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Spatial and temporal dynamics of *Ulva* assemblages in central San Francisco Bay, U.S.A.

By

Rosemary Romero

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Integrative Biology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor David R. Lindberg, Co-Chair
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Professor Erica Bree Rosenblum

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Abstract

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Harmful blooms of green macroalgae, known as green tides, have been increasing in frequency and intensity world-wide over the last decade. Composed mainly of the macroalgae, *Ulva*, these blooms occur in areas of low wave energy and high nutrient input from anthropogenic sources; they often result in massive die-offs in the impacted ecosystem. My dissertation addressed three key questions concerning the potential for green tides to occur in central San Francisco Bay: 1) what species of *Ulva* inhabit the bay and which of them have been identified as bloom-forming taxa in other locations? 2) does an overwintering, benthic bank of dormant propagules contribute to the rapid growth of spring *Ulva* populations? 3) does waterborne spore availability limit the recruitment of *Ulva* populations? Within central San Francisco Bay, I identified six species of *Ulva* using genetic barcoding, only four of which were previously reported within the bay. Several of these species are known to produce economically costly green tides in other regions of the world. While previous studies on the control of algal blooms focused on post-recruitment processes such as herbivory and space competition, I investigated two pre-recruitment processes: banks of benthic microscopic forms and supply of waterborne propagules. A multifactorial lab experiment demonstrated that banks of dormant benthic microscopic *Ulva* forms (i.e. gametes, zoospores or thallus fragments) survive winter incubation periods (12 weeks of darkness undisturbed at 11°C), but at a depressed growth rate. Successful recruitment from these banks is strongly influenced by seasonal increases in water temperature. A regular regime of water column sampling and laboratory culturing demonstrated that waterborne propagule supply varied with location within the central bay, and that the abundance of propagules in the water column varied strongly with season. Although water samples from all sites yielded recruits, sites north of the bay mouth yielded more *Ulva* recruits than southern sites during the spring recruitment period while sites south of the bay mouth yielded more recruits in fall. Together, this information indicates that propagule supply, hydrographic patterns that mediate dispersal, and environmental and physiological constraints on juvenile development impose important limitations on dynamics of green tide algae in the central San Francisco Bay. These interacting factors must be included in management as future global climate change is expected to stimulate more rapid development of spring green algal blooms.

Dedication

To my parents
Rosemary Romero-Keelan
Edward T. Keelan II
&
my grandmother
Rosa I. Romero

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INTRODUCTION

Understanding the causes of variation in the distribution and abundance of organisms in time and space is one of the fundamental goals of ecological research. A combination of biological and physical processes determines population dynamics and subsequent community organization. In general, these factors include competition, predation, parasitism, and mutualism as biological interactions and environmental factors such as nutrient and light availability, temperature, desiccation, and physical disturbance. The strength of recruitment from dispersing propagules can influence the relative importance of post-recruitment biological interactions (Lewin 1986, Underwood and Fairweather 1989). Incorporating propagule supply into ecological models is particularly important when considering sessile marine assemblages, where many organisms alternate between dispersing planktonic and sessile benthic stages. Areas with high settlement are saturated by propagules and community structure is more strongly influenced by post-settlement processes (Connell 1985, Gaines and Roughgarden 1985, Roughgarden et al. 1988). Alternatively, where settlement rates are low, oceanographic processes and offshore interactions limit recruitment and thus the degree to which post-settlement processes dictate distributions. Hence, variation in the supply of propagules can drastically alter community structure and dynamics. This phenomenon has been studied primarily in marine invertebrate populations, and much less so for marine macroalgae. Populations of early successional algal species that produce many propagules and disperse them widely are not thought to be limited by supply, but very little is known about propagule availability and the determinants of recruitment success in these species. These early successional species play a central role in the development of harmful algal blooms, also called green tides, which are receiving world-wide attention as they occur with increasing frequency in eutrophied coastal ecosystems.

Coastal ecosystems, including estuaries, are among the most productive and dynamic ecosystems on Earth. However, these ecosystems are undergoing ecological change at a remarkable rate. These changes are driven by overfishing, warming due to increases in atmospheric carbon dioxide, invasive species, coastal land use, and runoff of nutrients and toxins (Jackson 2008). Sixty-five percent of estuaries in the USA are classified as being moderately to highly eutrophic, with approximately 80% of highly eutrophic sites caused by nutrient supplementation from human activities (Clement et al. 2001). Symptoms of eutrophication include increased primary productivity in the form of algal blooms, accumulation of organic matter, and excessive oxygen consumption also known as hypoxia (Nixon 1995). As a result of these decreases in water quality, shifts from healthy seagrass and perennial macroalgae-dominated coastal ecosystems to those dominated by microalgae and ephemeral macroalgae have been observed (Valiela et al. 1997, Worm et al. 1999, Worm and Lotze 2006). Excessive nutrient loading also releases ephemeral macroalgae from consumer control as rates of productivity exceed consumption by herbivores (Worm et al. 1999, 2000, Lotze and Worm 2002, Worm et al. 2002). Similar patterns in algal production have been observed in coral reef ecosystems where human activities have increased nutrient loading and fishing has reduced populations of herbivorous fishes (Hughes 1994, Lapointe et al. 1996, Lapointe 1997, Valiela et al. 1997).

Ulva blooms, or green tides, are one example of macroalgal blooms that result in perennial macrophyte loss (Nielsen et al. 2004). Members of the genus *Ulva* are annuals commonly found on freshly denuded substrate from the subtidal to the uppermost littoral zone. Under eutrophic conditions, thalli become detached from their substrate and accumulate in enclosed embayments and on shores. *Ulva* blooms occur in coastal areas worldwide (Sfriso et al. 1992, Peckol and Rivers 1996, Pang et al. 2010). Their ability to rapidly colonized disturbed surfaces and fill eutrophic brackish waterways has led them to be considered a marine weed. While *Ulva* sp. are common in estuarine systems, the frequency, magnitude and economic impacts of nuisance blooms is highly variable.

The San Francisco Bay has a long history of anthropogenic disturbance. Some examples include conversions of wetland habitat to urbanized landscapes, episodic oil spills, species introductions from shipping vessel ballast and the seafood trade, manipulation of freshwater inflow, and nutrient enrichment (Carlton 1979, Nichols et al. 1990). Several of these have been identified as drivers of ecosystem change within the estuary (Cloern and Jassby 2012). Recent research indicates that *Ulva* abundance has reached levels that could be detrimental to seagrasses in some areas of the bay (Boyer and Wyllie-Echeverria 2010). In this dissertation, I address three questions concerning the potential for green tides in central San Francisco Bay. In chapter 1, I use a phylogenetic approach to identify species of *Ulva* commonly found in the central San Francisco Bay. I also discuss *Ulva* floristics within the bay, noting previously unreported species for the bay, several of which are known to produce nuisance blooms.

In chapter 2, I investigate benthic ulvoid propagule banks and their ability to overwinter in nearshore sediments of the central San Francisco Bay. I first demonstrate that benthic ulvoid microscopic stages accumulate in intertidal sediments throughout the nearshore of central San Francisco Bay. My data show, that current average winter temperatures are sufficiently low to slow growth of settled propagules and delay recruitment. I discuss the effects of seasonal temperature increases on the ability of these benthic propagules to contribute to spring recruitment. However, the importance of these overwintering propagule banks to spring recruitment pulses will be dependent on ocean chemistry and ocean circulation changes that result from anthropogenic induced global climate change and how these processes influence the San Francisco Bay (Iles et al. 2011). During winters with anomalously warm ocean temperatures *Ulva* recruitment is likely to occur earlier in spring and could lead to great accumulations of algal biomass over the subsequent summer.

In chapter 3, using an information theoretic approach, I developed statistical models to predict the suite of environmental and biological factors that best explains *Ulva* occurrence in the central San Francisco Bay. Location within the central San Francisco Bay and season of census were the most important factors in explaining variability in attached *Ulva* abundance, with benthic herbivore abundance and ammonium concentrations exerting strongly negative influences. Algal competitors exhibited moderately negative relationships while propagule supply had a moderately positive relationship with attached *Ulva* abundance. Together these approaches demonstrate that interacting factors cannot be overlooked in future management of estuarine and coastal ecosystems in the face of global change.

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CHAPTER 1 | A molecular assessment of *Ulva* diversity in central San Francisco Bay

Rosemary Romero, David Lindberg & Wayne Sousa
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ABSTRACT

Many marine algae lack morphological characteristics that can be reliably used to differentiate species. Since molecular species concepts have been applied to marine algae, cryptic species have been routinely detected. The ulvoids (sea lettuces) exemplify this challenge and species taxonomy has been poorly resolved. While *Ulva* species are often combined into a single functional group with other ephemeral foliose algae, studies incorporating molecular species concepts have revealed species specific differences in physiology, recruitment mechanisms, and interactions with higher trophic levels. Given the increased frequency of ulvoid blooms globally, and the accompanying potential for ecological disruption, this study set out to survey and identify the *Ulva* species found in central San Francisco Bay using the molecular barcode gene, *tufA*. This is the first study to use a molecular species concept in ulvoid identification within the San Francisco Bay. Sixty-two specimens were collected from six central bay localities between 2013-2015 and the *tufA* gene sequenced. These sequences were compared to 116 sequences available for the Ulvaceae in GenBank. Six OTUs were identified as *Ulva* species, two of which had not previously been reported for the San Francisco Bay. The more complete taxonomy presented here can guide future studies of *Ulva*, ulvoid blooms, and ulvoid introductions in San Francisco Bay and around the world.

INTRODUCTION

The accumulation of detached green macroalgae, also known as green blooms or tides, are stimulated by eutrophication producing many detrimental ecological conditions in embayments, including the loss of seagrass beds, as the bloom's rapid growth initially blocks light and their subsequent decay produces anoxic conditions (Nielsen et al. 2004, Han and Liu 2014). These conditions have led to shifts from communities of perennial macrophytes (rockweeds and seagrasses) towards low diversity assemblages dominated by a single genus of green macroalgae, *Ulva* Linnaeus (Valiela et al. 1997, Worm et al. 1999, Worm and Lotze 2006).The genus *Ulva* is widespread, and an ecologically important component of intertidal (Horn 1983) and subtidal (Shepherd and Hawkes 2005) habitats. Field collected individual thalli can form several simple morphologies including distromatic sheets known as blades, monostromatic tubes, and monostromatic tubes that flatten into distromatic blades near apex. All of these morphologies employ a discoid holdfast often with rhizoids for attachment to substrata (Abbott and Hollenberg 1976). Well adapted to respond to disturbance, the genus can tolerate a wide range of environmental conditions including varying temperatures, salinities and nutrients loads (Littler and Littler 1980, Fong et al. 1996). While this group has become notorious for the formation and negative impacts of green tides, several species have been used for biofuel production (Bikker et al. 2016), as bioindicators of pollution (Kozhenkova et al. 2006), for bioremediation (Sode et al. 2013) and extraction of bioactive products (antitumor, anti-inflammatory and antimicrobial to name a few) (Morelli et al. 2017).

