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FACTORS DRIVING DEMOGRAPHY AND TEMPORAL VARIABILITY IN PH OF THE
ACID WEED, *DESMARESTIA HERBACEA*

A Thesis
Presented to the
Faculty of
Moss Landing Marine Laboratories
California State University Monterey Bay

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
in
Marine Science

by
Angela T. Zepp
Fall 2017

CALIFORNIA STATE UNIVERSITY MONTEREY BAY

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FACTORS DRIVING DEMOGRAPHY AND TEMPORAL VARIABILITY IN PH OF THE
ACID WEED, *DESMARESTIA HERBACEA*

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ABSTRACT

Factors Driving Demography and Temporal Variability in pH
of the Acid Weed, *Desmarestia herbacea*

By

Angela T. Zepp

Masters of Science in Marine Science

California State University Monterey Bay, 2017

Demographic studies allow for a better understanding of how populations change over time and establish a baseline to examine how biotic and abiotic factors influence populations. The annual alga *Desmarestia herbacea*, or the acid weed, accumulates sulfuric acid within cell vacuoles, likely as a chemical defense mechanism. Whether intracellular pH varies among different life history stages is poorly understood. A *D. herbacea* population in the Stillwater Cove, California kelp bed was assessed for two years in order to measure how internal pH varies relative to demographics, season, grazing pressure and oceanography. The timing of both spring recruitment and fall senescence varied interannually. Sporophyte recruitment occurred during the upwelling season in mid-March, two weeks earlier than previously reported, and thalli reached maximum length during the Oceanic season, then senesced during the Davidson Current season. Maximum mean thallus length varied inversely as a function of density, with smaller plants present in 2015 when densities were higher. In contrast in 2016, individuals were significantly larger, their densities were lower, and the population senescence period extended much longer into January 2017. The ontogenetic shift in intracellular pH of *D. herbacea* varied with life history stage and was strongly seasonal in both years and may be driven by ocean temperature. Specifically, in 2016, the pH was highest during the recruitment season (1.38 ± 0.14), followed by a decline in pH during the growth period (0.60 ± 0.01), followed by an elevation during the senescence period (0.65 ± 0.02). Benthic invertebrate grazers had a strong, significant and negative effect on the early recruitment of *D. herbacea* both in permanent plots and an herbivore exclusion experiment. Plots with higher herbivore grazing pressure had significantly lower recruitment. Higher densities, and smaller individuals in 2015 may have been correlated with higher temperatures in 2015 associated with ENSO events. These findings suggest that despite inter-annual variability in demographic patterns, strong, seasonal shifts in intracellular pH may reflect ontogenetic shifts in chemical defense to protect vulnerable growth phases of life history.

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INTRODUCTION

Herbivory negatively impacts plant populations in both temperate and tropical ecosystems (Lubchenco & Gaines 1981, Watanabe & Harrold 1991, Haavisto 2016). Plants adapt to grazing pressure in a variety of ways in time and space (Lubchenco & Gaines 1981) by avoiding, tolerating or deterring herbivores (Hay & Fenical 1988). Direct defenses are supported by plant characteristics that deter herbivores such as mechanical protection on the surface of the plants (e.g., hairs, trichomes, thorns, spines, and thicker leaves) or production of toxic chemicals such as terpenoids, alkaloids, anthocyanins, phenols, sulfuric acid and quinones) that either deter, kill, or retard the development of the herbivores (Hanley et al. 2007). The evolution of these defenses has enabled plants to defend themselves against the loss of resources and energy, allowing for greater investment in reproduction and survival.

Defense strategies exist in marine algae and the most common defenses found in marine macroalgae are chemical (Duffy & Hay 1994, Agrawal 1998). Over 3,000 natural metabolites (e.g., terpenes, polyketides, nonribosomal peptides, alkaloids, shikimates) have been described in macroalgae (Haavisto 2016). Some of these metabolites act as constitutive defense mechanisms against consumers while others are induced in response to grazing (Pavia & Toth 2000, Amsler & Fairhead 2005, Baker et al. 2008, Amsler et al. 2014). A strong chemical found in select algal species is sulfuric acid. Species containing sulfuric acid exhibit a remarkably low intracellular pH (A. Zepp, unpublished data) which may act as an herbivore deterrent.

Defense mechanisms can be crucial in the survivorship of certain species. Demographic studies involving recruitment and growth are essential first steps to linking

population and community patterns with effectiveness of survivorship techniques. Demographic analyses have been performed on numerous kelp forest algal species including those on perennial kelps including *Laminaria* spp. (Chapman 1984, Kain & Jones 2009), *Macrocystis pyrifera* (Wheeler & North 1980, Graham 1997,) and *Pterygophora californica* (de Wreede 1986); fewer demographic studies exist for annual macroalgal species. Population dynamics have been studied for some annual kelps including *Alaria marginata* (McConnico & Foster 2005), *Pelagophycus porra* (Coyer & Zaugg-Haglund 1982), *Nereocystis luetkeana*, *Costaria costata* (Maxell & Miller 1996) and *Alaria nana* (Pfister 1992). These studies establish demographic patterns, valuable to examining inter and intraspecific interactions, however fewer are combined with studies on life history strategies related chemical defense, specifically.

Most species of the brown algal genus *Desmarestia* are annual and commonly found in temperate and polar waters (Moe & Silva 1981, Fig 1). *Desmarestia* spp. have microscopic zoospores, gametophyte and embryonic sporophyte stages, and a macroscopic sporophyte stage. Fertilization and growth of the microscopic stages occur in spring when recruit into juvenile sporophytes often in high densities (multiple sporophyte blade layers producing >100% cover 10-20 cm above the bottom in late spring). They often blanket the sea floor, particularly in areas where dominant canopy forming kelps had been removed by winter storms (Reed & Foster 1984, Dayton et al. 1992, Edwards 1998). Sporophytes rapidly grow during summer, reproduce in early-to mid-fall and then senesce and the fragile thalli entirely degrade and disappear with the onset of winter storms. (Edwards 1996). The disappearance of the macroscopic stage is attributed to a process called overwintering where microscopic gametophytes settle,

blanket the benthos and lie dormant during the winter months (Edwards 2000). This overwintering process allows them to persist during less optimal environmental conditions.

All species within the genus *Desmarestia* produce and store sulfuric acid in intracellular vacuoles (Eppley & Bovell 1958, McClintock et al. 1982, Sasaki et al. 1999) making them highly acidic (pH ranges from 1-6) relative to most other species of macroalgae (Fig 2). It was first hypothesized that low pH would make *Desmarestia* spp. less palatable to grazing herbivores (Eppley & Bovell 1958). If *Desmarestia* thalli are exposed to air, high water temperatures or physical damage, the vacuoles release the sulfuric acid, destroying the surrounding tissues (Pelletreau & Muller-Parker 2002). However, the role of sulfuric acid in the life history and ecology of *Desmarestia* is poorly understood. While the genus is found worldwide, there is limited information on how pH varies relative to life history stages and how intracellular pH fluctuates over time (Gagnon et al. 2013). One study described the temporal variation of intracellular pH of three species of *Desmarestia* (*D. herbacea*, *D. aculeata* and *D. viridis*) throughout their macroscopic life cycle and reported that the average internal pH of in the San Juan Archipelago, Washington, was 0.74, 6.32, 0.89 respectively (McClintock et al. 1982). These pH values remained constant throughout the growing season despite changes in thallus size. However, the sampling frequency was low and the study did not provide enough methodological detail to replicate. There is speculation regarding whether species of the genus *Desmarestia* have consistent pH through various life history stages or if it varies temporally.

The payoff for the energetic investment of concentrating sulfuric acid in *Desmarestia* is suggested to include an anti-herbivory defense, anti-fouling and allelochemical functions (Pelletreau & Muller-Parker 2002). The Optimal Defense Theory (ODT) states that an organism will allocate resources to defend itself in a manner that maximizes fitness (Cronin & Hay 1996). Valuable tissues and structures or those more vulnerable to attack, may be allocated greater levels of resources so the organisms benefit outweighs the cost of manufacturing and maintaining the defensive strategy. Such a shift in resource allocation was suggested by Gagnon et al. (2013) for a temperate Atlantic species of *Desmarestia*. They found that the annual temperature-mediated die-off of *D. viridis* sporophytes coincided with the synchronous release of zoospores and that growth *D. viridis* growth ceased approximately one week after the onset of acid release. That period was followed by a few days in which tissues underwent no growth, just discoloration prior to tissue sloughing. It was speculated that this senescence coincided with zoospores release (Gagnon et al 2013). However, the pH levels associated with this change were not measured. Both whether an ontogenetic shift in pH levels exists and the exact timing and mechanism of zoospore release relative to pH levels for *Desmarestia* species in general and *D. herbacea* specifically is still poorly understood.

The effectiveness of *Desmarestia*'s chemical defense against herbivores is under speculation. *D. viridis* is a species with a wide distributional range from Antarctica to the Arctic. Multiple studies have examined the ecological importance of extreme acid levels of *D. viridis* in the northern Atlantic Gulf of St. Lawrence (Gagnon et al. 2006), the Aleutians (Konar 2014), and the Arctic Ocean (Molis et al. 2009) and found that *D. viridis* is capable of limiting sea urchin distribution, movement, and grazing through its

high intracellular pH. Additionally, extensive work has been done on various Antarctic species (Moe & Silva 1977, 1989, Duffy & Hay 1994, Ankisetty et al. 2004, Fairhead et al. 2005, Gagnon et al. 2006, Amsler et al. 2014). All of these studies reported that *D. viridis* exhibits a chemical defense that deters grazing. The eastern Pacific species, *D. herbacea* is found from Alaska to South America (McClintock et al. 1982) yet no studies have examined pH variation relative to life history stages or if it chemically defends herbivorous grazing. However, field observations suggest that mature macroscopic stages of *D. herbacea* are susceptible to grazing at various life history stages (M. Edwards & personal observations). It is unclear if *D. herbacea* uses a chemical defense at different life history stages to ward off or reduce grazing pressure.

