0.261	0.794
UB_{day} vs UB_{night}	0.118	0.906	0.248	0.206
KC_{day} vs US_{day}	**4.53**	**< 0.001**	**7.35**	**< 0.001**
KC_{night} vs US_{night}	0.258	0.797	0.448	0.655
KC_{day} vs KB_{day}	**7.1**	**< 0.001**	**13.41**	**< 0.001**
KC_{night} vs KB_{night}	**3.15**	**0.002**	**6.25**	**< 0.001**
KB_{day} vs UB_{day}	0.118	0.906	1.07	0.286

Variation in seawater chemistry between the kelp interior and kelp edge

Although we observed the largest diel fluctuations in pH in the KC, the kelp edge (KE) on average had a higher pH during the day compared to the KC interior, but only by 0.05 units (Fig 5a, Table 2). pH at the KE reached as high as 8.38 during the day, and as low as 7.69 at night (Fig 5a, Table 2). The biggest difference between the KC interior and the KE came at night, where the KE had a pH 0.11 units higher than that of the KC (Fig 5a, Table 2). DO ranged from 7.32 $mg \cdot L^{-1}$ to 15.60 $mg \cdot L^{-1}$ at the kelp edge (Fig 5b, Table

2). Although DO was higher by 0.8 mg·L^{-1} in the KC compared to the KE during the day, DO was similar at the night in both habitats (Fig. 5b, Table 2). During comparable periods (13-29 July 2019), temperature explained only 10% of the variation in pH in the KE, and 18% of the variation in pH in the KC interior (Fig 5a, inset). Temperature explained a similar amount of variation in DO at both the KE (16%) and in the KC interior (15%) (Fig 5b, inset).

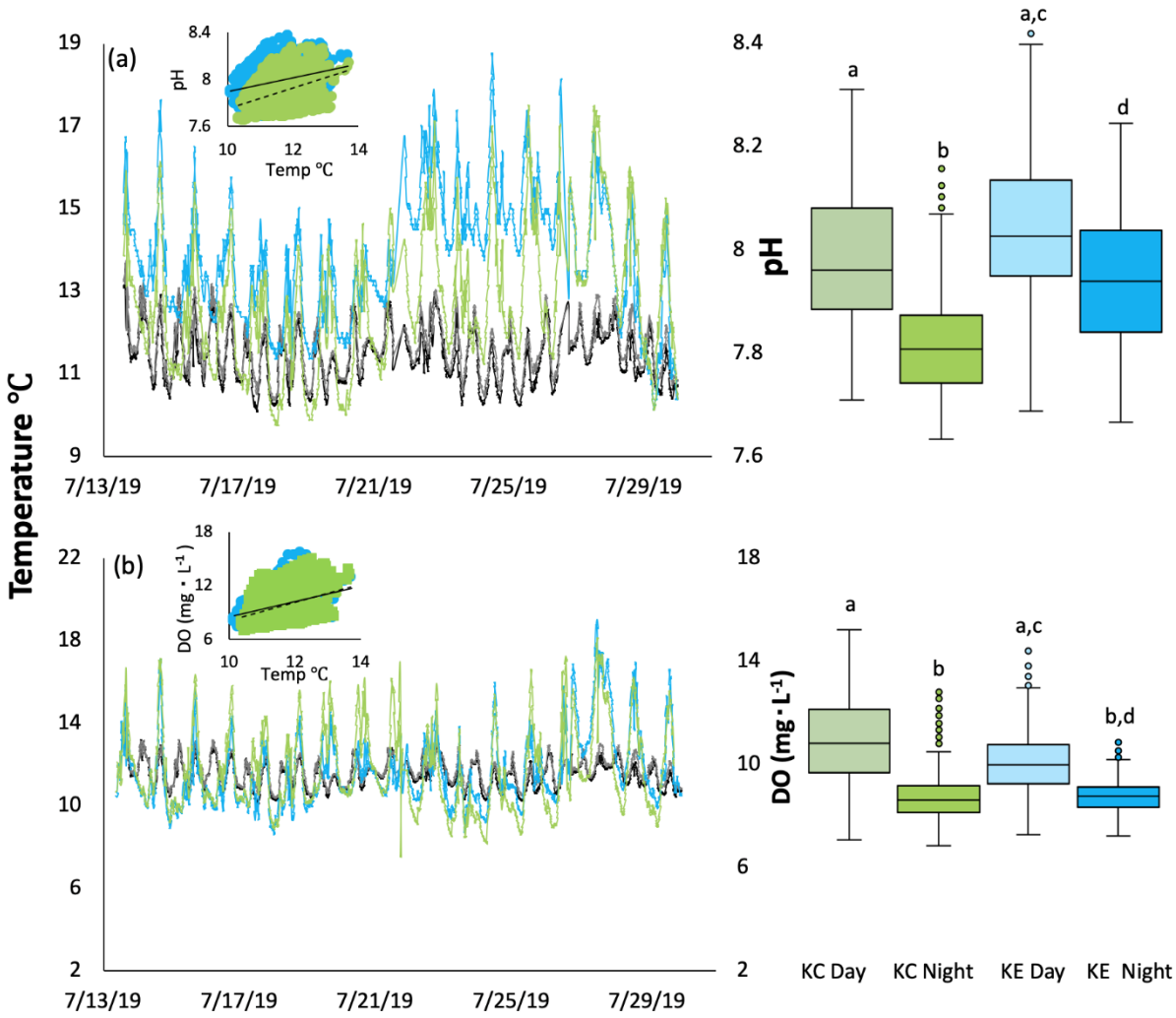

Figure 5. Spatiotemporal variation in pH and dissolved oxygen (DO) in kelp canopy (interior) and kelp edge. (a) includes fluctuations in pH and temperature through time (left), relationship between temperature and pH (inset), and average habitat-specific day and night pH's. (a) Kelp canopy interior (KC- light green) versus kelp edge (KE- light blue); temperature of KC is in grey and KE is in black. (b) includes fluctuations in DO and temperature through time (left), relationship between temperature and DO (inset), and average habitat-specific day and night DO's. (a) Kelp canopy interior (KC- light green) versus kelp edge (KE- light blue); temperature of KC is in grey and KE is in black. Letters denote significant pairwise differences ($P < 0.001$). R^2's for insets are: KC (light green) pH = 0.18, DO = 0.16 and KE (light blue) pH = 0.10, DO = 0.15.

Table 2. Day and night comparisons of both pH and dissolved oxygen between kelp canopy interior (KC) and kelp edge (KE). Bolded values denote statistical significance.

Comparison	pH *t*-value	pH *p*-value	DO *t*-value	DO *p*-value
KC_{day} vs KC_{night}	**3.79**	**<0.001**	**4.72**	**< 0.001**
KE_{day} v KE_{night}	**2.34**	**0.026**	**2.30**	**0.029**
KC_{day} v KE_{day}	1.27	0.213	1.49	0.148
KC_{night} v KE_{night}	**2.91**	**0.007**	0.09	0.930

Among-habitat variation in chlorophyll, flow, and light

There were no significant differences in average daily chl a concentration between habitats ($t = 0.157$, df = 12, $P = 8.78$). There were no significant effects of habitat (ANOVA; $F_{4,9} = 1.65$, $P = 0.246$) or the interaction between habitat and deployment depth on clod card dissolution rates (ANOVA; $F_{4,9} = 1.89$, $P = 0.196$). There was a significant effect of depth on clod card dissolution rate however (ANOVA, $F_{4,9} = 36.01$, $P < 0.001$). Clod cards deployed 1 m.b.s lost significantly more mass than those in the middle of the water column (Tukey HSD, $P = 0.012$), which lost significantly more mass than those 1 m.a.b. (Tukey HSD, $P = 0.003$). Light intensity during daylight hours was above 350 (lum $\cdot$ ft^{-2}) 25% of the time in the interior of the kelp canopy edge, 65% at the edge of the kelp canopy, and 80% at the urchin barren surface. In contrast, benthic habitats reach this threshold less than 1% of the time in the kelp interior, 30% in the kelp edge, and 64% in the urchin barren.

DISCUSSION

Our data indicate that small, fragmented bull kelp patches are capable of altering local seawater chemistry, but only the kelp canopy. The observed daily fluctuations in seawater chemistry recorded in and out of kelp patches were consistent with diel patterns of photosynthesis and respiration by bull kelp and similar in magnitude to those observed in large, intact kelp forests (Table 3). Thus, kelp forest habitats, regardless of size, are capable of modifying local seawater chemistry in a way that may have implications for calcifying marine organisms. We hypothesize that whether in the kelp interior, or at the kelp edge, frond density may be a good predictor of seawater chemistry dynamics within a kelp forest, regardless of the area or canopy coverage of the forest (Layton, C. et al. 2019).

pH, Ω aragonite, and Ω calcite all increased, and pCO_2 decreased, in the kelp canopy during the day relative to other habitats. The average daily pH differences between the surface and benthos of kelp fragments that we observed in our study (PB = 0.11, TH =0.09) were similar to those documented in central California's giant kelp forest (0.12; (Koweek, D. A. et al. 2017), whose main canopy forming kelp is *Macrocystis* (giant kelp) which is structurally different then bull kelp, as Macrocystis has photosynthetic blades that persist throughout the water column unlike bull kelp whose blades are aggregated at the surface. Similarly, pH differences in and outside of kelp fragments (PB and TH = 0.09) were similar to those found in Washington's large bull kelp forests (0.08 pH units). Similarly, Ω aragonite was higher and pCO_2 lower inside

than outside of kelp fragments (0.5 units and 131 µatm) to a similar degree to the large Washington kelp forests (0.2 units and 144 µatm; (Pfister, C. A., Altabet, M. A., & Weigel, B. L. 2019). In addition, although average daily differences in carbonate chemistry in and out of kelp fragments in our study were similar to differences in and out of large kelp forests, overall variation in pH was generally greater (kelp canopy 7.47-8.32) in our kelp fragments, than those recorded in large, intact kelp forests (Koweek, D. A. et al. 2017) ~7.7-8.33; (Kapsenberg, L., & Hofmann, G. E. 2016) ~7.88-8.12, (Takeshita, Y. et al. 2015)~7.78-8.12, (Hofmann, G. E. et al. 2011) ~7.7-8.25, (Frieder, C. A., Nam, S. H., Martz, T. R., & Levin, L. A. 2012) ~7.65-8.39; and (Pfister, C. A., Altabet, M. A., & Weigel, B. L. 2019) ~7.53-8.19).

