THE ABILITY OF *PHYLLOSPADIX* SPP., A PAIR OF INTERTIDAL FOUNDATION

SPECIES, TO MAINTAIN BIODIVERSITY AND AMELIORATE CO_2 STRESS IN

ROCKY SHORE TIDEPOOLS

By

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ABSTRACT

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SPECIES, TO MAINTAIN BIODIVERSITY AND AMELIORATE CO_2 STRESS IN
ROCKY SHORE TIDEPOOLS

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Ocean acidification (OA) is often demonstrated to have negative effects on
marine organisms, but less is known about whether marine organisms can mediate
OA effects. I examined relationships between surfgrass (*Phyllospadix spp.*), a
foundation species and tidepool biodiversity, and its ability to mediate fluctuations
in pH and dissolved oxygen (OA; DO) which are stressors in tidepools. I surveyed
tidepools in northern California, where I quantified biodiversity, pH, and DO, and
related those variables to surfgrass abundance. Laboratory and field experiments
manipulating CO_2 and surfgrass presence were done to examine surfgrass effects
on day/night pH and DO fluctuations in simulated and natural tidepools.
Intermediate surfgrass abundance was associated with the greatest tidepool
biodiversity in the field, suggesting amelioration of abiotic conditions up to
intermediate abundances, but exacerbated OA and DO stress at higher abundances.
In the lab, diel pH and DO fluctuations were highest in simulated tidepools that
contained surfgrass compared to pools without surfgrass, indicating the role of

surfgrass photosynthesis and respiration in modulating seawater chemistry. In the field, tidepool pH and DO were higher in the day and lower at night, consistent with results from the laboratory experiment. Interestingly, day/night fluctuations in pH were highest in tidepools with intermediate rather than high surfgrass abundance, suggesting the intriguing possibility that surfgrass modulates tidepool pH both directly via metabolic activity but also indirectly by facilitating macrophyte diversity at intermediate abundances. Taken together, these results suggest that surfgrass may act as a foundation species in tidepools, by mediating tidepool pH and influencing species diversity, which has important implications for the fate of these communities in the face of rapidly-changing global climates.

ACKNOWLEDGEMENTS

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GENERAL INTRODUCTION

Oceanic uptake of CO_2 diminishes effects of atmospheric carbon emissions on global climate change, however the result for global oceans is the increased dissolution of CO_2 in seawater – also known as 'ocean acidification' (OA; Orr *et al*., 2005). Acidification of seawater results in alteration of seawater chemistry, in which calcium carbonate saturation states are lowered, and ultimately affect the ability of calcifying organisms to produce and maintain their calcium carbonate skeletal structures (Doney *et al*., 2009).

Most OA research has focused on the effects of OA in open ocean systems, which are different from other marine ecosystems (Hofmann *et al*., 2011). Open oceans experience more stable conditions, when compared to more dynamic nearshore and coastal environments, like kelp forests, tidepools, estuaries, upwelling zones, and CO_2 vents (Hofmann *et al*., 2011). Further, most OA research has focused on how individual calcifying organisms will respond to OA stress (Harley *et al*., 2006; Gaylord *et al*., 2011). However, in order to understand how OA will affect marine ecosystems, we need to assess how OA will affect species interactions and the ability of whole communities to resist OA stress.

Foundation species have the ability to facilitate diverse community assemblages because they stabilize processes such as productivity, and ameliorate extreme environmental conditions (Dayton, 1972; Angelini *et al*., 2011; Ellison *et al*., 2005). They therefore structure communities, and promote ecosystem resilience (Orth *et al*., 1984;

Heck *et al*., 2003; Canion and Heck, 2009). Determining whether foundational species ameliorate OA stress will greatly increase our understanding of how both individual organisms, and whole communities, will respond to global change threats like OA.

Phyllospadix spp., intertidal seagrasses, more commonly known as surfgrasses, are foundation species within rocky shore ecosystems (Dethier, 1984; Shelton, 2010). Surfgrass alleviates thermal stress in tidepools within rocky intertidal ecosystems, acts as a nursery habitat for some species (e.g., juvenile California spiny lobsters – *Panulirus interruptus* and juvenile rockfish – *Sebastes* spp.), and stabilizes local community assemblages within tidepools (Engle, 1979; Shelton, 2010). Seagrasses function as refugia from pH stress, by modifying local pH by 0.2 – 0.7 units via photosynthesis and community metabolic processes (Frankignoulle and Distèche, 1984; Frankignoulle and Bouquegneau, 1990; Invers *et al*., 1997).

Not only does surfgrass act as foundation species within its environment, but other seagrasses in the same family locally buffer oceanic pH. The extent of pH regulation by the genus *Phyllospadix* has not been studied. This genus and the tidepools it inhabits provide an excellent system to (1) examine the role of surfgrass as a foundation species, and (2) determine whether surfgrass can increase tidepool community diversity via amelioration of stress from acidic seawater. My thesis aims to evaluate whether surfgrass increases species diversity (as predicted by foundation species theory) within tidepools along northern California coasts as well as examine the extent to which surfgrass can buffer local seawater from changing oceanic conditions.

CHAPTER 1: EFFECTS OF *PHYLLOSPADIX* SPP. ON COMMUNITY DIVERSITY

WITHIN ROCKY SHORE TIDEPOOLS

INTRODUCTION

Importance of Foundation Species

Research regarding the impacts of global climate change on marine ecosystems has focused primarily on the negative effects experienced at the organismal level (Harley *et al*., 2006). However, if we want to accurately depict and predict impacts of climate change on marine communities, more research is required to understand how changing oceanic conditions will alter species interactions that may play a large role in mediating the effects of changing environmental conditions on ecosystems (Harley, 2003; Gaylord *et al.*, 2015). Foundation species are organisms with disproportionately large effects on the surrounding community through the modification and amelioration of harsh environmental conditions (Dayton, 1972; Bruno and Bertness, 2001). Dayton (1972) recognized that foundation species are most often deemed competitive dominants within their community and provide most of the spatial structure. They typically occupy lower trophic levels, as opposed to keystone species which are usually top predators (Paine, 1966). Foundation species are thought to facilitate diversity, while providing crucial ecosystem services and stabilization within the community that they reside (Ellison *et al*., 2005; Bruno *et al*., 2003). It will therefore be critical to understand the role, both small and large, that these essential species play in a rapidly changing marine environment.

Impacts of Foundation Species, or Ecosystem Engineers within Ecosystems

Effects on species diversity and abundances

     It has been demonstrated that foundation species have a net positive effect over large, or landscape level, scales of an ecosystem, however, the effects that they provide at smaller scales have been shown to be variable (Jones *et al*. 1997; Jones *et al*., 2007). Jones *et al.* (2007) described these unique members of the community as physical ecosystem engineers – organisms that physically modify, maintain or create habitats, while regulating the availability of biotic/abiotic resources through physical state changes (directly or indirectly). Case studies regarding the impacts that such species have on species richness and abundances are in fact 'trivial to enormous' and not always positive (Jones *et al*., 1997; Jones *et al*. 2007). This conundrum lays the foundation for the variability that is observed with respect to the impacts that ecosystem engineers display at differing spatial scales. Jones *et al*. (2007) suggests that ecosystem engineers tend to have an overall positive effect on regional species richness and abundances through habitat diversification, however, the number of species that benefit from the engineering is often similar to the number that are negatively impacted (Jones *et al*. 2007).

Surfgrass as a Foundation Species/Ecosystem Engineer within Tidepools

The genus *Phyllospadix*

Surfgrasses in the genus *Phyllospadix* are the only seagrasses that have inhabited rocky shores – and they have been shown to play vital roles within this environment (Lobban and Harrison, 1994). The species within this genus are considered important foundation species in temperate rocky shore ecosystems and have profound effects on community structure (Shelton, 2010). For example, Shelton (2010) suggested that the modification of the thermal environment by surfgrass can influence tidepool community composition. The three-dimensional component that canopy species, like surfgrass, provide to the surrounding environment, creates microhabitats that alter the distribution of underlying organisms via protection from predation and stressful thermal conditions (Burnaford, 2004; Crain and Bertness, 2006; Shelton, 2010). Surfgrasses have been shown to occupy more canopy space within zones that they reside in than any other organisms inhabiting similar spaces in rocky shore systems (Turner, 1985). They are also considered competitive dominants because they outcompete other intertidal macrophytes for space by forming dense beds and preventing other organisms from invading (Turner 1985).

The mechanisms by which surfgrasses exclude other organisms are unknown. However, allelopathy, shading, whiplash, consumption of neighboring algae by associated invertebrates, and blocking propagules of other competitors, are thought to be some of the causes of exclusion and competition (Rosenthal *et al*., 1974; Dayton 1975;

Menge, 1976, Hruby and Norton 1979; Lubchenco, 1980; Deysher and Norton, 1982). Shelton (2010) noted that one of the most interesting findings from his surfgrass removal experiment was greater variation in community assemblages in the absence of surfgrass. Conversely, whether that was simply due to the stochastic process of the disturbance (removal) is unknown.

<div align="center">Study System</div>

California Current Large Marine Ecosystem (CCLME)

Surfgrasses and the tidepools they inhabit are found along rocky shores within the California Current Large Marine Ecosystem (CCLME), which spans from British Columbia, Canada to Baja California, Mexico in the eastern North Pacific Ocean basin. The CCLME is a diverse and dynamic system that yields abundant ecosystem goods and services including plentiful fisheries, recreation, tourism, energy production, climate regulation, pollution control, and transportation (NOAA-IEA), it constitutes one of the highest productivity regions in the world (Gruber *et al.,* 2012). The CCLME is controlled largely by upwelling that varies year-to-year bringing cold, nutrient rich, oxygen depleted, and CO_2 saturated water from ocean depths to surface waters (NOAA-IEA). Coastal ecosystems like the CCLME are essential regions that experience anthropogenic stress and are crucial places for research to examine how these effects are disturbing coastal and nearshore systems and the ecosystem services that they provide (Honig *et al.*, 2016).

Study Objectives

The objective for this chapter of my thesis is to examine the role of surfgrass as a foundation species and ecosystem engineer in naturally occurring tidepool communities along an understudied portion of the northern California coastline that lies within the CCLME. More specifically, I will examine the relationship between surfgrass abundance and biodiversity in tidepool communities at three rocky intertidal zones situated at sites that exhibit the topography of both boulder fields and rock benches. I predicted that surfgrass abundance will be positively correlated with greater biodiversity and species richness within naturally-occurring rocky shore tidepools.

MATERIALS AND METHODS

Study Sites

To determine whether surfgrass abundances are related to tidepool community structure, I quantified biodiversity within tidepools that varied with respect to the presence and abundance of surfgrass cover. The study took place at three sites on the Humboldt County coast in northern California, within the CCLME, and include Baker's Beach (BB), a large southwest facing boulder field in Trinidad, California (41.044º N, 124.123º W); Luffenholtz-North (LH) a small, exposed westward facing intertidal bench in Trinidad, CA (41.043º N, 124.122 º W); and Mussel Rock (MR), Cape Mendocino, CA (40.343º N, 124.362º W; Figure 1; Figure 2). The latter site is a large, expansive intertidal bench that has an overall higher elevation compared with the sites located in Trinidad. I chose these study sites to represent a gradient of upwelling along a dynamic and understudied segment of the northern California coastline. The last of the three sites, MR, is situated along the Lost Coast, south of Cape Mendocino, the westernmost point in California. This area is mostly natural, and development-free, lacking major highways and distinguished by the King Range mountains and wilderness area (US-DOI, Bureau of Land Management).

Figure 1. Locator site map of Humboldt County, located in northern California which is found within the northeast Pacific Ocean Basin. The Humboldt State logo indicates the location of the Humboldt State University campus, in Arcata, CA. Map created using Esri's ArcMap (10.5.1).
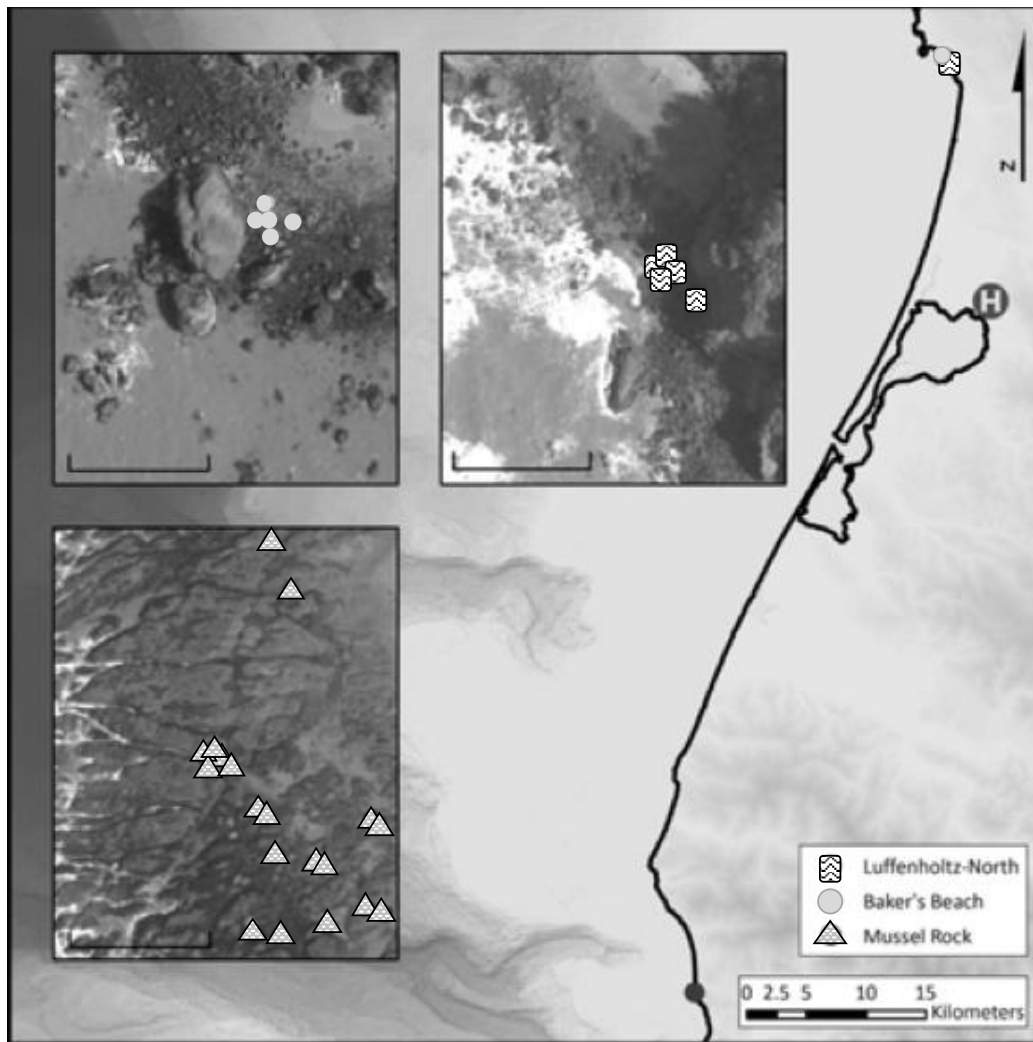
Figure 2. Site locator maps of each of the three study locations used for the duration of my thesis. From north to south, my study sites include: Baker's Beach (BB; 41.044º N, 124.123º W), Luffenholtz-North (LH; 41.043º N, 124.122 º W), and just south of Cape Mendocino, the final site – Mussel Rock (MR; 40.343º N, 124.362º W). Due to the close proximity of BB and LH, both sites are located at the blue point in the map. Maps were created using Esri's ArcMap (10.5.1) and GPS coordinates are averaged across each tidepool per site.