This study focuses on *Desmarestia herbacea* (Stackhous) J.V. Lamouroux, which occurs from the low intertidal to subtidal depths of 15 m and is widely distributed in the eastern Pacific (Abbott & Hollenberg 1976, Foster & Schiel 1985). *D. herbacea* is a seasonally abundant understory annual species in kelp forests, and becomes reproductive at the end of its life cycle (Edwards 2000). In central California kelp beds, some of the most conspicuous benthic grazers in central California kelp forests could directly influence *D. herbacea* survivorship. These include the common bat star (*Patiria miniata*), the turban snail (*Chlorostoma brunnea*), and red and purple urchins (*Mesocentrotus franciscanus* and *Strongylocentrotus purpuratus*). *P. miniata* are known generalists with a diet that commonly consists of drift algae, carrion, sponges, bryozoans and tunicates (Harrold & Pearse 1987). The omnivorous sea star grazes on the benthos including on gametophytes of the giant kelp, *M. pyrifera*, and the grazing can impact kelp survivorship into mature sporophytes (Leonard 1994). *C. brunnea* are abundant gastropod herbivores

in kelp forests (Watanabe 1984). M. Edwards (pers. comm.) noted from 5+ years of observation in central California kelp beds that *C. brunnea* were the most conspicuous grazer of *D. herbacea* sporophytes in fall. Sea urchins are another known grazer of macroscopic stages of *Desmarestia* sp. (Gagnon et al. 2006), despite the harmful effect of the acid inflicted on their Aristotle's lantern (Pelletreau & Muller-Parker 2002). It is unclear how herbivore grazing on different *D. herbacea* life history stages impact population dynamics and why *D. herbacea* sporophytes become more susceptible to grazing in the fall. Examining the relationship between pH and grazing pressure may help determine if *D. herbacea* recruitment is susceptible to the presence of herbivores.

The relationship between environmental factors and the recruitment, growth and mortality of marine macroalgae has been a focus of recent field studies in algal demography (Foster 1982; Mathieson 1982; Dayton 1985; Reed & Foster 1984; Schiel 1985; Deysher & Dean 1986; Santilices 1990; Underwood & Kennelly 1990). Biological factors such as grazing are less predictable (Dean et al. 1988; Leonard, 1994; Watanabe & Harrold, 1991) than environmental factors such as swell exposure (McLean 1962, Tegner & Dayton 1985, Harrold et al. 1988), temperature and nutrients (Jackson 1977; Zimmerman & Kremer 1984, Blain & Gagnon 2013), which vary seasonally. This current study sought to examine the relationship between environmental factors and to pH, demography and grazing pressure in the acid weed.

Knowledge on the status of *D. herbacea* population dynamics or intracellular pH variability in California is minimal. This current study aims to advance knowledge on the population dynamics, chemical defense, ontogenesis, and demographic distribution of *D. herbacea* in central California. The objectives of this study are to: (1) Establish the

demographic patterns (density, size, reproduction and time of first recruitment) of a *D. herbacea* population, (2) Determine if the internal pH of *D. herbacea* varies temporally and as a function of life history stage, (3) Determine if invertebrate grazing affects *D. herbacea* sporophyte recruitment and (4) Examine if *D. herbacea* pH changes as a function of abiotic factors (wave height, temperature).

METHODOLOGY

Study Site

All field research for this study occurred in a kelp forest in Stillwater Cove, Carmel Bay, California (36°34'N, 121°56'W) (Fig. 3). This cove is southwesterly exposed and is relatively protected from predominant northwest winter swells. The benthos is characterized by moderate-relief granite, sandstone and conglomerate terraces separated by cobble and sand channels at depths of 10–14 m. A persistent *Macrocystis pyrifera* surface canopy and mixed algal species understory is present throughout the year and represents a typical central California giant kelp forest (Foster & Schiel 1985). *Desmarestia herbacea* is a seasonally abundant understory species and the sporophyte recruitment is patchy, occurring in areas with reduced surface and understory canopy coverage (Edwards 1998). The thalli are generally distributed on bedrock and large boulders beginning in late March until around November when they senesce (Edwards 2000, personal observations).

Demography

To characterize *D. herbacea* demographics over time and relative to pH and grazing pressure, density and lengths of *D. herbacea* were measured in permanent plots on a raised terrace within Stillwater Cove at GPS location N 36° 33.636, W 121° 56.781 at a

depth of 13-14m. The terrace location was selected in June 2015 and had moderate *M. pyrifera* density (0.2 plants /m²) and recruitment of visible new *D. herbacea* sporophytes (occurring in early spring). Sixteen circular permanent plots denoted by a stainless-steel bolt in the center with a radius of 50cm (area = 0.785 m²) were haphazardly installed along a 30m line using the initial criteria that they contained at least 1 new recruit. Plots were monitored weekly from June 18th, 2015 until January 16th, 2017 for *D. herbacea* density and maximum thallus length. Common mobile grazers (sea star *P. miniata*, gastropod *C. brunnea*, and echinoids *Strongylocentrotus purpuratus* and *Mesocentrotus franciscanus*) within each plot were also counted. Randomly selected plants within the permanent plots were sampled to measure weekly internal pH (see pH section). *Timing of Recruitment*

M. Edwards (1998) reported that over five years the date of first recruitment for *D. herbacea* at this Stillwater Cove site occurred between April 4th-April 17th and hypothesized that the date for this recruitment window was annually consistent. To determine if this hypothesis held true, date of first visible recruitment at the permanent site was measured weekly throughout spring of 2016 at close intervals before, during and after this time window.

Specific Growth Rate

To determine if weekly tissue removal for the pH measurement influenced growth, specific growth rates were measured for a group of both trimmed and untrimmed plants for comparison. For both groups, thallus length was measured for eight individually marked plants around the permanent plot vicinity. Thalli were denoted by hammering a concrete nail next to the individual holdfasts. The maximum length (cm) of

each individual was measured weekly. A tissue sample (3-5 cm) was removed from these thalli for pH determination. The plants were followed from September 2016 to January 2017. The data for trimmed plants came from 8 individuals just outside of the permanent plots marked in the exact same way as described above, however no tissue was removed from these thalli for pH sampling thereby leaving them unmanipulated and referred to as untrimmed. Individuals were tracked from May 2016 to January 2017 to follow them through the senescent phase.

Specific growth rate, SGR, was calculated from the length data for each sporophyte during each interval separating two consecutive measurements with the equation: $((L_f - L_o)/L_o)/t$, where L_o and L_f are the initial and final average lengths of the frond (from the holdfast to the distal end of the frond), respectively, and t is the number of days between the two length measurements. Accordingly, SGR is expressed as a percentage of frond length per day ($\% \text{ day}^{-1}$) and reflects the change in length over a specific period, relative to the previous time period. A one sample t-test was used to examine if there was a significant effect of manipulative sampling on the individual's growth and natural senescence between manipulated and unmanipulated plants.

Fecundity

To determine when *D. herbacea* became reproductive in the fall, reproductive status was assessed from five individual thalli collected weekly from Stillwater Cove. Individuals 10-20 cm in length in 2015 were collected by hand and haphazardly chosen starting in both September 2015 and 2016 until plants disappeared for that year. Entire thalli were carefully collected in the site from an area not used for permanent plot surveys. The thalli were placed in individual Ziploc bags in seawater and transported in

the dark to Moss Landing Marine Laboratories. Each individual was rinsed for 60 seconds in sterile seawater, blotted dry gently and the wet weight and maximum length was recorded. All individuals were layered in glass baking pans with alternating layers of thallus and freshwater dampened paper towels. Once all collected samples are cleaned and layered, the pan was placed in the dark for a minimum of 3 hours at 10°C to induce spore release (Reed et al. 1991). This method is used for kelps and has been known to work for *Desmarestia* as well (M. Edwards, pers. comm.).

After incubation of thalli to encourage spore induction, 10-hole punches $\sim 1\text{ cm}^2$ were taken from random places along each thallus, avoiding the midrib. Punches from one thallus were collectively placed in individual petri dishes with a shallow layer of Provasoli enriched seawater (PES) (Provasoli 1968). The 5 weekly petri dishes, each representing one thallus, were incubated at 12°C, at an irradiance of $40\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and a 14:10 light/dark photoperiod to promote zoospore settlement. After 24 hours, each plate was examined for settled spores and then again on a weekly interval. Each dish's PES solution was changed weekly and maintained unless no visible gametophytes were seen after 4 weeks (Lüning & Neushul 1978).

Intracellular pH

To quantify the internal pH *D. herbacea* over time (\sim weekly), thallus tissue from one individual within each permanent plot ($n = 16$ per week) was randomly chosen each sampling time. A small ($\sim 3\text{-}5\text{ cm}$) piece of a lateral branch or the main thallus was trimmed off, avoiding the midrib if possible (Fig. 1). A preliminary study conducted in July 2015 on mature, non-reproductive sporophytes revealed that the intracellular pH of *D. herbacea* was not significantly different among the various thallus regions (overall

average and standard error = 0.62 ± 0.12 , midrid = 0.57 ± 0.62 , lateral branches = 0.64 ± 0.68 , branch tips = 0.59 ± 0.61 and holdfast = 0.68 ± 0.72 (K. Bartlett, unpublished data). All thallus tissue samples for measuring pH in this study were placed in individual plastic bags, brought to the surface, stored at approximately 52°F in the dark in a cooler filled with seawater to minimize vacuole lysing and brought to lab and tested 2-3 hours after collection in order to preserve the integrity of the samples. Each sample was divided to ~0.1g wet weight tissue. Each tissue sample was homogenized in 10mL of distilled water at room temperature (22°C) in a glass Kontes Duall tissue grinder and poured directly into a 10mL falcon tube. The pH of the liquid was read using a Beckman pH probe. The pH probe was calibrated with pH buffers 2 and 4 prior to reading samples. After each sample, the pH probe was rinsed with distilled water and dried with a paper wipe. The raw pH value did not take the weight of the sample into consideration and thus the pH was calculated by using the following equations (Sasaki et al. 1999).