Whereas we found evidence for photosynthetic modification of seawater chemistry in the kelp canopy, we did not find evidence of significant calcification in the kelp forest, or elsewhere. Calcification increases pCO_2 by depleting bioavailable CO_3 and therefore reducing TA (Dickson, A. G., Sabine, C. L., & Christian, J. R. 2011). We therefore expected that TA would have been depleted at the bottom relative to the canopy in both the kelp forest and the urchin barren due to calcification. However, the lack of differences in TA suggests that the chemical signature of calcification was small relative to water exchange (seawater mixing) in the kelp (and urchin) benthos. Although not significant, there was a slight trend for lower DIC in the kelp canopy compared to the kelp benthos, which is consistent with the decrease in pCO_2 in the kelp canopy. The relatively weaker decrease in DIC in the canopy compared to pCO_2 could be because DIC comprises CO_2, HCO^{3-}, and $CO3^{2-}$, and so if $CO3^{2-}$ (bioavailable calcium carbonate) is

added to the system in the benthos (e.g., due to the dissolution of calcifying invertebrates or algae in highly acidic benthic waters) then we would end up with a higher DIC than expected in the benthos, negating significant surface-benthos differences.

Although chl *a* concentration, which we used as a proxy for phytoplankton abundance, was higher in the kelp fragments than in the adjacent urchin barrens at both PB and TH in 2018, we observed no difference in chl *a* concentration between the kelp forest and urchin barren in PB in 2019. Regardless, we suggest that any effect of phytoplankton-driven modification of seawater chemistry should be small compared to the effects of kelp. For example, estimates of carbon uptake by phytoplankton (Pfister, C. A., Altabet, M. A., & Weigel, B. L. 2019) and published studies of *Macrocystis* and *Nereocystis* carbon fixation (Pfister, C. A., Altabet, M. A., & Weigel, B. L. 2019, North, W. J. 1994), suggest that carbon fixation by phytoplankton outside of kelp beds, (where it is not shaded by kelp or limited by nutrient competition with kelp) is relatively small compared with carbon fixed by canopy kelp (North, W. J. 1994). It follows then that photosynthetic modification of seawater in the canopy, where kelp reduce nutrient concentrations and shade the water column (Reed, D. C. & M. S. Foster. 1984, Gerard, V. A.1982), would be relatively low compared to the effects of kelp. We do note that recent studies have shown that carbon fixing microbes can also be abundant in the kelp canopies (Pfister, C. A., Altabet, M. A., & Weigel, B. L. 2019, Hugler, M., & S. M. Sievert 2011) and that productivity estimates based on chl *a* pigment concentrations or kelp biomass will be an underestimate. If kelp fragment canopies also increase microbial diversity relative to surrounding habitats, then they may also increase metabolic opportunities,

including carbon fixation by microbes (Pfister, C. A., Altabet, M. A., & Weigel, B. L. 2019, Weigel, B. L., & Pfister, C. A. 2019) Regardless of whether the kelp canopy *per se* is the sole driver of our observed seawater chemistry variation or microbes and phytoplankton contribute to the kelp canopy effect, fragmented kelp patches would seem to be capable of both directly and indirectly modifying their local chemical environment.

Non-biological processes (e.g., upwelling, water flow, etc.) can also contribute to variation in local seawater chemistry in and around kelp forests (Hauri, C. et al. 2009, García- Reyes, M., & Largier, J. 2010). For example, during periods of upwelling along the coast of California, kelp forests and adjacent coastal habitats are exposed to incursions of cold, nutrient rich, acidic and oxygen-poor water (Chan, F. et al. 2017, Feely, R. A. et al. 2018). pH, DO, and temperature are highly correlated in upwelled coastal water (Chan, F. et al. 2017, Feely, R. A. et al. 2018) and so if upwelling was driving the observed variation in seawater chemistry, we would expect to see these strong relationships between temperature and pH and DO maintained across habitats. However, examination of the relationships between temperature and both pH and DO, revealed that temperature explained significantly less variation in pH and DO in the kelp canopy than in other habitats. For example, seawater temperature explained 18% more of the variation in pH and 29% more of the variation in DO in the kelp benthos compared to the kelp canopy. Further, seawater temperature explained 44% more variation in pH in the urchin barren benthos compared to the kelp canopy. Taken together, these data suggest that the photosynthetic/respiratory activity of the kelp canopy play a more important role in driving local seawater dynamics than upwelling-driven temperature.

Another physical process that can play a large role in altering local seawater chemistry is water flow and mixing (Feely, R. A. et al. 2010), which depend on exposure to waves and currents, temperature, and depth (Koweek, D. A. et al. 2017) We controlled for depth effects by placing each sensor mooring at the same depth in both kelp and urchin barren habitats. We also attempted to keep our sampling locations as similar as possible in terms of wave and current exposure. Clod cards fastened to our sensor moorings lost an average of 75% of their mass in the kelp interior, 76% in the kelp edge, and 77% in the urchin barren, after 48 hours. Thus, we concluded that flow environments were roughly similar in all three habitats. Although the clod cards do not provide an actual measurement of how fast the water is moving in each habitat, they have been shown to be a reliable proxy for measuring flow, and so provide an understanding of which habitats experience more or less water motion compared to the others (Thompson, T. L., & Glenn, E. P. 1994). Because kelp can attenuate flow both along and across its boundaries [Jackson, G. A., & Winant, C. D. 1983, Rosman, J. H., Koseff, J. R., Monismith, S. G., & Grover, J. 2007, Gaylord, B.et al. 2007), we suggest that any local effects the kelp has on the local seawater chemistry are likely to persist – even in a fragment.

As with previous studies of large kelp forests (Frieder, C. A., Nam, S. H., Martz, T. R., & Levin, L. A. 2012, Koweek, D. A. et al. 2017, Pfister, C. A., Altabet, M. A., & Weigel, B. L. 2014), canopy-benthos differences in small kelp patches were evident from our data, and they are suggestive that photosynthesis/respiration played an important role in driving the seawater chemistry variability in the kelp canopy. However, unlike

previous observations of kelp forests where *Macrocystis* dominates (Koweek, D. A. et al. 2017), we did not see evidence of a strong photosynthetic effect in the understory algae, relative to adjacent urchin barren benthos, despite its strong presence. We provide four alternative hypotheses as to why. First, the resolution of our sampling (e.g., 1 m above the benthos) might have prevented us from being able to quantify an understory effect (e.g., (Koweek, D. A. et al. 2017)). Second, *Nereocystis* is very effective at creating a canopy habitat that inhibits high light intensity from reaching the bottom during the day, thus understory kelp may not be photosynthesizing during the day at a rate that significantly alters the local seawater chemistry. Indeed, we found that the kelp forest bottom received 11% less light intensity ($lum \cdot ft^{-2}$) than the kelp canopy. Thirdly, cold, acidic upwelled water did not mix vertically in our habitats and thus similar low pH's at the kelp forest and urchin barren benthos are due to benthic incursion of acidic upwelled seawater (Chan, F. et al. 2017, Feely, R. A. et al. 2018). Finally, similarly low pHs at the kelp and urchin barren benthos may be due to the presence of *Desmarestia* spp. (acid weed) in the understory of our kelp patches at both sites (*personal observation*). *Desmarestia* when disturbed, will release sulfuric acid, which can lower the pH of surrounding seawater (Molis, M., et al. 2009). Further studies are needed to distinguish among these alternative hypotheses.

As climate change-associated stressors continue to impinge on coastal habitats, kelp forests in northern California may continue to decline (Connell, S. D., & Russell, B. D. 2010, Provost, E. J. et al. 2017, Bindoff, N.L. et al. 2019), increasing the abundance of fragmented patches relative to intact forest habitat. Increases in forest fragmentation

leads to an increase in the ratio of perimeter to interior (i.e., edge) habitat, which can have implications for forest carbon dynamics (Reinmann, A. B., & Hutyra, L. R. 2017, De Paula, M. D., Costa, C. P. A., & Tabarelli, M., 2011, Chaplin-Kramer, R. et al. 2015). In the summer, when we sampled and when kelp canopies were well-developed, fronds at the forest's edge had larger, healthier-looking blades than those in the forest's interior, suggesting higher carbon fixation and growth rates in edge fronds. Light availability in the kelp forest's interior may also be low enough that nutrient uptake and photosynthesis is limited. Consistent with these observations, we found that during the day, seawater at the kelp edge was on average 0.05 pH units higher than seawater in the kelp interior. However, the biggest difference in pH between the kelp edge and interior was at night. On average pH was 0.11 units higher at the kelp edge at night than the interior. We hypothesize that higher pH's at the kelp edge are due to longer exposure to light at the kelp edge compared to the kelp interior. In support of this hypothesis, our data shows that light intensity during daylight hours was above 350 ($lum \cdot ft^{-2}$) 65% of the time at the kelp edge compared to 25% in the kelp interior.

Understanding the role of foundational species like kelp in creating OA refugia through metabolic activity is increasingly important to understand as OA, and its effects on marine organisms, is predicted to worsen (Hodgson, E. E. et al. 2018). Organismal growth, metabolism, and behavior have all been shown to be negatively affected in seawater with pH lower than 7.75. For example, sea urchin larvae are 7-13% smaller when exposed to pH below 7.7 (Pauline, C. Y., Matson, P. G., Martz, T. R., & Hofmann, G. E. 2011), and juvenile rockfish are 17% slower when pH drops to 7.5 (Hamilton, S. L.

et al. 2007). Our data indicate that seawater in the kelp canopy had a pH of 7.75 or greater, 75% the time; whereas seawater pH in adjacent urchin barren habitat was greater than 7.75 only 52% of the time. Thus, we hypothesize that the combination of physical and biological processes in kelp fragments may create natural refugia for canopy-dwelling organisms, with some caveats. First, because we sampled in the summer, when bull kelp would have the biggest effect on seawater chemistry (Springer, Y., Hays, C., Carr, M., & Mackey, M. M. 2007), this effect may be temporary and only during daytime during the spring and summer due to bull kelp being an annual species. Second, although animals may take advantage of periods of pH refugia from the kelp during the day, large fluctuations during day-night cycles in the kelp could create a stressful environment. However, if calcifying organisms can calibrate their calcification to periods of high pH (Wahl, M. et al. 2018), these diel and seasonal cycles could be critical. Subsequently, we need to monitor and understand kelp's ability to modulate local environments across multiple spatial and temporal scales if we are to effectively manage critical coastal ecosystems in the face of OA, climate change, and kelp deforestation.

Table 3. Comparison of seawater carbonate chemistry parameters between historically large and persistent kelp forests in a) central California and b) Strait of Juan de Fuca to kelp forest fragments at Portuguese Beach and Trinidad Harbor in northern California.