Tidepool Characterization

At each of the three study sites, I chose to survey tidepools during the spring tides on March 31 and April 1, 2017. Pools selected and marked for the study had to be isolated from sources of seeps, drainages, and other tidepools as much as possible. I selected five tidepools each at BB and LH and 19 pools at MR, totaling 29 tidepools for inclusion in this study. I measured the two longest perpendicular linear dimensions of each pool via a 50 meter transect tape and recorded ten depths along each dimension to estimate average pool depth. Linear measurements and depths were used to calculate the surface area (square meters) and volume (cubic meters, then converted to liters) of each pool. Emergent boulders in each pool were measured at their longest perpendicular linear dimensions to calculate their area, which was subtracted from the total surface area of the respective pool to obtain more accurate estimates of total pool volume (Table 1). The tidal elevation (meters above or below MLLW) of every pool was taken between April 27 and 29, 2017 with a CST/Berger Laser Level in relation to the low tide that morning. Low tide heights at each location (Trinidad Pier and Shelter Cove, CA) were then converted to the verified low tide (NOAA Tides & Currents) using the Crescent City, CA Station (the Trinidad Pier and Shelter Cove stations do not verify predicted low tides, which is why I used verified values for the low tides at the Crescent City, CA station, Station ID: 9419750). Pool elevations were then transformed to be relative to MLLW (mean lower low water) using the verified low tide heights and measured heights of each pool. For the northernmost site in my study, BB, the mean elevation of all five tidepools

was 0.09 meters below MLLW. Tidepools located at LH had a mean elevation of 0.38

above MLLW, and those at MR had a mean elevation of 0.86 above MLLW (Table 2). In

addition, percent cover of surfgrass in each pool was quantified, on the days that the

pools were established for the study, by visually estimating percent canopy cover of the

entire surface area of the pool and averaging estimates between myself and one or two

other independent observers to get a more accurate, and less biased estimate of canopy

cover. Percent cover was estimated again during the following low tide series and

averaged with initial percent cover estimates to obtain a more accurate approximation of

surfgrass canopy cover that was used for the study (Table 3).

Table 1. Physical attributes [length (meters), width (meters), average depth (meters), surface area (m^2), and volume (m^3 converted to liters)] of each tidepool across the three sites. Sites are arranged from north to south (BB = Baker's Beach, LH = Luffenholtz and MR = Mussel Rock).

Site/ Pool #	Length (m)	Width (m)	Average Depth (m)	Surface Area (m^2)	Volume (L)
BB1	7.34	4.65	0.07	14.95	2506.5
BB2	3.46	2.17	0.12	3.67	889.13
BB3	4.08	3.9	0.13	5.18	1998.18
BB4	2.43	2.07	0.09	1.49	468.02
BB5	4.4	1.35	0.07	1.22	437.18
LH1	4.26	2.02	0.18	3.24	813.63
LH2	1.37	0.96	0.07	1.03	60.75
LH3	1	0.55	0.07	1.63	31.89
LH4	0.82	0.77	0.26	0.46	148.23
LH5	1.2	0.75	0.06	0.62	20.75
MR1	2.35	1.55	0.34	2.86	1254.39
MR2	5.55	4.8	0.30	18.91	8046.85
MR3	4.6	2.5	0.60	9.03	6889.73
MR4	0.95	0.75	0.10	0.56	69.91
MR5	1.7	1.55	0.43	2.07	1120.45
MR6	0.99	0.64	0.34	0.5	217.33

Site/ Pool #	Length (m)	Width (m)	Average Depth (m)	Surface Area (m^2)	Volume (L)
MR7	3.3	2.4	0.09	6.22	731.28
MR8	4.5	1.2	0.11	3.82	572.72
MR9	1.6	0.95	0.12	1.19	189.31
MR10	2.15	1.9	0.03	3.02	103.83
MR11	1.2	1	0.05	0.76	62.25
MR12	3.2	1.5	0.10	3.77	465.39
MR13	4.5	1.15	0.22	3.92	1116.32
MR14	1.55	0.66	0.10	0.8	102.94
MR15	6.52	1.12	0.30	5.74	2180.2
MR16	4.44	2.23	0.35	7.78	3437.76
MR17	8.4	4.9	0.20	23.06	8246.7
MR18	4.88	1.6	0.13	6.13	1000.03
MR19	4.4	2.06	0.15	5.95	1389.29

Table 2. Tidal elevation of all tidepools across sites. Positive numbers indicate elevation above MLLW (meters), negative numbers indicate elevations below MLLW (meters). All elevation values are in respect to verified low tides at the Crescent City, CA tidal station and have been converted from feet to meters. Source: National Oceanic and Atmospheric Administration (NOAA) Tides & Currents, Crescent City Station (ID: 9419750).

Site/ Pool #	Elevation in Regards to MLLW (m)
BB1	-0.14
BB2	-0.15
BB3	0.02
BB4	-0.09
BB5	-0.07
LH1	0.36
LH2	0.26
LH3	0.62
LH4	0.36
LH5	0.32
MR1	1.71
MR2	1.77
MR3	1.15
MR4	1.26
MR5	1.15
MR6	1.39

Site/ Pool #	Elevation in Regards to MLLW (m)
MR7	0.19
MR8	0.14
MR9	0.5
MR10	0.53
MR11	0.52
MR12	-0.10
MR13	0.18
MR14	0.69
MR15	0.41
MR16	0.98
MR17	0.26
MR18	1.61
MR19	1.91

Table 3. Visual estimates of the percent canopy coverage of surfgrass for all pools at two-time periods at the start of the study.

Site/ Pool #	% Cover Surfgrass at Time 1	% Cover Surfgrass at Time 2	Average % Cover Surfgrass
BB1	25	55	40
BB2	5	25	15
BB3	40	65	52.5
BB4	10	10	10
BB5	0	0	0
LH1	40	75	57.5
LH2	85	85	85
LH3	95	95	95
LH4	0	5	2.5
LH5	80	60	70
MR1	40	70	55
MR2	10	15	12.5
MR3	75	80	77.5
MR4	60	90	75
MR5	5	8	6.5
MR6	0	0	0
MR7	40	90	65
MR8	30	80	55
MR9	30	55	42.5

Site/ Pool #	% Cover Surfgrass at Time 1	% Cover Surfgrass at Time 2	Average % Cover Surfgrass
MR10	80	60	70
MR11	85	75	80
MR12	75	65	70
MR13	2	8	5
MR14	5	5	5
MR15	20	35	27.5
MR16	0	0	0
MR17	70	85	77.5
MR18	60	45	52.5
MR19	15	15	15

Community Biodiversity Surveys

To determine whether surfgrass abundance was associated with biodiversity within tidepools along the northern California coastline, I estimated organismal diversity within all 29 study pools via point contact quadrat sampling. I completed biodiversity surveys during the same low tide series occurring from May 26 to 29, 2017. To maintain a similar sampling effort on a per pool basis, I calculated the number of quadrats for a given pool and ultimately, the number of points to be sampled before surveys began (Figure 3). Quadrats used for sampling were either $0.0625m^2$ or $0.5m^2$, depending on the

size of the pool to be sampled. The $0.0625m^2$ quadrats had 9 possible points, while the

$0.5m^2$ quadrats had 36 possible points. The number of quadrats used per pool was

dependent upon, and proportional to, the total surface area of the tidepool (Figure 13). I

used a layered sampling scheme, (similar to methods used by PISCO- the Partnership for

Interdisciplinary Studies of Coastal Oceans) to capture the three-dimensional component

(e.g., substratum, understory, canopy) of taxa within tidepools (Coastal Biodiversity

Survey Protocols, 2011). For each pool, there was the potential for collecting data from

layers A through D; where layer A was considered to be the top layer, or the canopy, B as

the second layer, and so on until the substrate was reached. Not all points on the quadrat

included all four layers, however, there was always the possibility for such cases.

Quadrats were aligned along the longest placed transect and a random number generator

was used to determine the position each quadrat was placed along the transect. Then, I

used the random number generator to determine how far to the left or right of the transect

I placed the quadrat for sampling. I repeated this process for each transect within a pool

and amongst pools at all three sites. Organisms were classified to lowest taxonomic rank,

when possible, or put into a functional group (e.g., 'biofilm' when the organisms were

microscopic and most likely diatoms, or 'articulated coralline' as opposed to identifying

to species, since they fill similar ecological niches within tidepools). I characterized the

communities within all 29 tidepools and observed 75 separate species/taxonomic groups

(Appendix A, B). The point contact cover of surfgrass was calculated per pool and used

for the percent cover estimates in all of the following analyses (Table 4).
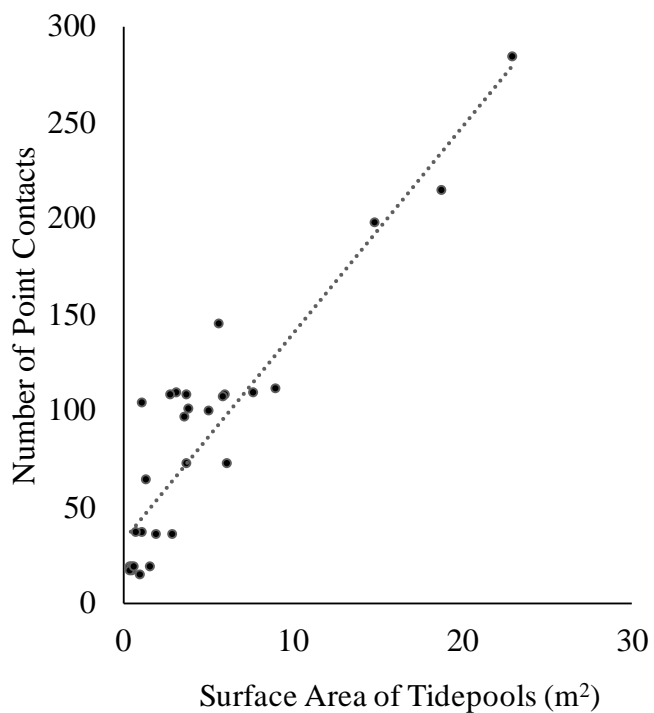
Figure 3. Relationship between surface area of tidepools and the number of point contacts per tidepool accounted for in the biodiversity surveys. Increasing the number of point contacts as function of pool surface area was done to keep sampling effort per pool proportional. Dotted line represents ordinary least squares best fit, $R^2 = 0.84$.

Table 4. Surface area (m^2) to volume (m^3) ratio of each pool and the percent cover of surfgrass calculated from the point contact quadrat data divided by the total number of points per pool.

Site	Pool #	Surface Area to Volume Ratio	Point Contact Percent Cover of Surfgrass
BB	1	5.96	39.59
BB	2	4.12	9.38
BB	3	2.59	46.46
BB	4	3.17	30.16
BB	5	2.78	0
LH	1	3.98	43.52
LH	2	17.00	42.86
LH	3	50.98	44.44
LH	4	3.07	5.56
LH	5	29.68	77.78
MR	1	2.28	63.55
MR	2	2.35	8.41
MR	3	1.31	65.45
MR	4	8.00	93.75
MR	5	1.85	0
MR	6	2.29	0

Site	Pool #	Surface Area to Volume Ratio	Point Contact Percent Cover of Surfgrass
MR	7	8.51	76.39
MR	8	6.67	61.68
MR	9	6.31	52.78
MR	10	29.04	31.43
MR	11	12.21	38.89
MR	12	8.10	84.72
MR	13	3.51	19
MR	14	7.81	13.89
MR	15	2.63	25.69
MR	16	2.26	0
MR	17	2.80	47.7
MR	18	6.13	50.47
MR	19	4.28	30.19

Data Analyses

Community composition

Tidepools were characterized in terms of their percent cover of surfgrass and their community composition. Several analyses examined the relationship between surfgrass abundance and the underlying community assemblages among pools. Non-metric

multidimensional scaling (NMDS) characterized and visualized community composition

among tidepools. NMDS was used instead of principal components analyses (PCA) since

there were numerous 0's within the community data set and NMDS makes few

assumptions about the nature of the data, so is well suited for a wide variety of data. Rare

species (taxa that occurred in less 5% of the total number of tidepools, n = 29) were

dropped from analysis. MRPP (Multi-Response Permutation Procedures) on Bray-Curtis

distances was used to test the hypothesis that there were no differences in tidepool

community composition among sites. I followed this analysis with MRPP pairwise

comparisons to assess which sites were different from another in regards to community

assemblages. MRPP analysis assumes that the distance measure adequately represents the

variation of interest in the data, sample units are independent, and the relative weighting

of variables was controlled prior to calculating the distance measure (Mielke, 1984;

Mielke and Berry, 2001; McCune and Grace, 2002). NMDS and MRPP analyses were

done using the *vegan* package (Oksanen *et al*., 2013) in R (R Core Team 2017).

Diversity metrics

I also calculated species richness, Simpson's diversity, and Simpson's dominance

indices for all tidepools across the study locations (Table 5). These indices were

calculated by using the raw percent cover data of each individual taxon (species or

functional group) per tidepool and then dividing by the sum of all individual's percent

cover. I then squared each value and used the appropriate diversity index equation

(Morris *et al.* 2014). Simple linear regressions and polynomial regressions were used to

evaluate associations between the percent cover of surfgrass and: species richness, Simpson's diversity, Simpson's dominance, invertebrate species richness, calcifying invertebrate species richness, and macrophyte species richness.

Table 5. Species richness, Simpson's Diversity, and Simpson's Dominance indices calculated from tidepool biodiversity data. Formulas from Morris et al. (2014) and Simpson (1949). See Appendix A and B for the list of all species.

Site	Pool #	Species Richness (# of Species)	Simpson's Diversity $(1 - \Sigma P^2)$	Simpson's Dominance $(1 / \Sigma P^2)$
BB	1	23	0.81	5.29
BB	2	23	0.83	5.73
BB	3	17	0.76	4.15
BB	4	16	0.85	6.56
BB	5	17	0.86	7.08
LH	1	18	0.85	6.46
LH	2	9	0.83	5.92
LH	3	7	0.79	4.74
LH	4	13	0.86	6.95
LH	5	7	0.69	3.28
MR	1	14	0.75	4.08
MR	2	20	0.80	4.92

Site	Pool #	Species Richness (# of Species)	Simpson's Diversity $(1 - \Sigma P^2)$	Simpson's Dominance $(1 / \Sigma P^2)$
MR	3	12	0.65	2.87
MR	4	11	0.72	3.55
MR	5	9	0.78	4.49
MR	6	8	0.83	5.74
MR	7	15	0.72	3.59
MR	8	16	0.73	3.67
MR	9	8	0.76	4.14
MR	10	10	0.83	5.94
MR	11	11	0.79	4.72
MR	12	9	0.50	2.00
MR	13	19	0.86	6.93
MR	14	10	0.82	5.54
MR	15	16	0.83	5.83
MR	16	10	0.68	3.12
MR	17	22	0.82	5.52
MR	18	13	0.70	3.37
MR	19	19	0.87	7.66