Hydrogen ions equation:

$$H^+ = \text{raw pH} * \text{volume of solution (mL)} / \text{weight of sample (g)}$$

Volume of solution is 10 mL of distilled water and the dry weight of the pulverized sample.

True pH equation

$$\text{True pH} = -\log [H^+]$$

A regression was performed between length and pH of *D. herbacea* over all time periods tested to test if sulfuric acid accumulates intercellularly with growth.

Biotic factors

To examine whether the *D. herbacea* internal pH varied as a function of grazing intensity, the density of *Chlorostoma brunnea*, *Patiria miniata*, *Strongylocentrotus purpuratus* and *Mesocentrotus franciscanus* within the permanent plots were recorded during each sampling period. To examine whether the grazers *P. miniata* and *C. brunnea* could impact microscopic stages and subsequent recruitment of *D. herbacea*, a caging experiment was conducted in Stillwater Cove in spring 2015. Prior to the recruitment period of visible sporophytes cages were established to compare to natural recruitment on the rocky reef. The treatments included: sea star inclusion cages, snail inclusion cages, grazer exclusion cages and natural reef control, no cage (n=4 replicates). Treatments were established on a rocky terrace adjacent to the permanent plot terrace. The working assumption was that the area was blanketed with microscopic *D. herbacea* gametophytes from the prior fall reproductive period, but with zero visible recruits. The experiment ran from March to May 2015. Two *P. miniata* specimens were enclosed within each sea star inclusion cage treatment (within range of typical bat star densities in the area, Leonard 1994, and data from this study, Fig. 8). Ten *C. brunnea* were enclosed within each snail inclusion cage treatment and the densities based on typical local densities (Watanabe 1984, data from this study). Grazer exclusion cages were cages not stocked with any invertebrate macrograzers and the mesh kept out macrograzers >1cm, no small ones were seen. Uncaged control plots termed ‘natural reef’ were adjacent to caging area. The twelve 0.25 x 0.25 x 0.15m² cages were constructed of PVC and vexar mesh (0.7mm). All cages were equipped with a 10cm mesh skirt around the bottom perimeter to create a more secure seal to the substrate and aided in excluding other organisms. Before

installing the cages, 1/4" stainless steel eyebolts were drilled around into the substrate 7/8" deep using a pneumatic drill with a 3/8" masonry drill bit. The eyebolts were held in place with z-spar putty and drywall anchors. Each cage required four eyebolts, one per side to anchor the cages to the seafloor. Prior to cage placement mobile invertebrates and macroscopic algae were removed from all plots by using a paint scraper. Stainless steel washers and masonry nails were hammered into the mesh skirt to further secure the cages.

The caging experiment commenced on March 12th, 2015, prior to the published *Desmarestia* recruitment period (Edwards 1998). Grazers were allowed to graze over the benthos in the cage and natural reef treatments for three weeks. The benthos in the cages and plots were visually checked weekly for any appearance of macroscopic *D. herbacea* recruits. During this three-week time, weekly population surveys were also conducted around Stillwater Cove at similar depths to assess the timing of annual recruitment. After three weeks, all cages were removed and all plots were surveyed for the initial number of individual *Desmarestia* recruits. Uncaged 'natural reef' control plots were sampled using a 0.25m² quadrat haphazardly thrown 10 times on the benthos surrounding the cages on the same terrace, at the same depth within 5m of the cage plots. The number of individual *D. herbacea* sporophytes that recruited into all treatments were recorded three weeks after cage removal.

A one-way ANOVA was used to compare sporophyte recruitment between treatments to examine the grazing impact of *C. brunnea* and *P. miniata* on *D. herbacea* recruitment relative to the herbivore exclusion and the 'natural reef'. A Tukey's HSD post hoc test was run to compare treatments. Recruitment data from the caging experiment were

shown to have unequal variances by a pre-analysis Bartlett's test. All data were 4th root transformed to account for this and reported results reflect the transformation.

To test for an effect of winter grazer intensity on subsequent 2016 spring recruitment into permanent plots in 2016, the total number of grazers counted per plot over were summed to calculate a proxy of invertebrate grazing intensity within each plot during the overwintering phase. The time over which the grazers were summed was after no visible individuals were seen (December 2015) until the date of the first visible recruitment (April 2016). A t-test was used to compare sum grazers between plots subsequently did not exhibit recruitment to those that did have recruitment in spring 2016.

Abiotic factors

Subsurface seawater temperature data were collected by a Sea-Bird Scientific SeapHOx deployed by Professor Scott Hamilton at 15m ~50m from the permanent plot. Temperature (°C) was collected every 15 seconds and then reduced to a daily average to produce a time series from April 2015 to January 2017. Gaps in the temperature data resulted from necessary sensor retrieval for data extraction in combination with rough deployment conditions. The first data gap from 6/18/2015 – 8/25/2015 were subsidized with University of California, Santa Cruz subsurface oceanic data. An acoustic doppler current profiler sensor (ADCP) located near the *Desmarestia* terrace 30m from the permanent plot site at (36° 33.293' N, 121° 56.487' W) was deployed at 20m and took temperature data every 15 seconds. The second gap (12/18/2015-2/23/2016) was subsidized by the NOAA Cabrillo surface buoy, station #46240 located at (36°37'35" N 121°54'25" W). These data were compared to subsurface temperature data and had no graphically significant differences. Average wave height data (m) was extracted from the

Coastal Data Information Program (CDIP) buoy station #185 located (36° 43' 22.26"N 122° 21' 2.77" W) and processed to daily wave height averages. Ocean temperature and pH are tightly correlated and thus temperature was used as a proxy for pH (Kroeker & Donham, unpublished data). Seasons were used to compare demographic data and were determined using MBARI's ten-year time series which determined that spring to early summer is 'Upwelling', late summer to fall is 'Oceanic' and winter is termed 'Davidson Current' (Chavez et al. 2000).

Lagged regressions between ocean temperature and intracellular pH and wave height and intracellular pH respectively were performed to examine whether a lagged effect of temperature or wave height on intracellular pH existed. The relationship between temperature, wave height and year on pH was examined using an ANCOVA with temperature and wave height as the covariates.

Statistical analyses

Data were analyzed using Python, JMP. Prior to each test, data were examined for homogeneity and for normality by graphical interpretation of residual plots. The appropriate transformations were applied to those data not meeting assumptions. All tests were analyzed at 5% confidence levels.

RESULTS

Demography

There was significantly higher density of *D. herbacea* in 2015 than in 2016 (Two-way ANOVA: $F_{2,186} = 8.199$, $p = 0.004$, Table 1A). Density was strongly seasonal, recruiting in spring and disappearing in late fall, and varied significantly between years with higher densities in 2015 ($7.67 \pm 2.61/m^2$) than in 2016 ($4.65 \pm 0.84/m^2$) (Fig. 4A).

There was higher density in 2015 that declined gradually until no plants were present in November. In 2016, density increased in early months but to a lower density and plateaued remaining consistent for almost 6 months of the year until November when all but two plants senesced. The last individuals persisted within permanent plots until January. A significant difference in *Desmarestia* density was found between seasons (Two-way ANOVA: $F_{1,186} = 15.770$, $p = < 0.001$). Oceanic months (July-October) had a more stable, lower density. Recruitment and natural senescent periods occurred during Upwelling (March-June) and Davidson Current (November-February) seasons respectively, when density was most variable. A Tukey HSD post-hoc test revealed significant differences between Upwelling and Davidson Current ($p = 0.0004$) and between Oceanic and Davidson Current ($p = 0.026$) meaning that most plants were not present during the Davidson Current season. Upwelling, which encompasses early recruitment, and Oceanic, which is the time of growth and density plateau, were nearly significant ($p = 0.058$). There was a significant interaction between Year*Season (Two-way ANOVA: $F_{2,186} = 3.996$, $p = 0.02$), driven by the higher density in 2015 compared to 2016.

Interannually, density was higher in 2015 and plants were generally smaller in length. 2016 showed a lower and more stable density but with larger overall lengths. A two-way ANOVA conducted to examine the effect of year and season on *Desmarestia* length during the same seasons described above (Fig. 4B, Table 1B) found a significant difference in thallus length between years (Two-way ANOVA, $F_{1,352} = 27.037$, $p = < 0.001$) and season (Two-way ANOVA, $F_{2,352} = 6.171$, $p = < 0.002$). There was also a significant interaction effect of both year and season on the length of *Desmarestia* (Two-

way ANOVA, $F_{2,352}=17.486$, $p = < 0.001$). The range of size of individuals measured in the plots followed in this study ranged from the smallest recruit observed at 1cm and the maximum length was 120cm. The largest individual measured in Stillwater Cove, outside of this permanent site, was measured at 130cm (A. Zepp, personal observation). The greatest average length was 61.93 ± 9.97 cm which occurred in August 2016.

Sporophyte recruitment occurred within permanent plots in late March in 2016 which is earlier than other previous studies performed within Stillwater Cove which found that the first recruitment consistently occurred between April 4-17 over the course of 5 years (Edwards 1998).

In 2016, recruitment occurred in only 10 of the 16 permanent plots. Invertebrate grazing intensity was significantly lower in plots that exhibited *D. herbacea* recruitment ($n=10$) while grazing intensity was almost twice as high in plots with no *D. herbacea* recruitment ($n=6$) ($t\text{-test}_{1,10} = 1.81$, $p = 0.01$, Fig. 10). The times compared were after no visible individuals were seen (December 2015) until the date of first visible recruitment (April 2016).

In order to ensure tissue removal would not alter the natural senescence of the *D. herbacea* population, trimmed and untrimmed plants were compared over the same time period. No detrimental effect of trimming was found on specific growth rate (One sample $t\text{-test}$, $df = 8$, $t = -1.356$, $p = 0.403$, Fig. 7). The period of untrimmed plants was August 2016 to January 2017. Trimmed plants were followed from November to January to capture the senescent phase.