A)

Variable	Comparison	Lover's Pont-Julia Platt (Koweek et 2017)	Portuguese	Trinidad	Average (PB & TH)
DIC	Canopy-Benthos	-70	-3.61	-72.61	-38.11
TA	Canopy-Benthos	-6	45.78	-38.77	3.505
pH	Canopy-Benthos	0.12	0.11	0.07	0.09
pCO2	Canopy-Benthos	-144	-130.87	-80.85	-105.86
Ω Ar	Canopy-Benthos	0.61	0.442	0.281	0.3615
Ω Ca	Canopy-Benthos	NA	0.688	0.437	0.5625

B)

Variable	Comparison	Strait of Juan de Fuca (Pfister et al. 2019)	Portuguese	Trinidad	Average (PB & TH)
DIC	Inside-Outside	NA	-7.03	116.19	54.58
TA	Inside-Outside	6.39	30.88	147.23	89.055
pH	Inside-Outside	0.087	0.08	0.08	0.08
pCO2	Inside-Outside	-167.1	-84.54	-51.49	-68.015
Ω Ar	Inside-Outside	0.195	0.346	0.338	0.342
Ω Ca	Inside-Outside	0.307	0.539	0.529	0.534

CHAPTER 2: EFFECTS OF CURRENT AND FUTURE PREDICTED UPWELLING ON URCHIN CONDITION, SURVIVAL, AND GRAZING

INTRODUCTION

A recent 'perfect storm' of multiple environmental stressors has caused dramatic reductions (> 90% loss in canopy cover) in historically large kelp forests along the northern California coast (Rodgers-Bennet 2019). As a result, these once diverse ecosystems have been converted to species-depauperate, and potentially stable, urchin barrens (Filbee-Dexter & Scheibling 2014, Steneck et al. 2002, Steneck 2020). This dramatic loss of kelp has resulted in cascading and detrimental effects on other organisms and the vital ecosystem services provided by kelp forests. For example, the associated mass decline (80% mortality) of abalone (*Haliotis* spp.) populations, has resulted in the closure of an estimated $44M recreational fishery (Reid et al. 2016). The loss of kelp has been attributed to the recent recurrence of marine heat waves, the disappearance of the predatory sea star *Pycnopodia helianthoides*, and the subsequent increase in purple sea urchins (*Strongylocentrotus purpuratus*), the major grazer of kelp (Rogers-Bennett 2019). Because sea urchins have played a major role in the destruction of these kelp forests (Steneck 2020), it is of the utmost importance that we gain a greater understanding of how current and future environmental conditions will shape this critical interaction.

One way the bull kelp-purple urchin interaction may be affected is through the process of coastal upwelling. Upwelling is a process that brings cold, nutrient-rich, hypoxic water with high concentrations of CO_2 (and low pH) to nearshore coastal

environments seasonally. Upwelling thus simultaneously changes multiple environmental factors that can affect species interactions. Although theory predicts that species interactions in marine benthic communities vary predictably with upwelling (Menge and Menge 2013), effects of individual abiotic stressors associated with upwelling are variable and can be-species specific. For example, slight decreases in water temperature associated with upwelling can dramatically reduce the effects of the keystone predatory sea star, *Pisaster ochraceus*, on mussels on rocky shore. In estuarine food webs, low but nonlethal dissolved oxygen concentrations can either greatly increase or decrease predation (Breitburg et al. 1997). Further, in some regions, present-day upwelling can deliver water that is as acidic as that predicted for global ocean averages 50-100 years from now (Hauri et al. 2009) and such low pH conditions have been shown alter competitive interactions between calcareous algae and kelp, shifting rocky reefs to kelp-dominated habitats (Connell and Russell 2010).

Whereas most studies have focused on the effects of individual abiotic factors associated with upwelling on species interactions (Moulin et al. 2014, Siikavuopio et al. 2007), few have examined the effects of these factors in concert (but see Low 2018). Even fewer have examined how future predicted variation and temporal variability in these factors will influence species interactions (but see Low and Micheli 2018 for a notable exception). However, during upwelling, changes in temperature, DO, and pH are highly correlated in coastal systems, and upwelling happens intermittently, over scales of days to weeks (Huyer 1983, Frieder et al. 2012). Therefore, to understand how current and future upwelling conditions will affect key species interactions it is necessary to

examine combinations of factors, acting together under natural temporal scales of variability.

Upwelling plays a defining role in the biogeography, ecology, and productivity of the California Current Large Marine Ecosystem (CCLME (Feely et al. 2018, Chan et al. 2017, Orr et al. 2005). Upwelling is characterized by strong northwest winds that drive net surface waters offshore via Eckman transport (Feely et al. 2016). Generally speaking, upwelling dominates during the spring and early summer months in the CCLME and dissipates in the late summer and fall, before the start of winter storms (Feely et al. 2016). However, the magnitude of current day upwelling conditions varies regionally along the CCLME (Chan et al. 2017). Off the coast of northern California, between Cape Blanco and Cape Mendocino, upwelling can last as few as three days and as long as 12, with a high range in variability (Iles et al. 2012). On average, upwelling periods are shorter than non-upwelling periods in this region and in 2018, Trinidad Harbor (located between Cape Blanco and Mendocino) experienced pH levels below 7.8 49% of the time, DO below 8.0 mg $\cdot L^{-1}$ 44%, of the time, and temperature below 10°C 23% of the time during the upwelling season (CeNCOOS).

Recent climate models predict that nearshore coastal regions are expected to experience more frequent (Bakun et al., 2015), prolonged (Wang et al., 2015), and intense (Bakun, 1990; Snyder et al., 2003) periods of upwelling. Iles et al. (2012) showed that annual mean duration of upwelling increased by 26-86% from 1967 to 2010 in regions of Oregon and northern California, and recent models predict that upwelling will increase as a result of increasing in coastal winds due to global climate change (García-

Reyes & Largier 2010). Along with changes in the duration of upwelling, current models predict that by 2100, global ocean pH will decrease to 7.7 (IPCC 2014), DO will decrease between 0.6-2.0% from current levels (IPCC 2014), and sea surface temperatures will increase by 2-3°C (IPCC 2014) under current anthropogenic climate change (IPCC 2014, Rykaczewski and Dunne, 2010; Somero et al., 2016). Further, anomalous marine heat waves have become more frequent, longer lasting, and more intense in the past few decades (Frölicher et al. 2018). In general, coastal habitats along the CCLME are predicted to experience an increase in the duration and magnitude of coastal upwelling, thus having potentially important effects on marine life.

As northern California faces the deforestation of many kelp forests, it is crucial to better understand how both current and future-predicted upwelling may impact the grazing rates and survival of arguably the largest threat to kelp forests, purple sea urchins (*Strongylocentrotus purpuratus*). The rapid decline in kelp forest habitat and concomitant increase in urchin barrens has provided a unique opportunity to test the role sea urchin condition may play in mediating the effects of present-day fluctuations and predicted future levels of acidification, hypoxia and temperature. In a 4-week mesocosm experiment, I manipulated both the duration and strength of upwelling to test the effects of present-day and future-predicted upwelling on the survival and grazing rates of purple urchins (*Strongylocentrotus purpuratus*) from kelp forest and urchin barren habitats. By designing a mesocosm experiment that cycled between upwelling and non-upwelling and manipulated multiple stressors, my study aims to provide better insight into the bull kelp-

purple sea urchin interaction and how it is affected by present-day conditions and under future climate scenarios.

METHODS

Collection and maintenance of experimental organisms

Using SCUBA, I collected experimental urchins from an urchin barren at Baker Beach (41.03′N, 124.07′W), and a kelp forest located in Trinidad Harbor (41.07'N, 124.08'W) in July 2019. To insure there was a difference in the condition of the urchins from each habitat, I dissected a subset of 30 urchins from each habitat, and a total of 40 urchins from each habitat for the experiment. The condition of each urchin was measured by calculating their gonad index, defined by: ((blotted wet mass of gonad)/ (total wet body mass) X 100) (Conor 1972). Individual urchins selected for the experiment were transported to the Telonichier Marine Laboratory (TML) where they were immediately weighed (blotted wet weight) and measured (test diameter) and then placed in individual treatment chambers (see Experimental Design below). Urchins were starved for 24 hours and allowed to acclimate to their chamber prior to the start of the experiment. Kelp forest urchins were fed bull kelp (*Nereocystis luetkeana*), which was collected subtidally in Trinidad Harbor either the same day or the day before the urchins were to be fed in the experimental trials. Urchin barren urchins were feed *Corallina* spp., which were collected intertidally from Cape Mendocino (40°2′N 124°2′W) due to their abundance and ease of collection there. I made sure to feed the urchins the same *Corallina spp*. species that were present in the urchin barren habitat where I collected them.

Experimental Design

Overview

I did a laboratory mesocosm experiment to examine how current and future-predicted upwelling affected the grazing rates, growth, and reproductive condition of kelp forest and urchin barren urchins. I manipulated both the duration of upwelling and non-upwelling events, as well as the magnitude of pH, temperature and dissolved oxygen, to represent current day and future-predicted upwelling and non-upwelling conditions. Each treatment consisted of two upwelling/non-upwelling cycles lasting 15 days each, thus the duration of the experiment was a total of 30 days. Although each upwelling/non-upwelling cycle was 15 days, the proportion of days of upwelling varied across treatments (see 'Treatments' below).

Experimental Set-up

I established four experimental treatments: starting with present day durations of both upwelling and non-upwelling, with present day magnitudes of pH, DO, and temperature, then by adjusting both the duration of upwelling and the magnitudes of pH, DO, and temp I created 3 future conditions (explained in further detail below). Each treatment was manipulated in separate 208 L insulated reservoir tanks, supplied with flow-through sea water and maintained via float valves. For each treatment there were 5 replicate 80L insulated tanks drawing from their corresponding treatment reservoir. Each replicate tank had four cylindrical, perforated PVC columns that housed one urchin each, for a total of 2 urchin barren and 2 kelp forest urchins per tank and 10 urchins for each combination of treatment and habitat. Each tank had an individual manifold that allowed

me to control the water flow to each cylinder, assuring similar flows rates for each urchin within each tank. To help maintain treatment levels, each reservoir was fitted with two pumps. The first pump helped with vertical mixing, while the second pumped helped circulate treated water to a manifold from which a small fraction was diverted into each individual treatment tank manifold; the remaining water was circulated back to the reservoir. This partially closed system enhanced the stability of treatment conditions between the reservoir and treatment tanks. When switching between upwelling and non-upwelling periods, conditions (pH, DO, and temperature) in the treatment tanks matched reservoirs within 120 min.