RESULTS

Tidepool Community Composition

The NMDS analysis of community composition among tidepools indicated that species assemblages at BB and LH were distinct from one another, while the species composition at MR overlapped with both BB and LH. (2D stress = 0.16, dissimilarity metric = Bray-Curtis distances, data = standardized; Figure 24). NMDS also revealed that the tidepools at LH had the tightest clustering, after removing rare species, or most similar species assemblages across tidepools, while MR had the widest spread of community assemblages, encompassing similar species to both of the other sites. BB had an overall higher abundance of invertebrates (e.g., polychaete worms, sponges, bryozoans, gumboot chitons [*Cryptochiton stelleri*], snails, crabs, sea stars, and isopods) along with a few vertebrate species (e.g., sculpins; *Oligocottus maculosus*). LH had a greater diversity of red algae, compared to BB, (e.g., *Constantinea simplex*., *Cryptopleura ruprechtiana*., *Mazzaella splendens*., *Plocamium pacificum*., *Ptilota filicina*., and other rare filamentous reds) and also contained the green alga *Ulva* spp. and the brown algae *Laminaria* spp. and *Egregia menziesii*. MRPP analysis indicated that the three study locations differed significantly in terms of tidepool community structure (BB: $\delta = 0.6352$, n = 5, LH: $\delta = 0.5286$, n =5, and MR: $\delta = 0.5556$, n = 19; distance matrix = Bray-Curtis, A = 0.065, $P = 0.002$, number of permutations = 999). Pairwise comparisons indicated that BB and LH tidepool communities were significantly different from each

other (A = 0.1424, observed δ = 0.58, expected δ = 0.68, P = 0.007, number of permutations = 999), BB and MR tidepool communities were significantly different from one another (A = 0.03, observed δ = 0.57, expected δ = 0.59, P = 0.02, number of permutations = 999), and LH and MR were significantly different from one another (A = 0.04, observed δ = 0.55, expected δ = 0.57, P = 0.01, number of permutations = 999).
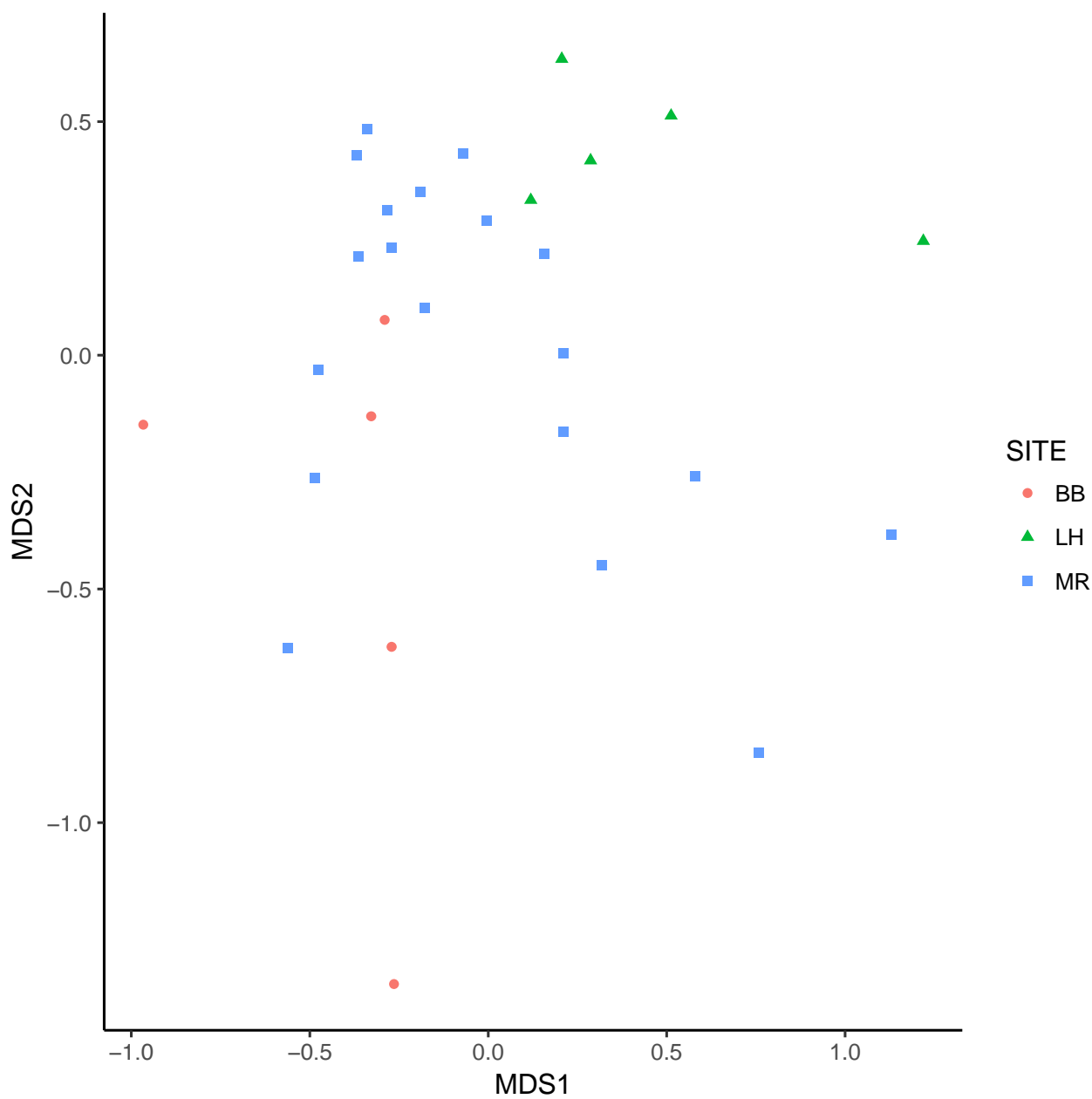
Figure 4. Non-metric multidimensional scaling plot (NMDS) of biological communities across all tidepools and grouped by site. Stress of NMDS = 0.16; dissimilarity metric = Bray-Curtis distance; data were standardized, rare species (< 5% occurrence across all

tidepools) were removed from data. SITE CODES: BB = Baker's Beach, LH =
Luffenholtz, and MR = Mussel Rock.

Tidepool Biodiversity Metrics

There was no relationship between surfgrass cover and overall species richness in tidepools. Although there was a non-significant trend toward a unimodal or hump-shaped relationship, with species richness greatest at approximately 33 percent cover of surfgrass (polynomial regression, $R^2 = 0.11$, $P = 0.21$; Figure 5). Simpson's diversity index, which combines species richness and abundances of the observed taxa (Morris *et al*. 2014), indicated that at lower coverage of surfgrass per pool ($< 25\%$), diversity increased with increasing surfgrass cover. Highest diversity was observed at an intermediate abundance of surfgrass coverage (~25%), then decreased, and was lowest at the highest percent cover of surfgrass that was observed across pools (polynomial regression, $R^2 = 0.53$, $P = $ 5.94e-05; Figure 5). Simpson's Dominance values peaked at approximately 20 percent surfgrass cover and decreased until reaching the highest values of surfgrass cover that were observed (polynomial regression, $R^2 = 0.47$, $P = 0.002$; Figure 5). When surfgrass coverage was low (20% or less) in a given pool, dominance values increased, meaning that certain taxa were more numerous than other competitors when surfgrass coverage was low or absent within a pool. Linear regression of invertebrate abundances on surfgrass cover indicated no significant relationship between surfgrass coverage and invertebrate abundance (simple linear regression, $R^2 = 0.07$ $P = 0.16$; Figure 6). The relationship between surfgrass cover and macrophyte species richness followed a similar

pattern to that of the relationship between surfgrass cover and overall species richness; at

intermediate levels of surfgrass presence (~45%), macrophyte richness peaked, although

this relationship only approached statistical significance ($R^2 = 0.17$, $P = 0.087$; Figure 7).
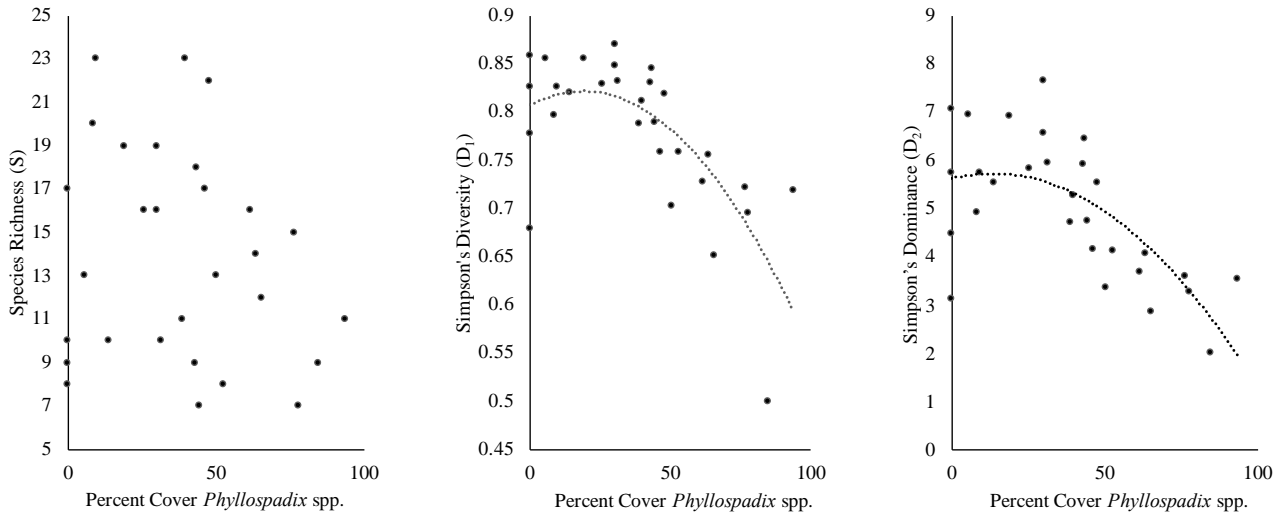
Figure 5. Relationships between surfgrass and (a) species richness, (polynomial

regression, $R^2 = 0.11$, $P = 0.21$), (b) Simpson's Diversity index, (polynomial regression,

$R^2 = 0.53$, $P < 0.001$), and (c) Simpson's Dominance index, (polynomial regression, R^2

$= 0.47$, $P < 0.005$) across all tidepools at the three study locations. Dotted lines represent

ordinary least squares fit.
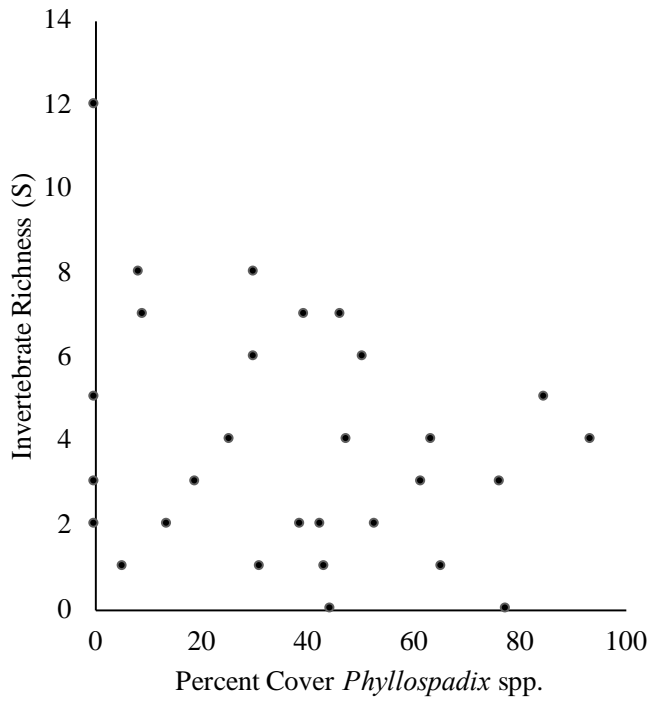
Figure 6. Relationship between surfgrass cover and invertebrate species richness (simple linear regression, $R^2 = 0.07$, $P = 0.16$).
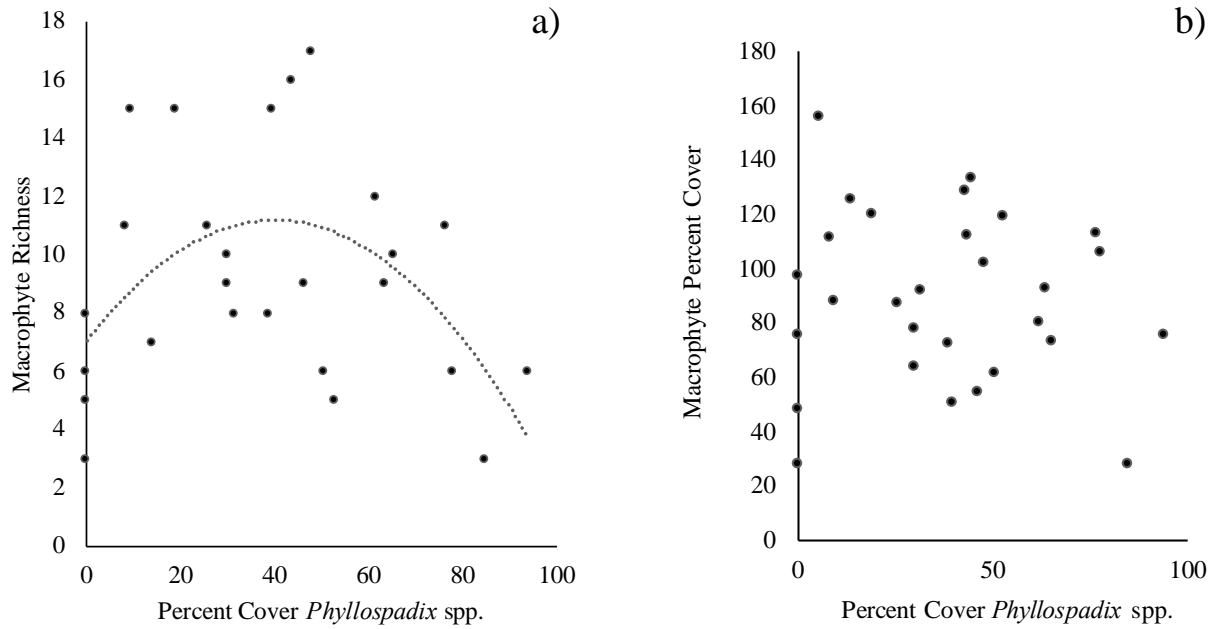
Figure 7. Relationship between surfgrass cover and (a) macrophyte species richness

(polynomial regression, $R^2 = 0.26$, $P < 0.03$) and (b) macrophyte percent cover

(polynomial regression, $R^2 = 0.05$, $P = 0.5$). Dotted lines represent ordinary least squares

fit.

DISCUSSION

My findings indicate that as surfgrass cover of a tidepool increased from 0-40%, there was a concomitant increase in macrophyte richness, suggesting that surfgrass has positive effects on macrophyte diversity at intermediate densities. Such patterns are consistent with surfgrass acting as a foundation species. Surfgrass has been categorized as a foundation species – those with large, yet extremely important roles in determining the functionality and structure in ecological communities – in rocky intertidal ecosystems along the west coast (Shelton, 2010). In support of this characterization, it has been shown that surfgrass ameliorates harsh environments, while creating structure and habitat for fellow community members (Turner, 1985; Shelton, 2010). It has also been shown to provide thermal stress relief to underlying organisms by forming a dense canopy and ultimately shading tidepools (Dethier, 1984; Shelton, 2010). For example, Dethier (1984) noted that surfgrass has the ability to keep tidepools approximately 2-7°C cooler than pools without surfgrass, creating a markedly different microclimate. Surfgrass also has the ability to act as refugia for organisms seeking protection from predation (Dethier, 1984; Bruno and Bertness, 2001).

With all of the documented benefits that surfgrass provides to its surrounding community, the expectation is that tidepools with higher surfgrass abundances should support greater biodiversity. Instead, higher surfgrass abundance was actually associated with a decline of community diversity and richness of other macrophytes, and invertebrates. Decrease in biodiversity in pools with high surfgrass cover suggests that at

high density, surfgrass may be outcompeting other macrophytes for light, nutrients, and space. Surfgrass has been shown to play the role as a strong competitor within rocky shore communities. For example, on rocky shores in the northeastern Pacific, surfgrass is a competitive spatial dominant within its habitat range (Dethier, 1984; Turner, 1985; Jones *et al.*, 1997; Menge *et al.*, 2005). Turner (1985) also demonstrated that surfgrass recruits to pre-existing beds, which could further generate a strong and sustained dominant presence within tidepools. Turner (1985) also suggests that allelopathy and whiplash by the long blades, may play other important factors in limiting the recruitment of other species in tidepools dominated by surfgrass. My results support the notion that surfgrass may have negative competitive effects on other tidepool macrophytes, and potentially negative disturbance-related impacts on overall species diversity, especially at high abundances.

Dominance values per pool in the study were also calculated and provided similar evidence to that of the species richness and diversity data, indicating that when surfgrass coverage was low, dominance values were highest. This suggests a hypothesized mechanism that other community members were able to compete for space and available resources when surfgrass was not found within pools or had not outcompeted other macrophytes. I saw this within some pools that had much higher elevations above MLLW (specifically at MR; Table 2), and the assemblages were much more dominated by diatom, or biofilm crusts as opposed to fleshy or calcifying alga. Such patterns were also noted by Dethier, (1984).

Interestingly, tidepool invertebrate diversity was lower at higher abundances of surfgrass, a pattern that deviates from expectations if surfgrass is in fact acting as a foundation species within the tidepools they inhabit. The definition of foundation species is that they provide structure in a community via stabilization and regulating ecosystem processes, including habitat and resource provisioning. Divergence from these expectations that I found tidepools could be due in part to the fact that surfgrass does not represent a food resource, at least as much as other tidepool macrophytes do, for invertebrate grazers (except for the surfgrass limpet, *Tectura palacea*, which specializes in grazing on blades of surfgrass in the low intertidal; Vermeij, 1992).

Thermal stress is an important feature that surfgrass has been found to ameliorate in tidepool communities. Previous studies by Tuner (1985) and Dethier (1984), conducted experiments in Oregon and Washington, where summertime low tides are later in the day than northern California, which allows for surfgrass to have a larger, and more beneficial effect on underlying communities in relieving temperature stress. At times when the physical environment is considered more stressful for the biological assemblages in tidepools, surfgrass may also be able to increase the pH and DO levels of seawater, as seen in other seagrasses in other ecosystems.