To examine if *D. herbacea* adheres to the Optimal Defense Strategy and reallocates energy from producing sulfuric acid towards becoming reproductive was

attempted but was unsuccessful. No gametophytes were seen in lab cultures throughout this two-year study thus fecundity could not be assessed.

Intracellular pH

The intracellular pH trend of *D. herbacea* was low throughout the study with an average pH of 0.75. The pH was strongly seasonal during both years (Fig. 4B & Fig. 5). In 2016 where the pH throughout the recruitment, growth and senescence period was followed, the pH was highest during the recruitment period (1.38 ± 0.14), followed by a decline in pH during the growth period (0.60 ± 0.01), followed by an elevation during the reproductive/senescence period (0.65 ± 0.02). This trend differed in 2015 where there was a much more dramatic increase in pH during the final senescence period (0.97 ± 0.16). A two-way ANOVA conducted to examine the effects of year and season on *Desmarestia* intracellular pH (Table 1C) revealed that there was not a significant difference in pH between years (two-way ANOVA, $F_{1,73} = 1.153$, $p = 0.286$) but there was a significant difference between season due to a temporal change in pH over the growth season (two-way ANOVA, $F_{2,73} = 5.179$, $p = 0.008$). The significant interaction between season and year indicates that the slopes of the linear regressions of pH differed between years and therefore pH is changing over time at different rates between years (ANOVA, $F_{2,73} = 10.87$, $p = < 0.001$). Upwelling, which encompasses early recruitment had a higher pH in comparison to the Oceanic period which remained stable and consistent. Recruitment and natural senescent periods occurred during Upwelling and Davidson Current seasons respectively, when pH was most variable. There was an elevated pH during the final two months of life history stages (October and November) in 2015. There was less of an increase in pH during the senescent phase in 2016-2017 (Fig.

4). A Tukey HSD post-hoc test revealed significant differences between Upwelling and Davidson Current ($p = 0.05$). There was a significant interaction between Year*Season (Two-way ANOVA: $F_{2,73} = 10.87$, $p = 0.0001$). There was a significant relationship between intracellular pH and thallus length of *D. herbacea* ($F_{1,230}=15.42$, $p=0.0001$, $r^2=0.06$) therefore acid does accumulate as individuals grow (Fig. 6).

Biotic factors

Invertebrate grazers were consistently present in the permanent plots over the course of this study and density ranged from a high of 13.86 ± 0.18 to a low of 2.14 ± 0.68 individuals/plot. Some plots were more rugose therefore providing cracks and crevices for invertebrates to reside in, while other plots had little relief and generally lower invertebrate densities. Overall, the grazing intensity throughout the duration of this study was highly variable (Fig. 8). Within the plots, purple urchins (*Strongylocentrotus purpuratus*) dominated and made up over 85% of the grazers. A repeated measures ANOVA was used to compare the density of each invertebrate group over time. Invertebrate density varied significantly over time with the exception of sea stars in 2015 (Table 4). This test shows that invertebrate grazers are consistently present and highly variable over time. Urchins were seen actively grazing on *D. herbacea* starting in October of both years. Sessile invertebrates were associated with the surface of *Desmarestia* with bryozoan recruitment observed directly on the lower portions of the thalli during the senescent phase.

Herbivore inclusion experiment

Benthic invertebrate grazers were shown to have a strong, significant and negative effect on the early recruitment of *Desmarestia* as detected using an herbivore exclusion

experiment (Fig. 9). Highest recruitment was measured with the exclusion of all grazers. Sea star presence had the greatest effect on recruitment during the cage inclusion experiment. Snail inclusion was significantly different from grazer exclusion but did not differ significantly from natural reef. While sea stars remained in the cages throughout the incubation, over all 4 snail inclusion cages, ~5 snails escaped. It is difficult to determine if the herbivores actively grazed on the microscopic sporophytes or if their mobility on the benthos disturbed recruitment. Genuculate coralline algae were removed in all caged plots which also may have affected recruitment since coralline algae is one of the prime substratum for both *D. herbacea* (Edwards 1998). When all grazers were excluded from the caged plot, there were significantly higher densities of *D. herbacea*, despite having removed all the substratum. Visible recruitment occurred in the natural reef surveys during the cage incubation indicating that natural recruitment occurred without disturbance or manipulation. A one-way ANOVA used to assess the grazing impact of the gastropod, *C. brunnea* and sea star, *P. miniata* on *D. herbacea* (between treatments, ANOVA_{3,12} = 3.796, p = 0.040) and a Tukey's post hoc test revealed a significant difference between the cage exclusion and star inclusion (p = 0.026). More *D. herbacea* recruits were seen in the all grazer exclusion than the star inclusion. Natural reef had a significantly lower density than the grazer exclusion likely due to exposure to grazers. There was seven times more recruitment in the herbivore exclusion treatment (n = 117/0.25m²) than in the natural reef population (n = 16/0.25m²). A post hoc test showed that there was no significant difference between snail inclusion and natural reef.

Abiotic Factors

To assess the relationship between oceanographic conditions and intracellular pH a lagged regression was conducted. Statistical significance increased with days and had the strongest significance 7 days prior to taking the pH tissue samples ($p = 0.0018$, Table 2). Wave height had no significant effect on pH but wave height had the strongest, but insignificant effect two days ($p=0.127$) before samples were taken (Table 2). The following analyses used the strongest lagged regressions as a proxy for the appropriate amount of days to average the oceanographic data. A two-way ANCOVA was used to examine whether there was an effect of oceanographic temperature, wave height or year on weekly intracellular pH (Fig. 5, Table 3). The random factors in the two-way ANCOVA analysis were temperature, wave height and year, and the interaction terms used were Temperature*Year and Wave Height*Year. There was a significant effect of temperature on pH (ANCOVA, $F_{1,40} = 22.21$, $p=0.001$) as well as a significant effect of year on pH (ANCOVA, $F_{1,40} = 7.605$, $p=0.009$). The non-significant interaction between temperature and year indicates that the slopes of the linear regressions of temperature did not differ between years and therefore pH is changing over time at roughly the same rate between years (ANCOVA, $F_{1,40}=3.497$, $p =0.069$). Wave height however, did not have an effect on pH (ANCOVA, $F_{1,40} = 0.193$, $p < 0.66$). Showing that there is no evidence that patterns in pH with respect to wave height are consistent between years (ANCOVA, $F_{1,40}=0.412$, $p =0.525$).

DISCUSSION

Desmarestia herbacea density, length and recruitment varied inter-annually and large and small scale oceanic changes likely had an important influence on algal demographics

during the course of this study. The oceanic and atmospheric processes in the Pacific cause large-scale, low frequency changes leading to important inter annual variability such as ENSO events (Tegner 1984, Dayton et al. 1992). In 2015, the first year of this study ENSO events with elevated sea surface were observed (McPhaden 2015) with cooler waters subsequently in 2016. This may have influenced the significant differences seen in inverse relationship between *D. herbacea* thallus length and density between 2015 and 2016. The inverse relationship between density and size revealed a classic plant-population tradeoff. In 2015, plants were more dense but smaller in size. Conversely, in 2016 plants were less dense but larger in size.

The inter-annual variability in size and density data may reflect juvenile sporophytes being subjected to density-dependent mortality. Major declines in sporophyte density coincided with the period of the greatest change in length which indicates that as plants grow rapidly, they interfere with survivorship of neighbors (McConnico & Foster 2005). Larger plants suppress the growth of smaller individuals at increased densities due to competition for space, light and nutrients (Reed 1991; Creed et al 1996). These dynamics could play an integral role in population demographics. The measured annual variation in thallus length relative to density could be attributed to self-thinning. *D. herbacea* lacks pneumatocysts and therefore is free to whip around the holdfast. This can have a negative physical effect as larger individuals may dislodge smaller individuals entirely. There is also an entanglement risk with being large with no vertical buoyancy. Light and nutrients may have reduced smaller individuals due to larger individuals smothering or shading smaller plants.

Timing of recruitment in annual species can vary due to duration of light, nutrients and temperature (Edwards 1998). During this study, recruitment within permanent plots occurred in late-March 2016 and was seen outside of plots, within Stillwater Cove in early March 2016. These findings are different from reports from M. Edwards in which he found that *D. herbacea* recruited around April 4th-17th consistently throughout his 5-year study (Edwards 1998) at the same site. This study shows that timing of recruitment could also be influenced by the density and intensity of grazers.

No evidence was found for a negative impact of trimming on growth rates since manipulated plants grew and senesced at the same rate as those that were not manipulated. Thus *D. herbacea* appears to be able to withstand tissue loss without releasing its acid or senescing. This result suggests that the techniques used in this study for collecting tissue samples did not induce a stressful, self-destructive response relative to growth (Gagnon 2006). Since *Desmarestia* is notoriously intolerant to physical stress, this result also indicates that slight physical disturbances over time do not negatively impact its growth or natural senescence. Further study is needed to test for the upper stress threshold that *Desmarestia* can withstand without releasing its intracellular acid.

Intracellular pH

The temporal variability in intracellular pH measured over two years in this study supports the idea that pH changes with life history stage (Gagnon et al. 2013). The previous study showed that *D. viridis* continuously accumulated acid (0.4 to 0.99 until the convergence of abiotic controls, primarily sea temperature which may have trigger synchronous death of individuals (Gagnon et al. 2013). While my current study did not measure a similar pattern in intracellular pH, a clear pattern was found that pH varied

with life history stage and accumulates with growth. A rise in pH was measured in fall of 2015, similar to the Gagnon (2016) study, however, a dramatic increase in pH was not measured at the end of 2016. *D. herbacea* therefore, appears to have a more variable acid accumulation and release pattern as *D. viridis*. The value of my results is that I followed a population for two years compared to their one-year study and found interannual variability in the signal. My study suggests that more expanded research, combined with measuring oceanography may indicate that the story is complex and that pH trends may be influenced by both demographic and abiotic factors. Young *D. herbacea* recruits have a lower acidity level likely because the sulfuric acid has not accumulated yet. As plants grow in length, they accumulate more acid, therefore reducing their pH, however lack of fall senescence in 2016 may have complicated this trend.