To lower seawater pH, carbon dioxide (industrial grade, Eureka Oxygen Supply) was bubbled in through 60mm fine-pore air diffusers raising pCO_2 and lowering the pH. Each reservoir had one pH probe integrated with a temperature sensor (WTW pH 3310, Loligo Systems) through which pH and temperature were monitored at 1 s intervals. pH was monitored and logged using Loligo Systems CapCTRL software for Windows. Hysteresis for each pH treatment was set to ±0.01 pH units, so if treatment dropped below or above the desired set point by 0.01, gas was added or shut off. pH probes were calibrated throughout the duration of the experiment using a three-point calibration. Dissolved oxygen was manipulated by bubbling in pure nitrogen gas (N_2, industrial grade, Eureka Oxygen Supply) through 60mm fine-pore air diffusers. Each reservoir had one ocular DO probe (Vernier Software and Technology) that was controlled by an Arduino system that continuously monitored and recorded dissolved oxygen in mg $\cdot L^{-1}$ at 1s, 15m, and every hour. Water temperature was controlled using a series of aquarium

chillers, heaters, and heat pumps. To achieve an integrated hourly measurement for pH, temperature and DO, I averaged the one second readings across each hour, for an hourly integrated average.

All treatment conditions were monitored and recorded continuously throughout the duration of the experiment. Discrete water samples were collected approximately weekly for a total of 7 times during the experiment to verify treatment conditions and to calculate carbonate chemistry parameters. All samples were collected in 350ml amber glass bottles to minimize light exposure and were immediately poisoned with 100 µL of saturated mercuric chloride ($HgCl_2$) to halt any biological activity; thus, no metabolic processes from organisms in the sample could alter the seawater chemistry, allowing the sample to reflect the water properties at the time of collection. All samples were processed to measure pCO_2 and DIC via gas equilibration and stripping, respectively, followed by infrared detection, after Bandstra et al. (2006), and modified for discrete samples as in Hales et al. (2004). To ensure accuracy, gas and liquid standards included the full range of values for ocean seawater: primary gas standards had a precision of ±1% with nominal pCO_2 of 200, 800, and 1500 ppm; liquid standards were prepared to a precision of six significant figures with nominal DIC of 1800, 2200, and 2500 mol/kg. Samples were run only when the calibration curve for both gas and liquid standards was highly linear ($R^2 \geq 0.999$). Calibrations curves for both DIC and pCO_2 were created before and after each batch of samples; values calculated using the before and after calibration curves never differed by more than 3.9%.

Treatments

Treatment 1 (current day conditions), including the duration of upwelling and seawater chemistry parameters (pH, DO and temperature) were based on CeNCOOS data from Trinidad Harbor from May-September 2018 (CeNCOOS). The CeNCOOS data showed that upwelling events in Trinidad Harbor lasted, on average, for four days, while non-upwelling periods lasted an average of approximately 11 days. Upwelled seawater temperatures averaged 8°C, with a pH of 7.60, and DO of 5.0 mg $\cdot L^{-1}$. During non-upwelling periods, conditions were set to 12°C, a pH of 7.90, and DO was allowed to come to equilibrium with the atmosphere. The other three treatments were variations based on future predicted upwelling and non-upwelling conditions. A recent study (Iles.et al 2012) showed that the annual mean duration of upwelling increased by 26-86% from 1967 to 2010. Thus, our rationale was to adjust the duration of upwelling in our Current Day treatment by 26-86% to create two future-predicted upwelling treatments (Future 1 and 2). We established a fourth treatment (Future 4), which is a 'worst-case' scenario treatment whose upwelling duration was increased by 150%. Magnitudes of temperature, pH, and DO within treatments were manipulated based on climate change predictions released by the IPCC (2014). Temperatures for non-upwelling periods were increased by 2°C in each future treatment, whereas upwelling temperatures were increased by only 1°C per treatment; mimicking how temperature in surface waters (non-upwelling periods) are predicted to warm faster than colder upwelled water. For treatment 2 (future 1) upwelling parameters were 9°C, 7.4 pH units, and 4.0mg $\cdot L^{-1}$ DO, while non-upwelling

was 14°C, 7.7 pH units, and DO was allowed to come to equilibrium with the atmosphere. Treatment 3 (future 2) upwelling parameters were 10°C, pH-7.3, and DO at 3.0 mg $\cdot L^{-1}$, while non-upwelling was 16°C, pH-7.6, and DO was allowed to equilibrate with the atmosphere. Finally, Treatment 4 (future 3) upwelling parameters were 11°C, pH-7.2, and DO of 2.0 mg $\cdot L^{-1}$, while non-upwelling was 18°C, pH-7.5, and DO was allowed to come to equilibrium with the atmosphere. DO was allowed to come to equilibrium during non-upwelling events in all four treatments because the atmosphere and ocean are constantly exchange gases at the sea surface tending toward equilibrium, and the diffusion of oxygen is heavily dependent on pH and temperature. Thus, by manipulating pH and temperature and leaving oxygen unmanipulated, I better mimicked natural conditions (Figure 6, Table 4).

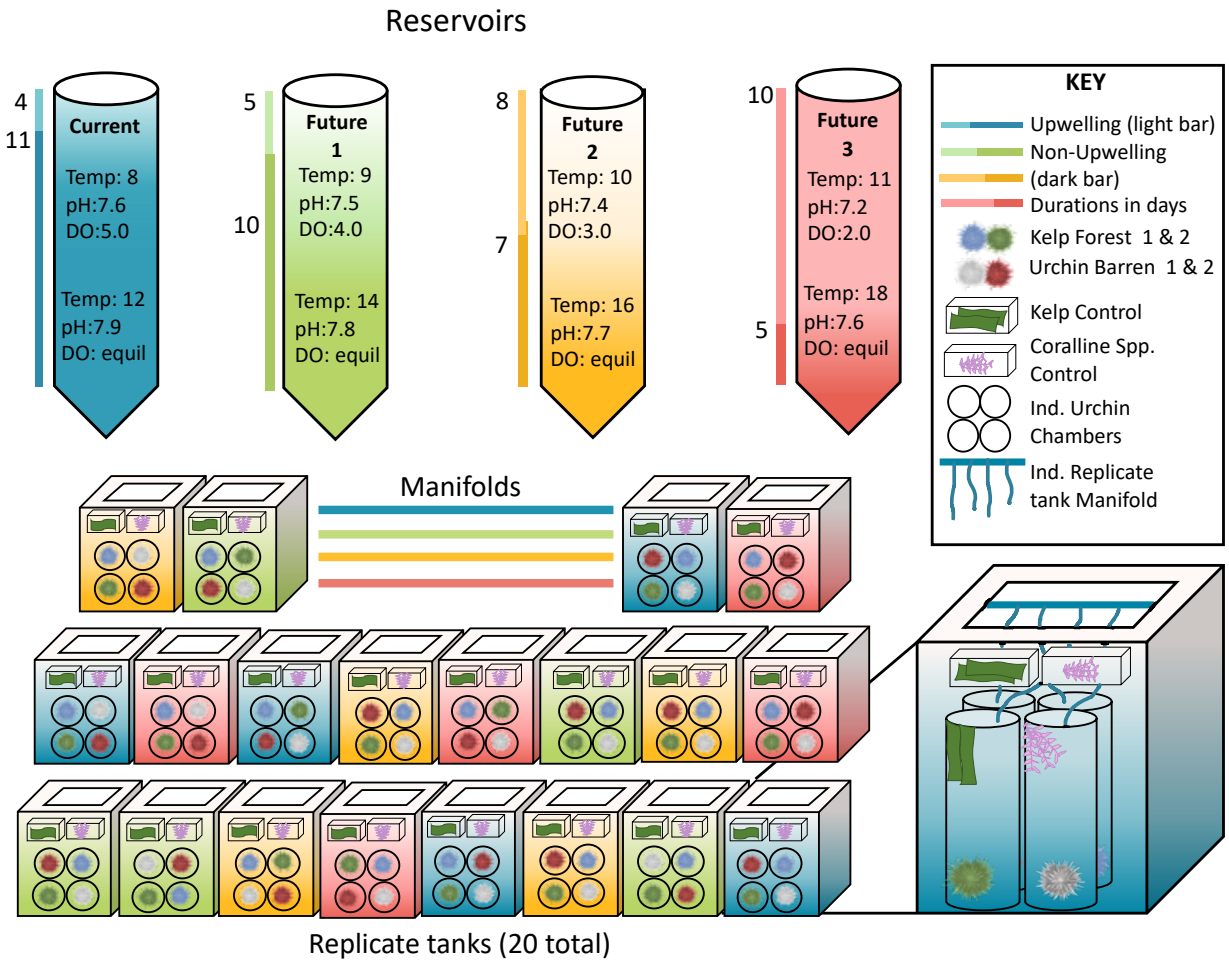

Figure 6. Schematic of mesocosm experimental design. Seawater chemistry parameters for each treatment are (L-R): reservoir 1-Current Day (blue), reservoir 2-Future 1 (green), reservoir 3-Future 2 (orange), reservoir 4-Future 3 (red) (n=4). Both upwelling and non-upwelling conditions in regard to seawater parameters are listed in each corresponding reservoir, with upwelling on top and non-upwelling on the bottom. Colored bars located on the side of each reservoir represent the duration of each upwelling and non-upwelling period in days. Lighter colors represent upwelling and darker colors represent non-upwelling. Random distribution of treatments and urchin condition are depicted among

each replicate tank (replicate tank n=20, urchin condition per treatment n=10). See key in figure for additional components of each replicate tank.

Table 4. Desired, achieved, and associated carbonate chemistry parameters for each treatment in the mesocosm experiment. Achieved conditions are time integrated averages and numbers in parentheses represent standard deviations. U Cond denoted upwelling condition, U=Upwelling and NU=non-upwelling, Equil=Equilibrium, Treat=treatment.