My findings that intermediate abundances of surfgrass have positive effects on tidepool community diversity are consistent with what Jones *et al*. (1994 and 1997) classify as an ecosystem engineer (a term which is sometimes used interchangeably with foundation species) – organisms that physically modify, maintain, and create habitat and structure within their community, not actually via food resources, but by modulating

abiotic stressors in the environment (Jones *et al*., 1994 and 1997). Jones *et al.* (1994 and 1997) describe ecosystem engineers as having positive effects at the landscape level, however, for the effects that they provide the local community, the number of taxa that benefit may be similar to the number of taxa that suffer negative consequences from their presence. Miller *et al*. (2018) argued that many dominant plants have been considered foundation species by ecologists without much supporting evidence. For example, giant kelp, *Macrocystis pyrifera*, has long been considered a marine foundational species in temperate subtidal kelp forest communities, but the quantitative evidence to support this claim is minimal (Miller et al. 2018). Miller et al. (2018) suggests that while giant kelp indeed has positive effects on surrounding species richness, most of the qualities that would classify it as a foundation species were in fact the indirect effects of its physical structure (primarily shading) rather than providing food resources. I argue that surfgrass may have the same type of engineering effects on its environment as giant kelp, at least up to intermediate abundances. However, I also suggest that at higher than intermediate abundances, the engineering effects of surfgrass on tidepool communities become negative for overall diversity.

Tidepools for my survey were chosen to represent not only a gradient of surfgrass coverage but also to represent different levels of exposure to waves and other physical processes. For example, the diversity of red algae was much higher at LH than the other two sites, while BB had much more abundant invertebrate communities, and tidepool sculpins in most of its pools. These differences hypothetically could be due to a latitudinal gradient of recruitment due to upwelling zones and Cape Mendocino, which is

a major biogeographic boundary between the two regions (Connolly *et al*., 2001), or the dissimilarities in topography of the three site locations.

The CCLME has been surveyed extensively in terms of the coastal oceanographic processes that take place on seasonal and yearly scales. Historically, oceanographers typically viewed community dynamics stemming from productivity, nutrients, and vertical/horizontal transport, ('bottom-up' processes) whereas intertidal ecologists most often viewed predation or grazing ('top-down' forces) as the drivers of community structure (Duggins et al., 1989; Persson et al., 1992). More recent studies have focused on the interconnectivity between the two environments, and the large-scale processes that connect the two systems (Menge *et al*., 1997). Menge *et al.* (1997) found that rocky intertidal benthic assemblages were, in part, effected by oceanographic patterns off of the Oregon coastline, and explained some of the variation in community structure and dynamics. Despite some site-specific differences in oceanographic processes and physical stresses, the significant association between surfgrass abundance and tidepool community composition among across sites suggests that the effects of surfgrass on tidepool communities across regional scales is strong and robust to oceanographic and physical differences.

This study was particularly important in describing patterns of association between surfgrass and tidepool community structure along the northern California coastline, which is, for the most part, understudied. Relative to other marine macrophytes, the effects of surfgrass on underlying communities, and its role as a foundational species has also been understudied. My results suggest that surfgrass can

positively affect tidepool biodiversity up to a point (i.e., intermediate abundance), but that

it may have negative effects on community diversity at higher abundances. It is possible

that with a larger sample size of tidepools, and a broader latitudinal range of survey sites,

I may have observed different relationships between surfgrass abundance and tidepool

biodiversity. Future research could examine the relationship between surfgrass abundance

and tidepool biodiversity along a wider biogeographical range, across a variety of

habitats (e.g., wave-protected vs. wave-exposed sites), and whether the different surfgrass

species (*P. torreyi, P. scouleri, and P. serrulatus*) play similar or varying roles within

tidepools in regards to community assemblages. It would also be valuable to assess the

potential for other tidepool macrophytes to fill the vital role that surfgrass plays in

structuring tidepool communities in rocky shore systems. For example, Shelton (2010)

suggested that kelps, *Egregia menziesii* and *Saccharina sessile*, have the size and

morphology to possibly fill similar functional roles (e.g., mediating temperature fluxes in

tidepools) as surfgrass in tidepools, but kelps typically have lower temperature tolerances

than surfgrass, and so may not be able to successfully establish in higher elevation pools.

It would be interesting to examine patterns between the abundances of these kelp species

and tidepool community diversity to see if they follow a similar pattern as the

associations between surfgrass abundance and tidepool diversity.

It will also be crucial to understand the role of surfgrass in structuring tidepool

communities under global climate change scenarios (e.g., sea surface temperature

increase, and ocean acidification), and the role that surfgrass may play in ameliorating

these anthropogenic stressors. It has been shown that seagrass meadows are in fact one of

the most efficient ecosystems in sequestering carbon, however, if disturbed or degraded,

they can leak carbon back into the environment and accelerate rates of global climate

change (Nellemann *et al*., 2009; Macreadie *et al*., 2014). The potential for marine

macroalgae and other marine vegetation as carbon sinks has raised a great deal of interest

in terms of fixing global carbon, when it has been shown that they contribute

approximately 50% to 71% of carbon sequestration in ocean sediments (Nellemann *et al*.,

2009; Chung *et al*., 2009). On a smaller, more localized scale, surfgrass could be another

potential player in sequestering carbon, since some species, notably *Phyllospadix*

scouleri, can reside in sediment-rich substrates (Turner, 1985). In the next chapter of my

thesis, I investigate the potential for surfgrass to consume CO_2 and increase seawater pH

and dissolved oxygen levels in naturally occurring and simulated tidepools.

CHAPTER 2: EXAMINING THE EFFECTS OF PHYLLOSPADIX SPP. IN

AMELIORATING ABIOTIC STRESS WITHIN ROCKY SHORE TIDEPOOLS

INTRODUCTION

Effects of Ocean Acidification on Marine Systems

Additions of human-induced carbon, in the form of greenhouse gases, into the earth's atmosphere are strongly connected with an increased uptake of CO_2 by global oceans (Kelly *et al.* 2011; Kwiatkowski *et al.* 2016). It is estimated that oceanic pH has decreased by approximately 0.1 units since the preindustrial era and is projected to further decrease by 0.07-0.33 units by 2100 (Caldeira and Wickett, 2003; Bopp *et al.* 2013). Although marine uptake of CO_2 is thought to dampen the overall effects of global climate change, absorption of that much carbon by the ocean and subsequent ocean acidification (OA) may have strong, negative repercussions that will be difficult to predict (Fabry *et al.* 2008).

OA is anticipated to have negative consequences for marine biota, primarily calcifying organisms, on a global scale (Kwiatkowski *et al.* 2016). These organisms predominantly reside in coastal and nearshore ecosystems, which are characterized by extremely variable pH and associated carbonate chemistry, which vary over temporal and spatial scales (Hoffman *et al.* 2011; Mercado and Gordillo 2011; Duarte *et al.* 2013). Although a loss of biodiversity is forecasted to occur as a result of ocean acidification, effects on whole species assemblages will be much more problematic and difficult to assess in terms of the direct and indirect effects of OA (Garrad *et al.*, 2014). In coastal ecosystems, specifically rocky shore communities and estuaries, pH regulation is

enhanced via metabolic activity and physical processes that naturally take place (Duarte *et al*. 2013). It has also been described that biological communities and population density largely control oxygen concentrations and pH within tidepools (Ganning, 1971).

Effects of Seagrasses on Ocean pH

It has been shown that seagrasses and other marine macro-autotrophs fix carbon mainly via C_3 photosynthesis, and that most are not saturated at current oceanic dissolved inorganic carbon levels (Borum *et al*., 2005; Koch *et al*., 2013). It is understood that seagrasses and macrophytes predominantly utilize CO_2 and that under elevated CO_2 regimes predicted by climate change models they will likely increase their photosynthetic output and growth rates under future conditions (Koch *et al.* 2013). For example, Hendricks *et al.* (2014) found that the seagrass *Posidonia oceanica* buffered the local carbonate chemistry in seagrass meadows at varying times throughout the year, while displaying strong diel patterns driven by primary production. It has also been established that seagrass beds and other productive communities may act as ocean acidification 'refugia' – areas where pH and associated aragonite saturation states are elevated compared to surrounding source seawater (Semesi *et al*. 2009; Kleypas *et al*. 2011; Manzello *et al.* 2012; Unsworth *et al*. 2012; McLeod *et al*. 2013; Hendriks *et al*. 2014; Camp *et al*. 2016b). The majority of this work, however, has been done based on seagrasses in soft substrates (Unsworth *et al*. 2016; Mongin *et al*. 2016). It is within reason to assume that seagrasses within the genus *Phyllospadix* may be able to fill similar roles in coastal/nearshore rocky intertidal ecosystems as other seagrasses. It has been

shown that species within the genus *Zostera*, the sister genera to *Phyllospadix*, are physiologically similar, and with increasing amounts of CO_2 in surrounding seawater, *Zostera* has demonstrated the ability to increase photosynthesis and flowering output (Invers *et al*. 1997; Palacios and Zimmerman, 2007). Therefore, more research is needed to understand the spatial and temporal mitigation variability of surfgrass, which is a competitive dominant in rocky shore tidepool ecosystems.

Tidepools as a Model System to Test Effects of Surfgrass on Seawater pH and DO

Rocky shore tidepools experience widely fluctuating levels of pH, dissolved oxygen (DO), and temperatures daily. Respiration in tidepools dominates at night, when photosynthesis is diminished, and the consumed O_2 is replaced by CO_2. Hence, pH and DO levels decrease at night, when temperature is also lower, and when respiration dominates photosynthetic activity. Alternatively, during daytime low tides, tidepools experience excess photosynthesis from primary producers, which leads to a drop in CO_2 (increase in pH) during a time when temperature is also increasing (Pörtner 2008). The natural daily variation in pH, DO, and temperature conditions that occur within tidepools create a model system to examine the effects of added CO_2 in natural communities, and the potential role that surfgrass (*Phyllospadix* spp.) takes in buffering local communities from abiotic stressors associated with climate change.

Rocky shore communities have been well-described in terms of the physical and biotic processes that ultimately control local patterns of community assemblages (Menge and Farrell, 1989; Paine, 1994; Menge *et al*. 1997). In terms of community structure,

biological communities residing in tidepools are not nearly as well-known as other rocky

intertidal habitats (Metaxas and Scheibling, 1993). Underwood (1981), even suggested

that tidepools are not necessarily representative of rocky intertidal habitat since they are

submerged during low tides, and do not display extreme changes with vertical

distribution, though the degree as to which fluctuations occur are exacerbated with

vertical intertidal height (Metaxas and Scheibling, 1993). Nevertheless, tidepools and the

adjoining emergent substrata communities experience a wide range in conditions due to

tidal cycles each day, and tidepools could potentially serve as refuges for surrounding

community members during stressful times (Metaxas and Scheibling, 1993). Fluctuations

that occur within tidepools are larger and more stressful than subtidal ecosystems, with

changes in temperature and salinity that can be fairly extreme during daytime low tides

(Metaxas and Scheibling, 1993). It has also been shown that pH, dissolved oxygen, and

alkalinity fluctuate daily in tidepools, largely in part due to the biological processes that

occur; namely photosynthesis and respiration (Pyefinch, 1943; Ganning, 1971; Green,

1971; Daniel and Boyden, 1975; Morris and Taylor, 1983). The amplitude of these

fluctuations varies vertically and horizontally within a given pool, with the volume and

the elevation of the pool, and have also been described to vary diurnally and to a lesser

extent, seasonally (Ganning, 1971; Metaxas and Scheibling, 1993). Metaxas and

Scheibling (1993) also conclude that it is 'virtually impossible' for two naturally

occurring tidepools to be similar in terms of physical characteristics (volume, depth,

elevation, etc.) which suggests that any detectable influence of biological components of

the tidepool (e.g., surfgrass) on seawater chemistry across this variable backdrop, will suggest important and robust effects.

Study Objectives

My objective in this chapter, was to quantify the effect of *Phyllospadix* spp. on seawater chemistry (pH and DO) in simulated and natural tidepools. Specifically, I determined whether the presence of surfgrass is able to counteract, via photosynthetic activity, the addition of [CO_2] in (1) artificially-simulated tidepools and (2) naturally-occurring tidepools; in order to explore relationships between surfgrass abundance and pH within tidepools across my three study locations. I hypothesize that surfgrass presence will mediate CO_2 and subsequent pH and DO stress within artificially generated and naturally occurring tidepools in the following experiments.

MATERIALS AND METHODS

Laboratory Manipulations

I did a three-way, fully-factorial laboratory experiment to test the effects of CO_2-addition, surfgrass presence, and macroalgal (two types of red algae) presence on seawater chemistry dynamics in simulated tidepools at the Humboldt State University Telonicher Marine Laboratory (TML).

Collection and maintenance of experimental organisms

Experimental organisms for the simulated tidepools (hereafter referred to as mesocosms) were collected from Baker's Beach, Trinidad, CA (41.05º N, 124.13º W), due to ease of accessibility from TML. This site was formerly used for community surveys and *in situ* field manipulations and encompasses the diversity of species across all three study sites from the overall project. All species were collected by hand prior to the start of each of the trials, placed in buckets, and brought back to the lab. The species represented in each mesocosm consisted of the two dominant surfgrass species across all sites: *Phyllospadix scouleri* and *P. torreyi* (hereafter, surfgrass), two dominant fleshy red alga groups found across all sites: *Neorhodomela larix,* and calcifying articulated corallines (*Bosiella* spp., *Corallina* spp. and *Calliarthron* spp.). I also incorporated *Tegula funebralis*, the black turban snail, the dominant calcifying invertebrate found across tidepools during my survey. *Tegula funebralis* was incorporated to fill the role of

respiring animals within natural tidepools to create more realistic pH and DO fluxes within each experimental tidepool (n = 48).

For surfgrass, the entire clonal rhizome and associated shoots were scraped from the substrate. Previous studies (Marin-Guirao *et al.* 2011) have shown that larger rhizome fragments survive longer in laboratory experiments and therefore I adopted this protocol to promote adequate growth and photosynthetic conditions of surfgrass in my experiment. All red algal species were scraped from the substrate at the point of the holdfast in order to keep individuals intact for the duration of the experiment. Each of the organisms was placed in their respective mesocosm and left to acclimate to laboratory conditions for three days prior to running each of the trials. One black turban snail (~ 2-4 cm in diameter) was enclosed in a mesh float cage within each mesocosm to prevent them from grazing on any of the macrophytes during the experiment. The snails were fed pre-weighed (~2 grams) *Mazzaella* spp. in the float cages in order to keep them satiated during the experiment. All remaining organisms were weighed prior to being placed into corresponding mesocosms. The abundance of each species used in experimental tidepools was derived from prior field survey data and was meant to resemble natural field abundances. The biomass of surfgrass (~434 grams), red algae (~18 grams *N. larix* and ~14 grams articulated corallines), *T. funebralis,* and associated *Mazzaella* spp. were standardized to keep the photosynthetic and respiration outputs consistent across the simulated tidepools.

Experimental set-up and design

The experiment crossed two levels of pH (ambient [mean = 7.87 $\pm$ 0.036 SE] and reduced [7.50 $\pm$ 0.007 SE]), with the presence and absence of surfgrass, and the presence and absence of red algae (*Neorhodomela larix* and articulated corallines). The experimental set-up consisted of six separate seawater tables (122 cm x 62 cm) with access to flowing seawater from the marine lab's recirculating seawater system. Each treatment combination was randomly assigned to mesocosms (15 L plastic bucket) within each spatial block (sea table), so that there was one replicate mesocosm per treatment in each of the six sea tables (Figure 8). Because the space designated for my experimental set-up only allowed for six replicate buckets per treatment at a time, I ran the experiment in two replicate temporal blocks for a total of 12 replicate mesocosms per treatment combination.

Since the experiment took place inside the laboratory, full-spectrum grow lights (Sylvania – T8, 2,800 Lumens, 32 Watt, 5000K bulbs) in 12 light fixtures were placed over the entire row six of sea tables to ensure appropriate growth and photosynthesis of macrophytes throughout the experiment. Each light fixture hung approximately 0.46m above the experimental tidepools. The mean quantity of light in Humboldt Bay, Humboldt County for the months that this experiment ran is approximately 500 μmol m^2 s^{-1} (data from the photosynthetically active radiation [PAR] sensor located in Humboldt Bay, CeNCOOS). The average output of the lights used in my experiment was 352.8 μmol/(m^{2}s) at the water level of each mesocosm (Li-Cor Quantum Sensor, LI-190); although this value is lower than the natural average, it is well within the natural range of

values observed in Humboldt Bay. The entire experimental area (from light fixtures to sea tables) was covered with black plastic and light fixtures were kept on timers to incorporate a consistent photoperiod regime for optimal growing conditions. 'Daylight' hours, or the time throughout the day that the lights were on, were increased from approximately 11 hours per day (normal photoperiod for Humboldt County during winter months) to 14 hours per day in order increase the photosynthetic yield and maximize growing conditions for all photosynthetic species used in the experiment.