While it was not possible to evaluate the timing of reproduction relative to oceanography and pH in this study, it would be informative to our understanding of *Desmarestia* demographics to determine if an increase in fecundity requires the accumulation or production in sulfuric acid to cease. The general assumption is that reproductive structures should have higher levels of defenses than vegetative parts (Steinberg 1984, Tuomi et al. 1989, Poore 1994, Van Alstyne et al. 1999). Old vegetative tissues in seaweeds have previously been found to contain higher concentrations of chemical defenses than young growing apices, although it has been assumed that the optimal defense theory (ODT) predicts the opposite (Cronin & Hay 1996). This study saw a slight increase in pH during the anticipated reproductive phase (October-December), therefore decreasing the level of chemical defense. Further research would be

needed to test the specific fitness value of each portion of *D. herbacea* to determine if this alga adheres to the optimal defense theory.

Biotic factors

This study showed that the presence of herbivores, whether through active grazing or general movement along the benthos negatively impacted the microscopic stages and subsequent recruitment of *Desmarestia* populations. Leonard (1992) observed that within Stillwater Cove, the bat star *Patiria miniata* effectively grazed 100% of the available substrate every 90 days, and predicted that this grazing killed all *Macrocystis pyrifera* gametophytes. Grazing pressure on algal recruits has direct negative impacts the demography of the population. Densities of grazers for cage inclusions were based on natural populations seen throughout this study. The caging experiment indicated that sea stars can affect early recruitment while snails had little effect. Further examination of grazer impacts including testing for an effect of sea urchins on recruitment could broaden the understanding of grazer effects on *Desmarestia* populations in general.

This study measured a negative effect of grazer presence on microscopic sporophytes likely due to physical dislodgement or grazing. Young *Desmarestia* sporophyte recruits are erect or vertically oriented off the bottom and exhibit a lower acidity level, which should make them more vulnerable to grazers. However, grazing was never witnessed on young recruits through the duration of this study. Once they reach a length of ~20 cm they lose their vertical structure and lay down. It's possible that they grow upright while they accumulate sulfuric acid and once they achieve a certain size their weight drags them down making them structurally more vulnerable but more chemically defended. They appeared to be most susceptible to grazing once their thallus length

extended enough (~20cm) to be whipped into urchin-dwelling cracks. Konar (2000) found a similar opportunistic grazing relationship of *D. viridis* and *D. herbacea* to the urchin, *Strongylocentrotus droebachiensis*.

The holdfast of *D. herbacea* is relatively small in comparison to the rest of the thallus, but is generally not physically stressed from wave action due its lack of vertical structure. Instead this alga whips around the holdfast in tandem with water movement. While *Desmarestia* releases acid under stress, the pH may not be affected by wave height unless the individual is dislodged from the substrate. Herbivores opportunistically catch and consume *D. herbacea* as it whips near the crevices they live in (A Zepp, personal observations). Grazers were never seen eating the holdfast which could potentially dislodge the thallus, therefore affecting density. Thus, density declines measured in this study are likely due to senescence. There was only one sighting of *P. miniata* actively grazing on *D. herbacea* and a few cases of *Chlorostoma* and another snail, *Lithopoma* directly on the thallus. The recruitment of bryozoans onto thalli only occurring during the senescence period in this study may indicate close thallus associations only as pH increases but could also be due to the rigidity of the thallus.

Plant pigment in this study varied over time and as plants begin to senesce during the Davidson Current (Nov.-Feb.) period there was a slight color change in the tips of the thallus blades and a spike in pH (~1). While this decrease in acidity might be related to senescence or faculty, it could also be attributed to urchin grazing. While *D. herbacea* may not be the primary food source for urchins, they do consume larger individuals despite the negative physiological consequence of their Aristotle's Lantern feeding apparatus degrading due to the acidity (Pelletreau and Muller-Parker 2002). It is unclear

if urchins opportunistically consume *D. herbacea* when they are large enough to ‘catch’ long enough thalli from within their sheltered crevices, or if they are less deterred by the decrease in acidity in the last months of *D. herbacea*’s life stages.

Abiotic Factors

Survivorship and timing of zoospore release could have suffered in 2015 due to the anomalous warm waters, thereby affecting the subsequent recruitment of new individuals in 2016. If the anomalous temperature increase did have a negative effect on zoospore production or survivorship, waters warming in the future due to climate change would have a substantial negative effect on the persistence of *D. herbacea*. No studies have looked at *Desmarestia* zoospore survivorship therefore, further research would be needed to support this hypothesis. The warm water blob phenomenon began in late 2013 and persisted along the west coast until summer 2015 (Leising et al. 2015). Waters warmed ~3°C above average which slowed the flow of nutrients from the deep ocean, reducing the productivity of coastal ecosystems. This warm water pulse combined with the ENSO of 2015-2016 augmented for abnormally high temperatures and cascading oceanographic effects. *Desmarestia* notoriously releases its acid and become green when exposed to a temperature threshold. This study suggests that *D. herbacea* is more tolerant than other *Desmarestia* species to a wider temperature range and fluctuation (~9-16.5 °C) and higher threshold (16°C) without releasing its own acid. This differs from other species such as *D. viridis* off the coast of Newfoundland which has shown to have a thermal threshold of ~12°C. (Blain & Gagnon 2013).

CONCLUSIONS

This study describes the temporal and demographic pattern of how the acid weed, *Desmarestia herbacea*, chemically defends itself through lower pH. It also clarifies the abiotic factors such as wave height and oceanographic temperatures, and biotic factors such as grazing, may attribute to demographic or pH fluctuations which change relative to *D. herbacea* life history phase.

This study tracked the demography of a population of *D. herbacea* through two years. Density and lengths were highly variable between sampling years and seasons. The intracellular pH was slightly higher in the initial life history stage which may allude to smaller individuals building up sulfuric acid. The pH level spiked at the final life stage while populations were senescing.

Elevated water temperature anomalies from the “warm water blob” and the ENSO in 2015 may attribute to the differences in density and lengths of *Desmarestia*. There was not a difference in intracellular pH between years, indicating that temperature doesn’t seem to affect acid production or accumulation and wave height did not have an effect on intracellular pH.

Biotic factors such as grazing did have a significant effect on *Desmarestia* recruitment or survivorship. The presence of snails and stars had a negative impact on the microscopic stages therefore reducing the recruitment of *Desmarestia* populations in grazer inclusion experiments. Urchins were the primary grazer on macroscopic sporophytes throughout the duration of this study and were able to utilize this remarkably acidic alga for food despite its low pH levels. Bryozoans utilized *D. herbacea* as a substrate in its later life stages.

Further studies of *Desmarestia* are necessary to determine the tradeoffs that may exist between acid production, resource allocation to the various primary and secondary life processes such as photosynthesis and reproduction, and morphological adaptability. Specifically, investigations of how nutrient levels and ongoing changes in ocean temperature may interfere with these processes must be conducted to better determine and anticipate changes in populations of *Desmarestiales* and their effects on other aspects of the marine ecosystem.

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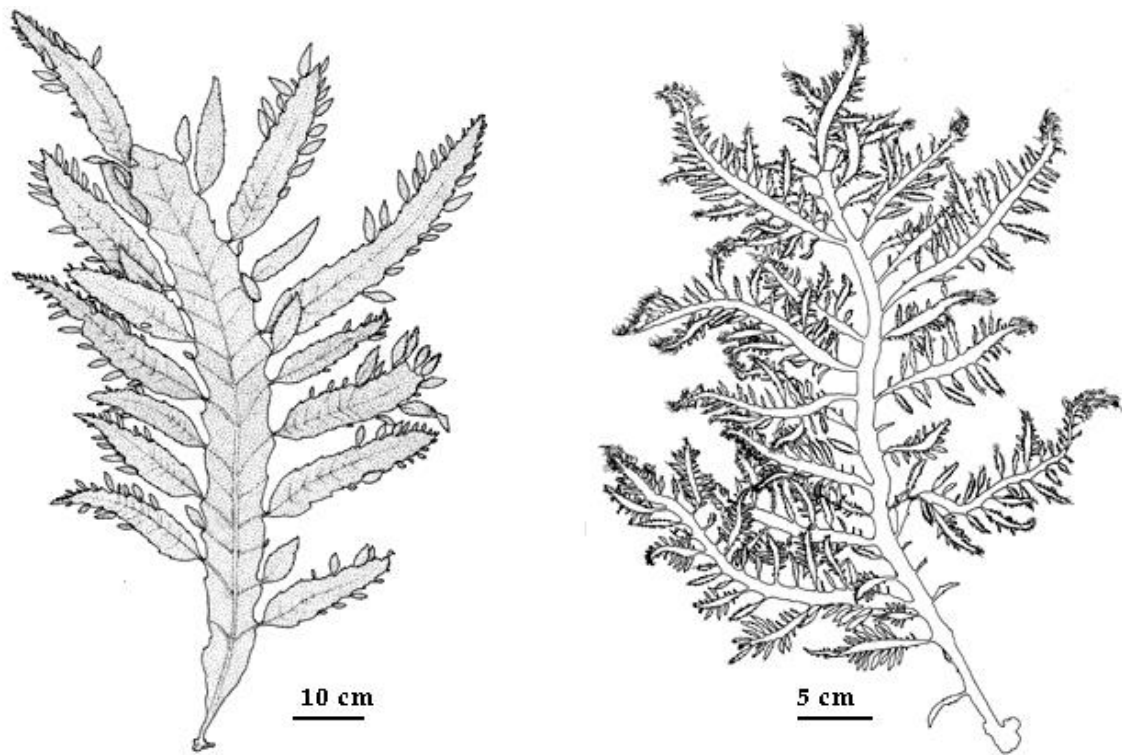


Figure 1. Variation in thallus morphology of *Desmarestia herbacea* (adapted from Paul Silva and the Regents of the University of California 2003).