			Desired Conditions			Achieved Conditions			Total Carbonate Parameters		
Treat	U Cond.	Duration	pH	DO	Temp (°C)	pH	DO	Temp (°C)	pCO_2	TA	Ω Calcite
1	NU	11	7.9	Equil	12	7.87 (0.02)	9.28 (0.12)	11.7 (0.39)	696.64	199.35	1.67
1	U	4	7.6	5	8	7.58 (0.03)	4.96 (0.20)	8.2 (0.55)	1233.57	195.26	0.86
2	NU	10	7.8	Equil	14	7.81 (0.05)	8.73 (0.12)	13.7 (0.42)	812.29	201.70	1.63
2	U	5	7.4	4	9	7.41 (0.04)	4.01 (0.17)	9.4 (0.55)	1943.07	198.05	0.58
3	NU	7	7.7	Equil	16	7.71 (0.03)	8.61 (0.15)	15.9 (0.21)	685.02	201.50	2.01
3	U	8	7.3	3	10	7.29 (0.07)	3.00 (0.31)	10.4 (0.65)	1840.61	198.53	0.82
4	NU	5	7.6	Equil	18	7.63 (0.06)	8.27 (0.44)	17.6 (0.60)	951.89	208.42	1.79
4	U	10	7.2	2	11	7.25 (0.05)	2.09 (0.39)	11.0 (0.52)	2020.91	199.10	0.79

Response Variables

Urchin Morphometrics

To quantify how current and future upwelling impacted urchin growth, mortality, and reproductive condition, all urchins were measured prior to the start of the experiment by measuring both blotted total wet mass (g) and test diameter (mm), before and after the experiment. Throughout the duration of the experiment, I recorded mortality among treatments for both kelp forest and urchin barren urchins. If an urchin died, I replaced it immediately and recorded its final wet mass and test diameter. I dissected each urchin at the end of the experiment and calculated its gonad index ((blotted wet mass of gonad)/ (total wet body mass) X 100) (Connor 1972). Because quantifying urchin gonadal index requires lethal dissection, prior to the experiment I obtained an average initial gonad index for kelp forest and urchin barren urchins by dissecting 30 of each, allowing me to calculate the difference in gonad index due to treatment effects.

Grazing Rates

To assess whether the grazing rates of kelp forest and urchin barren urchins were differentially impacted by current and future upwelling, I measured grazing throughout the duration of the experiment. To maintain the natural condition of each urchin and the natural state of food present in each habitat, kelp forest urchins were given *Nereocystis,* while urchin barren urchins were given *Corallina* spp.*ad libitum.* Algae were placed at the top of each individual chamber to force urchins to expend energy to graze. This was

an attempt to create the more realistic scenario where urchins in declining kelp forest have shifted from sheltering in place to active foraging. Both the kelp and coralline algae were dried using a manual centrifuge (equally spun 5 times) and weighed to the nearest 0.01 g before being offered to urchins in the experiment. To capture differences in grazing between upwelling and non-upwelling events, grazing rate measurements were made in proportion to the duration of each event. For example, the current day non-upwelling duration in Treatment 1 is 11 days, so I took 3 grazing measurements during those 11 days, compared to the 1 day of grazing data I took during the 4 days of upwelling. Final algal weights were obtained the same way as the initial weights; algae were dried using a manual centrifuge (equally spun 5 times) and weighed to the nearest 0.01 g. To account for autogenic changes (degradation or growth) in the experimental algae, I subtracted the change in weight from each replicate by the change in the control algae in each treatment tank.

Statistical Analysis

To access the differences in urchin condition between urchins collected from the kelp forest and urchins collected in barrens, I used an analysis of covariance (ANCOVA) with urchin habitat as a fixed factor and total wet mass as a covariate. Because upwelling and non-upwelling periods within each treatment were not independent of one another, I first analyzed the effect of upwelling regime on urchin grazing within each treatment with a paired t-test. To examine temporal trends in urchin grazing across treatments I used a repeated measures ANOVA, crossing treatment with time. In both cases, I did

separate analyses for kelp forest and urchin barren urchins. Residuals were checked for normality visually and homoscedasticity via Levene's test; all data met assumptions and so were not transformed. To assess the difference in wet mass growth and change in gonad index among treatments for kelp forest urchins that survived to the end of the experiment, I used a mixed model ANOVA with treatment as a fixed factor, and tank as random factor. All analyses were done in R (R (v3.6.1; R Core Team R. 2013).

RESULTS

Habitat differences in urchin condition and survival

Urchins collected from kelp forests differed from urchin barren urchins with regard to gonad condition. Interactive effects of habitat and urchin wet mass on gonad wet mass showed in general that larger kelp forest urchins have proportionally more gonads than urchin barren urchins of a similar size. (Appendix 1: ANCOVA, $F_{3,26} = 19.32$, $P < 0.0001$; Fig. 2). Both kelp forest and urchin barren urchins also differed in their survival among treatments throughout the experiment (Fig. 7). In total, four kelp forest urchins died compared to 60 urchin barren urchins. For kelp forest urchins, no deaths occurred in the Future 3 treatment, but 1 death occurred in both Current Day and Future 2 treatments, and 2 deaths in Future 1 treatment. Among urchin barren urchins, Future 3 had the highest number of deaths (n=20) compared to the other 3 treatments (Current Day = 12, Future 1 = 11, and Future 2 = 17). In general, as acidity, anoxia, and temperature increased, the number of deaths increased for urchin barren urchins, but not for kelp forest urchins (Fig. 7).

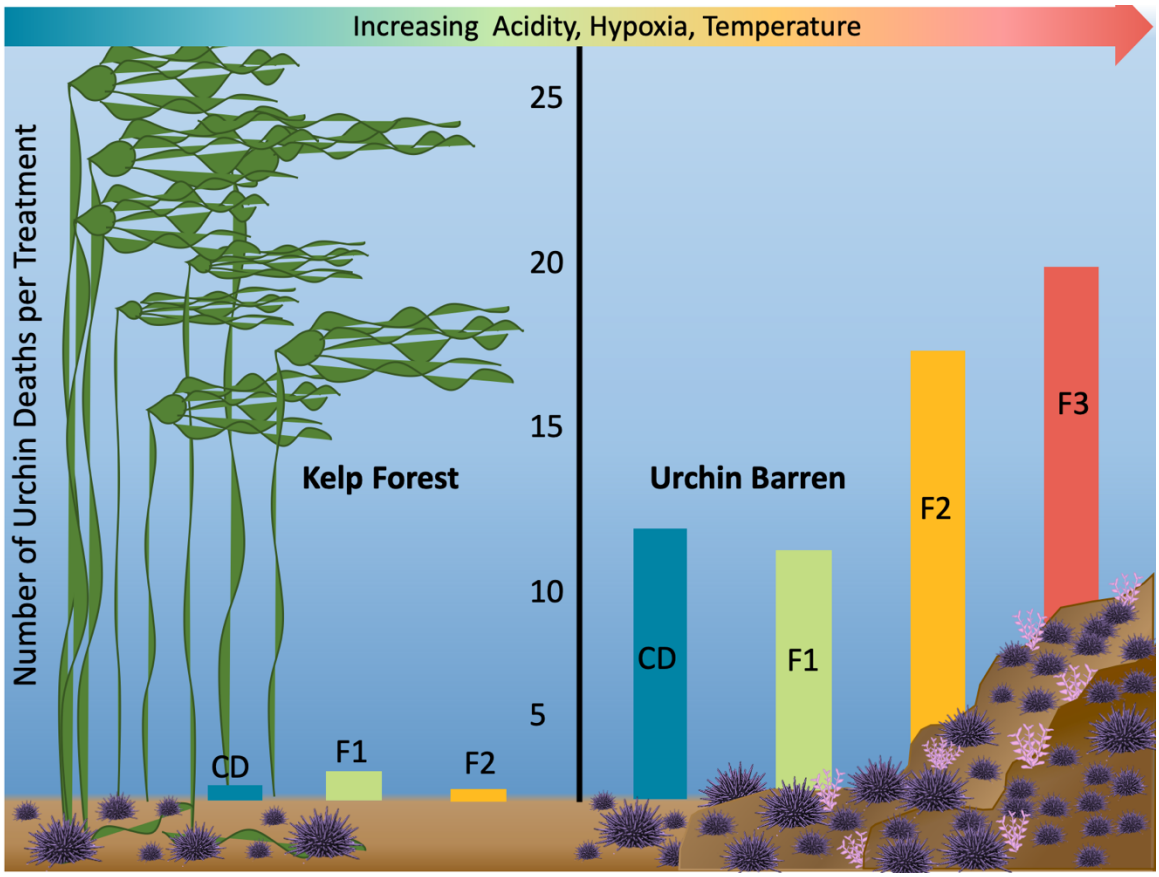

Figure 7. Number of urchin deaths per treatment for both kelp forest and urchin barren urchins throughout the duration of the experiment (30 days). Blue bars represent Current Day treatment (CD), green represents Future 1 (F1), orange represents Future 2 (F2), and red represents Future 3 (F3).

Urchin Grazing

The effect of upwelling on both kelp forest and urchin barren urchins differed among treatments (Fig. 8a). During upwelling events grazing rates decreased for kelp forest urchins for Current Day ($t = 2.56$, df = 29, $P = 0.016$), Future 2 ($t = 2.48$, df = 39, P

= 0.018) and Future 3 (t = 2.58, df = 19, P = 0.018), but not for Future 1 (t = 0.75, df = 49, P = 0.456). Grazing decreased by 76% and 66% in both Future 2 and 3 during upwelling, respectively (Fig. 8b). For urchin barren urchins, grazing rates decreased significantly for Future 1 (t = 3.41, df = 49, P = 0.0001) and Future 2 (t = 5.69, df = 39, P = 0.0001) by 50 and 85% respectively, while Current Day (t = 1.50, df = 29, P = 0.144) and Future 3 (t = 0.48, df = 19, P = 0.632) grazing rates decreased by 51 and 55%, but they were not significant (Fig. 8a). Although urchin barren urchins were fed *Corallina* spp. rather than bull kelp, they grazed less overall, but proportionally, grazing reductions caused by upwelling were similar to those of kelp forest urchins (Fig. 8b).