- SG + algae ambient	- SG + algae +CO_2
+ SG - algae ambient	+ SG + algae +CO_2
+ SG + algae ambient	control +CO_2
+ SG - algae +CO_2	control ambient

Figure 8. Schematic of one spatial block (sea table) of the laboratory experiment, with each of the eight treatment combinations randomly interspersed and repeated once per block. Each treatment combination was replicated a total of six times across six spatial blocks (six sea tables) and over two temporal blocks, for a total of 12 replicates per treatment combination.

Manipulation of abiotic environmental conditions in mesocosms

 I manipulated pH in experimental tidepools by bubbling CO_2 directly into header

tanks (61cm x 36cm). Each header tank was responsible for supplying continuously

flowing seawater (flow rates into each mesocosm = ~3.5 mL per second), either ambient

or reduced pH, to eight randomly assigned mesocosms within their respective treatments.

Three header tanks distributed ambient seawater, while the other three delivered reduced

pH seawater to mesocosms in order to decrease pH from ambient levels. The pH of the

seawater in the manipulated header tanks was controlled by a Digital Aquatics

Reefkeeper CO_2 dosing-system (Wilcox-Freeburg *et al*., 2013) and was set to maintain

pH at 7.5, levels that are observed not only on the outer coast of Humboldt County when

upwelling events are occurring (Trinidad Shore Station; CeNCOOS), but also regularly

recorded in tidepools during my field surveys. pH probes were located inside

manipulated header tanks to monitor pH and ensure that the aforementioned pH range

was met.

 The pH and DO in mesocosms served as my response variable. pH and DO

measurements were recorded via hand-held multimeter and probe (Hach handheld probe

pHC101, LDO101) seven times per day during temporal block 1 (three times during

nighttime hours, and four times during daytime hours), and six times per day during

temporal block 2 (twice during nighttime hours and four times during daylight hours) to

quantify the differences among treatments and between day and night (periods of high

photosynthesis and respiration, respectively). The entire experiment ran over a span of 18

days (including acclimation time before each of the temporal blocks); the first temporal

block ran for a total of eight days from January 24 to February 1, 2018, and the second

temporal block ran for four days from February 5 to 8, 2018.

Physical Parameters in Surveyed Tidepools

Surveys of abiotic conditions in tidepools across sites

During the community biodiversity surveys described in the previous chapter, I

measured abiotic variables in each of the 29 surveyed tidepools during two separate low

tide series occurring on June 8 and 13, 2017 and June 23-24, 2017. Using a handheld

multimeter and probe (Hach LDO101, pHC101, HM Digital pH meter PH-200 probes),

and a refractometer (Aqueous Lab Portable Refractometer), I measured pH, DO (mg/L),

temperature (°C) and salinity (oo/0) in each pool across all three study sites (BB, LH, and

MR), and in the adjacent ocean. Measurements were taken during the early morning low

tides and broken down into two separate time periods: 'nighttime' and 'daytime'

sampling events. 'Nighttime' abiotic parameters were collected between the hours of

3AM – 8AM (during which times the tidepools were still under 'dark' conditions, and

respiration clearly dominated abiotic processes in all tidepools), and from 8AM – 1PM

when organisms just started, or had been photosynthesizing for multiple hours out of the

day (Table 6).

Table 6. Surveyed tidepools from each study site with: sampling events, nighttime and daytime pH measurements, and the adjacent ocean pH. Numbers next to the site codes indicates whether the measurements were taken in sampling event 1 or 2. BB and LH were measured in the same day, and thus BB was sampled during dark conditions and LH was sampled during day conditions for both sampling events. MR was sampled in a similar fashion for both sampling events, however the order of tidepools sampled was reversed and thus the tidepools that experienced night/day conditions are different in each sampling event.

Site/ Sampling Event	Pool #	Time of day	Tidepool pH	Ocean pH
BB1	1	Night	7.60	7.89
BB1	2	Night	7.60	7.89
BB1	3	Night	7.54	7.89
BB1	4	Night	7.57	7.89
BB1	5	Night	7.57	7.89
BB2	1	Night	7.47	7.73
BB2	2	Night	7.42	7.73
BB2	3	Night	7.44	7.73
BB2	4	Night	7.43	7.73
LH1	1	Day	7.98	7.97
LH1	2	Day	7.91	7.97
LH1	3	Day	7.80	7.97

Site/ Sampling Event	Pool #	Time of day	Tidepool pH	Ocean pH
LH1	4	Day	7.83	7.97
LH1	5	Day	7.84	7.97
LH2	1	Day	7.80	7.86
LH2	2	Day	7.67	7.86
LH2	3	Day	7.78	7.86
LH2	4	Day	7.77	7.86
LH2	5	Day	7.80	7.86
MR1	1	Night	7.70	8.13
MR1	2	Night	7.95	8.13
MR1	3	Night	7.89	8.13
MR1	4	Night	8.00	8.13
MR1	5	Night	7.88	8.13
MR1	6	Night	7.85	8.13
MR1	7	Day	8.29	8.13
MR1	8	Day	8.38	8.13
MR1	9	Day	8.60	8.13
MR1	10	Day	8.49	8.13
MR1	11	Day	8.40	8.13
MR1	12	Day	8.33	8.13
MR1	13	Day	8.41	8.13
MR1	14	Day	7.88	8.13

Site/ Sampling Event	Pool #	Time of day	Tidepool pH	Ocean pH
MR1	15	Day	8.24	8.13
MR1	16	Day	8.19	8.13
MR1	17	Day	8.49	8.13
MR1	18	Day	8.26	8.13
MR1	19	Day	8.42	8.13
MR2	1	Day	8.53	8.08
MR2	2	Day	8.29	8.08
MR2	3	Day	8.12	8.08
MR2	4	Day	8.13	8.08
MR2	5	Day	8.07	8.08
MR2	6	Day	8.04	8.08
MR2	7	Day	8.17	8.08
MR2	8	Day	8.30	8.08
MR2	9	Night	7.91	8.08
MR2	10	Night	7.48	8.08
MR2	11	Night	7.50	8.08
MR2	12	Night	7.64	8.08
MR2	13	Night	7.61	8.08
MR2	14	Night	8.02	8.08
MR2	15	Night	7.58	8.08
MR2	16	Night	7.68	8.08

Site/ Sampling Event	Pool #	Time of day	Tidepool pH	Ocean pH
MR2	17	Night	7.56	8.08
MR2	18	Night	7.38	8.08
MR2	19	Night	7.34	8.08

In-situ Manipulations of Surfgrass and CO_2 Within Natural Tidepools

Field manipulations

In addition to the initial characterization of all experimental tidepools, I performed two separate *in situ* field manipulations. Field manipulations were carried out to assess the effect of CO_2 additions and surfgrass presence on seawater chemistry within natural tidepools. I manipulated tidepools across all three study locations in the summer of 2017 (sampling event 1) and then again in the fall of 2017 (sampling event 2). The first sampling event ran for five days at each of the three sites from July, 2017 to August, 2017. Northern California has mixed semidiurnal tides, and the larger and more accessible spring and summer low tides occur in the early morning. Therefore, sampling event 1 took place at the end or directly after prolonged darkness, when respiration should dominate over photosynthetic activity within tidepools; therefore, sampling event 1 is hereafter referred to as the 'nighttime' sampling event. I repeated the experiment at each of the three sites in October and November, 2017 when the larger and more accessible of the mixed semidiurnal low tides switched from early mornings to

afternoon/evenings. This sampling event took place at a time when tidepools had been exposed to daylight conditions for the entire day and macrophyte photosynthetic activity should dominate over respiration within each of the pools. Sampling event 2 is therefore referred to as the 'daytime' sampling event.

Twelve previously surveyed tidepools with the smallest volumes were selected from each of the three study sites to be used as my experimental units. The aforementioned tidepools were selected due to their small volumes in an effort to increase likelihood of successfully altering pH through experimental manipulations. Four of the 12 pools were randomly selected to have their surfgrass left intact (percent cover of surfgrass = 30.16, 44.44, 77.78, 93.75), in five of the remaining pools, I manually removed all surfgrass. The last three pools were used as 'control' pools since they did not have pre-existing coverage of surfgrass. Tidepool pH was manipulated in all experimental pools by bubbling in CO_2 using *in situ* self-contained watertight boxes (described in the next paragraph). Seventeen additional tidepools, which were too large to effectively manipulate, served as un-manipulated reference pools (Table 7).

Each tidepool received CO_2 via a sealed Pelican dry box (Pelican – 1010 Micro Series Dry Case), which was filled with ~400 grams of sugar, 100 mL of distilled, deionized water, ~5 grams of yeast, and ~0.25 grams of $NaHCO_3$ (Gillis, 2014), which released the CO_2 produced by the yeast's respiration into the pool. Each Pelican case came equipped with an automatic pressure purge valve that I drilled a hole into and screwed a valve fitted with water sealing tape in order to (1) maintain the watertight capabilities of the box, and (2) secure airline tubing to the end exposed to the environment. Airline tubing

was then fitted with an airstone and fastened via zip ties into corresponding CO_2-addition tidepools for the duration of each cycle of the experiment. After creating the sugar/yeast mixture in the lab, dry boxes were brought into the field and deployed immediately, after recording the initial pH, DO, and temperature of each pool (serving as the baseline with which to compare the post-manipulation water chemistry in the tidepools; Appendix C and D). Boxes were installed with galvanized steel plumber's tape that was fastened to the rock via plastic dry wall anchors and stainless-steel washers and screws. The number of CO_2 boxes per pool depended on the volume of the pool; the larger the volume, the more boxes were necessary to change pool pH. The boxes varied on a per-pool basis from two to six boxes per pool, to ensure similar effects of CO_2 additions. Surfgrass that was removed from each tidepool was brought back to the lab and weighed, and the relationship between tidepool volume and surfgrass biomass was used to derive the biomass of surfgrass that I would use for the subsequent laboratory experiment portion of my thesis.

Table 7. The distribution of treatment allocation to experimental tidepools for both field manipulations ('daytime' and 'nighttime'). Each treatment, per pool, was consistent for both sampling events. 'Reference' refers to control pools that were too large, volumetrically, to experimentally manipulate effectively; 'Surfgrass intact' indicates surfgrass coverage that was kept intact for the duration of the experiment; 'Removal of surfgrass' indicates surfgrass that was removed from the specified pool; '+CO_2' indicates CO_2 was added to pools.

Site/Pool#	Treatment
BB1	reference
BB2	reference
BB3	reference
BB4	surfgrass intact, +CO_2
BB5	control, +CO_2
LH1	reference
LH2	removal of surfgrass, + CO_2
LH3	surfgrass intact, +CO_2
LH4	removal of surfgrass, + CO_2
LH5	surfgrass intact, +CO_2
MR1	reference
MR2	reference
MR3	reference
MR4	surfgrass intact, +CO_2

Site/Pool#	Treatment
MR5	control, $+ CO_2$
MR6	control, $+ CO_2$
MR7	reference
MR8	reference
MR9	removal of surfgrass, $+ CO_2$
MR10	reference
MR11	removal of surfgrass, $+CO_2$
MR12	reference
MR13	reference
MR14	removal of surfgrass, $+ CO_2$
MR15	reference
MR16	reference
MR17	reference
MR18	reference
MR19	reference

Data Analyses

Laboratory experiment

The effects of CO_2 addition, surfgrass presence, red algal presence, and their interactions on mesocosm pH and DO over time were analyzed with separate 3-way, fully-factorial, repeated-measures ANOVA. I also used a 3-way ANOVA to test for

treatment effects on averaged day-night differences in pH and DO among treatments. Prior to analyses, I visually assessed adherence to the assumption of normality using qq-plots and I assessed the assumption of homogeneity of variance using Levene's Test.

<u>Surveys of abiotic conditions within tidepools</u>

I examined the associations between surfgrass abundance and tidepool pH during nighttime and daytime sampling events. First, I pooled nighttime pH measurements and daytime pH measurements and tested for differences in average tidepool pH between daytime (LH and MR) and nighttime (BB and MR) sampling events using Welch's t-tests. Next, I visually inspected the relationships between surfgrass abundance and nighttime and daytime pH. After concluding that the relationship was parabolic, I used a 2nd order polynomial regression to statistically analyze the relationship. Lastly, to assess whether surfgrass abundance in tidepools was associated with changes in tidepool pH relative to oceanic pH, I subtracted the adjacent ocean pH from each tidepool pH to attain a pH difference. I then analyzed the relationship between surfgrass abundance and pH differences with a 2nd order polynomial regression (for both day and night). I only used MR pools for the daytime analysis and the tidepools from MR and BB for the nighttime analysis. The reasoning behind leaving out the daytime measurements at LH was due to the fact that pools at this site were very different from the pools at MR and BB in terms of the dominant macrophyte diversity, as indicated by the NMDS analysis in chapter 1 (Figure 4).

Field manipulations

I used multiple analyses to assess the effects of added CO_2 and the removal of surfgrass on seawater chemistry and associated abiotic parameters of experimental tidepools. I ran separate 1-way repeated-measures ANOVAs to statistically test for any treatment (combination of CO_2-addition and surfgrass removal) effects on tidepool pH over time for each of the sampling events (daytime and nighttime). Pairwise comparisons were then used to assess which of the treatment combinations were significant. Lastly, I ran separate one-way ANOVAs to test the effect of treatment on the differences between the initial and final pH over time for each sampling event (daytime and nighttime).

RESULTS

Physical Characteristics of Tidepools

<u>Laboratory experiment</u>

<u>Change in pH/DO over time.</u> Repeated-measures ANOVA indicated significant effects of surfgrass, CO_2, time, header tank, and the interactions between surfgrass and CO_2 and time and CO_2 on mesocosm pH (Table 8). All treatments containing surfgrass yielded stronger day/night fluctuations in pH, regardless of CO_2 additions (Figure 9). Treatments containing surfgrass but no algae, and treatments containing both surfgrass and algae were significantly different from treatments that contained only algae or the photoautotroph-free controls (Figure 9). The mean pH of mesocosms containing only red algae had far less dramatic day/night fluctuations in pH than treatments that included only surfgrass or both surfgrass and red algae, regardless of the addition of CO_2 (Figure 9; Table 8).