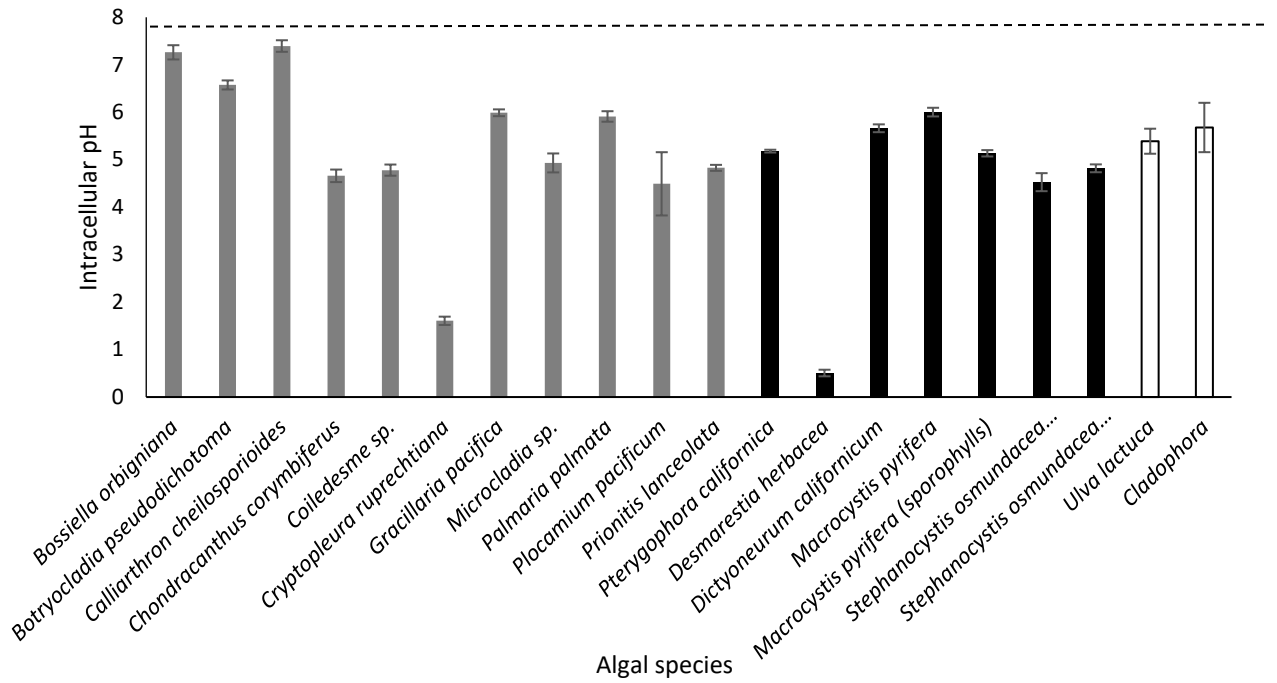


Figure 2. Mean (\pm SE) intracellular pH of local intertidal and subtidal algal species collected in July 2015 & 2016 within the Monterey Bay region (n=3). Species of Rhodophyta (gray bars), Ochrophyta (black bars), and Chlorophyta (open bars). The dashed line represents the average pH of seawater (MBARI buoy data retrieved from <http://www.cencoos.org/data/buoys/mbari/m1ph>).



Figure 3. Map of research site in the Stillwater Cove kelp bed, Carmel Bay, California. The star represents the location permanent plots.

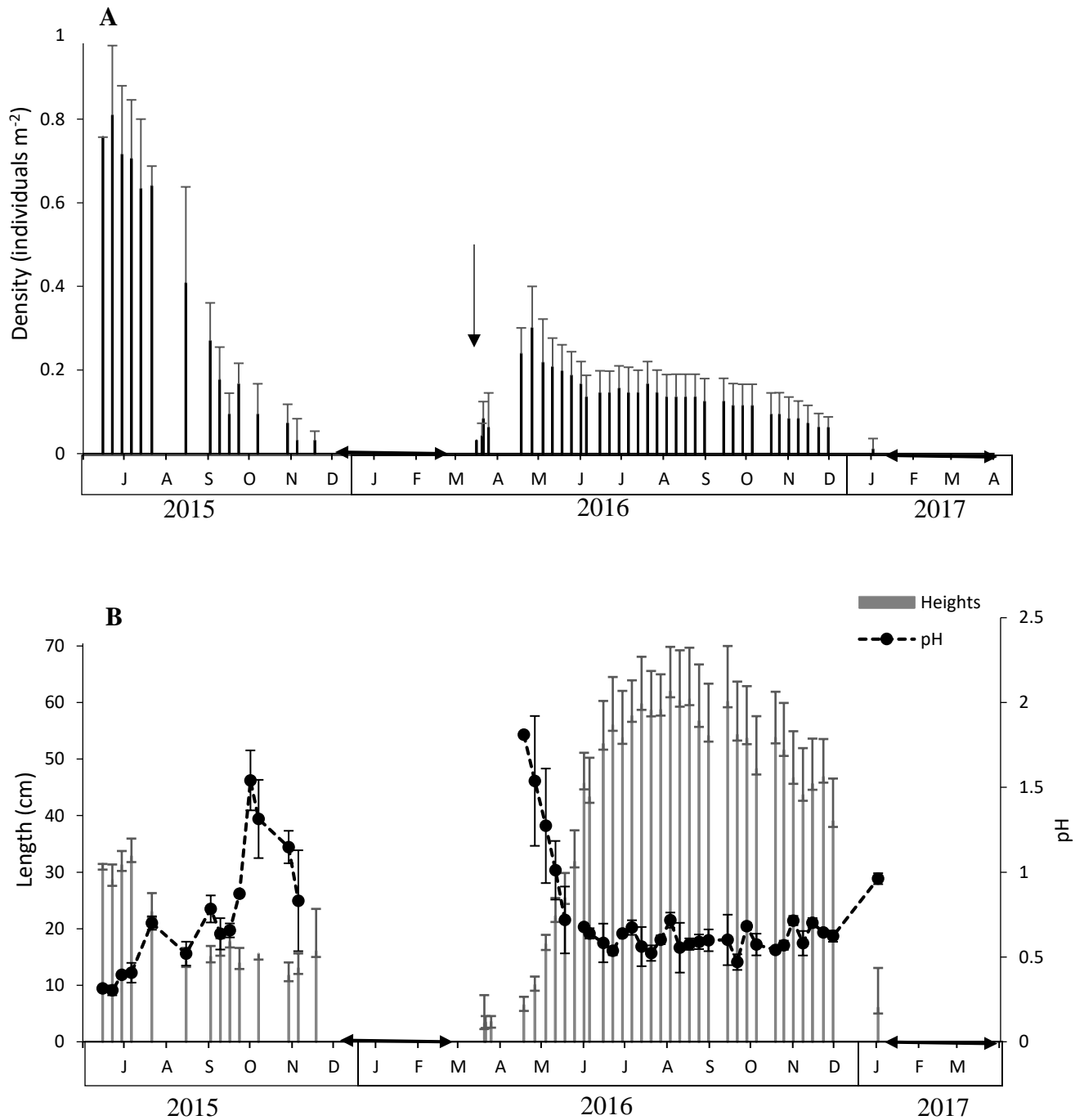


Figure 4. Mean \pm SE of (A) density and (B) average length and average intracellular pH of *Desmarestia herbacea* sporophytes in permanent plots within Stillwater Cove between June 2015 and April 2017. The arrow in A represents the date of initial recruitment on March 16, 2016 within permanent plots in 2016.

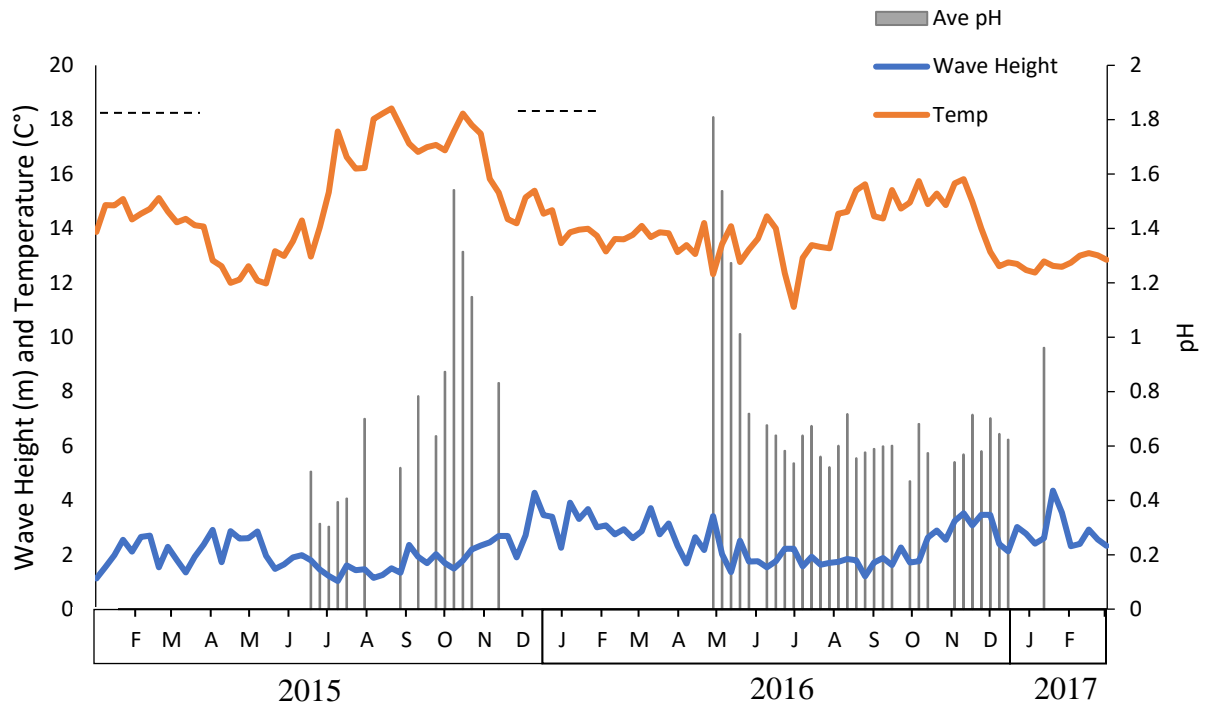


Figure 5. Temporal variation in the mean (\pm SE) intracellular pH of *Desmarestia herbacea*, subsurface seawater temperature (C°) and wave height (m). Dashed lines indicate subsidized data from NOAA or UCSC.