Despite the ecological and economic importance of *Ulva* at a global scale, there have been few detailed regional studies of this group using molecular species concepts. This study focused on the central San Francisco Bay, CA region as this region is highly impacted by anthropogenic disturbance, contains most of the hard substrata in the SFB for macroalgal attachment and because salinity varies temporally across a wide range, this region had the potential to support a greater diversity of cryptic *Ulva* species (Josselyn and West 1985). The San Francisco Bay is one of the most heavily impacted estuaries in the world in terms of anthropogenic impacts because it is the final destination of most of California's agricultural drainage through the Sacramento-San Joaquin River Delta and is surrounded by urban development, both potential sources of eutrophication. Besides urban runoff, this center of commercial shipping has endured oil spills and ecological upheaval resulting from species introductions (Silva 1977, Carlton 1979, Josselyn and West 1985, Nichols et al. 1990). In spite of these anthropogenic alterations, the bay is home to shorebirds, serves as a nursery to commercially important species (fish and crabs), and provides habitat for threatened and endangered species (e.g., harbor seals, steelhead and Chinook salmon, and the California clapper rail)(Cloern and Jassby 2012). These important ecological functions would be impacted by increases in the magnitude of green tides within the bay.

Green tides are one of the most conspicuous effects of eutrophication on estuaries. The magnitude of green tide biomass in estuaries varies greatly worldwide and is not necessarily directly correlated to attached biomass. For example, in the San Francisco Bay, attached *Ulva* species dominate spring and summer intertidal assemblages and persist at low levels in fall and winter throughout the central bay (Romero, unpublished data). These attached populations contribute to periodic green tides with a maximum reported biomass of 1200 g · m^{-2} wet weight (Boyer and Wyllie-Echeverria 2010), just over half the critical amount observed to negatively impact eelgrass beds in Bodega Bay, CA approximately 76 km to the north (2000 g · m^{-2} wet weight; (Olyarnik 2008). Green tide biomass reported for other California estuaries is highly variable between estuaries as well as between years (Newport Bay > 1000 g wet weight · m^{-2}; (Kamer et al. 2001), Elkhorn Slough: 2244 g wet weight · m^{-2}; (Hughes et al. 2011), and Bodega Bay 4000 g wet weight · m^{-2}; (Olyarnik 2008). These central California blooms are small compared to the striking biomass accumulations reported for Qingdao, China (>1 million tons wet weight over 13,000–30,000 km^2) (Leliaert et al. 2008, Sun et al. 2008, Liu et al. 2013), several European countries including Ireland and Brittany (2164 tons; (Wan et al. 2017) and 100,000 tons; (Smetacek and Zingone 2013). These blooms can have important local economic influences; for example, the 2008 Qingdao bloom cost $100 million USD in algal removal to open waterways. Many of the species reported to dominate green tides globally have been reported from the California coast by morphological surveys, including *Ulva rigida* in Ireland, *U. intestinalis* in the Baltic Sea, *U. prolifera* and *U. compressa* in China yet little work has been done to confirm the species composition of California's nuisance blooms.

Distinguishing among *Ulva* species is critical because some species may be more associated with nuisance blooms than others. However, differentiating *Ulva* species solely based on morphological characteristics is unreliable as members exhibit simple morphologies with characters that vary within species and overlap across species (Tan et al. 1999, Blomster et al. 2002, Hayden et al. 2003, O'Kelly et al. 2010). Traditionally, *Ulva* species have been considered to be functionally redundant and little effort was made to identify species in ecological research. Those who have attempted to apply morphological species concepts in conjunction with ecological studies have identified ecophysiological differences between species (Beach et al.

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1995, Fong et al. 1996, Nelson et al. 2008, 2010). However, the application of molecular species concepts have revealed an abundance of cryptic species (Blomster et al. 2002, Guidone et al. 2013), species-specific tolerances to a variety of environmental factors (Liu et al. 2012, Song et al. 2015), variation in chemical composition (de Pádua et al. 2004), and a variety of ecological interactions (Guidone et al. 2010, 2012).

Several genetic markers have been used to differentiate *Ulva* species, including ITS, *rbc*L, and *tufA*. Evidence of divergent copies of ITS (Saunders and Kucera 2010) and low levels of genetic diversity in *rbc*L (Heesch et al. 2009), for the Ulvophyceae led to the development of the *tufA* barcode for use within this group of green algae in particular (Saunders and Kucera 2010). This barcode is often used in conjunction with the *rbc*L gene because of the historical use of *rbc*L in this group. However, recent surveys utilizing the *tufA* barcode in North America and Australasia have greatly increased the amount of *tufA* data available for comparison and have demonstrated its usefulness in distinguishing species within this class (Saunders and Kucera 2010, Kirkendale et al. 2013, Saunders 2014).

Considering the accumulating evidence of ecophysiological differences among *Ulva* species and the projected environmental impacts from climate change as well as future anthropogenic manipulation of the bay-delta complex, the application of a molecular species concept in this region is long overdue. The objectives of this study were to utilize the molecular *tufA* barcoding marker to estimate molecular species richness of *Ulva* within central San Francisco Bay and compare these results to previous estimates of California ulvoid biodiversity. Not only will correct species resolution allow the accurate estimation of potential shifts in ecological interactions, it will also facilitate a better evaluation of their potential for use as biofuels, and their use as bioindicators.

MATERIALS AND METHODS

Field Collection.

The San Francisco Bay system can be divided along increasing levels of salinity into three main sections: the North Bay (including Suisun Slough and San Pablo Bay), the South Bay (a marine lagoon), and central bay region that connects the North and South Bays to the Pacific through the Golden Gate (Figure 1). To estimate *Ulva* diversity in potential attached source populations within the bay, five sites within the central San Francisco Bay (from here forward SF bay) were selected for sampling. The central bay has ample rocky substrate for attachments, both natural and artificial along with the highest concentration of ports with greatest potential for species introductions. Algal specimens were hand collected at the Romberg Tiburon Center (37° 53' 30.912" N., 122° 26' 49.518" W) in September 2013, February, April, and July of 2014. Specimens were collected from four additional sites in November 2015: Point Potrero, Richmond, CA (37° 54' 27.0504" N, 122° 22' 26.0724" W), Point Isabel, Richmond, CA (37° 53' 56.418" N, 122° 19' 31.332" W), Berkeley Marina, Berkeley, CA (37° 51' 45.492" N, 122° 18' 53.49" W) and Ballena Bay Alameda Island (37° 45' 50.04" N, 122° 17' 0.378" W). A 50m transect was placed in the exposed intertidal, halfway between the highest observed macroalgae and the waterline and oriented parallel to the water line during full moon low tides to maximize the exposed collection area. The entire exposed area was searched and five individuals of each *Ulva* morphotype (expanded distromatic blade, narrow distromatic blade, monostromatic tube, and monostromatic tube that flattens into a blade) found were collected noting approximate

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location, date, and substratum. Hence, a maximum of 20 individuals were collected from a given site and date combination if all four morphologies were encountered. Tubular morphologies, including blades with tubular bases, were difficult to get sequences from due to their small size and are underrepresented in the sampling. Many of these specimens were smaller than 5cm in length and ranged from 1-5mm in width. For this reason, most of the specimens collected that yielded adequate sequences were from blades with abundant tissue. Specimens were transported back to the lab in a cooler with ice and stored at 4°C and processed within one week of collection.

Laboratory specimen processing.

Each specimen was assigned a unique collection number, rinsed with fresh tap water to remove excess salts, sediment, and diatoms. Specimens were dried on herbarium paper and portions of the thallus were removed with a clean razor blade and either frozen at -20°C or placed in silica gel for later DNA extraction.

DNA extraction, amplification, and sequencing.

DNA was extracted from either frozen or dried algal material using Qiagen DNeasy Plant and MoBio PowerPlant® Pro DNA Isolation kits following manufacturers protocol and separating two 50µl elutions. Dried algal material was prepared for DNA extraction following procedures outlined by Guidone (2013) in conjunction with the MoBio kit and PowerLyzer24. Double-stranded amplification of the plastid encoded gene *tufA* was performed in 20 µl reactions in a BioRad (Hercules, CA, USA) thermocycler, using the primers developed by Saunders and Kucera (2010) and an optimized profile. Each reaction consisted of 2 µl 10 x buffer with 15mM $MgCl_2$, 2 µl 25mM $MgCl_2$, 1.6 µl of 2.5mM each dNTPs, 1 µl each primer, 4 µl of 1 M Betaine, 0.160 µl T. aq (Amplitaq Gold or Roche) and 1 µl each undiluted or diluted DNA extract (1:10). Amplification profiles consisted of an initial denature at 94°C for 5 minutes followed by 35 cycles of 94°C for 1 minute, annealing at 47.5°C for 2 minutes, extension at 72°C for 3 minutes and then a final extension at 72°C for 7 minutes for *tufA*. Specimens producing multiple bands were amplified with a lowered final $MgCl_2$ concentration of 2.0 using touchdown PCR that included an initial denature at 94°C for 3 minutes followed by phase 1, consisting of 15 cycles of 94°C for 30 seconds, 45 seconds of annealing at temperatures ranging between 63°C-49°C (annealing temperature decreased by 1°C with each subsequent cycle in phase 1) and extension of 1 minute. Phase 2 consisted of 20 cycles of denature for 30 seconds at 94°C, annealing for 45 seconds at 55°C and extension of 1 minute at 72°C and a final extension at 72 for 5 minutes before holding at 4°C. Amplicons were visualized on 1% Agarose I Gels to confirm successful amplification prior to sequencing. PCR clean up and Sanger sequencing were performed by the University of California, Berkeley Sequencing Facility.

Successful forward and reverse sequences were aligned and edited using Geneious v. 10.1.3 (2005-2017 Biomatters Ltd). The single consensus sequences were aligned to representative *tufA* sequences of Ulvaceae species retrieved from GenBank using SUMAC v. 2.21 (Freyman 2015). When possible, these sequences were supplemented with 4 additional *tufA* sequences spanning geographic and temporal variation for each species represented and aligned using Geneious ® 10.1.3 (Appendix A.1).

Alignment and molecular analysis.

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The final data matrix consisted of a 794 bp sequence for *tufA* for 178 specimens. Sixty-two of these were collected from San Francisco Bay as a part of this study, and the remaining 116 were reference sequences downloaded from GenBank representing 33 taxa (including 1 outgroup taxon of the order Ulotrichales). Maximum likelihood phylogenetic analyses were performed using RAxML – HPCv.8 (Stamatakis 2014) on XSEDE using the CIPRES Portal Science Gateway v. 3.1 (Miller et al. 2010) with rapid bootstrapping (1000 iterations, GTRCAT substitution model). The resulting phylogenetic tree was rooted by hand in Figtree v.1.4.3 on the branch connecting the outgroup, *Acrosiphonia coalita* to the ingroup of the order Ulvaceae.

RESULTS

Molecular assessment of species richness

A total of 23 unique *tufA* sequences were found among the 62 specimens collected in central San Francisco bay (from here forward "SF"). The *tufA* gene region resolved these specimens into seven OTUs (A-G) within a monophyletic *Ulva* clade (72%) and a single monophyletic clade sister to *Blidingia marginata* (OTU H) (Figure 2). Inclusion of the recently described, *Ulva ohiohilulu*, resulted in three strongly supported clades UI (77%), UII (89%), and *U. ohiohilulu* (100%) grouped sister to UI (65% bootstrap support for UI with *U. ohiohilulu*) within the larger monophyletic *Ulva* clade (Figure 2). The UI clade (77%) included *Ulva flexuosa*, *Ulva ovata* (as *Enteromorpha*), *Ulva iliohaha*, *Ulva erecta*, *Ulva sp.* VRTC0022, *Ulva californica*, *Ulva tanneri*, *Ulva torta, Ulva procera*, *Ulva stenophylla*, *Ulva linza*, *Ulva prolifera*, *Ulva shanxiensis*, *Ulva gigantea*, *Ulva ohnoi*, *Ulva fasciata*, *Ulva rigida*, *Ulva laetevirens,* and *Ulva taeniata*. The UII clade (89%) included *Ulva australis*, *Ulva lactuca*, *Ulva arasakii*, *Ulva lobata*, *Ulva intestinalis*, *Ulva compressa*, and *Ulva howensis*. These UI and UII clade designations are consistent with results of (Kirkendale et al. 2013) who used *rbc*L to confirm nomenclature assignments. The outgroup, *Acrosiphonia coalita*, was distantly related to all *Ulva* as well as all *Blidingia*. Consistent with Kirkendale et al. (2013), all *Umbraulva* grouped sister to the *Ulva* clade with moderate support (66%) and *Ulvaria obscura* more distantly related to these two lineages (100%). OTU H was composed of four of the SF bay specimens (99%) sister to *Blidingia marginata* and are assigned the name *Blidingia* spp. due to long branch lengths.