Dissolved oxygen was also altered by the presence of surfgrass (Figure 9). Repeated-measures ANOVA indicated that only surfgrass and time had significant effects on mesocosm DO (Table 9). Changes in dissolved oxygen in experimental mesocosms generally mirrored changes in pH, thus the mesocosms containing surfgrass had greater day/night fluctuations in DO than algae-only, or control mesocosms (Figure 9; Table 8).

a)

+sg,+algae,+CO2
+sg, +algae, ambient
-sg, +algae, +CO2
-sg, +algae, ambient
+sg, -algae, +CO2
+sg, -algae, ambient
control (+CO2)
control (ambient)

b)

+sg, +algae, +CO2
+sg, +algae, ambient
-sg, +algae, +CO2
-sg, +algae, ambient
+sg, -algae, +CO2
+sg, -algae, ambient
control, +CO2
control, ambient
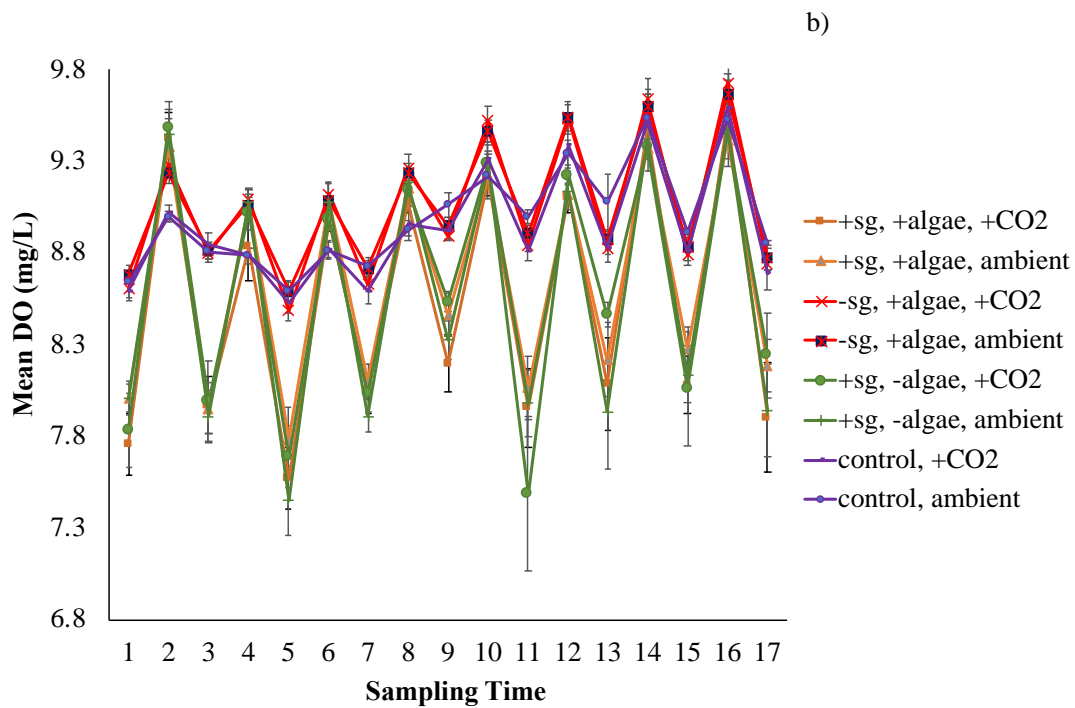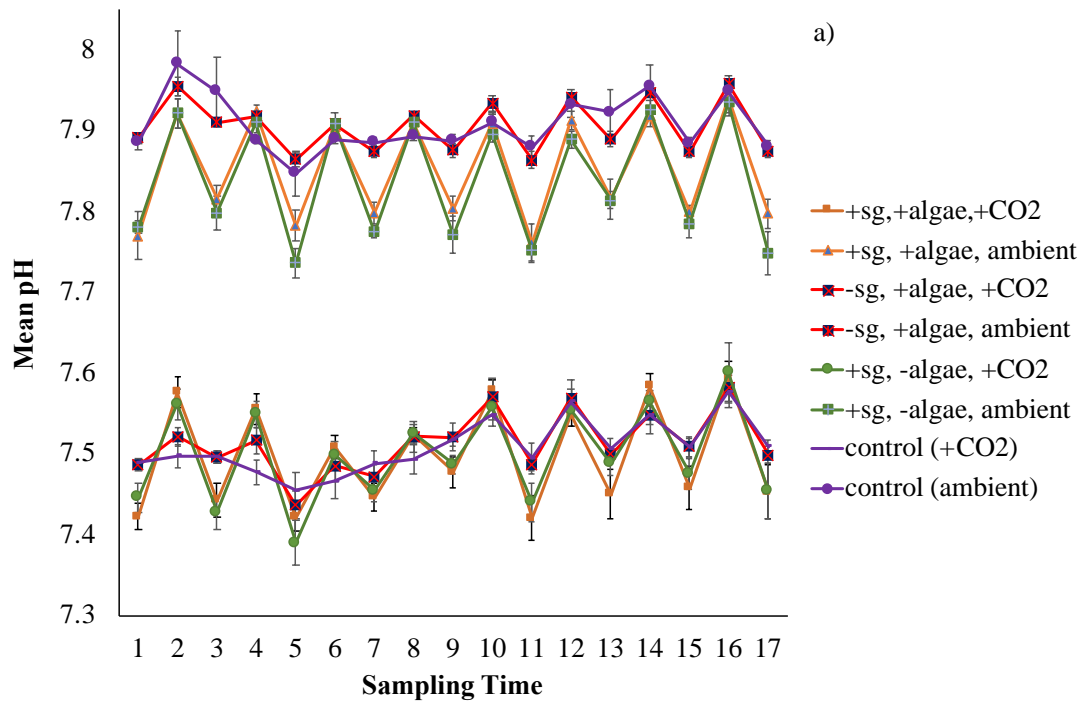
Figure 9. Mean (a) pH and (b) dissolved oxygen (mg/L) over time in the laboratory experiment. Error bars are ± 1 SE. TREATMENT KEY: all dotted lines represent treatments with added CO_2, while solid lines represent the ambient treatments (no CO_2 added). +/- signs indicate when there was the presence or absence of 'sg' = surfgrass or red algae = *Neorhodomela larix* and articulated coralline algae. Controls did not include either groups of seaweed.

Table 8. Model summary for the 3-way, fully-factorial, repeated-measures ANOVA analyzing the effects of surfgrass (SG – presence or absence), algae (presence or absence), time (eight- day time span of the experiment), and CO_2 (presence or absence in mesocosms) on mesocosm pH. Bolded *P*-values indicate statistical significance ($P <$ 0.05).

Treatment	df	*F*	*P*
Surfgrass (SG)	1	69.64	**< 0.001**
Algae	1	1.41	0.24
Time	1	14.18	**< 0.001**
CO_2	1	8385.62	**< 0.001**
Header tank	4	5.49	**< 0.001**
SG*Algae	1	0.004	0.95
SG*Time	1	0.03	0.86
Algae*Time	1	0.08	0.78
SG*CO_2	1	31.27	**< 0.001**

Treatment	df	*F*	*P*
Algae*CO$_2$	1	0.04	0.84
Time*CO$_2$	1	11.14	**< 0.001**
SG*Algae*Time	1	0.02	0.88
SG*Algae* CO$_2$	1	0.51	0.47
SG*Time*CO$_2$	1	1.06	0.3
Algae*Time*CO$_2$	1	0.55	0.46
SG*Algae*Time* CO$_2$	1	0.07	0.79

Table 9. Model summary for the 3-way, fully-factorial, repeated-measures ANOVA analyzing the effects of surfgrass (SG – presence or absence), algae (presence or absence), time (eight- day time span of the experiment), and CO$_2$ (presence or absence in mesocosms) on dissolved oxygen (DO) in the laboratory experiment. Bolded *P*-values indicate statistical significance ($P < 0.05$).

Treatment	df	*F*	*P*
Surfgrass (SG)	1	131.54	**< 0.001**
Algae	1	2.39	0.12
Time	1	43.01	**< 0.001**
CO$_2$	1	0.88	0.35
Header tank	4	2.01	0.09
SG*Algae	1	3.22	0.07
SG*Time	1	1.65	0.2

Treatment	df	*F*	*P*
Algae*Time	1	0.07	0.79
SG*CO$_2$	1	0.023	0.88
Algae*CO$_2$	1	0.27	0.6
Time*CO$_2$	1	0.04	0.84
SG*Algae*Time	1	0.41	0.52
SG*Algae* CO$_2$	1	0.77	0.38
SG*Time*CO$_2$	1	0.28	0.6
Algae*Time*CO$_2$	1	0.01	0.95
SG*Algae*Time* CO$_2$	1	0.17	0.68

Day/night pH and DO differences. In general, day/night fluctuations in pH and

DO were similar among treatments, however, mesocosms under ambient conditions (no

added CO$_2$) exhibited slightly larger day/night pH fluctuations on average than

mesocosms with added CO$_2$ (Table 10). Surfgrass presence had a significant effect on

diel pH fluctuations, whereas algae did not (Table 10; Figure 10). Surfgrass on its own

and the interaction of surfgrass and algae presence had statistically significant effects on

DO, indicating that both surfgrass and algae had similar effects on day/night dissolved

oxygen fluctuations, however algae on its own did not significantly alter diel fluctuations

in DO (Table 11; Figure 10).

Differences in pH (day − night)

a)

- control, ambient
- control, +CO2
- +sg, -algae, ambient
- +sg, -algae, +CO2
- -sg, +algae, ambient
- -sg, +algae, +CO2
- +sg, +algae, ambient
- +sg, +algae, +CO2

0   0.05   0.1   0.15   0.2
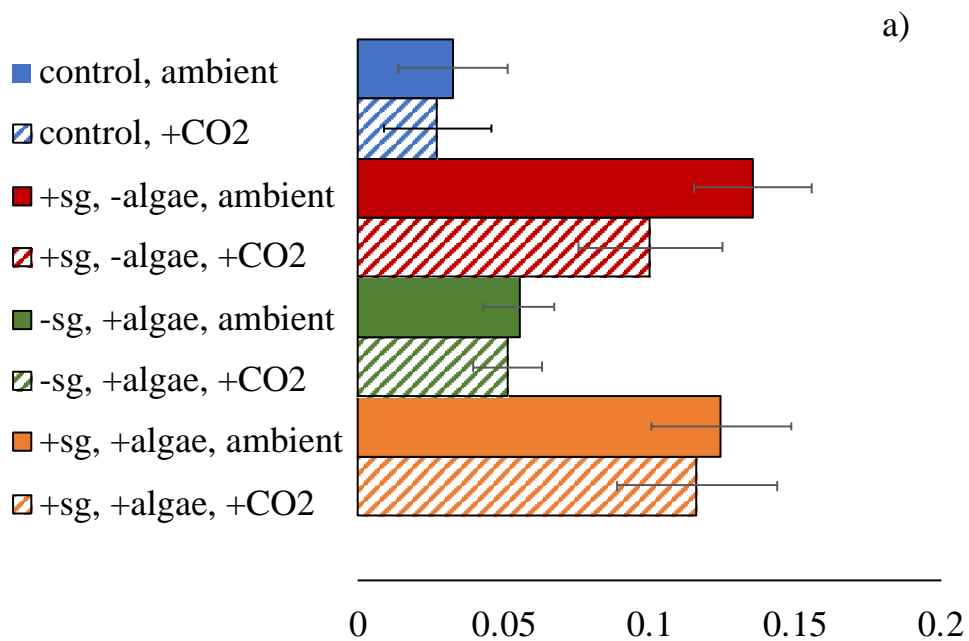
Differences in DO (day − night)

b)

- control, ambient
- control, +CO2
- +sg, -algae, ambient
- +sg, -algae, +CO2
- -sg, +algae, ambient
- -sg, +algae, +CO2
- +sg, +algae, ambient
- +sg, +algae, +CO2

0   0.5   1   1.5   2
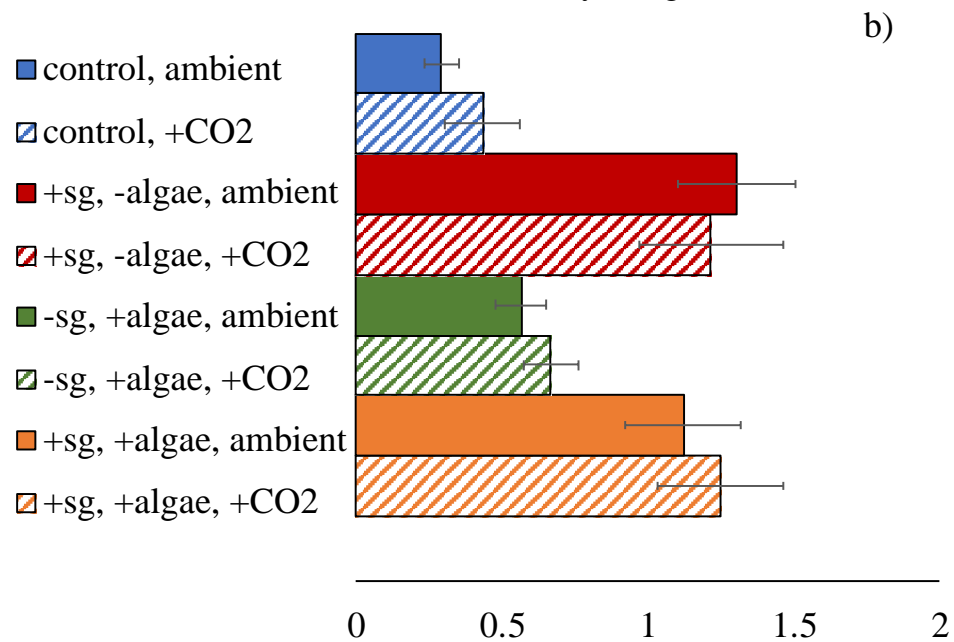
Figure 10. Day-night differences in (a) mean pH and (b) mean DO in the laboratory experiment. Error bars are ± 1 SE. Note that the scale is one order of magnitude larger for the DO plot. TREATMENT KEY: all hashed bars represent treatments with added CO_2, while solid bars represent the ambient treatments (no CO_2 added). +/- signs indicate when there was the presence or absence of 'sg' = surfgrass or red algae = *Neorhodomela larix* and articulated coralline algae. Controls did not include either groups of seaweeds.

Table 10. Model summary for the 3-way ANOVA analyzing the effects of surfgrass (SG – presence or absence), algae (presence or absence), time of day (day vs. night measurements), and CO_2 (presence or absence) effects on day-night pH differences in the laboratory experiment. Bolded *P*-values indicate statistical significance ($P < 0.05$).

Factor	df	*F*	*P*
pH	1	4.37	**0.04**
Surfgrass (SG)	1	78.23	**< 0.001**
Algae	1	1.12	0.29
pH*SG	1	0.73	0.39
pH*Algae	1	0.585	0.44
SG*Algae	1	1.89	0.17
pH*SG*Algae	1	0.088	0.77

Table 11. Model summary for the 3-way ANOVA analyzing the effects of surfgrass (SG – presence or absence), algae (presence or absence), time of day (day vs. night measurements), and CO_2 (presence or absence) effects on day-night DO differences in the laboratory experiment. Bolded P-values indicate statistical significance ($P < 0.05$).

Factor	df	*F*	*P*
pH	1	0.34	0.56
Surfgrass (SG)	1	89.68	**< 0.001**
Algae	1	0.91	0.34
pH*SG	1	0.34	0.56
pH*Algae	1	0.33	0.56
SG*Algae	1	4.02	**< 0.05**
pH*SG*Algae	1	0.27	0.6

Abiotic conditions of surveyed tidepools in the field

Average tidepool pH differed between daytime and nighttime sampling events (Welch's two sample T-test, $P < 0.05$; Figure 11). Tidepool pH showed a parabolic relationship with surfgrass cover. Tidepool pH was highest in daytime and lowest at nighttime in pools with between 40-50% surfgrass cover (daytime: 2nd order polynomial regression, $R^2 = 0.4$, $P = 0.009$; nighttime: 2nd order polynomial regression, $R^2 = 0.33$, $P = 0.05$]; Figure 12). Differences between tidepool pH and adjacent ocean pH also showed a parabolic relationship with surfgrass cover. Positive differences between tidepool pH and adjacent ocean pH (i.e., tidepools were less acidic than adjacent ocean waters) in

daytime were also greatest in tidepools with between 40-50% surfgrass cover (daytime:

2nd order polynomial regression, $R^2 = 0.38$, $P = 0.01$. Conversely, negative differences

between tidepool pH and adjacent ocean pH (i.e., tidepools were more acidic than

adjacent ocean waters) at nighttime were greatest in tidepools with between 40-50%

surfgrass cover. (nighttime: 2nd order polynomial regression, $R^2 = 0.31$, $P = 0.07$]; Figure