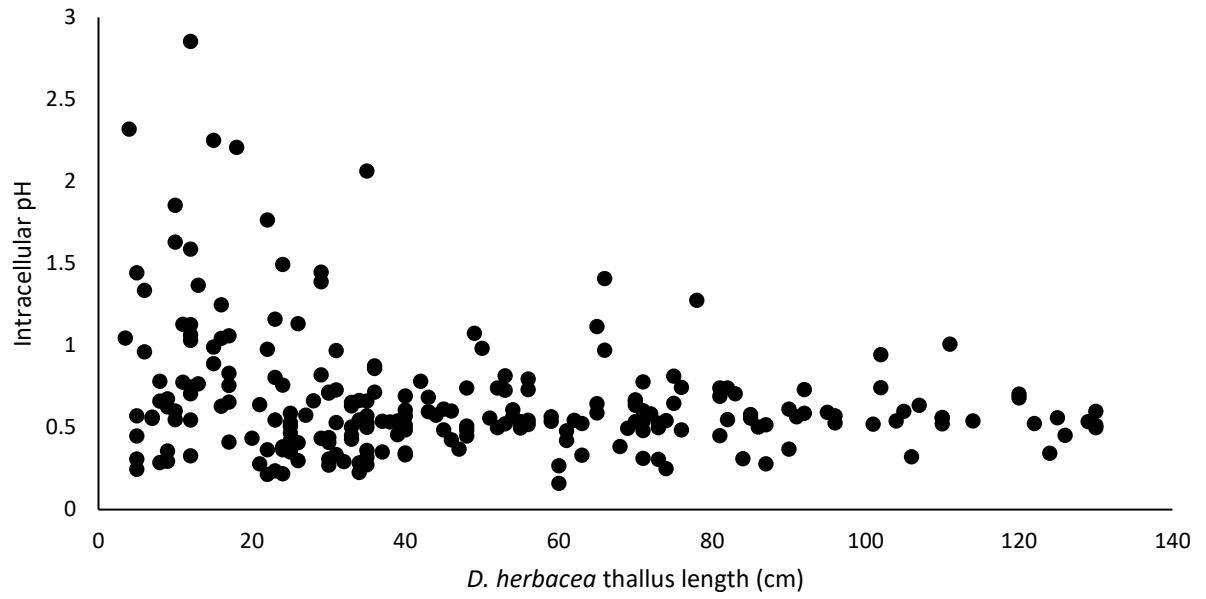


Figure 6. Relationship between intracellular pH and thallus length of *D. herbacea* (n=274) for all time periods (June 2015-January 2017) ($F_{1,230}=15.42$, $p=0.0001$, $r^2=0.06$)

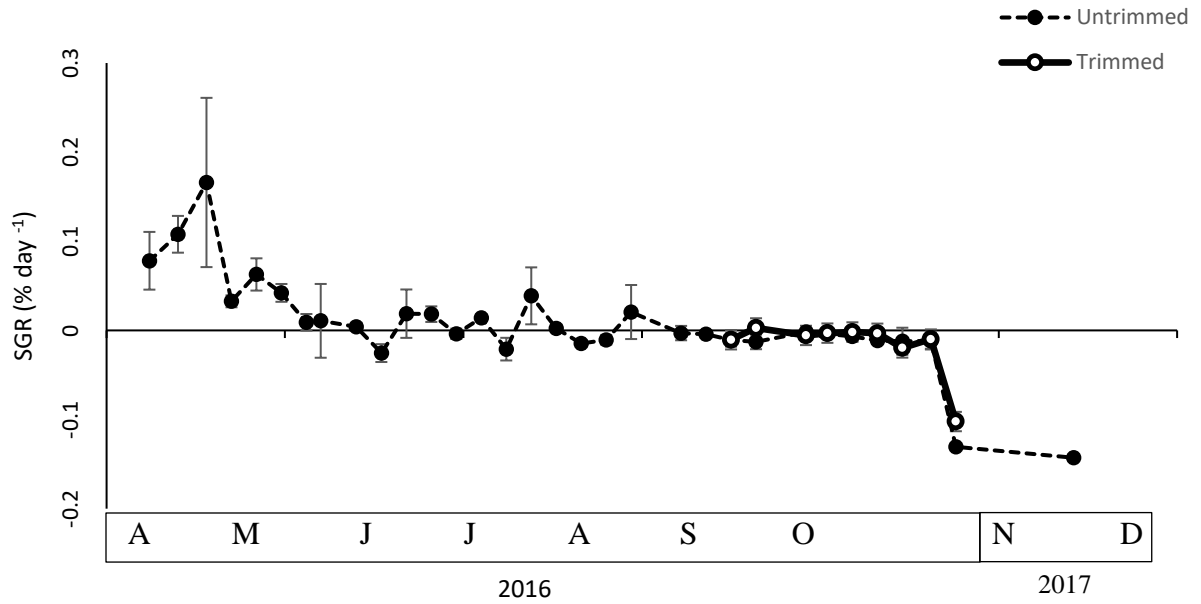


Figure 7. Testing for the effect of plant trimming on growth. Mean (\pm SE) specific growth rate (SGR) of trimmed (sample taken for pH measurement) vs. untrimmed *D. herbacea* individuals from August 18, 2016-January 17, 2017. The gray line represents untrimmed plants, left undisturbed (Initiated with $n = 8$, sample size gradually decreased ($n = 1$) as plants senesced). The black line represents trimmed plants which had a small tissue sample removed (~ 5 -10cm) for weekly pH analysis (initial $n = 11$) for trimmed plants and gradually decreased ($n = 2$) as plants senesced.

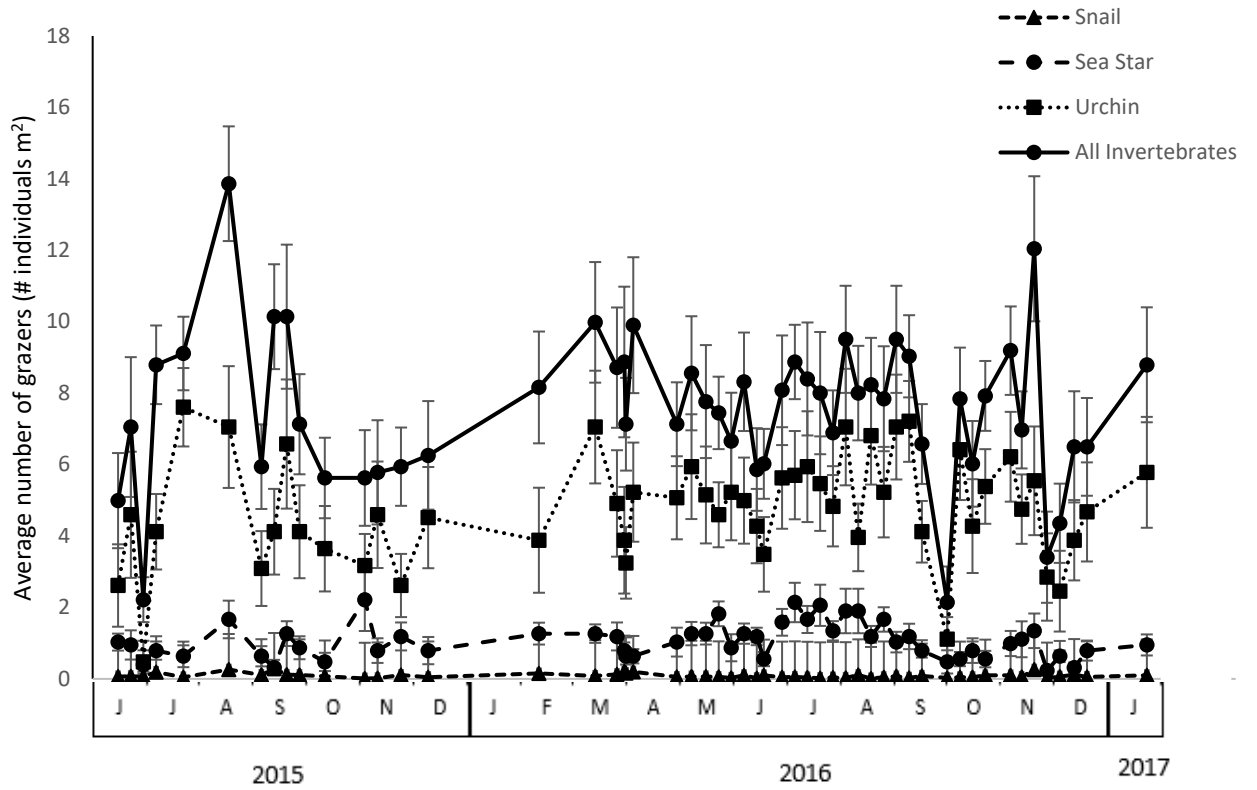


Figure 8. Temporal average invertebrate (urchins, snails, sea stars and cumulative grazers) density in permanent plots (n=16, proxy for grazing).