Greater than 75% of SF specimens were distributed among four of the seven OTUs resolved by *tufA* (Figure 2, Table 1). Nearly all OTUs (Figure 2; A, B, C, E, F) were strong to moderately supported (≥73%) monophyletic groups with the exception of two lineages, OTUs B and D. OTUs A, C, E, (all 100%) and F (94%) were identified as *Ulva australis*, *Ulva compressa*, *Ulva lobata*, and *Ulva procera*, respectively. Specimens previously identified as *Ulva pertusa* in GenBank formed a strongly supported monophyletic clade (100%) with *U. australis*. These two species have been synonymized (Couceiro et al. 2011), thus OTU A was assigned the name *U. australis*. OTU B grouped with *Ulva californica* specimens in a moderately supported monophyletic clade (73%) nested within a weakly supported polyphyletic clade that included six SF specimens (VBKM0258, VBKM0255, VPTI0268, VBKM0260, VPTP1136, and VRTC0022), *Ulva flexuosa* (OTU D), *Ulva erecta*, *Ulva iliohaha*, and *Enteromorpha ovata*. Five of these six SF specimens grouped moderately (55%) with *Ulva flexuosa* specimens within OTU D and are tentatively identified as *Ulva flexuosa* (Figure 2; Table 1). The sixth of these specimens, VRTC0022 was weakly supported (21%) sister to *U. californica* and was assigned the name *Ulva* sp. OTU F included all *U. procera* specimens along

with European and Chinese *U. linza* accessions and a single Chinese *U. prolifera* accession (Appendix A.1). All other *U. linza* of Northeastern Pacific origin and *U. prolifera* accessions from the North Atlantic included in the analyses were differentiated as distinct species with strong support (100% for each clade). This result is consistent with those of previous studies using *rbc*L and ITS (Tan et al. 1999, Hayden and Waaland 2004). For this reason, SF specimens within OTU F were identified as *Ulva procera*.

Spatial patterns of species composition.

Ulva species were identified from all SFB sites and in all seasons at Tiburon. However, species composition of *Ulva* assemblages varied among the five sites (Figure 3). Increased temporal resolution at the Tiburon site yielded a similar number of OTUs (5) as a single collection from the remaining sites (4 OTUs at each site) with the exception of Alameda Island. Only a single species was collected from Alameda Island in November 2015. However, *Ulva lobata* and *Ulva* sp. (VRTC0022) were collected from Tiburon and were not collected from any of the other sites surveyed.

DISCUSSION

This study provides resolution of *Ulva* relationships at several taxonomic and spatial scales. At the broadest scale, 25 ulvoid lineages were resolved by integrating new *tufA* data collect from SFB with data from GenBank. Several of these lineages, *Ulva taeniata*, *Ulva ridgida*, *Ulva laetevirens*, and *Ulva flexuosa* require further sampling and taxonomic scrutiny to clearly delineate their relationships within the genus. For example, only two *Ulva taeniata* specimens were available in GenBank, one of which grouped with moderate support (58%) sister to a strongly supported clade (97%) that included *U. rigida* and *U. laetevirens* while the second formed a polytomy (67%) with *U. reticulata* and *U. beytensis* within the strongly supported (91%) *U. ohnoi* clade. Other clades apparently lacking sufficient data to provide further resolution include an *Ulva iliohaha* and *U. erecta* clade, (95% bootstrap support), that is sister to *U. tanneri* (no support), but only represented by a single sequence. These relationships do, however, support those of Spalding et al. (2015) in their initial descriptions of mesophotic Hawaiian *Ulva* species (*U. iliohaha* and *U. ohiohilulu*).

Floristics of San Francisco Bay.

Detailed sampling through the SFB demonstrated that at least 6 *Ulva* OTUs are found in this region. Of these 6 OTUs, two grouped with *Ulva* species previously reported from California and SFB using morphological assessments but these species were absent from subsequent molecular surveys of the North East Pacific (*Ulva compressa* and *Ulva flexuosa*) (Table 1). Alternatively, *Ulva australis* (OTU A) and *Ulva procera* (OTU F) were not previously reported within the SFB and were only recently reported for California when molecular species concepts were applied (Table 1). OTU's A and F are discussed in further detail below. One specimen, VRTC0022 was ambiguously placed sister to the *Ulva californica* clade (OTU B) with weak bootstrap support (21%) and within OTU D. This is the only SFB collected specimen that remains unidentified. Review of sequence data for VRTC0022, revealed several ambiguous bases that likely contributed to the uncertainty the placement of this specimen.

This survey focused on five exemplar regions of the central San Francisco Bay, but was not exhaustive and previous surveys have documented other *Ulva* species within the central SFB and throughout the estuary (Table 1). Increased temporal and spatial resolution are necessary to further investigate why some species were not observed at all sites. Seasonal changes in timing of recruitment and species-specific physiological tolerances to changes salinity gradients due to winter increases in precipitation could result in seasonal variability in species composition at these sites. Surveys of other habitat types including mudflats, and salt marsh habitats could reveal increased diversity of *Ulva* species as well. Further sampling of the North and South Bays would likely confirm the presence of *Ulva expansa*, an alga previously only reported for salt marsh habitats within the bay (Silva 1979). Free floating tubes are also common in lower salinity habitats and were not sampled in this study; however, *tufA* did tentatively detect *Ulva flexuosa* an alga commonly found free floating in estuaries as well as attached. SFB *U. flexuosa* specimens primarily consisted of attached tubes and tubes that flattened into blades at the apex. Other typically tubular species previously detected from the bay include *Ulva intestinalis*, *U. prolifera*, and *U. linza*. Although these species can also be found growing attached to rocks or wood, further sampling of detached assemblages could confirm their presence. Additionally, no tubular *Ulva* was collected from Alameda Island in November 2015. Tube shaped *Ulva* is much more difficult to find in riprap assemblages than the larger more conspicuous blades, therefore representation of this morphology in this dataset is low. It is likely that with additional sampling across the tubular morphologies and habitat types, more species would be detected.

In addition to the need for further sampling, additional genetic data will be useful for further refining the taxonomy of ulvoids. Three species of *Ulva* previously reported for California (*Ulva clathrata, Ulva expansa*, and *Ulva pseudocurvata*), two of which have been reported for San Francisco Bay (*U. expansa* and *U. clathrata*), are lacking *tufA* sequences in GenBank (Table 1). *Ulva clathrata*, (morphological identification) is a tube forming species known to bloom on mudflat habitats of the Richardson Bay, a small embayment within San Francisco Bay (Shellum and Josselyn 1982). As mentioned above, mudflat habitats were not sampled in this study and the only unidentified specimen was of blade morphology. While the ability to identify this species was hindered, collection of this species was unlikely. Hayden and Waaland (2004) proposed that *Ulva* with expanded blade morphologies collected on the northeastern Pacific coast previously classified as *U. expansa*, *U. fenestrata*, *U. lactuca*, and *U. lobata* were all in fact *U. lobata* based on similarities in thallus habit and ecology as detailed in Setchell and Gardner's (1920) original description of *U. lobata*. Of these taxa, *Ulva fenestrata* is currently considered a synonym of *Ulva lactuca* (Hayden et al. 2003) and type specimens of *U. lactuca* have matched molecular signatures of *U. fasciata* (O'Kelly et al. 2010). *Ulva lactuca* (UII) and *Ulva fasciata* (UI) were resolved as distinct taxa (100%, 99%), in different *Ulva* clades. GenBank accessions for *Ulva lactuca* included northeastern Pacific and northwestern Atlantic specimens while those for *U. fasciata* were predominantly from Australia. Further sampling of SFB or sequencing of type specimens would be necessary to sort this out considering that only *U. lobata* was encountered in this study and other surveys in central California have not reported *Ulva expansa*. Several other currently accepted *Ulva* species (i.e. *Ulva stipitata*) also lack *tufA* sequences in GenBank; the addition of these taxa and sequencing of type material of *Ulva expansa* could better resolve some of the ambiguous identifications (*Ulva flexuosa* and *Ulva sp.* VRTC0022).

Identity of green tide species

The application of the tufA barcode in conjunction with specimens from a wide geographic range, has allowed us to resolve some inconsistencies with regards to the relationships between the common green tide forming species: Ulva linza, Ulva procera, and Ulva prolifera (also known as the LPP complex). Hayden and Waaland (2004) also reported inconsistencies resolving a monophyletic Ulva linza clade using both rbcL and ITS gene regions. They found that Ulva linza specimens collected in northern California grouped with weak support to U. linza, U. procera, and U. prolifera specimens of European and Japanese origin. Their results strongly supported a clade including European U. linza and U. procera specimens regardless of distinct morphological differences. In this study, European U. linza grouped with U. prolifera from China and U. procera from the northeastern Pacific, north Atlantic, and Australia (93%).

Ambiguities in resolving *Ulva linza*, *Ulva procera*, and *Ulva prolifera* using *rbc*L have become apparent to those working to identify the sources of nuisance green tides. Prior to the recommendation of *tufA* as a barcode, the source alga of the 2008 Qingdao blooms was identified as *Ulva prolifera* but taxonomic studies employing *rbc*L found that it fell within the LPP complex with *Ulva linza*, *Ulva procera*, and *Ulva prolifera* (Leliaert et al. 2009). Guidone et al. (2013), employing *rbc*L and ITS, detected tubular *Ulva sp.* in the green tides of Narragansett Bay, RI that grouped strongly within the LPP complex and matched the genotypes of the Qingdao strains. Inclusion of specimens from a wider geographic range for *U. linza*, *U. prolifera*, and *U. procera* combined with strong bootstrap support for these clades in this study, indicates that these particular ambiguously placed *U. linza* and *U. prolifera* specimens within the *Ulva procera* clade may be misidentifications and supports the usefulness of this barcode for resolving species within this genus. In fact, Gabrielson et al. (Gabrielson et al. 2012) provisionally assigned the 2008 Qingdao alga to *Ulva procera* in their key to the seaweeds and Seagrasses of Southeast Alaska, British Columbia, Washington, and Oregon. Further sampling of these taxa across a broader geographic range, including the UK, Germany, China, and eastern USA using the *tufA* gene region in conjunction with secondary barcodes would be necessary to eliminate any potential influence of biogeographic variation on these relationships and confirm the identity of the Qingdao green tides (see Appendix A.1 for accession numbers of GenBank specimens).