12).
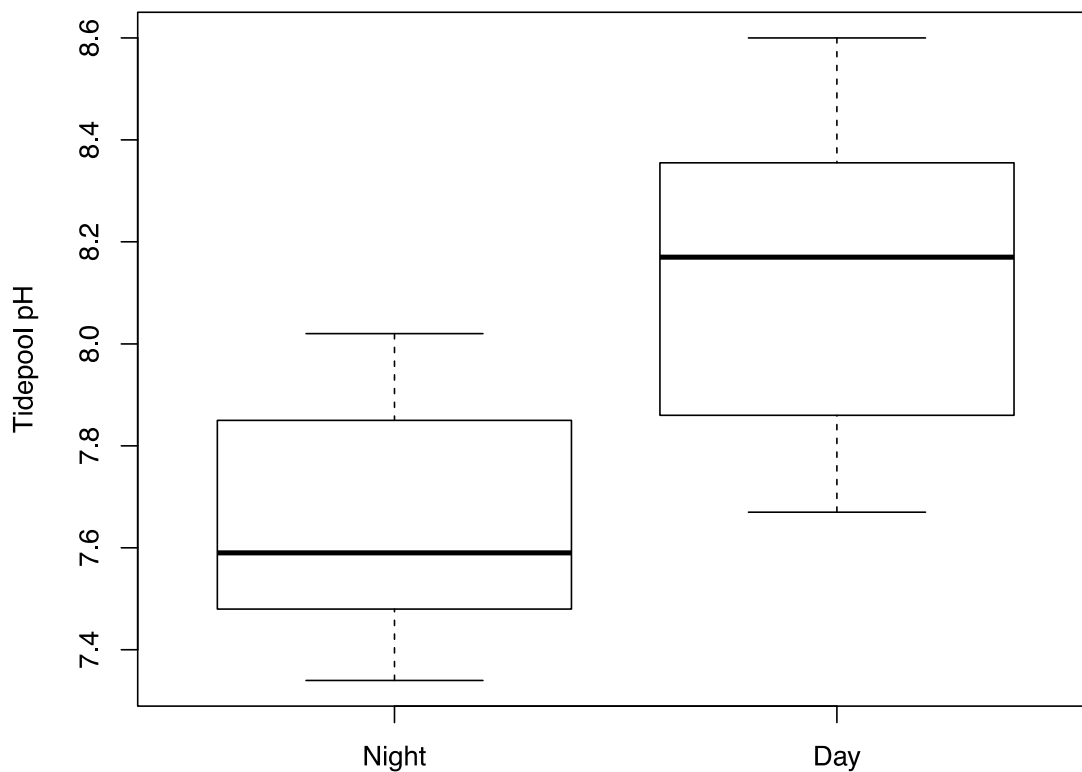
Figure 11. Differences between daytime and nighttime pH in natural tidepools between

my three sampling sites. Sampling event 1 occurred between July 8 and 13, 2017 and

sampling event 2 occurred between July 23-24, 2017. BB and LH were sampled in the same day for both sampling events, however, BB was sampled first for both events, and thus under 'nighttime' conditions, whereas LH was sampled during 'daytime' conditions. Tidepools at MR were sampled prior to sunrise, and after sunrise for both sampling events, however, the order in which tidepools were sampled was switched for the second sampling event. (Welch's t-test was used to test for differences in average tidepool pH between 'daytime' and 'nighttime' sampling events; $P < 0.001$).
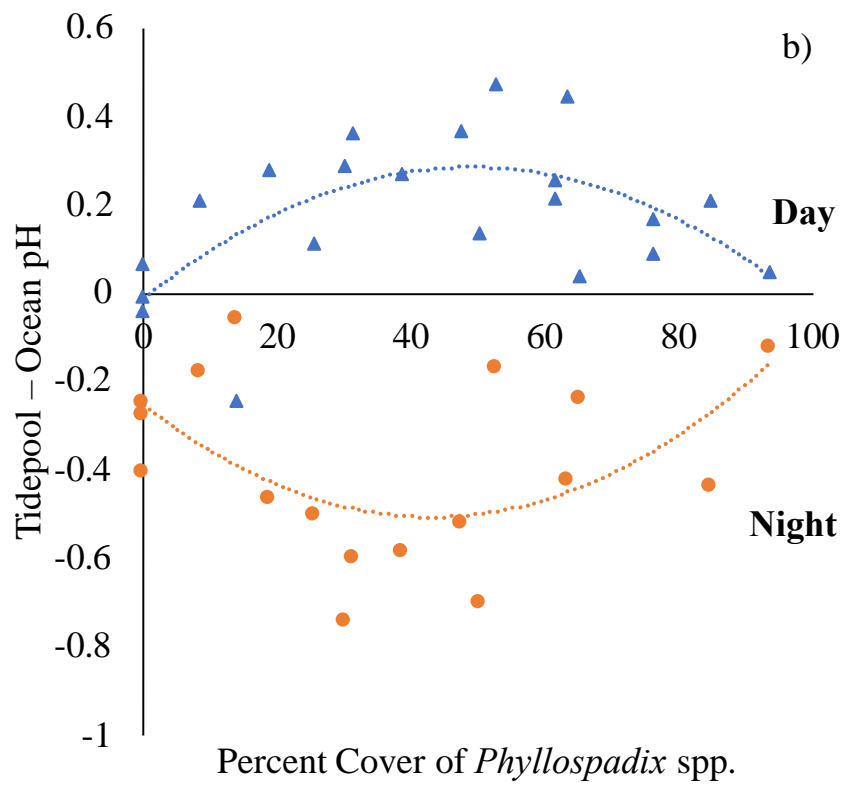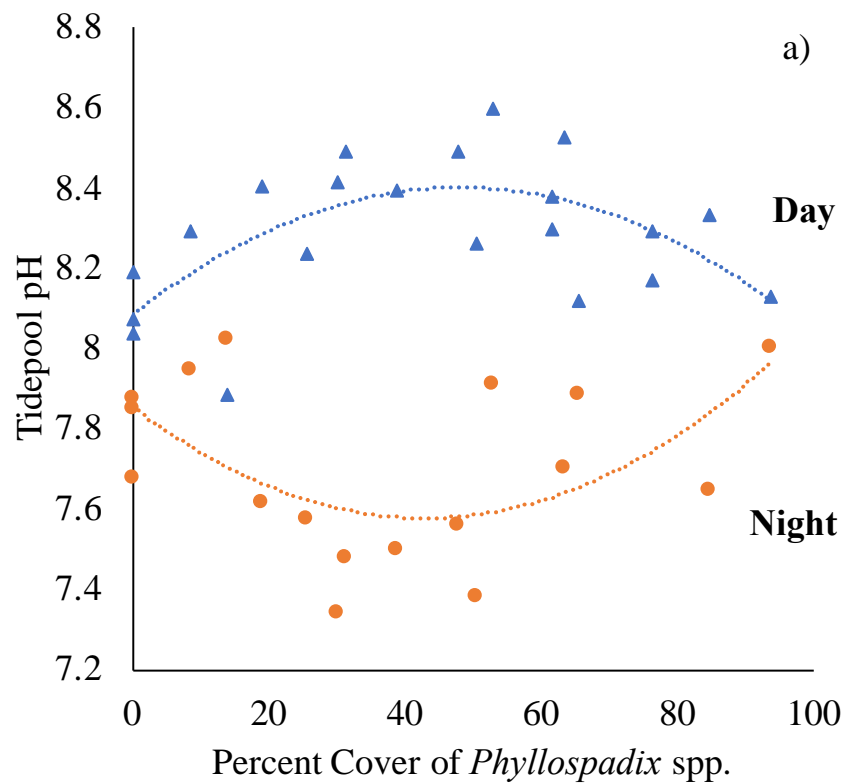
Figure 12. (a) Tidepool pH during the daytime ($R^2 = 0.4$, $P = 0.009$) and nighttime ($R^2 = 0.33$, $P = 0.05$) sampling events, and (b) differences between tidepool pH and ocean pH during the daytime ($R^2 = 0.38$, $P = 0.01$) and nighttime sampling events ($R^2 = 0.31$, $P = 0.07$) as a function of surfgrass abundance. Lines are best-fit 2nd order polynomial regressions.

Field manipulations

I found that time was a significant factor in both the nighttime and daytime one-way repeated-measures ANOVA (Figure 13; Table 12). Nighttime pH changed significantly over time in the experimental tidepool treatments (Figure 13). Daytime pH dropped over time in all pools, regardless of experimental treatment, which suggests that CO_2 additions did not affect the overall pH of manipulated pools, and that the ocean pH had a significant effect on the tidepool pH (Figure 13). In the one-way repeated-measures ANOVA for the nighttime sampling event, I also found that treatment was a significant factor in the model. Pairwise comparisons were used to test among the four treatments within the nighttime sampling event to determine which treatments differed significantly, in terms of pH over time, from one another. Since I had a limited number of replicate pools (due to site and volume constraints), I did not correct for the experiment-wide error rate as this would render these comparisons too conservative to detect potentially real biological differences between treatment pools. For each pairwise combination, treatments that differed significantly (using $P < 0.05$) from one another were: (1) the intact treatment (surfgrass left intact in experimental pools and CO_2 was added in) and

reference pools (which had varying amounts for surfgrass coverage but were not manipulated in any way; $P= 0.004$), (2) the intact and removal pools (pools that naturally had surfgrass present, but was removed; $P < 0.05$), and lastly, (3) the intact and control pools (which were naturally devoid of any surfgrass presence; $P = 0.05$). All other pairwise combinations showed no statistical significance.

Differences between the final and initial pH of all treatment pools for both sampling events shows that surfgrass removal pools had the highest differences (Figure 13; though not statistically significant; $P > 0.05$ for both daytime and nighttime sampling events).
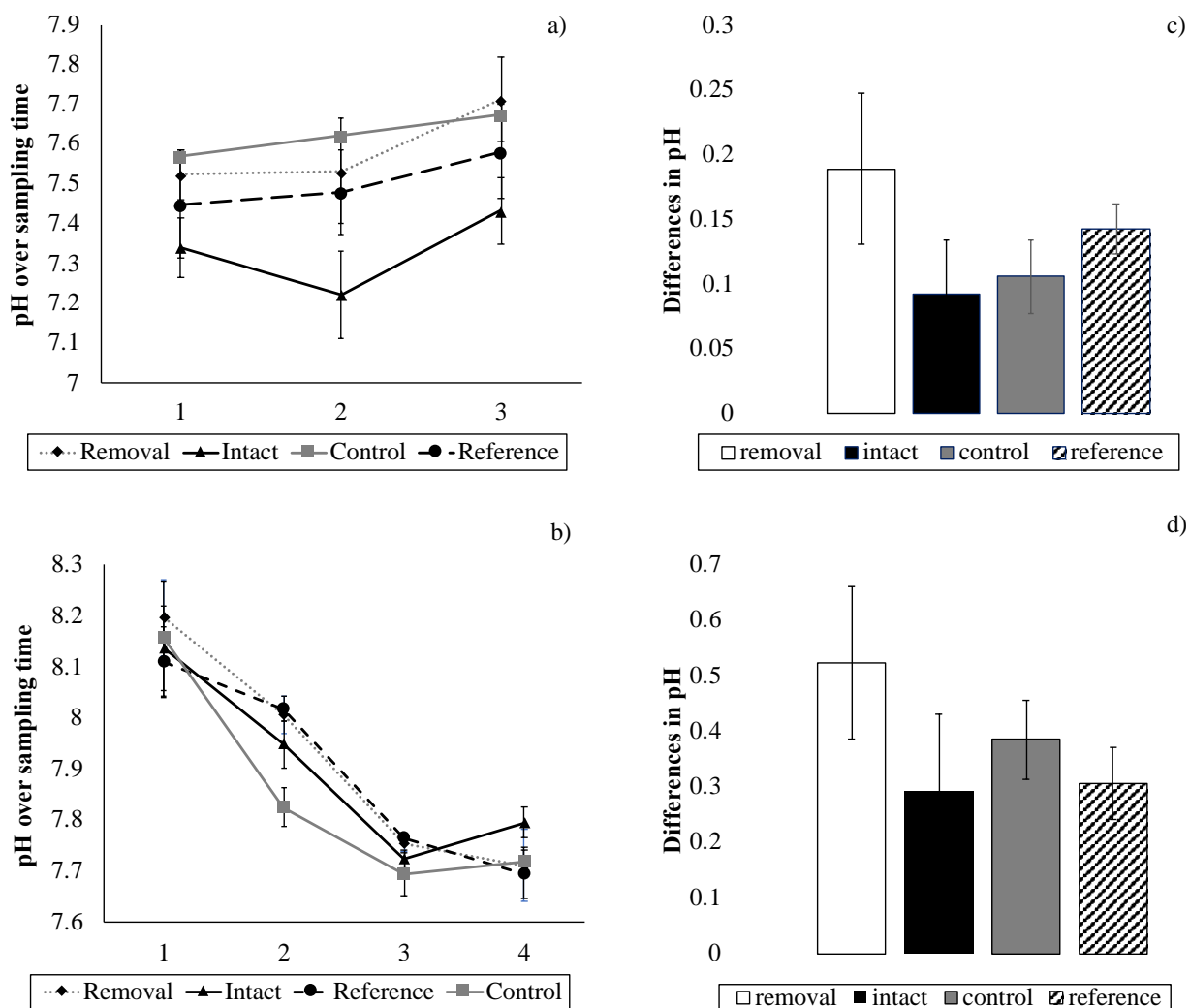
Figure 13. Nighttime (a) and Daytime (b) change in average pH over time and the differences in average pH between initial and final pH in the *in situ* manipulations (nighttime [c] and daytime [d]). TREATMENT KEY: Removal = surfgrass removal pools; Intact = pools in which surfgrass was left intact; Control = tidepools naturally without surfgrass that had CO_2 added; and Reference = surrounding pools were not manipulated in any way but were near manipulated pools.

Table 12. Model summary for the 1-way repeated-measures ANOVAs used to analyze variation in average pH over time in manipulated and un-manipulated pools in (a) nighttime and (b) daytime sampling in situ field manipulations. Bolded *P*-values indicate statistical significance ($P < 0.05$).

Factor	df	*F*	*P*
A) Nighttime Sampling Event	-	-	-
Treatment	3	16.18	**< 0.001**
Time	1	12.917	**< 0.001**
Site	2	28.902	**< 0.001**
Treatment*Time	3	0.418	0.74
B) Daytime Sampling Event	-	-	-
Treatment	3	1.07	0.37
Time	1	129.82	**< 0.001**
Site	2	2.59	0.083
Treatment*Time	3	1.1	0.36

Table 13. Model summary for the 1-way ANOVAs used to analyze variation in pH differences (final pH – initial pH) over time in manipulated and un-manipulated pools in (a) nighttime and (b) daytime sampling in situ field manipulations.

Factor	df	*F*	*P*
A) Nighttime Sampling Event	-	-	-
Treatment	3	1.041	0.403
B) Daytime Sampling Event			
Treatment	3	1.77	0.196

DISCUSSION

The outcome of my laboratory experiment indicated that surfgrass has the potential to strongly affect diurnal pH and DO conditions via photosynthesis and respiration. In my experimental mesocosms, treatments that contained surfgrass yielded the largest differences between day and night pH among all treatments. On the other hand, red algae alone (not in the presence of surfgrass) either had non-significant or much smaller effects on day/night fluctuations in pH and DO in mesocosms. These data suggest that surfgrass may be able to 'buffer' pH in natural tidepools, during the day when photosynthesis dominates over respiration. However, at nighttime, when respiration dominates, surfgrass actually produces much lower pH and DO values, which could deleteriously affect some calcifying tidepool organisms.

Although surfgrass had strong effects on day/night pH fluctuations in simulated tidepools, the effect brought about by the CO_2 additions were much stronger than the effect surfgrass could produce on the local pH conditions. This means that while surfgrass modulated pH within each CO_2 treatment, it did not increase the pH in mesocosms with experimentally manipulated CO_2 to ambient pH levels. While surfgrass is capable of modulating day/night pH fluctuations, it did not have the ability to bring CO_2-addition seawater up to the pH levels of ambient seawater in my experiment – suggesting that its ability to buffer natural tidepools from predicted future pH change could be limited.

My findings that tidepool pH was significantly different between daytime and nighttime sampling across sites was consistent with my laboratory results and with expectations based on previous work. It has been previously documented that photosynthesis by marine macrophytes dominates in tidepools during the day, when CO_2 is drawn out of the water column and replaced with oxygen (increasing pH and dissolved oxygen levels). Alternatively, at night respiration by the majority of organisms within them dominates tidepool metabolism, adding CO_2 back into the system at a time when oxygen is also being consumed, producing large drops in both pH and dissolved oxygen. Morris and Taylor (1983) observed large fluctuations in temperature, pH, DO, and salinity in tidepools during tidal cycles throughout the day along with fluctuations associated with seasonal changes. It has also been noted that seawater has much greater concentrations of inorganic carbon than freshwater and terrestrial systems (Burris, 1977), which may play crucial roles in determining the dynamics of photosynthesis/respiration in tidepool microhabitats. I observed that tidepool pH and oceanic pH are highly correlated though the fluctuations in both pH and DO were much more intense in all tidepools observed than the ocean readings taken during the same time.

The results of my field data showed that during the day, tidepools with intermediate abundance of surfgrass were least acidic, and conversely, at night those same tidepools were the most acidic. This outcome reveals that the largest oscillations in tidepool pH conditions were in pools with intermediate surfgrass abundance. This finding is counterintuitive to previous expectations that the largest pH differences within tidepools should occur in tidepools with the highest abundances of surfgrass, where we

would expect to see the highest rates of surfgrass photosynthesis and respiration occurring. Intraspecific competition, in the field, could be a potential explanation for this finding due to the fact that high quantities of surfgrass shoot density may create a limited light environment, and ultimately inhibit photosynthetic activity and the buffering effect of surfgrass on pH/DO conditions in tidepools. However, the parabolic relationship observed between surfgrass abundance and pH parameters in tidepools may in fact be due to the association between surfgrass and other photosynthetic macrophytes in tidepools. As conveyed in chapter one, I found that the highest levels of macrophyte diversity occurred at intermediate levels of surfgrass abundance. The combination of greater abundances of surfgrass and higher macrophyte diversity could lead to the largest photosynthetic output within tidepools and vice versa in terms of respiration with lower pH conditions.