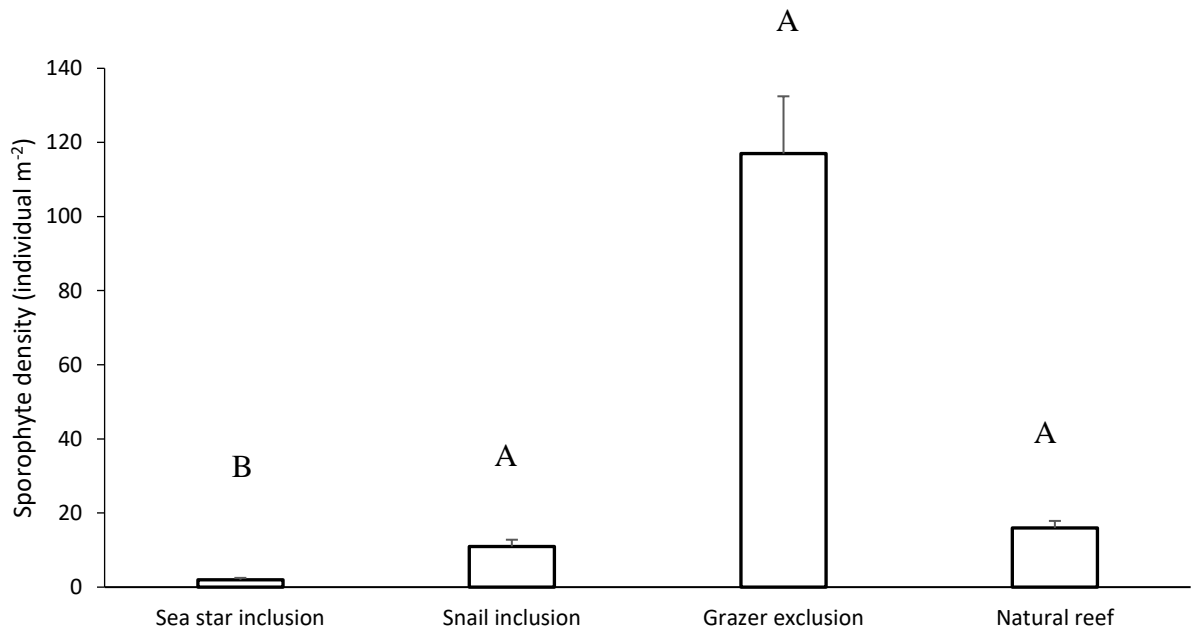


Figure 9. Herbivore impacts on *Desmarestia herbacea* sporophyte recruitment. Mean (\pm SE) density of *D. herbacea* sporophytes recruited in a field cage experiment after 24 days (March 3, 2015- March 27, 2015) of undergoing treatment exposure; sea star inclusion (*P. miniata*), snail inclusion (*C. brunnea*), cage control (grazer exclusion), natural control (natural reef). Cages enclosed *P. miniata* and *C. brunnea* while the cage control excluded all grazers (between treatments ANOVA_{3,12} = 3.796, $p = 0.040$, letters represent post hoc test differences between cage and stars $p = 0.026$).

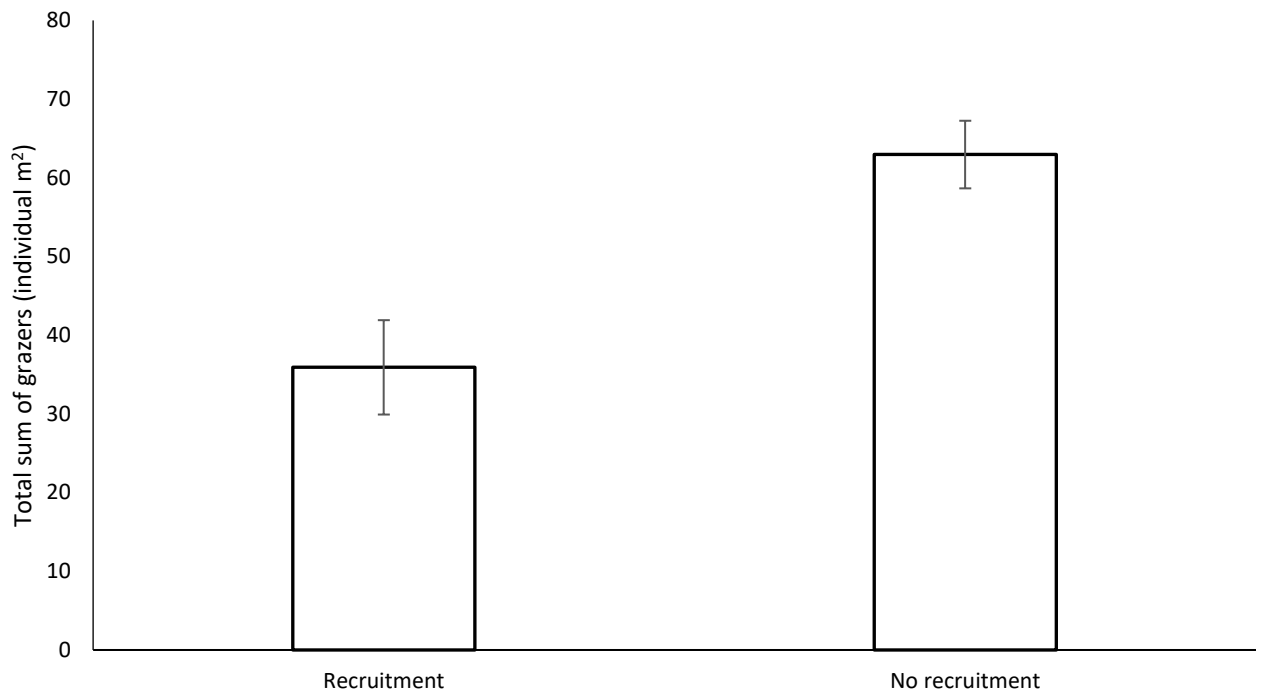


Figure 10. *Desmarestia herbacea* sporophyte recruitment success as a function of grazing intensity in permanent plots. The sum of all grazers in permanent plots between the senescence period (2015) and recruitment point (2016) ($t\text{-test}_{1,10} = 1.81$, $p = 0.01$).

Table 1. Results of a two-way ANOVA comparing the effect of year (2015 and 2016) and season (Upwelling: Mar.-June, Oceanic: July-Oct., Davidson Current: Nov.-Feb.) on the density, length and intracellular pH of *Desmarestia herbacea*.

A. Density of *Desmarestia herbacea*

	df	F	p-value
Year	1,186	8.1988	0.0004*
Season	2,186	15.7703	0.0001*
Year*Season	2,186	3.9955	0.0200*
Tukey HSD Multiple Comparisons Test			p-value
Upwelling	V	Davidson Current	0.0004*
Oceanic	V	Davidson Current	0.0255*
Upwelling	V	Oceanic	0.0587

B. Length of *Desmarestia herbacea*.

Year	1,352	6.1712	0.0023*
Season	2,352	27.0372	0.0001*
Year*Season	2,352	17.4855	0.0001*
Tukey HSD Multiple Comparisons Test			p-value
Oceanic	V	Upwelling	0.8842
Oceanic	V	Davidson Current	0.9907
Davidson Current	V	Upwelling	0.9939

Year	1,73	1.1533	0.2864
Season	2,73	5.1794	0.0079*
Year*Season	2,73	10.8704	0.0001*

C. Intracellular pH of *Desmarestia herbacea*.

Tukey HSD Multiple Comparisons Test			p-value
Davidson Current	V	Upwelling	0.0512
Davidson Current	V	Oceanic	0.1737
Oceanic	V	Upwelling	0.4371

Table 2. Lagged regressions on the effect of various time intervals (1-7 days) between average temperature and wave height within Monterey Bay versus intracellular pH of *Desmarestia herbacea*. The underlined time intervals with the highest r^2 value were used for ANCOVA in Table 3 [7 days for temperature, 2 days for wave height].

Time interval	Temperature p-value	Temperature r^2	Wave Height p-value	Wave Height r^2
1	0.8668	0.0007	0.3300	0.0243
2	0.7597	0.0024	<u>0.1272</u>	0.0586
3	0.5024	0.0116	0.1299	0.0578
4	0.2039	0.041	0.1436	0.0540
5	0.0511	0.094	0.1950	0.0426
6	0.0098*	0.1591	0.3939	0.0186
7	<u>0.0018*</u>	0.2222	0.5601	0.008

Table 3. Results of a two-way Analysis of Covariance (ANCOVA) testing for effects of ocean temperature (time lag = 7 days) and wave height (time lag = 2 days) and year on intracellular pH of *Desmarestia herbacea*.

	df	F	p-value
Ocean Temperature	1,40	22.2085	<.0001*
Wave Height	1,40	0.1963	0.6604
Year	1,40	7.6048	0.0092*
Temp*Year	1,40	3.4971	0.0699
Wave Height*Year	1,40	0.4120	0.5251

Table 4. Results of Repeated Measures ANOVAs of invertebrate grazer densities through time. The time periods used were June 2015 through December 2015 and February 2016 through January 2017 following when *Desmarestia* individuals were present.

	Both years	2015	2016
All herbivores	F _{7.72,115.74} = 3.56 p = 0.0012*	F _{5.42,81.23} = 4.82 p = 0.0005*	F _{6.87,103.14} = 3.24 p = 0.0418*
Urchins	F _{8.14,122.2} = 2.41 p = 0.0184*	F _{4.92,73.79} = 3.08 p = 0.0145*	F _{7.19,107.2} = 2.16 p = 0.0418*
Sea stars (<i>P. miniata</i>)	F _{9.2,138} = 1.94 p = 0.0496*	F _{3.99,59.9} = 2.02 p = 0.1033	F _{9.098,136.48} = 2.05 p = 0.0378*
Snails (<i>Chlorostoma</i> sp.)	F _{6.11,85.47} = 4.79 p = 0.0003*	F _{6.138,92} = 7.67 p = 0.0001*	F _{486,68.1} = 3.59 p = 0.0066*