Although green tides in the SFB occur at low biomass on the global scale, several notorious green tide producing lineages have been reported for the region. The *tufA* barcode revealed four OTUs present in SFB that are known to produce green tides worldwide including *Ulva australis* (as *U. pertusa*), *U. compressa*, *U. procera*, and *U. flexuosa* (Guidone et al. 2013). *Ulva australis*, through morphological and molecular identification, has been attributed with green tides in New England (Maine/New Hampshire, USA: (Hofmann et al. 2010), Japan (Kawai et al. 2007), and Korea (Sidharthan et al. 2004). Green tides of both NW and NE Atlantic have included *Ulva compressa* (British Isles: (Taylor et al. 2001), Finland: (Leskinen et al. 2004), Maine/New Hampshire, USA: (Hofmann et al. 2010), Rhode Island, USA: (Guidone et al. 2013)), while *Ulva flexuosa* has only been reported to contribute to blooms in the Rhode Island, USA (Guidone et al. 2013). Finally, as mentioned above, the *Ulva procera* genotype from this study matches the genotype responsible for the massive green tides recurring since 2008 in Qingdao China. Given the frequency of known green tide lineages present in the SFB, further research needs to be done to explain why green tides in this region are so benign and under what conditions they could become a greater nuisance.

Potential species invasions

Given the heavily invaded status of San Francisco Bay, we expected to detect potentially recent *Ulva* invasions. Previous work by Hayden and Waaland (2004) first reported *Ulva australis* (as *Ulva pertusa* Kjellman) in Southern California (Orange and San Diego Counties), then in Punta Baja, Baja California, Mexico (Aguilar-Rosas et al. 2008) and most recently it was reported in Monterey, California by Saunders (2014). This study documents a new northernmost record for this species on the Pacific coast of North America with high abundance, as even with a small sample size it was found at three of the 5 bay sites (Figure 3). This alga is thought to be of Asiatic origin and has been introduced to North America, Europe, and Australia. The exact method of introduction is unknown but possible vectors identified for European introductions by Couceiro et al. (2011) including attachment to boat hulls (Stegenga 2007), ballast water (Flagella et al. 2010), and through the shellfish industry where seaweeds are often used as packaging material are all likely to have introduced this species in California as well. *Ulva procera* was unreported for the California coast prior to 2010 at which point two specimens were collected on the outer coast of central California (Saunders 2014). Specimens grouping with *U. procera* were collected at all but one location within the central SFB indicating that this species is also well established in the region (Figure 3). More detailed taxonomic work including morphology and herbarium collections is needed to determine if these species are introduced or cryptic diversity revealed by the application of a molecular species concept.

In closing, the *tufA* barcode detected four *Ulva* lineages previously reported for the San Francisco Bay region based on morphological traits and two lineages, *Ulva australis* and *U. procera*, previously reported for California but not within the bay. The former represents a new northernmost record. Given that morphological characteristics are not reliable for species identification for this genus, this study improves our knowledge of *Ulva* diversity within SFB. However, these identifications must be confirmed through further sequencing of a secondary barcode (*rbcL*) and data generation from herbaria specimens previously collected by Silva (1979) within the bay. Of particular interest are species known to contribute to green tides worldwide in order to monitor their potential impacts on SFB ecosystems. Several species known to produce green tides were confirmed at multiple locations in the SFB. However, continued monitoring of the four known nuisance bloom species is necessary to better understand the conditions under which these species produce nuisance green tides. This study is the first, to use genetic barcoding to identify *Ulva* species in SFB and provides preliminary data towards future prospects for the use of SFB *Ulva* in biomedical research and bioremediation. Inclusion of a broad range of taxonomic data recently available for the *tufA* barcode region elucidated potential species misidentifications among these lineages as well. The genetic information provided by this study, though focused on one of three major ecoregions of the San Francisco Bay delta complex, improved our understanding of *Ulva* diversity in central California and can be utilized in conjunction with ecophysiological studies, environmental monitoring, resource extraction and management planning.

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TABLES

Table 1. *Ulva* species reported from the California coast (Abbott and Hollenberg 1976, Hayden and Waaland 2004) and San Francisco Bay (SFB) (Silva 1977). Nomenclature reflects recent changes based on Guiry and Guiry (2017) and Hayden et al (2003).

California		San Francisco Bay			OTU classification of SFB specimens from this study
Abbott and Hollenberg (1976)	Hayden and Waaland (2004)*	Silva (1977)	Josselyn and West (1985)	Genus species	
•[a]				*Blidingia dawsonii* (Hollenberg & I.A.Abbott) S.C.Lindstrom, L.A.Hanic & L.Golden	
				Blidingia marginata (J.Agardh) P.J.L.Dangeard ex Bliding	H
•		•		*Blidingia minima* (Nägeli ex Kützing) Kylin	
•				*Blidingia minima var. subsalsa* (Kjellman) Scagel	
•		•	•	*Blidingia minima var. vexata* (Setchell & N.L.Gardner) J.N.Norris	
				Blidingia subsalsa (Kjellman) Kornmann & Sahling ex Scagel et al.	
	•[b]			*Ulva australis* Areschoug	A
•[c]	•	•[c]	•[c]	*Ulva californica* Wille	B
•[d]		•[d]	•[d]	*Ulva clathrata*[1] (Roth) C.Agardh	
•[e]		•[e]		*Ulva compressa* Linnaeus	C
•		•		*Ulva expansa*[1] (Setchell) Setchell & N.L.Gardner	
•	•			*Ulva fasciata* Delile	
•[f]		•[f]		*Ulva flexuosa* Wulfen	D
•[g]	•	•[g]	•[g]	*Ulva intestinalis* Linnaeus	
•[h]			•	*Ulva lactuca* Linnaeus	
•[i]	•	•[i]	•[i]	*Ulva linza* Linnaeus	
•	•	•	•	*Ulva lobata* (Kützing) Harvey	E
•[j]				*Ulva nematoidea* Bory	
•[k]	•	•[k]	•[k]	*Ulva prolifera* O.F.Müller	
	•			*Ulva pseudocurvata*[1] Koeman & Hoek	
•	•			*Ulva rigida* C.Agardh	
•	•			*Ulva stenophylla* Setchell & N.L.Gardner	
•	•			*Ulva taeniata* (Setchell) Setchell & N.L.Gardner	
	•			*Ulva tanneri* H.S.Hayden & J.R.Waaland	
				Ulva procera (K.Ahlner) Hayden, Blomster, Maggs, P.C.Silva, M.J.Stanhope & J.R.Waaland	F

* Indicates molecular species concept in association with morphological assessment. Superscripts refer to original nomenclature as follows: [a]*Percursaria dawsonii*, [b]*Ulva pertusa*, [c]*Ulva angusta*, [d]*Enteromorpha clathrata*, [e]*Enteromorpha compressa*, [f]*Enteromorpha flexuosa*, [g]*Enteromorpha intestinalis*, [h]*Ulva dactylifera*, [i]*Enteromorpha linza*, [j]*Ulva costata*, [k]*Ulva prolifera*.
[1]Taxa lacking *tufA* data in Genbank.

FIGURE CAPTIONS

Figure 1. Map of the study location with locations of specimen collections labeled in bold and marked (●). Map courtesy of Cassandra J. Hansen 2018.

Figure 2. Phylogram of *Ulva tufA* sequence data with SF Bay specimens in bold font. Bootstrop support values ≥50 are above branches unless referred to in the text. Specimens grouping within clades that do not match their Genbank species name are labeled with Genbank species name next to their Genbank accession number.

Figure 3. Species composition of attached *Ulva* assemblages at the five collection sites. Species representing less than 10% of the *Ulva* assemblage are not labeled with values. *Ulva* species ID's based on a molecular species concept using the *tufA* barcode region.

FIGURES

Figure 1.
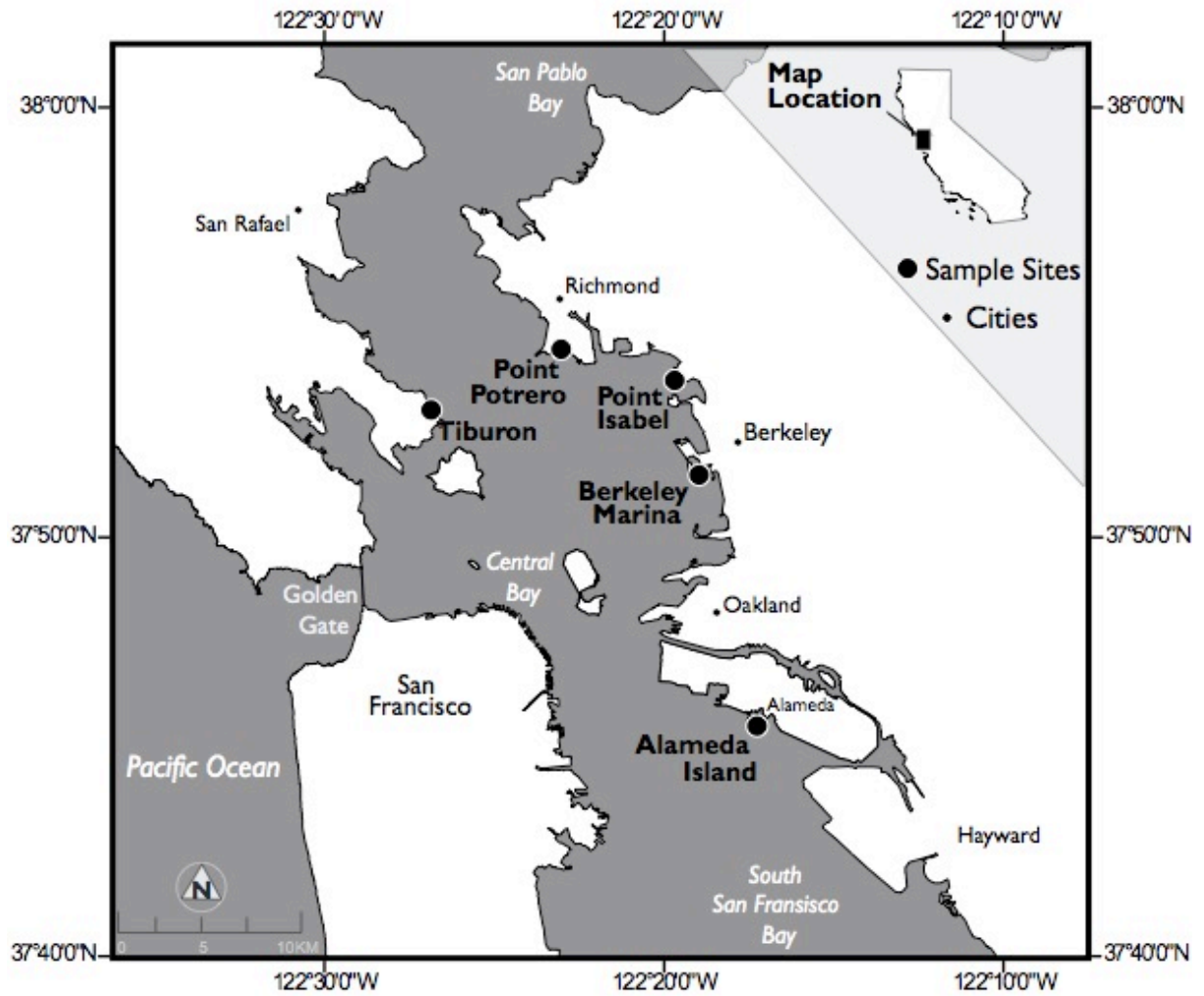
Figure 2.

Figure 3.