The additions of CO_2 into tidepools for the field manipulation component of the overall project did not alter pH in my manipulated pools, which is evidenced by similar increases and decreases over time between CO_2 manipulated pools and unmanipulated reference pools for the two sampling events. The reason behind this failure could be that the pools were volumetrically too large to effectively change the tidepool pH using the CO_2 dosing apparatuses that I implemented. Another reason could be that the water temperature in the manipulated tidepools were cold enough to reduce the metabolic rate of the yeast and thus its ability to generate CO_2. For example, Gillis (2014) found that warmer temperatures yielded higher CO_2 output rates from similar apparatuses. Yet another reason could be that the dosing boxes were strapped to the substrate to prevent

movement during shifts in the tidal cycle, but the solution within the boxes bubbled at higher rates when shaken. Although these CO_2 apparatuses have been previously successful in manipulating tidepool pH (Gillis, 2014; Sorte and Bracken, 2015), those manipulations were done on tidepools a fraction of the size that I used for my study. It also appears that flooding by adjacent oceanic water had a much larger impact on the experimental pools and swamped out any effects that the CO_2-dosing apparatuses could have produced.

The nighttime sampling event in my field manipulation did produce a significant treatment effect, however; indicating significant differences in pH between the intact pools and reference pools, control pools and removal pools. Specifically, during the nighttime sampling events, tidepools that had intact surfgrass had significantly lower pH over time compared with the other three treatments. These findings are consistent with my laboratory experiment and field survey results, in that in the presence of surfgrass, diel fluctuations in pH in tidepools are more dramatic.

During the daytime sampling event, pH declined in all pools, concurrently with a drop in adjacent oceanic pH. However, reference tidepools and experimental tidepools with intact surfgrass showed significant trends toward smaller decreases in pH compared with surfgrass removal pools and pools naturally devoid of surfgrass. Similarly, during nighttime sampling, intact surfgrass pools showed a non-significant trend for a larger decrease in tidepool pH after CO_2-addition than surfgrass removal pools. This trend, if significant, would also be consistent with my laboratory experiment, in which simulated

tidepools containing surfgrass had lower nighttime pH than those simulated pools without surfgrass.

Taken together, my results from this chapter indicate that surfgrass provides a means for CO_2 sequestration in a time of rapidly changing oceanic conditions, however this can only be said during times of photosynthetic output. The amount of CO_2 that surfgrass puts back in the system during times of respiration may be even more consequential for other organisms. These findings follow in line with other studies regarding the effects of biological production and metabolism in the lens of extreme abiotic conditions observed during diurnal fluctuations. These trends may be more apparent in rocky intertidal systems due to the fact that the residence time of seawater in tidepools are more prolonged when compared to open oceans, or even seagrass meadows. I hypothesize that more diverse tidepools may actually be better suited for climate change events due to the less extreme abiotic conditions that they provide for surrounding communities.

FINAL CONCLUSIONS

To conclude, I want to revisit the two main questions that I addressed in my thesis. First, I wanted to investigate the relationship between surfgrass abundance and species diversity in rocky shore tidepool communities to understand the potential and foundational role that surfgrass plays in these systems. Rather than a consistently positive relationship between surfgrass abundance and species diversity, as might be predicted by the theory surrounding foundation species (Dayton, 1972), I found that very high surfgrass abundance actually decreases species richness and diversity in tidepools, and maximum tidepool diversity occurred at intermediate levels of surfgrass abundance (~40% cover). These results suggest that surfgrass may play an increasingly positive role from low to moderate abundances, but an increasingly negative role at higher abundances. Such variable effects of foundation species, namely ecosystem engineers, have been documented at different scales, or across environmental gradients, but not across foundation species abundances. Future work should focus on the mechanisms that drive the patterns of association between surfgrass and species diversity in tidepools. Two potential mechanisms driving the positive effects of surfgrass on tidepool diversity at intermediate levels may be: 1) surfgrass's ability to mediate fluctuations in pH and dissolved oxygen, thus buffering tidepool organisms from stress, and 2) the sweeping action of surfgrass blades, which, like in a lot of large kelps, may act as an agent of disturbance and suggest that interference competition could be a significant factor influencing community composition across tidepools in rocky shore systems.

The potential mechanisms by which surfgrass can influence tidepool community structure bring me to my second question: 'to what extent can surfgrass mediate pH and DO conditions in tidepools?'. My findings suggest that surfgrass presence leads to more extreme day/night fluctuations in both pH and DO conditions; buffering pH during the day but lowering pH at night. Interestingly, day/night pH fluctuations were most extreme at intermediate levels of surfgrass abundance, suggesting the intriguing possibility that surfgrass modulates tidepool pH both directly via metabolic activity but also indirectly by facilitating macrophyte diversity at intermediate abundances. Taken together, these results suggest that surfgrass may act as a foundational species in tidepools, in part by mediating tidepool pH, and influencing species diversity, which has important implications for the fate of these communities in the face of rapidly-changing global climates. Future work should focus on mechanistically linking the modulating effects of surfgrass on tidepool chemistry with patterns of association between surfgrass abundance and tidepool community diversity.

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APPENDICES

Appendix A. List of all algae found at the three study locations. Rare reds were species that occurred infrequently and were put into the same functional group.

Baker's Beach (BB)	Luffenholtz (LH)	Mussel Rock (MR)
Corallina spp.	*Corallina* spp.	*Corallina* spp.
Bosiella spp.	*Bosiella* spp.	*Bosiella* spp.
Calliarthron spp.	*Calliarthron* spp.	*Calliarthron* spp.
Dilsea carnosa	*Constantinia* spp.	*Ceramium* spp.
Egregia menziesii	*Cryptopleura* spp.	*Chondracanthus* spp.
Crustose coralline	*Egregia menziesii*	*Cryptopleura* spp.
Encrusting red algae	Crustose coralline	Crustose coralline
Gellidium spp.	Encrusting red algae	Encrusting red algae
Juvenile reds	Filamentous red algae	Filamentous red algae
Mastocarpus spp.	Juvenile Mazzaella spp.	*Gellidium* spp.
Mastocarpus gametophyte	Juvenile red algae	Juvenile *Mastocarpus* spp.
Mazzaella spp.	Juvenile Ulva spp.	Juvenile *Mazzaella* spp.
Neorhodomela larix	*Laminaria* spp.	Juvenile *Prionitis* spp.
Odanthalia floccosa	*Mastocarpus* gametophyte	Juvenile red algae
Phyllospadix scouleri	*Mazzaella* spp.	*Mastocarpus* spp.
Phyllospadix torreyi	*Neorhodomela larix*	*Mastocarpus* gametophyte
Prionitis spp.	*Plocamium pacificum*	*Mazzaella* spp.
Rare reds	*Phyllospadix scouleri*	*Neorhodomela larix*

Baker's Beach (BB)	Luffenholtz (LH)	Mussel Rock (MR)
Turfy red algae	*Phyllospadix torreyi*	*Neogastroclonium subarticulatum*
Smithora naiadum	*Ulva* spp.	*Odanthalia floccosa*
		Osmundea spp.
		Phyllospadix scouleri
		Phyllospadix torreyi
		Prionitis spp.
		Rare reds
		Smithora naiadum
		Soranthera ulvoidea

Appendix B. List of all invertebrates, and sculpins that were found amongst tidepools at each of the three sites. Species were

classified to lowest taxonomic information as possible.

Baker's Beach (BB)	Luffenholtz (LH)	Mussel Rock (MR)
Anthopleura elegantissima	*Anthopleura xanthogrammica*	*Anthopleura elegantissima*
Biofilm/Diatom crust	*Leptasterias hexactis*	*Anthopleura xanthogrammica*
Bryozoans	*Pugettia producta*	Biofilm/Diatom crust
Cryptochiton stelleri	*Semibalanus balanoides*	Limpets
Dermasterias imbricata		*Littorina littorea*
Idotea spp.		*Mopalia spp.*
Limpets		*Nucella lamellosa*
Littorina littorea		*Oligocottus maculosus*
Margharites spp.		*Pachygrapsus* spp.
Oligocottus maculosus		*Pagurus samuelis*

Baker's Beach (BB)	Luffenholtz (LH)	Mussel Rock (MR)
Pachygrapsus spp.		*Pugettia producta*
Pagurus samuelis		*Semibalanus balanoides*
Semibalanus balanoides		*Stronglyocentrotus purpuratus*
Serpulid polychaete		*Tegula funnebralis*
Spirorbid polychaete		
Sponges		
Tegula funnebralis		

Appendix C. Table of abiotic measurements taken per pool during sampling event 1 of the field manipulations. 'AM'
indicates that these measurements were taken during the early morning low tides under 'dark' conditions and are referred to as
the nighttime samples.

SITE/ POOL#	pH1 am	pH2 am	pH3 am	DO1 am	DO2 am	DO3 am	temp1 am	temp2 am	temp3 am	Ocean pH	Ocean DO	Ocean temp
BB1	7.33	7.43	7.54	1.14	1.06	2.46	12.10	13.80	13.10	7.84	6.72	11.90
BB2	7.32	7.45	7.51	1.02	1.08	2.13	12.22	13.77	13.16	7.84	6.72	11.90
BB3	7.38	7.43	7.55	1.25	1.23	2.79	12.30	13.72	13.10	7.84	6.72	11.90
BB4	7.42	7.39	7.42	1.06	0.73	1.18	12.26	13.77	13.21	7.84	6.72	11.90
BB5	7.42	7.47	7.51	2.49	2.09	2.82	11.98	14.00	13.28	7.84	6.72	11.90
LH1	7.49	7.37	7.52	4.42	5.71	8.48	11.65	10.95	11.40	7.80	9.75	11.04
LH2	7.50	7.35	7.49	4.57	4.02	8.38	12.30	11.48	11.83	7.80	9.75	11.04
LH3	7.21	7.11	7.30	2.50	2.17	5.59	12.10	11.46	11.78	7.80	9.75	11.04
LH4	7.34	7.16	7.48	1.63	1.57	6.67	11.80	11.11	11.59	7.80	9.75	11.04
LH5	7.29	7.10	7.37	2.03	1.50	5.04	12.00	11.19	11.63	7.80	9.75	11.04
MR1	7.83	7.92	NA	6.16	7.09	NA	13.24	13.40	NA	8.12	8.57	11.96
MR2	7.80	7.94	NA	5.60	6.36	NA	13.31	13.56	NA	8.12	8.57	11.96

SITE/ POOL#	pH1 am	pH2 am	pH3 am	DO1 am	DO2 am	DO3 am	temp1 am	temp2 am	temp3 am	Ocean pH	Ocean DO	Ocean temp
MR3	7.70	NA	7.79	4.57	NA	8.51	12.20	NA	13.35	8.12	8.57	11.96
MR4	7.44	7.29	7.65	5.13	2.47	6.36	12.73	13.08	12.90	8.12	8.57	11.96
MR5	7.69	7.72	7.82	4.52	4.76	7.53	12.52	12.43	13.16	8.12	8.57	11.96
MR6	7.60	7.68	7.70	5.22	5.61	5.76	12.84	12.63	12.48	8.12	8.57	11.96
MR7	7.58	NA	7.69	3.81	NA	5.27	11.78	NA	12.34	8.12	8.57	11.96
MR8	7.54	NA	7.58	3.69	NA	4.03	11.54	NA	12.46	8.12	8.57	11.96
MR9	7.65	7.81	7.98	4.72	6.49	9.76	11.89	12.69	13.48	8.12	8.57	11.96
MR10	7.41	7.52	NA	2.39	5.01	NA	11.93	13.03	NA	8.12	8.57	11.96
MR11	7.45	7.52	7.67	3.08	3.89	6.28	12.47	13.50	14.10	8.12	8.57	11.96
MR12	7.57	NA	7.71	3.64	NA	5.46	11.74	NA	12.36	8.12	8.57	11.96
MR13	7.59	7.82	NA	3.29	5.89	NA	11.66	12.36	NA	8.12	8.57	11.96
MR14	7.67	7.84	7.94	4.32	6.20	9.03	11.94	12.90	13.15	8.12	8.57	11.96
MR15	7.52	7.67	NA	1.99	4.32	NA	12.84	12.78	NA	8.12	8.57	11.96
MR16	7.48	7.70	NA	1.47	4.42	NA	12.60	12.97	NA	8.12	8.57	11.96
MR17	7.46	7.70	NA	1.82	5.58	NA	12.53	12.77	NA	8.12	8.57	11.96

SITE/ POOL#	pH1 am	pH2 am	pH3 am	DO1 am	DO2 am	DO3 am	temp1 am	temp2 am	temp3 am	Ocean pH	Ocean DO	Ocean temp
MR18	7.46	7.60	NA	1.87	3.53	NA	12.80	13.16	NA	8.12	8.57	11.96
MR19	7.46	7.56	NA	1.50	3.03	NA	13.17	13.51	NA	8.12	8.57	11.96

Appendix D. Table of abiotic measurements taken per pool during sampling event 2 of the field manipulations. 'PM' indicates that these measurements were taken during the afternoon/evening low tides under 'daytime' conditions and are referred to as the daytime samples.

SITE/ POOL#	pH1 pm	pH2 pm	pH3 pm	pH4 pm	DO1 pm	DO2 pm	DO3 pm	DO4 pm	temp1 pm	temp2 pm	temp3 pm	temp4 pm	Ocean pH	Ocean DO	Ocean temp
BB1	8.01	8.03	7.78	7.62	9.55	8.31	4.74	4.67	10.84	10.89	11.48	13.82	8.10	8.92	11.65
BB2	7.93	8.13	7.76	7.68	8.42	7.64	4.00	4.26	11.36	11.36	11.60	13.67	8.10	8.92	11.65
BB3	7.85	8.02	7.80	7.75	8.48	7.54	5.30	5.37	11.62	11.65	11.76	13.85	8.10	8.92	11.65
BB4	7.79	7.98	7.77	7.85	4.94	4.71	3.45	3.60	10.96	10.95	11.65	13.65	8.10	8.92	11.65
BB5	7.92	8.09	7.81	7.63	8.82	8.41	6.04	4.27	10.56	10.82	11.54	13.82	8.10	8.92	11.65
LH1	8.26	8.06	7.77	7.88	10.83	13.11	9.27	7.55	11.44	11.00	11.12	10.67	8.16	8.97	11.20
LH2	8.25	8.11	7.74	7.89	13.08	16.12	8.34	6.80	11.70	10.88	11.07	10.17	8.16	8.97	11.20
LH3	8.19	8.08	7.73	7.84	8.76	9.31	5.39	6.23	11.75	10.61	11.06	10.26	8.16	8.97	11.20
LH4	8.14	8.04	7.70	7.87	8.53	8.97	5.89	6.10	11.50	10.60	11.08	10.23	8.16	8.97	11.20
LH5	8.15	8.06	7.70	7.88	9.03	9.79	6.46	6.60	8.10	10.74	11.03	10.37	8.16	8.97	11.20
MR4	8.33	7.96	7.78	7.80	12.23	8.84	7.54	7.07	12.43	11.57	10.45	10.94	NA	NA	NA
MR5	8.37	7.92	7.76	7.76	10.70	8.04	6.98	6.11	12.38	11.48	10.32	10.90	NA	NA	NA

SITE/ POOL#	pH1 pm	pH2 pm	pH3 pm	pH4 pm	DO1 pm	DO2 pm	DO3 pm	DO4 pm	temp1 pm	temp2 pm	temp3 pm	temp4 pm	Ocean pH	Ocean DO	Ocean temp
MR6	8.27	7.86	7.74	7.75	11.74	8.36	7.21	7.30	12.40	11.40	10.45	10.80	NA	NA	NA
MR7	8.04	7.79	7.65	7.69	9.61	8.20	7.83	7.53	11.88	11.49	10.60	10.90	NA	NA	NA
MR9	8.34	7.90	7.76	7.49	10.95	7.61	6.77	5.84	11.79	11.30	10.11	10.52	NA	NA	NA
MR10	8.24	7.99	7.72	7.53	6.93	6.63	4.75	2.48	11.86	11.15	9.97	10.47	NA	NA	NA
MR11	8.42	7.97	7.75	7.56	10.06	7.90	6.33	4.10	12.19	11.38	10.12	10.50	NA	NA	NA
MR14	8.10	7.92	7.77	7.82	9.06	7.31	6.89	6.99	11.71	11.15	10.18	10.84	NA	NA	NA
MR15	8.15	7.94	7.74	7.60	8.16	7.20	6.89	4.30	12.30	11.57	10.42	10.82	NA	NA	NA