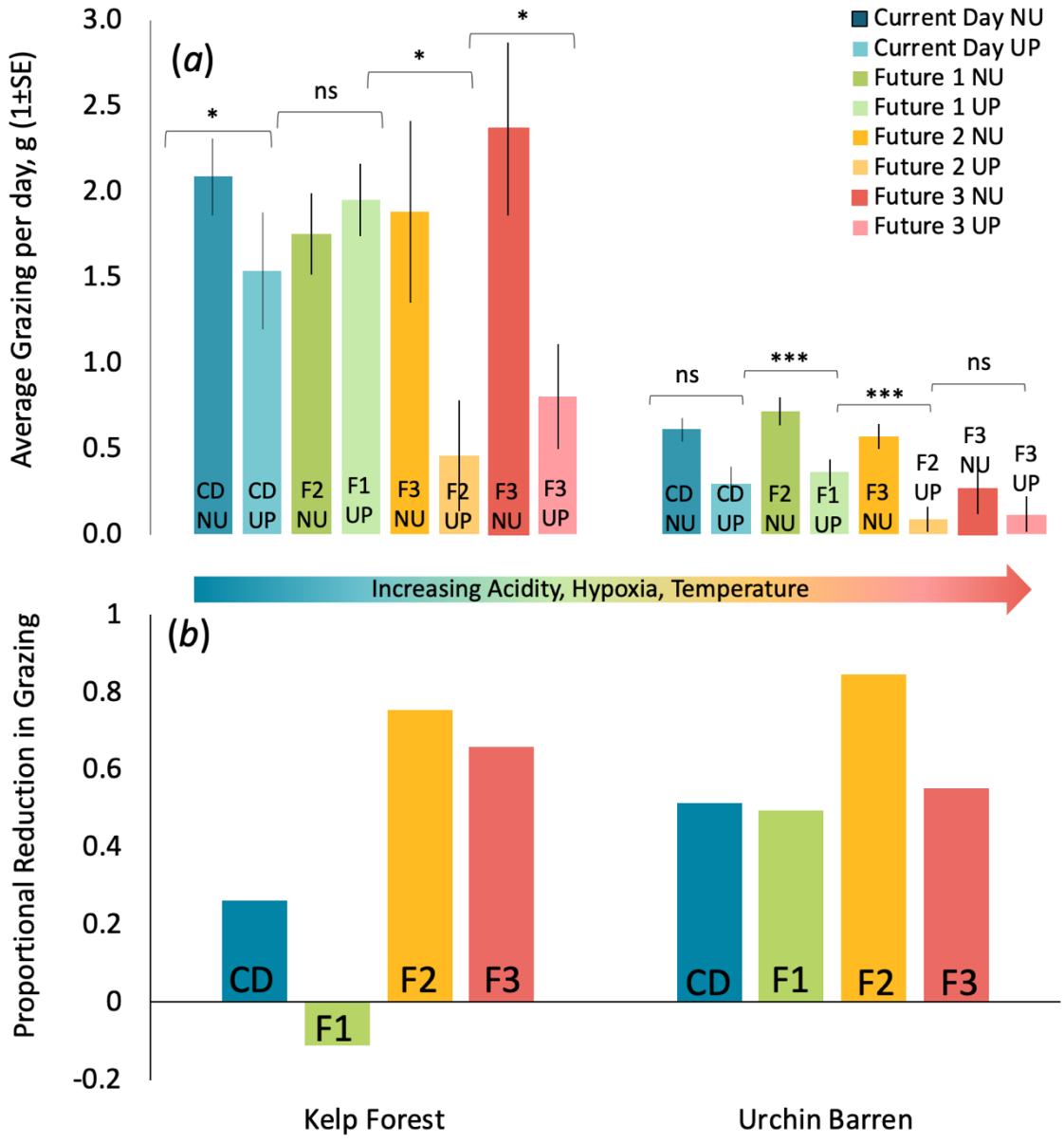

Figure 8. A) Average grazing per day for both urchin condition (kelp forest and urchin barren) and upwelling condition (non-upwelling-NU and upwelling-UP) for all 4 treatments(CD-Current day, F1-Furture 1, F2-Furture 2, F3-Furture 3. Error bars represent standard error (1±SE). Statistically significant t-tests are indicated by asterisks

(* for $p < 0.02$; *** for $p < 0.0002$). B) Proportional reduction in grazing for both urchin condition (kelp forest and urchin barren) for all 4 treatments.

The effect of treatment on kelp forest urchin grazing depended on upwelling period (RM-ANOVA, Treatment*Time $F_{9,354} = 4.27$, $P < 0.001$) (Fig. 9a). I found no significant differences in urchin grazing among treatments during the first non-upwelling event (simple effects ANOVA, $F_{3,106} = 1.00$, $P = 0.396$) and the first upwelling event ($F_{3,86} = 1.03$, $P = 0.382$). In contrast, I found significant differences among treatments during the second non-upwelling ($F_{3,46} = 6.08$, $P = 0.001$) and upwelling events ($F_{3,116} = 11.79$, $P < 0.0001$) (Fig. 9a). During the second non-upwelling event, urchins in Future 2 treatments grazed more than urchins under Current Day conditions (Tukey's HSD, $P = 0.004$) and Future 1 conditions (Tukey's HSD, $P = 0.008$). During the second upwelling event, urchins in Future 2 and Future 3 treatments each grazed less than urchins in Current Day and Future 1 treatments (Tukey's HSD, All P's < 0.002) (Fig. 9a).

For urchin barren urchins there was no significant interaction between treatment and time (RM-ANOVA, Treatment*Time $F_{9,354} = 1.60$, $P = 0.113$), though general linear models often fail to statistically detect real biological interactions (Wahlsten 1990). Thus, I acknowledged a trend where urchins grazed less in Future 3 and 4 treatments during the first non-upwelling period and the last upwelling period (Fig. 9b). In addition, urchin barren urchins appeared to graze less in Future 3 than other treatments regardless of upwelling regime (Fig. 9b). There was no significant treatment effect ($F_{3,354} = 1.87$, $P = 0.197$), but there was a significant effect of time ($F_{3,354} = 7.72$, $P = 0.006$). All urchins

grazed more during non-upwelling periods than upwelling periods (Tukey's HSD, all P's < 0.01), except when comparing both of the second round of upwelling events (Tukey's HSD, both P's > 0.25; Fig. 9b). Also, urchin barren urchins grazed less during the first upwelling event than the last upwelling event (Tukey HSD, $P = 0.005$; Fig. 9.b).

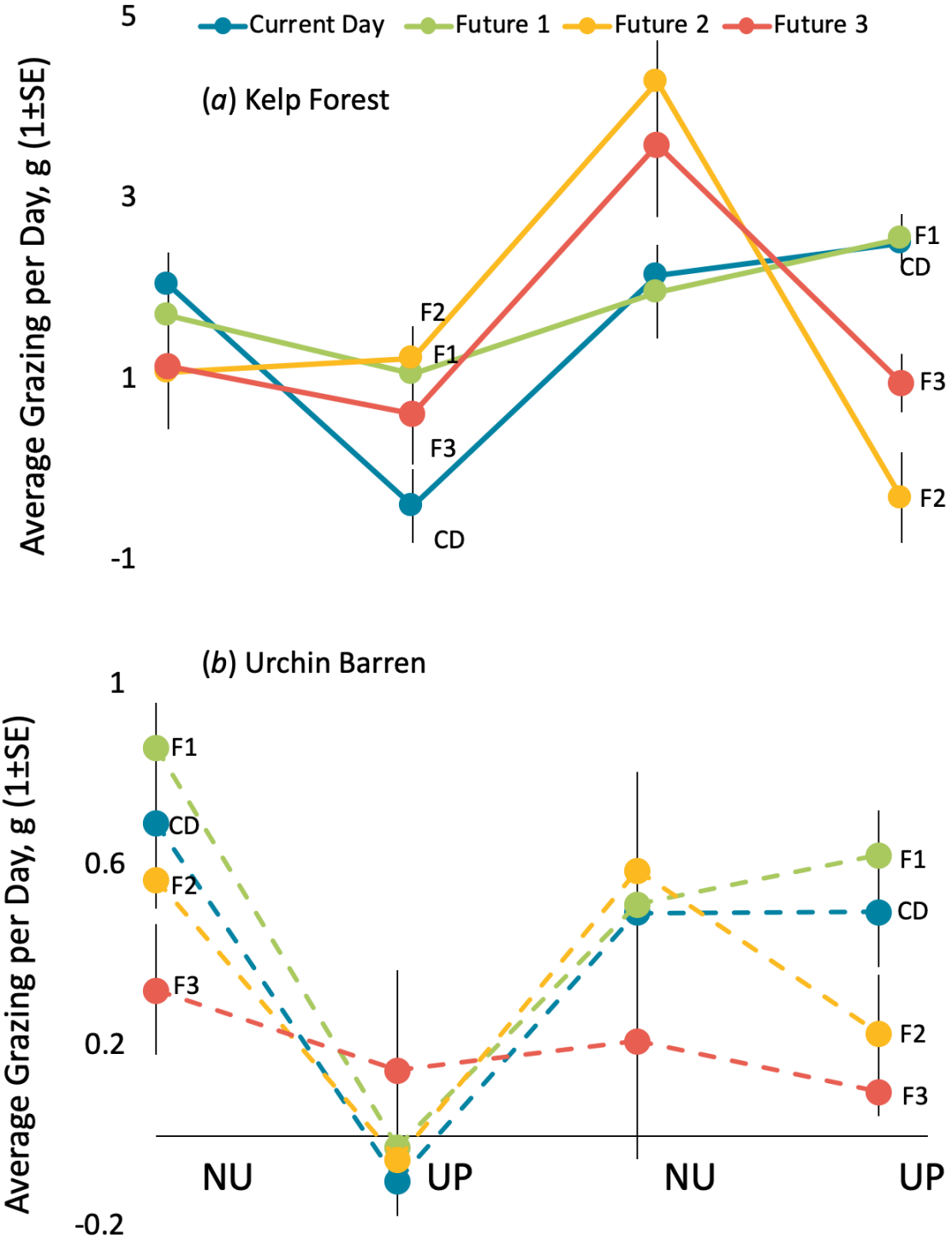
Current Day
Future 1
Future 2
Future 3
(a) Kelp Forest
Average Grazing per Day, g (1±SE)
5
3
1
-1
F2
F1
F3
CD
F1
CD
F3
F2
(b) Urchin Barren
Average Grazing per Day, g (1±SE)
1
0.6
0.2
-0.2
F1
CD
F2
F3
F1
CD
F2
F3
NU
UP
NU
UP

Figure 9. Interaction plot of treatment and time effects on urchin grazing for A) kelp forest urchins and B) urchin barren urchins. NU-Non-upwelling, UP-upwelling, CD-Current Day, F1-Future 1, F2-Future 2, F3-Future 3. Error bars represent standard error (1±SE).

Urchin Morphometrics

Due to high mortality rates and the replacement of urchin barren urchins during the experiment, I only analyzed wet mass growth and gonad index change for kelp forest urchins that survived throughout the duration of the experiment (Fig. 10a & b). There was no significant difference in wet mass growth among treatments (ANOVA, $F_{3,26} = 2.46$, $P = 0.08$; Fig. 10a). However, there was a clear non-significant trend that showed that as treatments increased in acidity, anoxia, and temperature, total wet mass of urchins decreased from Future 1 to Future 2, and Future 3 treatments. Treatment had a significant effect on gonad index (ANOVA, $F_{3,26} = 2.89$, $P = 0.05$; Fig. 5=10b). The change in gonad index in urchins exposed to Future 3 conditions was significantly lower than that of kelp forest urchins in the Future 1 treatment (Tukey's HSD, $P = 0.031$), however there were no differences in gonad index when comparing among other treatments (Tukey's HSD, all P's > 0.330). Similar to the results for wet mass growth, I saw a trend that as treatments increased in acidity, anoxia, and temperature, the increase in gonad index of kelp forest urchins decreased in both Future 2 and 3, relative to Current Day and Future 1 treatments (Fig. 10b).