Composition of attached *Ulva* species in central San Francisco Bay (%)
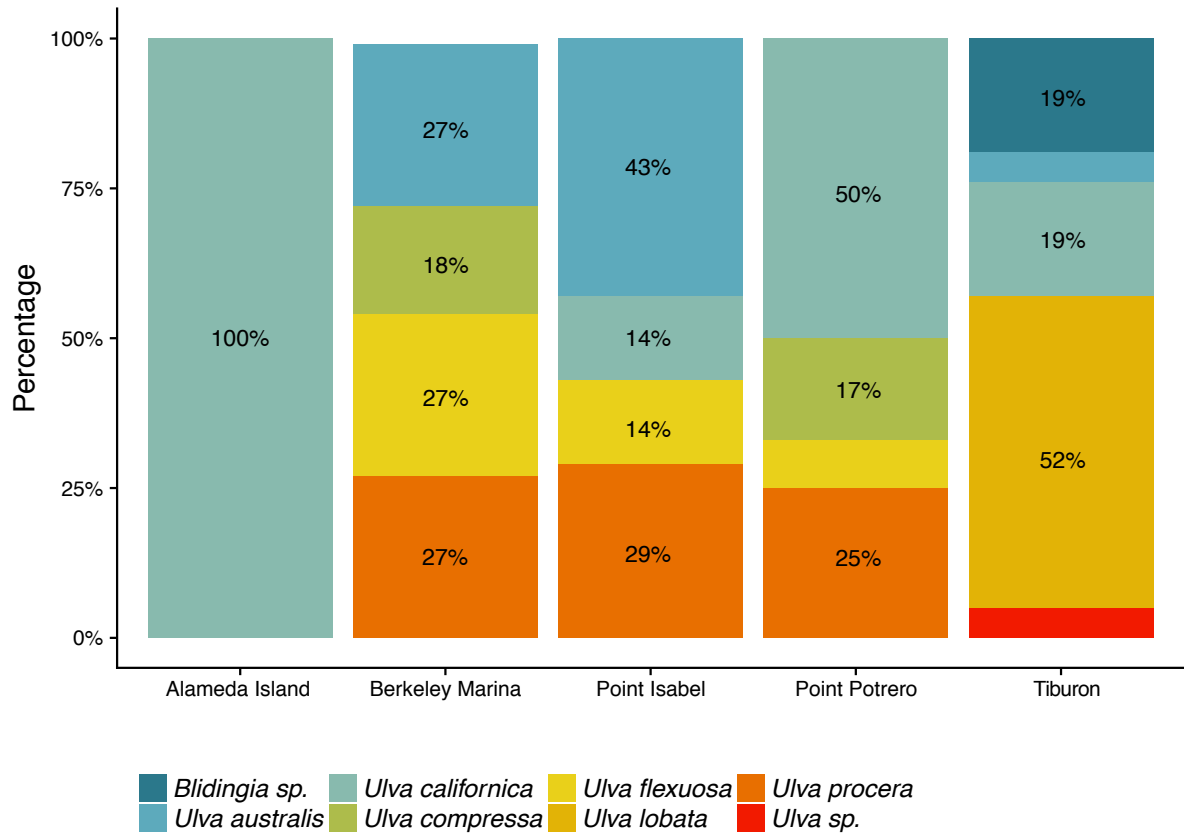
Appendix A.1–Supplemental Tables

Table S1. GenBank accessions used in analyses. [1]Accessions removed from final analyses if unidentified and not matching any SFB specimens.

Ocean Region	Locality	Name	GenBank No.	VoucherID
NW Atlantic	Canada: New Brunswick, Letete exposed biodiversity site, Bay of Fundy	*Blidingia marginata*	HQ610237.1	
NE Pacific	Canada: British Columbia, Butze Rapids, Prince Rupert	*Blidingia marginata*	HQ610238.1	
NE Atlantic	Germany: Schleswig-Holstein, Heiligenhafen	*Blidingia marginata*	KT290276.1	
NW Atlantic	Canada: Newfoundland and Labrador, Deer Arm, Bonne Bay	*Blidingia minima*	HQ610239.1	
NE Atlantic	Germany: Mecklenburg-Vorpommern, Wohlenberg	*Blidingia minima*	KT290281.1	
NE Atlantic	United Kingdom	*Blidingia minima*	EF595343.1	
NE Pacific	Tiburon, San Francisco Bay	*Blidingia sp.*		VRTC0015
NE Pacific	Tiburon, San Francisco Bay	*Blidingia sp.*		VRTC0016
NE Pacific	Tiburon, San Francisco Bay	*Blidingia sp.*		VRTC0027
NE Pacific	Tiburon, San Francisco Bay	*Blidingia sp.*		VRTC0029
		Enteromorpha ovata	KC661429.1	
		UNVERIFIED: Ulva[1]	KC411833.1[1]	
		Ulva arasakii	AB561079.1	
NE Pacific	Tiburon, San Francisco Bay			VRTC0013
NE Pacific	Berkeley Marina, San Francisco Bay			VBKM0251
NE Pacific	Berkeley Marina, San Francisco Bay			VBKM0252
NE Pacific	San Francisco Bay			Bio1B01
NE Pacific	Point Isabel, San Francisco Bay			VPTI0258
NE Pacific	Point Isabel, San Francisco Bay			VPTI0257
NE Pacific	Berkeley Marina, San Francisco Bay			VBKM1201
NE Pacific	Point Isabel, San Francisco Bay			VPTI0264
NE Pacific	Canada: British Columbia, Bamfield, Seppings Island	*Ulva australis*	HQ610378.1	
SW Pacific	Australia: Western Australia, Pt. Peron	*Ulva australis*	JN029252	
SW Pacific	Australia: Tasmania, Tinderbox (Fiona's Point)	*Ulva australis*	JN029270	
NE Pacific	Canada: British Columbia, Stephenson Pt., Nanaimo	*Ulva australis*	HQ610379	
CW Pacific	China	*Ulva australis*	KC411857	
		Ulva beytensis	JF918547.1	
NE Pacific	Tiburon, San Francisco Bay	*Ulva californica*		VRTC0006
NE Pacific	Tiburon, San Francisco Bay	*Ulva californica*		VRTC0007
NE Pacific	Tiburon, San Francisco Bay	*Ulva californica*		VRTC0021
NE Pacific	Tiburon, San Francisco Bay	*Ulva californica*		VRTC0023

Ocean Region	Locality	Name	GenBank No.	VoucherID
NE Pacific	Alameda Island, San Francisco Bay	*Ulva californica*		VBBA1207
NE Pacific	Alameda Island, San Francisco Bay	*Ulva californica*		VBBA1208
NE Pacific	Point Potrero, San Francisco Bay	*Ulva californica*		VPTP1138
NE Pacific	Point Potrero, San Francisco Bay	*Ulva californica*		VPTP1137
NE Pacific	Point Potrero, San Francisco Bay	*Ulva californica*		VPTP1135
NE Pacific	Point Potrero, San Francisco Bay	*Ulva californica*		VPTP1133
NE Pacific	Alameda Island, San Francisco Bay	*Ulva californica*		VBBA1205
NE Pacific	Alameda Island, San Francisco Bay	*Ulva californica*		VBBA1206
NE Pacific	Point Potrero, San Francisco Bay	*Ulva californica*		VPTP1132
NE Pacific	Point Potrero, San Francisco Bay	*Ulva californica*		VPTP1131
NE Pacific	Alameda Island, San Francisco Bay	*Ulva californica*		VBBA0251
NE Pacific	Alameda Island, San Francisco Bay	*Ulva californica*		VBBA0252
NE Pacific	Alameda Island, San Francisco Bay	*Ulva californica*		VBBA0253
NE Pacific	Alameda Island, San Francisco Bay	*Ulva californica*		VBBA0254
NE Pacific	Alameda Island, San Francisco Bay	*Ulva californica*		VBBA0255
NE Pacific	Alameda Island, San Francisco Bay	*Ulva californica*		VBBA0256
NE Pacific	Alameda Island, San Francisco Bay	*Ulva californica*		VBBA0257
NE Pacific	Point Isabel, San Francisco Bay	*Ulva californica*		VPTI0269
NE Pacific	USA: False Bay, San Juan Island, WA	*Ulva californica*	AY454401.1	
NE Pacific	Canada: British Columbia, Ridley Island (south of coal terrminal), Prince Rupert	*Ulva californica*	HQ610279	
NE Pacific	USA: California, Pigeon Point Lighthouse	*Ulva californica*	KM255003	
NW Atlantic	Australia: Victoria, St. Kilda, intertidal man-made boulder wall	*Ulva californica*	JN029283	
Mediterranean Sea	Italy:Adriatic Sea	*Ulva californica*	HE600173	
NE Pacific	Berkeley Marina, San Francisco Bay	*Ulva compressa*		VBKM0261
NE Pacific	Point Potrero, San Francisco Bay	*Ulva compressa*		VPTP1118
NE Pacific	Point Potrero, San Francisco Bay	*Ulva compressa*		VPTP1142
NE Pacific	Berkeley Marina, San Francisco Bay	*Ulva compressa*		VBKM0259
Mediterranean Sea	Italy:Adriatic Sea	*Ulva compressa*	HE600184.1	

Ocean Region	Locality	Name	GenBank No.	VoucherID
NE Pacific	Canada: British Columbia, Bamfield, `Sparlingia Pt.`, Bradys Beach	*Ulva compressa*	HQ610292	
NW Atlantic	Canada: New Brunswick, Lepreau exposed biodiversity site, Bay of Fundy	*Ulva compressa*	HQ610286	
SW Pacific	Australia: New South Wales, Dolphin Point, on rock platform	*Ulva compressa*	JN029289	
NE Pacific	USA: California, Sea Lion Point North (frontside), Point Lobos State Reserve	*Ulva compressa*	KM255037	
		Ulva erecta	KC661427.1	
SW Pacific	Australia: New South Wales, Green Island, on rock platform	*Ulva fasciata*	JN029299.1	
		Ulva fasciata	NC_029040	
		Ulva fasciata	KT882614	
SW Pacific	Australia: New South Wales, Algae Hole North, Lord Howe	*Ulva fasciata*	JN029306.1	
SW Pacific	Australia: New South Wales, Old Gulch, Lord Howe I.	*Ulva fasciata*	JN029305.1	
NE Pacific	Point Isabel, San Francisco Bay			VPTI0268
NE Pacific	Berkeley Marina, San Francisco Bay			VBKM0255
NE Pacific	Berkeley Marina, San Francisco Bay			VBKM0258
NE Pacific	Point Potrero, San Francisco Bay			VPTP1136
Mediterranean Sea	Italy:Adriatic Sea	*Ulva flexuosa*	HE600177.1	
SW Pacific	Australia: New South Wales, Manyana Beach rock platform	*Ulva flexuosa*	JN029308	
NE Pacific	Canada: British Columbia, Backeddy Resort	*Ulva flexuosa*	HQ610296	
CW Pacific	South Korea: Cheju-do, Jeju, Seongsan	*Ulva flexuosa*	JN029309.1	
SW Pacific	Australia: Western Australia, Blackwall Reach, Swan River	*Ulva flexuosa*	JN029307.1	
NW Atlantic	Canada: New Brunswick, Lepreau exposed biodiversity site, Bay of Fundy	*Ulva gigantea*	HQ610297.1	
NW Atlantic	Canada: New Brunswick, Lepreau exposed biodiversity site, Bay of Fundy	*Ulva gigantea*	HQ610300	
NW Atlantic	Canada: New Brunswick, Lepreau exposed biodiversity site, Bay of Fundy	*Ulva gigantea*	HQ610298	