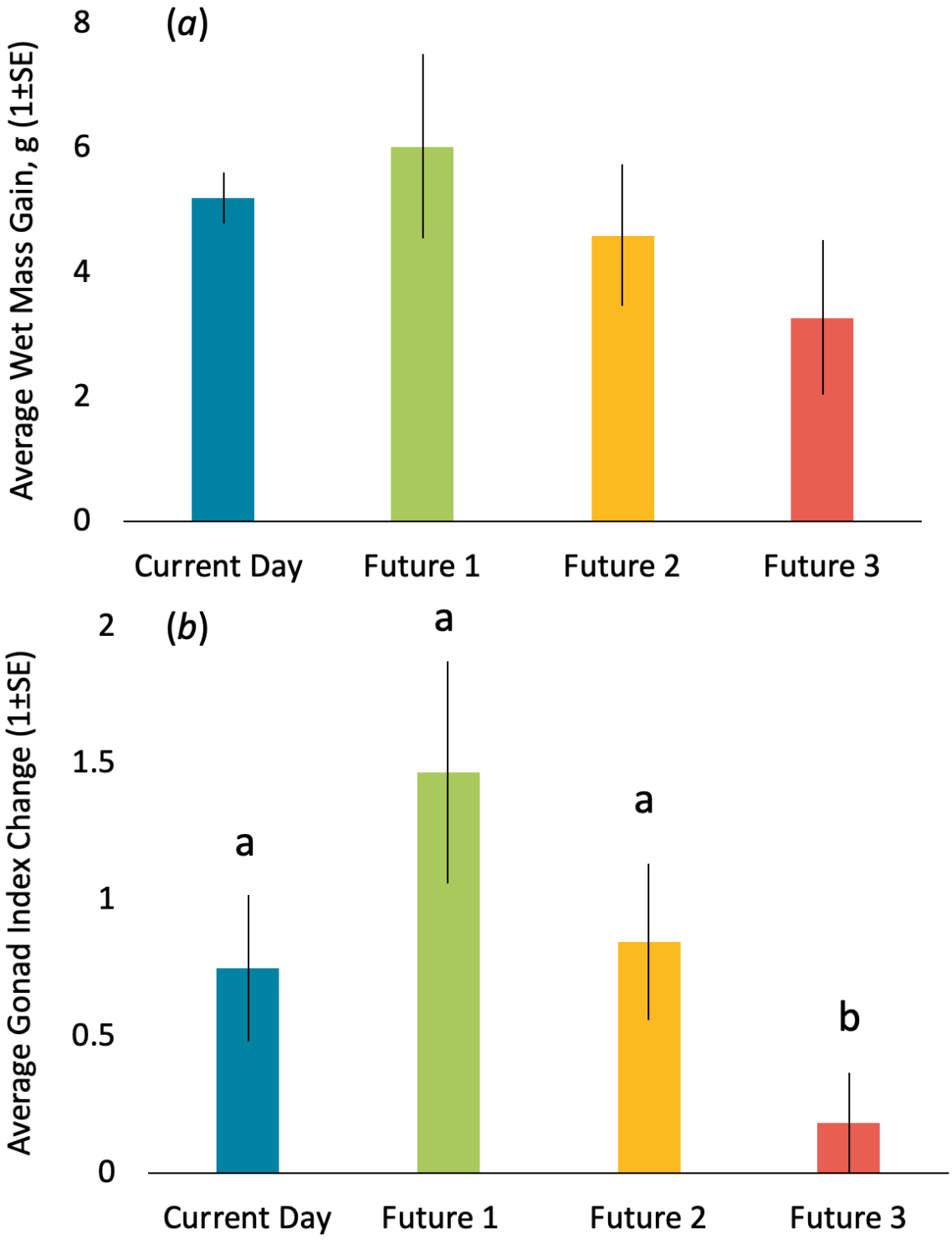
(a)
Average Wet Mass Gain, g (1±SE)
8
6
4
2
0
Current Day
Future 1
Future 2
Future 3
(b)
Average Gonad Index Change (1±SE)
2
1.5
1
0.5
0
a
a
a
b
Current Day
Future 1
Future 2
Future 3

Figure 10. A) average wet mass gain and B) average gonad index change for all kelp forest urchins that survived the duration of the experiment. The first bar on the left is Current Day, second bar is Future 1, third bar is future 2, and fourth bar on the right is Future 3.

DISSCUSSION

Effects of climate change on organisms and ecosystems are complex, involving changes in both the magnitude and duration of multiple physiological stressors. For example, upwelling-driven hypoxia tends to occur in phase with low temperature and low pH, which can each have direct impacts on sea urchin metabolism, grazing, calcification, and reproduction. Therefore, it is important to consider multiple responses to multiple stressors to understand impacts of climate change on populations and ecosystems. Our results showed condition-dependent susceptibility in purple urchins when exposed to an increased magnitude and duration of abiotic stressors associated with climate-induced changes in upwelling for both current and future predicted coastal ocean conditions. We found that in kelp forest urchins, grazing was most affected by upwelling events and gonad development was affected most by distant future conditions. In urchin barren urchins, grazing was sensitive both to exposure to upwelling events, but also more generally to near and distant future conditions. These results have potentially important population- and ecosystem-level consequences as overall kelp forest urchins are more resilient to current and near future upwelling stress, than urchin barren urchins.

With a lack of natural predators and the recurrence of marine heatwaves, the occurrence of urchin barrens may continue to increase and persist. Our results highlight that as urchin barren urchins become more common, we may see increasingly higher rates of mortality compared to kelp forest urchins as the climate changes. Although the precise cause of mortality differences in our experiment cannot be known due to the

integrated nature of our treatments, treatment conditions or an artefact of the experimental design are the two possible causes. We hypothesize that mortality differences are due to the interactive effects of treatment conditions and urchin condition. However, observed differences in kelp forest and urchin barren urchin mortality may be driven in part by food quality (Larson et al. 1980). In general, when food is of high quality, organisms may be able to compensate for environmental stressors that would suppress performance under low quality food conditions (e.g., Thomsen et al. 2013). For example, prior studies examining the combined effects of food availability and pH variation in invertebrates have found that *ad libitum* feeding can help ameliorate the negative consequences of pH variation alone (e.g. Melzner et al. 2011, Thomsen et al. 2013). Studies have also shown correlations between changes in C:N ratio, a good metric of nutritional value and feeding rate (Stiling and Cornelissen 2007, Falkenberg et al. 2013, Cruz- Rivera and Hay 2000).

Specific to urchins, previous studies have shown that the effects of elevated temperature and pCO_2 on purple sea urchins can be partially ameliorated by resource quality. For example, purple sea urchins feeding on higher-quality diets have been shown to have significantly higher growth and feeding rates than those on poorer quality diets (Brown et al. 2014). In healthy bull kelp forest habitats, abundant *Nereocystis* provides a high-quality food source for urchins, whereas in urchin barrens, high quality food is scarce or absent and urchins must rely on poor-quality coralline algae (Rogers-Bennett et al. 2011, Filbee-Dexter and Scheibling 2014). In an effort to simulate and maintain more realistic and ecologically relevant differences between our kelp forest and urchin barren

urchins, we fed kelp forest urchins bull kelp and barrens urchins coralline algae, and these different diets were likely to play a strong role in the mortality patterns we observed. In sea urchins, a diet of kelp has been shown to accelerate reproductive maturation and growth rate, thus enhancing gonad production relative to a diet of coralline algae (Meidel and Scheibling 1999). Differences in kelp forest and urchin barrens sea urchin responses in our study highlight that environmental stress often imposes metabolic trade-offs on organisms and that an organism's condition or state, which will be a product of their resource environment, will strongly influence how well it can balance those tradeoffs.

Although the nature of our experimental design and the integrated nature of our treatments, did not allow us to precisely indicate which upwelling-associated stressor had the largest effect on urchin mortality, grazing, growth, and gonad production, our results are consistent with previous work. In general, it has been shown that increasing temperatures increase metabolic activity, thus leading to higher grazing rates in many organisms (Brown et al. 2004). We hypothesize that increased temperatures during non-upwelling may be the driving factor behind why we saw higher grazing rates for both kelp forest urchins and urchin barren urchins during non-upwelling periods. Most relevantly, recent work by Low (2018), has shown that when exposed to low temperatures, low pH, and low DO separately, low temperature and DO had significant negative effects on purple urchin respiration and grazing rates, but low pH did not; however, when combined, pH, temperature and DO can have interactive effects. For example, low temperatures and low DO together had larger combined effects on urchin

respiration and grazing than either did alone (Low 2018). In contrast, when low DO or low temperature was combined with low pH, low pH seemed to lessen the effects of temperature and DO on urchin respiration and grazing (Low 2018). When all three were combined, low pH was not enough to compensate for combined negative effects of low temperature and low DO (Siikavuopio et al. 2007, Low and Micheli 2018). Because we saw a large reduction in grazing during upwelling conditions, and with the results previously discussed, it is reasonable to believe that low temperature and low DO in our future treatments were mainly responsible for a decrease in grazing for both kelp forest urchins and urchin barren urchins.

In addition to the reduction in grazing rates under upwelling conditions, we also saw a decrease in gonad production for kelp forest urchins in future treatments. Increased metabolism might explain the lower gonad indices in urchins held under future conditions, as higher metabolic costs would leave less energy available for gonad production and energy storage. Metabolic rates increase with temperature across a wide range of organisms (Brown et al. 2004) and increased metabolic costs associated with elevated pCO_2 have been observed in sea urchins (Spicer et al. 2011, Catarino et al. 2012). Sea urchin reproductive processes may also be sensitive to higher-frequency patterns of variability in sublethal hypoxia. For example, sea urchins that spend longer periods of time in ambient oxygen conditions tend to produce larger gonads (Low and Micheli 2018). Other studies have also observed decreases in gonad size under elevated pCO_2 (Siikavuopio et al. 2007).