Ocean Region	Locality	Name	GenBank No.	VoucherID
NW Atlantic	Canada: New Brunswick, Letete exposed biodiversity site, Bay of Fundy	*Ulva gigantea*	HQ610299.1	
SW Pacific	Australia: New South Wales, Far Rocks, Signal Point, Lord Howe	*Ulva howensis*	JN029310.1	
SW Pacific	Australia: New South Wales, Far Rocks, Signal Point, Lord Howe	*Ulva howensis*	JN029311.1	
SW Pacific	Australia: Western Australia, Emu Beach Holiday Park	*Ulva howensis*	JN029318	
SW Pacific	Australia: New South Wales, Far Rocks, Signal Point, Lord Howe	*Ulva howensis*	JN029312.1	
SW Pacific	Australia: New South Wales, Narrawallee Beach rock platform	*Ulva howensis*	JN029315.1	
N Pacific; mesophotic	USA: Hawaii	*Ulva iliohaha*	KT932976.1	
NE Pacific	USA: False Bay, San Juan Island, WA	*Ulva intestinalis*	AY454399.1	
NW Atlantic	USA: Maine, Cape Elizabeth, near Portland	*Ulva intestinalis*	HQ610323	
NE Pacific	Canada: British Columbia, Bamfield, Dixon I.	*Ulva intestinalis*	HQ610316	
NW Atlantic	Canada: New Brunswick, New River Beach, Bay of Fundy	*Ulva intestinalis*	HQ610319	
NW Atlantic	USA: Rhode Island, Hazard Ave., Narragansett	*Ulva intestinalis*	HQ610308	
NW Atlantic	USA: Maine, End of public road, Starboard	*Ulva lactuca*	HQ610325.1	
NE Pacific	USA: California, Pigeon Point Lighthouse	*Ulva lactuca*	KM255044	
NE Pacific	Canada: British Columbia, Pachena Beach, Bamfield	*Ulva lactuca*	HQ610326	
NW Atlantic	Canada: New Brunswick, Harrington Cove exposed biodiversity site, Grand Manan	*Ulva lactuca*	HQ610335	
NW Atlantic	USA: Rhode Island, Governor Sprague Bridge 17, Narragansett	*Ulva lactuca*	HQ610357	
NW Atlantic	Canada: New Brunswick, Kouchibouguac lagoon seagrass beds	*Ulva laetevirens*	HQ610428.1	
NW Atlantic	Canada: New Brunswick, Kouchibouguac lagoon seagrass beds	*Ulva laetevirens*	HQ610428	
SW Pacific	Australia: New South Wales, North Brighton intertidal man-made boulder wall	*Ulva laetevirens*	JN029322	

Ocean Region	Locality	Name	GenBank No.	VoucherID
SW Pacific	Australia: Western Australia, Windy Harbour	*Ulva laetevirens*	JN029327.1	
NW Atlantic	USA: Holly Pond, Stamford, Connecticut	*Ulva laetevirens*	JQ048943.1	
NE Atlantic	United Kingdom: East Cornwall, Greenaway	*Ulva linza*	EF595300.1	
ortheast Pacif	Canada: British Columbia, Otter Point, near Sooke, Vancouver Island	*Ulva linza*	HQ610367	
ortheast Atlan	Germany: Schleswig-Holstein, Heiligenhafen	*Ulva linza*	KT290273.1	
NE Pacific	USA: California, Bird Rock, Pacific Grove	*Ulva linza*	KM255053.1	
NE Pacific	USA: California, Stillwater Cove, Pebble Beach	*Ulva linza*	KM255042.1	
CW Pacific	China	*Ulva linza*	KC411858	
NE Pacific	Tiburon, San Francisco Bay			VRTC0008
NE Pacific	Tiburon, San Francisco Bay			VRTC0014
NE Pacific	Tiburon, San Francisco Bay			VRTC0017
NE Pacific	Tiburon, San Francisco Bay			VRTC0018
NE Pacific	Tiburon, San Francisco Bay			VRTC0019
NE Pacific	Tiburon, San Francisco Bay			VRTC0030
NE Pacific	Tiburon, San Francisco Bay			VRTC0039
NE Pacific	Tiburon, San Francisco Bay			VRTC0040
NE Pacific	Tiburon, San Francisco Bay			VRTC0041
NE Pacific	Tiburon, San Francisco Bay			VRTC0042
NE Pacific	Canada: British Columbia, Bamfield, Wizard I.	*Ulva lobata*	HQ610369.1	
NE Pacific	USA: Washington	*Ulva lobata*	KX281918.1	
NE Pacific	USA: California, Stillwater Cove, Pebble Beach	*Ulva lobata*	KM255061.1	
NE Pacific	USA: California, Santa Cruz (Four Mile)	*Ulva lobata*	KM255006.1	
NE Pacific	Canada: British Columbia, Bamfield, Dixon I.	*Ulva lobata*	HQ610376.1	
N Pacific; mesophotic	USA: Hawaii	*Ulva ohiohilulu*	KT932977.1	
N Pacific; mesophotic	USA: Hawaii	*Ulva ohiohilulu*	KT932985.1	
N Pacific; mesophotic	USA: Hawaii	*Ulva ohiohilulu*	KT932983.1	
N Pacific; mesophotic	USA: Hawaii	*Ulva ohiohilulu*	KT932979.1	
N Pacific; mesophotic	USA: Hawaii	*Ulva ohiohilulu*	KT932978.1	

Ocean Region	Locality	Name	GenBank No.	VoucherID
SW Pacific	Australia: New South Wales, Narooma, outer bar on intertidal rocks	*Ulva ohnoi*	JN029328.1	
Gulf of Mexico	FloridaBay, USA	*Ulva ohnoi*	KU561325.1	
SW Pacific	Australia: Western Australia, Cozy Corner (Knobby Pt.)	*Ulva ohnoi*	JN029335.1	
SW Pacific	Australia: New South Wales, North Head Gutters, Lord Howe	*Ulva ohnoi*	JN029331.1	
SW Pacific	Australia: New South Wales, Algae Hole North, Lord Howe	*Ulva ohnoi*	JN029333.1	
Mediterrane an Sea	Italy:Adriatic Sea	*Ulva pertusa*	HE600186.1	
Mediterrane an Sea	Italy:Adriatic Sea	*Ulva pertusa*	HE600189.1	
Mediterrane an Sea	Italy:Adriatic Sea	*Ulva pertusa*	HE600188.1	
Mediterrane an Sea	Italy:Adriatic Sea	*Ulva pertusa*	HE600187.1	
Mediterrane an Sea	Italy:Adriatic Sea	*Ulva pertusa*	HE600190.1	
NE Pacific	Point Potrero, San Francisco Bay	*Ulva procera*		VPTP1752
NE Pacific	Point Potrero, San Francisco Bay	*Ulva procera*		VPTP1771
NE Pacific	Point Potrero, San Francisco Bay			VPTP1134
NE Pacific	Berkeley Marina, San Francisco Bay			VBKM0257
NE Pacific	Berkeley Marina, San Francisco Bay			VBKM0265
NE Pacific	Berkeley Marina, San Francisco Bay			VBKM0264
NE Pacific	Canada: British Columbia, Butze Rapids, Prince Rupert	*Ulva procera*	HQ610386.1	
NW Atlantic	USA: Maine, End of public road, Starboard	*Ulva procera*	HQ610390	
SW Pacific	Australia: Tasmania, Hells Gates (beach to north)	*Ulva procera*	JN029337.1	
NE Pacific	USA: California, Santa Cruz (Four Mile)	*Ulva procera*	KM254997.1	
NW Atlantic	Canada: New Brunswick, Harrington Cove exposed biodiversity site, Grand Manan	*Ulva procera*	HQ610387.1	
NE Atlantic	United Kingdom: Westerness, Gortenachullish/Eilean Ighe	*Ulva prolifera*	EF595301.1	
NW Atlantic	Canada: New Brunswick, `Cottonii` Creek, near Letete (Maine border)	*Ulva prolifera*	HQ610398	
NW Atlantic	Canada: Newfoundland and Labrador, St. Paul, Bonne Bay	*Ulva prolifera*	HQ610394.1	
N Atlantic	Iceland	*Ulva prolifera*	EF595334.1	

Ocean Region	Locality	Name	GenBank No.	VoucherID
Hudson Bay	Canada: Manitoba, East shore Churchill River, S of SeaNorth	*Ulva prolifera*	HQ610396.1	
CW Pacific	China	*Ulva prolifera*	KC411848	
		Ulva reticulata	JF918548.1	
Mediterranean Sea	Italy:Adriatic Sea	*Ulva rigida*	HE600178.1	
Mediterranean Sea	Italy:Adriatic Sea	*Ulva rigida*	HE600179.1	
Mediterranean Sea	Italy:Adriatic Sea	*Ulva rigida*	HE600181.1	
CW Pacific	China	*Ulva shanxiensis*	KJ617036.1	
NE Pacific	USA: Friday Harbor Laboratories, San Juan Island, WA	*Ulva sp.*[1]	AY454400.1[1]	
NE Pacific	Canada: British Columbia, Bamfield, Seppings I.	*Ulva stenophylla*	HQ610433.1	
SW Pacific	Australia: New South Wales, Lake Conjola boat ramp	*Ulva stenophylla*	JN029341	
NE Pacific	USA: Washington	*Ulva stenophylla*	KX281916.1	
NE Pacific	USA: Washington	*Ulva stenophylla*	KX281913.1	
NE Pacific	Canada: British Columbia, Pachena Beach, Bamfield	*Ulva stenophylla*	HQ610435.1	
		Ulva taeniata	KC661445.1	
		Ulva taeniata	KC661451.1	
NE Pacific	USA: California, Sea Lion Point South, Point Lobos State Reserve	*Ulva tanneri*	KM255002.1	
NE Pacific	Canada: British Columbia, Botanical Beach, Port Renfrew, Vancouver I.	*Ulva torta*	HQ610436.1	
SW Pacific	Australia: New South Wales, Narrawallee Beach rock platform	*Ulva torta*	JN029342.1	
SW Pacific	Australia: Tasmania, Snug Park	*Ulva torta*	JN029343.1	
NE Pacific	Canada: British Columbia, Kye Bay, Vancouver Island	*Ulva torta*	HQ610438.1	
SW Pacific	Australia: South Australia, Port Lincoln, intertidal man-made boulder wall	*Ulva torta*	JN029340.1	
CW Pacific	South Korea: Cheju-do, Channel between Little & Big Munseom Islands	*Umbraulva japonica*	JN029344.1	
N Pacific; mesophotic	USA: Hawaii	*Umbraulva kaloakulau*	KT932971.1	
N Pacific; mesophotic	USA: Hawaii	*Umbraulva kuaweuweu*	KT932968.1	
SW Pacific	Australia: Western Australia, Pt. Peron	*Umbraulva sp.*	JN029347.1	
SW Pacific	Australia: New South Wales, Malabar Reef, Lord Howe I.	*Umbraulva sp.*	JN029348	

Ocean Region	Locality	Name	GenBank No.	VoucherID
NW Atlantic	Canada: New Brunswick, SE of Beaver Harbour in SCUBA Bay, Bay of Fundy	*Ulvaria obscura*	HQ610405	

CHAPTER 2 | Evidence of an overwintering *Ulva* propagule bank in San Francisco Bay, CA, U.S.A.

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ABSTRACT

Blooms of the ephemeral macroalgae, *Ulva* spp., negatively affect coastal ecosystems and are typically observed seasonally in spring and summer. Benthic microscopic *Ulva* spp. forms contribute to nuisance spring blooms following periods of seasonal ice cover but have not been documented for the central California coast. These overwintering stages can promote populations of ephemeral species by buffering the negative effects of unfavorable environmental conditions, competition, and herbivory. This study focused on survival of overwintering propagules in intertidal sediments of central San Francisco Bay as a mechanism contributing to spring recruitment pulses. Sediment samples (three 50g samples/site) were collected from three central bay sites and incubated in an overwintering simulation (enriched media for 12 weeks of darkness at mean winter temperature). Incubations were aliquoted into a 6-week common garden experiment to test for the effects of temperature on recruitment of overwintering sediment propagule banks at three temperature treatments (simulating winter mean, summer mean, or summer high temperatures). Sediments cultured under all three conditions produced *Ulva* recruits after 6 weeks in culture, with strong site differences and the seasonal temperature regime imposing a marked effect on early recruitment rates. Winter treatments yielded mostly microscopic (<1mm) recruits through the sixth week. Summer treatments produced many macroscopic recruits, with summer mean temperature treatments yielding the greatest density of recruits · cm^{-2}. These results demonstrate that propagules not only survive winter incubation periods, but that success of recruitment is influenced by seasonal increases in water temperature. After 4 weeks in culture, recruits in the two warmer treatments were twice the size of recruits grown in the winter treatment. However, by the sixth week, mean recruit length in the mean summer treatment had surpassed the mean length of recruits in the summer high treatment. In light of recent increases in the frequency of harmful algal blooms, understanding the overwintering abilities of microscopic algal propagule banks and how they contribute to spring recruitment pulses will be useful in predicting future bloom events.

1. INTRODUCTION

Blooms of opportunistic macroalgae are often associated with anthropogenic eutrophication as bloom species are characterized by fast growth and benefit from nutrient enrichment (Raffaelli and Poole, 1998). Variation in extent, distribution and species composition of blooms under similar levels of nutrient enrichment make it difficult to predict blooms based solely on nutrient loading (Smetacek and Zingone, 2013). These sudden proliferations are commonly referred to as green tides when they are composed of chlorophyte (green) algae and can have major ecological impacts on coastal systems (Raffaelli and Poole, 1998). Remediation of nutrient inputs and hydrography is

often not sufficient to mitigate and prevent blooms from reoccurring (Lowthion et al., 1985; Sfriso et al., 1992; Yabe et al., 2009). While changes in nutrient inputs and hydrography are important, it has become clear that other abiotic and biotic factors, including early developmental stages, contribute to bloom recurrence (Smetacek and Zingone, 2013).

Propagule supply is a potentially important driver of bloom initiation that has not been sufficiently investigated (but see (Bellgrove et al., 2004)). There are at least two important sources of the algal propagules that colonize unoccupied hard substrate from the water column. Many are released into the water column by adult thalli, and then dispersed by currents some distance from the parental population. However, others are resuspended from a persistent, benthic spore or fragment bank by some form of disturbance to the sediment prior to dispersal and resettlement (Liu et al., 2012). The latter phenomenon is the focus of this study; the importance of the planktonic spore supply on *Ulva* population dynamics is examined in Chapter 3.

Inherent to the concept of a benthic propagule bank for marine algae is the assumption that propagules persist for some time in an undeveloped or slowly developing state until the onset of conditions favorable to successful development. This life history feature would be particularly advantageous in strongly seasonal environments in which there is an alternation of favorable/benign and unfavorable/harsh conditions for juvenile establishment and growth. At temperate and higher latitudes, winter is a period of harsh conditions (e.g. low temperature, low light, high wave forces, and ice or sand scour), whereas spring and summer offer favorable conditions for settlement, growth, and reproduction (e.g. warmer temperatures and higher light levels).

Many seaweeds are capable of temporarily suspending growth over a wider variety of life history stages (Hoffman and Santelices, 1991). Three main components of these banks of microscopic benthic forms (BMBF), as categorized by Chapman (1987) include: 1. Perennial microscopic life history stages, such as microscopic gametophytes and sporophytes, coexisting with macroscopic forms, 2. Developmental stages (i.e. propagules: germlings, prostrate discs or filaments) with the ability to survive stressful conditions through suspended growth and 3. Recently germinated seaweed propagules with direct development (i.e. apomixis) (Santelices et al., 1995). The composition of BMBFs is thought to be dependent on seasonal changes in fertility and the supply of propagules; it often resembles the surrounding algal community (Hoffman and Santelices, 1991; Santelices et al., 1995). Algal propagules can include zoospores, gametes, fragments, zygotes and microscopic germlings (i.e. germinated spores). These propagules can have suspended growth or direct development and the longevity of these stages for several species have been observed to be 3-8 months with a slightly decreased range (2-7 months) in low light or complete darkness (Hoffman and Santelices, 1991).

Many organisms exhibit the ability to temporarily suspend growth and development during periods of stressful environmental conditions (Brock et al., 2003; Hairston, 1996; Pake and Venable, 1996; Venable, 2007). The term "dormancy" is used to describe this phenomenon, but reaching consensus on a rigorous, generally accepted definition has been challenging (J. M. Baskin and C. C. Baskin, 2004; Finch-Savage and Leubner-Metzger, 2006; Keeley et al., 1987). In organisms with complex life histories such as

insects, plants, zooplankton, and algae; dormancy characteristics are restricted to specific stages in the life history (i.e. plant seeds and cysts of brine shrimp). Overwintering benthic microscopic stages of macroalgae can function in a manner analogous to seed banks of terrestrial plants (Chapman, 1987; Hoffman and Santelices, 1991) in that they provide escapes from herbivory (Blanchette 1996) and from seasonal fluctuations in factors including competition, disturbance, temperature as well as quantity and quality of light and nutrients (Lotze et al., 1999; 2000).

Recent increases in the frequency and pervasiveness of harmful algal blooms has reinvigorated research on the importance of overwintering banks and their role in nuisance bloom formation. Green tides, in particular, are predominantly caused by the cosmopolitan genus *Ulva* and these blooms are predicted to continue to increase with climate change as a result of the opportunistic nature of these organisms. *Ulva* species are eurythermal, able to tolerate a wide range of temperatures. However, temperature ranges for optimal growth varies within the genus. For example, the sheet or blade forming species, *Ulva lactuca,* can survive temperatures in the range 0-28°C, but exhibits optimal growth in the narrow range of 10-15°C for (Lüning, 1990). In contrast, tube forming species such as *Ulva intestinalis* (formerly *Enteromorpha intestinalis*) can survive from 0-30°C but its optimal growth range is twice that of *U. lactuca*, (10-20°C) (Lüning, 1990). Temperature, salinity and light have been demonstrated as important factors controlling the motility of *Ulva* propagules (Christie and Shaw, 2007; Jones and Babb, 1968). The combined effects winter conditions (low temperature, prolonged darkness, and increased precipitation) likely contribute to the abilities of *Ulva* species to overwinter in benthic banks.

Overwintering BMBFs have been identified as important to the formation of spring *Ulva* blooms in northern latitudes experiencing seasonal ice cover. These BMBFs are composed of spores (Schories, 1995), germlings (Lotze et al., 1999), and fragments of adult thalli (Kamermans et al., 1998). Release of spores and gametes in *Ulva* spp. in general has been linked to seasonal increases in temperature, however they are thought to be capable of reproduction year-round (Lüning et al., 2008; Niesenbaum, 1988). Shifts from weekly propagule releases in summer to biweekly releases in spring and fall are thought to be a response to unfavorable environmental conditions (Lüning et al., 2008). Spores of several tubular *Ulva* spp. of the Wadden Sea are capable of overwintering for up 10 months at 5°C and subsequent germination occurs at 15°C when exposed to increased light and nutrients (Schories, 1995). Viable spores can be found up to 5cm depth in sediment with the greatest abundances in the top 3cm. These germlings can develop into plantlets directly on a single sand grain with grain sizes >500um supporting the greatest abundances of plantlets (Schories, 1995). Lotze et al. (1999) found that tubular bloom forming *Ulva* species in the Baltic Sea require temperatures between 10-15°C to germinate from BMBFs overwintering on rocky surfaces. They concluded that temperature was an important factor in transitioning from the propagule bank to the germling stage and herbivory and nutrients played stronger roles in the transition from germlings to adults (Lotze et al., 1999). Recent investigations in the Yellow Sea of China have identified ulvoid BMBFs in the shallow sediments adjacent to Qingdao Bay, the location of annually recurring green tides(Liu et al., 2012). Molecular work revealed that the species responsible for these nuisance blooms is capable of overwintering in intertidal sediments (Liu et al., 2012). Given the evidence that BMBFs are important to the

development of spring green tides following seasonal freezing temperatures and their discovery in temperate regions with nuisance green tides, we wanted to know if BMBFs were present in the San Francisco Bay. We tested for the presence of BMBFs in the shallow sediments of central San Francisco Bay and the effects of seasonal temperature increases on their growth after recruitment. Specifically, we wanted to know if these BMBFs had the ability to overwinter in the bay and if this was a potential mechanism contributing to spring ulvoid recruitment pulses.

2. MATERIALS AND METHODS

2.1 Study sites and sample collection

Sediment samples were collected from the intertidal adjacent to the Estuary & Ocean Science Center, Tiburon, CA (37° 53' 30.912" N, 122° 26' 49.518" W), Point Potrero, Richmond, CA (37° 54' 27.0504" N, 122° 22' 26.0724" W), and Point Isabel, Richmond, CA (37° 53' 56.418" N, 122° 19' 31.332" W) in November of 2016 during low tide (Figure1). These locations are characterized by steep to moderately sloping uneven terrain dominated by natural boulder fields and "rip rap" concrete slabs, and face east, southwest and west, respectively, towards the bay. Point Potrero and Point Isabel are located on the east side of the bay while the Tiburon site is located across the bay to the west, on the eastern side of the Tiburon peninsula. The Tiburon site has a strong freshwater current that moves water from the San Pablo Bay south to the Golden Gate, and experiences regular wave action from the Larkspur ferry wake. The Point Potrero site is located near a major port and is somewhat protected by a jetty that extends from Brooks Island to the southwest. Lastly, Point Isabel Regional Shoreline is located adjacent to a dog park with increased pedestrian foot traffic and faces west to the central bay. Samples were collected from all sites in the same 24hr tide cycle. At each site, a 50m transect was haphazardly placed parallel to the water level and a total of 3 sediment samples (50g w.w./each) were collected using a garden trowel from exposed sediment (top 5 cm; a mix of silt, sand, gravel and shell fragments) within 1m of MLLW, each at 0m, 25m and 50m positions of the transect. Samples were transported to the lab in coolers and stored at 4°C (< 24hrs) until they were aliquoted into sterile incubation containers.

2.3 Overwintering incubation

Upon return to the lab, 50g of each sediment sample was aliquoted into separate 2L Erlenmyer flasks. These incubation flasks were then filled to the 2L mark with F/2 enriched seawater media (NCMA, omitting Na_2SiO_3 $9H_2O$, adding 1g/L GeO_2 to prevent diatom growth, 30-32ppt salinity, from here forward "F/2") and maintained at 11°C (average winter water temperatures for SF bay 2008-2014, data obtained for Tiburon from the Central and Northern California Ocean Observing System or CeNCOOS), in complete darkness for 12 weeks to simulate overwintering for an entire season.