Our experiment also did not allow us to distinguish whether the duration or intensity of upwelling was driving urchin performance. As duration of upwelling increased, we saw a decrease in urchin grazing for both kelp forest and urchin barren urchins, and a decrease in urchin gonad development for kelp forest urchins. However, because the increase in duration was also coupled with increases in acidity, hypoxia, and temperature we could not distinguish individual impacts by upwelling duration *per se*. Further studies are needed to determine whether changes in just the duration of upwelling would produce similar results, or potentially have synergistic effects with upwelling intensity on urchin performance.

Although the complexity in our experimental design did not allow us to isolate the individual effects of individual upwelling stressors on urchins, our results are novel and important as individual factors associated with upwelling are never independent in nature. Our results indicate that exposure to the multiple stressors associated with upwelling could have important implications for sea urchin populations and kelp forest ecosystem structure and dynamics. Smaller gonads in sea urchins lead to reduced reproductive capacity (e.g., decreased fertilization and recruitment; Rogers-Bennett et al. 1995, Wahle and Peckham 1999), especially at lower urchin densities (i.e., Allee effects; Quinn et al. 1993). Further, urchins' gonads are a main target for predators because of their nutritional value (Eurich et al. 2014), so decreased gonad production could also reduce energy transfer to higher trophic levels. In addition, urchin grazing is critical to shallow subtidal ecosystems, and its effects could influence kelp forest dynamics and 'tipping points' between alternate stable states (Steneck et al. 2013, Filbee-Dexter and Scheibling 2014).

Therefore, present-day spatial differences in the temporal pattern of upwelling exposure and future projected changes in upwelling exposure regimes could produce population-level effects (through reproduction) and ecosystem-level effects (through grazing reductions and trophic energy transfer) via impacts on just one or two particularly sensitive organism-level responses, such as grazing and gonad production.

As climate change alters the magnitude and duration of physiological stressors in ecosystems, subpopulations of organisms will be differentially affected (Marcel et al. 2003, Parker et al. 2011). It is therefore necessary to examine the effects of different exposures under realistic patterns of variability, and how organismal condition mediates these effects. Our study showed that exposure to increased intensity and duration of upwelling (as predicted by climate models) has negative impacts on grazing and gonad development in ecologically important purple sea urchins, and that these impacts are mediated by the urchins' condition. Further, the observed responses of *S. purpuratus* to multiple stressors could not have been predicted from single stressors. For example, studies examining the impact of a single stressor (e.g., elevated temperature) likely would have produced results opposite to what we observed under conditions more likely to occur in the future (i.e., elevated temperature, hypoxia, and pCO_2). This highlights the importance of understanding the combined effects of multiple stressors on marine organisms (Kroeker et al. 2017). To our knowledge, our study is the first to test the effects of variable exposure to multiple stressors and how organismal condition can mediate responses to these stressors in a naturally variable coastal upwelling system.

Future studies should test the effects of variable exposure to multiple stressors and their possible ecosystem-level consequences under field conditions.

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APPENDIX

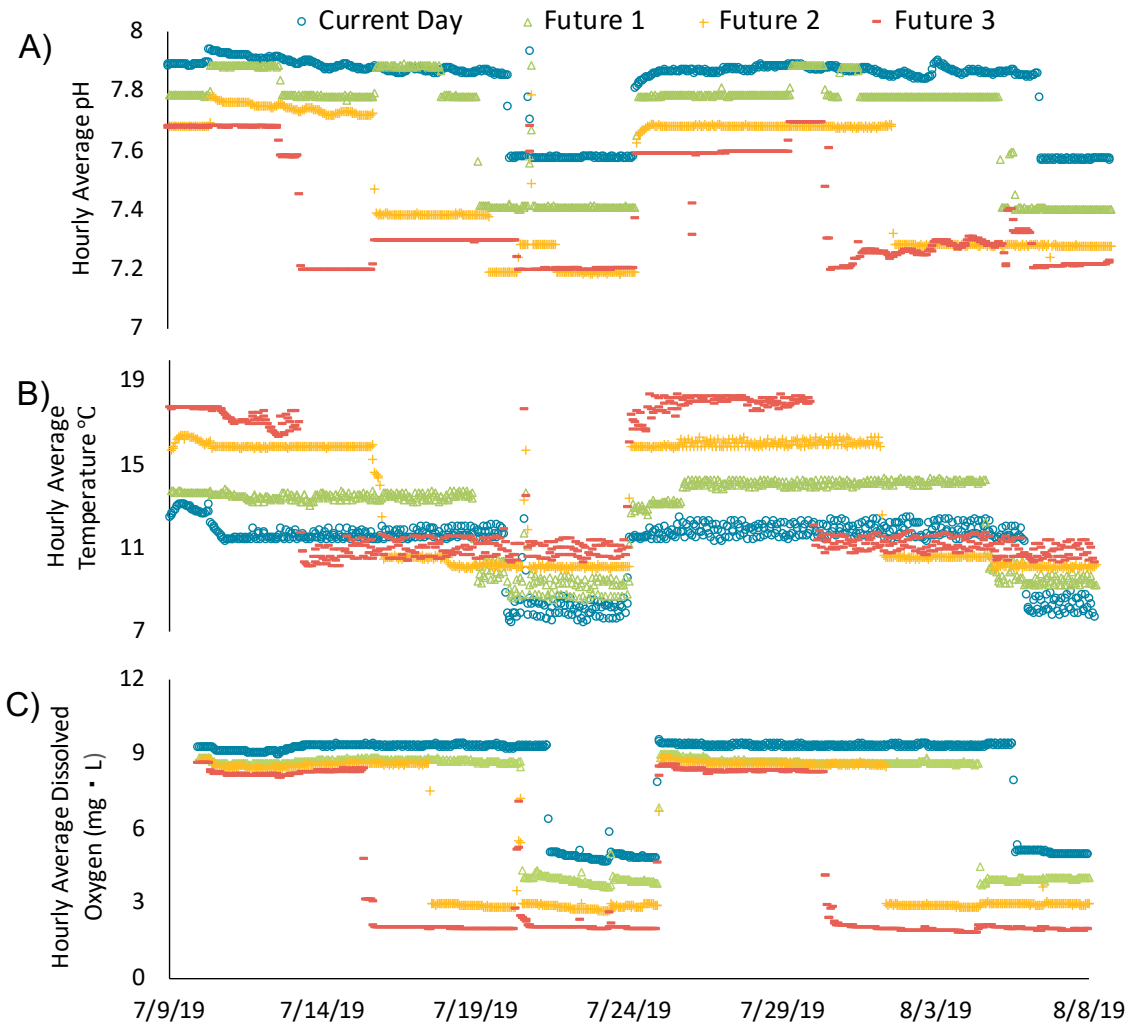

Figure 1. Averaged hourly readings for a) pH b) dissolved oxygen (mg $\cdot L^{-1}$) and, c) temperature (°C) per treatment for the duration of the lab experiment. Current Day is open circles, future 1 is open triangles, future 2 is the cross symbol, and dash lines is future 3.

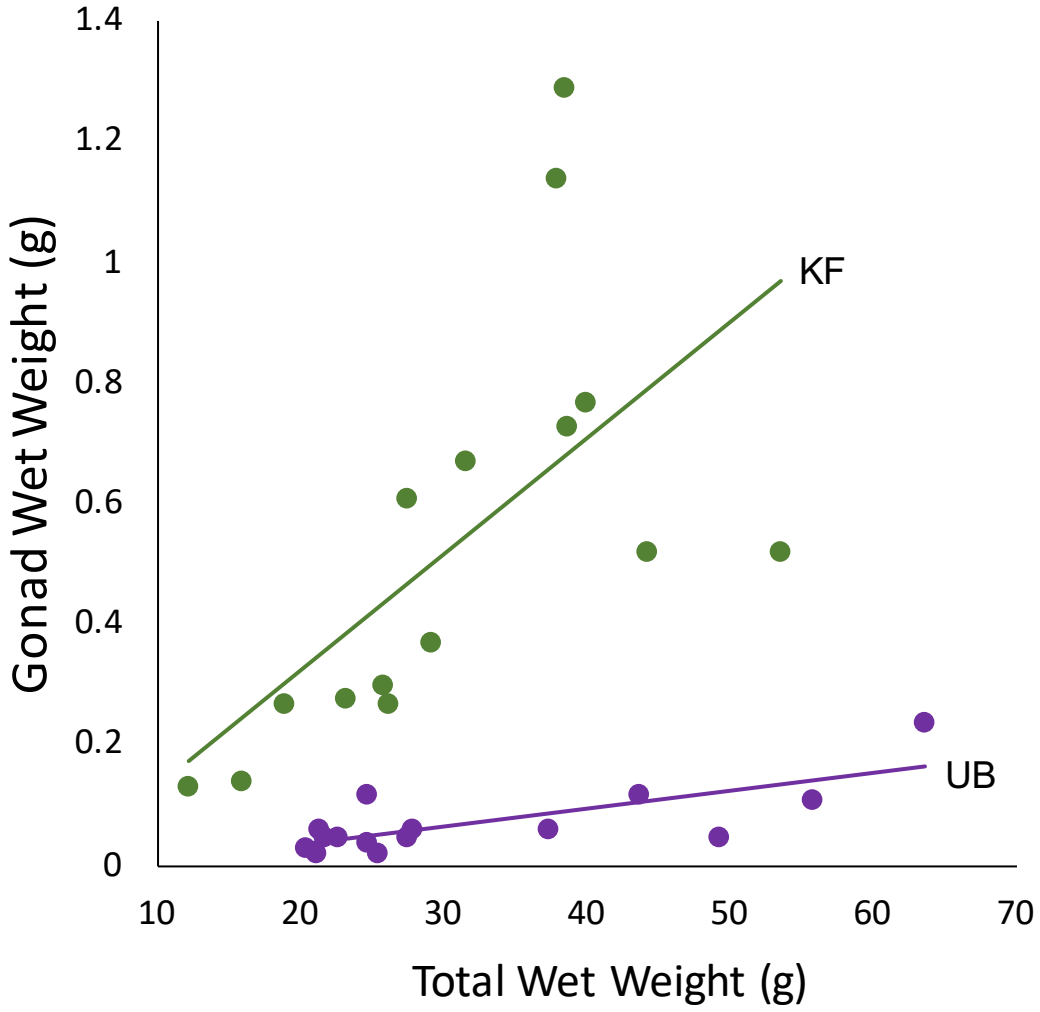

Figure 2. Relationship between total wet mass (g) and gonad wet weight (g) for both subset populations of urchins collected from a kelp forest (R^2=0.40) and urchin barren (R^2=0.56). Green (lighter color) represents urchins collected in a kelp forest and purple (darker color) represents urchins collected in an urchin barren. KF-Kelp forest and UB-Urchin Barren.