2.4 Pre-incubation viability cultures

To determine the initial abundance of algal propagules available prior to entering the overwintering incubation, aliquots were cultured after allowing the incubations to

settle for 24 hours. Each flask was stirred with a sterile serological pipette and 3 subsamples of 50mL from each of the 9 incubations was aliquoted into vented culture flasks (Nunc™ EasYFlask™ 75cm^2 Nuclon™ Delta Surface, ThermoFisher Scientific Cat.No.156499). Culture flasks (3 x 9 = 27) were maintained at 17°C (mean summer temperature), mean irradiance of 28 μmol photons · m^{-2} · s^{-1}, and 12-hour day light regime. Culture media was replaced with 50mL of F/2 seven days following culture initiation and then weekly for 4 weeks. In week five of culture, all *Ulva* individuals visible in photos, from here forward referred to as recruits (≥0.025 mm in length), were enumerated, and gross morphology noted.

2.5 Post-overwintering temperature treatments

Following the simulated overwintering period, all incubations were cultured under each of three temperature treatments representing winter mean: 11°C, summer mean: 17°C, and summer high: 25°C, mean irradiance of 28 μmol photons · m^{-2} · s^{-1}, and 12-hour day light regime for 6 weeks; recruits were visible in the warmer treatments by week 4. Temperature treatments were determined using water temperature data collected at EOS (CeNCOOS) from 2008-2014. A pilot experiment including a treatment approximating the winter low temperature of 6°C resulted in no recruitment (e.g. no recruits large enough to identify as *Ulva*) across all sites, thus this treatment was excluded from this experiment. Each incubation was divided into 50mL aliquots and added to sterile mason jars (0.236 L) each containing a single 60mm x 15mm petri dish (Corning 351007) to facilitate enumeration and photographing of recruits. Each of the nine incubations was replicated across all three temperatures (3 samples x 3 sites x 3 temperatures x 4 tanks/temperature = 108 jars; Figure 2).

All petri dishes were photographed using a Canon 5D Mark III camera with 100mm macro lens and both 22mm and 30mm extension tubes mounted on copy stand and remote shooting with Canon EOSUtility software at 2-week intervals. Recruits were enumerated and measured from photographs using ImageJ (Schneider et al. 2012). For individual recruits that were present in weeks 6 or 4 but not visible in images taken at prior sampling intervals, a small value (0.001 mm), well below the smallest individual measured (0.025 mm), was assigned in order to be able to calculate means and variances for earlier sampling dates. This small value was used to represent "present but not detectable" by the measuring method as these individuals had to be present at week 2 and week 4 in order to be measured in week 6 jars as artificial seawater was used in the experiment, preventing the introduction of new propagules. For the purposes of this study, and to prevent potential overestimation of recruitment, all recruit clumps were analyzed here as single individuals (Figure 3).

2.6 Statistical analyses

Experimental design was partially nested with tank treated as a random factor nested under the fixed temperature treatment (n=4) to address spatial heterogeneity within the cold room specifically due to lighting over the tanks. Site was fully factorial fixed factor within tank(temperature)(Winer 1971). Differences in density and length of recruits due to temperature and site following the overwintering incubation were tested using ANOVA (α = 0.05, Type III SS, JMP Pro 13). Specific differences among

individual tanks or any interaction effects including tank number were not of interest. Assumptions of normality and equal variances were evaluated through visual inspection of residuals and Cochran's C test of residuals (Underwood 1997). Density and length values were transformed when necessary to improve normality and address heteroscedasticity using one of the following equations (see tables for details on when each was used):

(1) $Y' = Log_{10}(Y + 1)$ (Keough 2002) and

(2) $Y' = \sqrt{(Y + \frac{3}{8}}$ (Zar 1999).

Planned *post hoc* multiple comparisons of significant main effects were tested using Tukey HSD ($\alpha = 0.05$, JMP Pro 13.0.0) on the transformed data.

3. RESULTS

3.1 Pre-incubation viability of sediment propagule banks

Cultures aliquoted from all incubation flasks before entering the incubation treatment yielded viable recruits indicating that viable *Ulva* propagules were present in all samples collected in November 2016. Although, viable propagules were present in all samples, variation in the abundance of propagules collected from these three sites was observed. Variation in ulvoid recruitment observed after 5 weeks of culture under mean summer temperatures is summarized in Figure 4. Sediment samples collected from Point Isabel (1.965 ± 0.3147 recruits · cm^{-2}) yielded in the greatest pre-incubation recruitment density (mean) when compared to Tiburon (1.407 ± 0.4167 recruits · cm^{-2}) and Point Potrero (0.6777 ± 0.3168 recruits · cm^{-2}) (Figure 4, Table 1).

3.2 Recruitment from overwintering benthic banks of microscopic forms

The ability of benthic ulvoid propagules to overwinter was estimated from the density of ulvoid recruits observed after 6 weeks in culture following a 12-week overwintering incubation. Presence of recruits following the incubation was used as an indication of survival of overwintering. Relative survival and mean lengths of surviving recruits in the different temperature treatments was used to understand the effects of seasonal variation in temperature and future increases in mean seawater temperature on recruitment and growth from BMBFs. Variation in relative survival and mean length of BMBF recruits across sites and temperature treatments was observed and is discussed in detail below.

Benthic propagules from all sites survived the overwintering period (12 weeks of darkness undisturbed at 11°C, Figure 5). Recruitment under the winter treatment (11°C) was depressed when compared to both warmer treatments, mean summer (17°C) and summer high (25°C) across all sites after 2 and 4 weeks (Figure 5a & b), however this effect was disappeared after 6 weeks in culture (Figure 5c). Location of sample collection (site) had a strong effect on recruitment densities in all treatment combinations, except for the winter treatment (11°C) at 2-weeks. In fact, benthic propagules from all sites exhibited low recruitment densities after 2 weeks under mean winter conditions (Table 2; Figure 5a). With the exception of the winter treatment at 2-weeks, BMBF from

Point Potrero yielded the greatest densities of recruits across all post-incubation temperatures followed by Point Isabel and Tiburon, respectively (Tables 2 & 3; Figure 5).

3.3 Effects of temperature on growth of post-overwintering recruits

Recruit size under mean winter temperature (11°C) was depressed when compared to both warmer treatments, mean summer (17°C) and summer high (25°C) (Figure 6; Tables 2 & 4) across all sites following 2 and 4-week culture intervals. After six weeks, recruit lengths were on average shorter in both mean winter and summer high treatments when compared to the mean summer treatment (17°C). Recruit size (length in mm) ranged from 0.025 to 164.7 mm over the duration of the experiment. The longest individual was a branched tube measured in week 4 in the 25°C treatment that was twice as long as all other individuals measured at the 4-week time point. By the week 6 measurement, all other recruits had caught up to this individual, which had decreased in length to 117.7 mm. Several individuals measured in week 6 had decreased in length. This decrease often coincided with biofilm overgrowth in the 25°C treatment but this particular individual was not overgrown by biofilm. This change in the temperature effect on recruit length over different culture intervals indicates that low temperatures initially slow recruit growth and summer high temperatures result in decreased recruit length later in the recruitment stage.

A significant effect of the Tank*Site[Temperature] interaction was observed for all time intervals only for the length analyses. This interaction represents error associated with heterogeneity across the tank "blocks" of the experiment and is dependent on the site from which the sediments were collected. This is not surprising considering the strong site effects observed for mean recruit lengths. Similarly, the Tank[Temperature] interaction for week 2 was also significant indicating variation across blocks.

4. DISCUSSION

Cultures and a multifactorial lab experiment demonstrated that accumulations of microscopic *Ulva* propagules are abundant in intertidal sediments of the central SF Bay and survive winter incubation periods. Water temperature strongly influenced the growth rates of recruits that had undergone an overwintering simulation. We propose that seasonal decreases in water temperature are important for slowing growth, facilitating the accumulation of propagules in intertidal sediments, and seasonal increases in water temperature provide an opportunity for surviving benthic ulvoid propagules to recruit and dominate rocky shores within the bay in spring.

4.1 Viable benthic Ulva propagule banks

Benthic banks of microscopic *Ulva* spp. forms are common in the nearshore sediments of the central SF Bay and are a possible recruitment mechanism for spring blooms. These microscopic forms are able to survive at least 12 weeks of burial (i.e. darkness) at current mean winter temperatures. No visible recruits were present in the incubation flasks at the end of the incubation period. Upon transfer to the experimental treatments, any early developmental stages present were microscopic.

4.2 Survival and recruitment post overwintering

Recruitment density and size of ulvoids developing from intertidal sediments was controlled by the combined effects of temperature and collection location within the central San Francisco Bay with temperature effects more pronounced earlier in recruitment. Combination of low temperature and no light suppressed development over the twelve weeks of incubation. Ulvoid unicells (gametes and zoospores of *Ulva fasciata* and *U. flexuosa*) exhibit increased respiration relative to adult thalli, likely attributable to the metabolic demands of motility (Beach et al., 1995). Additionally, photosynthesis is less efficient ($< \alpha$) in recently settled zoospores (*Ulva fasciata*) than adult thalli (Beach et al., 1995). This increase in metabolic demands due to motility would have been most apparent in the incubation period while the propagules were light starved. The combination of increased metabolic demands, low photosynthetic efficiency, and potentially a decrease in photosynthetic output at colder temperatures, could explain the low densities observed in the winter treatment after 2 weeks compared to much higher densities observed after 6 weeks in culture. There is also some evidence that lower temperatures negatively affect adhesion abilities of *Ulva* zoospores. For example, the number of bound zoospores released from *Ulva compressa* increases 3-fold (150 mm^{-2} to 450 mm^{-2}) with temperatures increasing from 5°C to 25°C (Callow et al., 1997). Given the absence of a temperature effect on recruitment densities after 6 weeks in culture, differential adhesion across the temperature treatments was not observed in this experiment. Alternatively, the increasing recruitment densities observed in the present study across all temperatures over time indicates that the temperature effect on recruitment density was driven by slowed growth (increased metabolism and decreased production) as opposed to a decrease in adhesion abilities and settlement densities. Recruits were likely present prior to the 6-week census but at a size that was too small to be detected in the photographs.

More recruits were detected in the warmer temperatures after two weeks and this is likely because they were on average larger, and more easily visible in the images, than those in the cold treatment. Differences in recruitment densities were more strongly influenced by collection location of the propagule banks than temperature and this effect was apparent at all time points. All three sites had propagules that survived overwintering, but some sites yielded greater numbers of recruits than others. Spatial variation in abundance of benthic propagules surviving overwintering was also observed for benthic propagule banks of the Yellow Sea, China by Liu et al. (2012). Possible explanations for these patterns of spatial variation in propagule bank performance are described in detail in section 4.4 below.

4.3 Growth of overwintered recruits

Deviations from summer mean temperature negatively affected growth of newly established recruits from the benthic propagule bank. Recruits grown at mean winter temperatures exhibited slower growth at early developmental stages while those in the summer high treatment were more strongly affected during the 4 to 6-week time interval. This slowed growth at colder temperatures indicates that low temperatures retain propagules in a microscopic size longer and facilitate accumulation within intertidal sediments. Lüning et al. (2008) demonstrated that seasonal changes in photoperiod

reduce the periodicity of reproduction in *Ulva pseudocurvata* from weekly to biweekly in fall and winter. Since decreases in photoperiod coincide with seasonal decreases in water temperature, these low temperatures facilitate the accumulation and retention of propagules in intertidal sediments during periods of decreased reproductive output providing a source for spring recruitment once conditions improve (light and temperature).

Temperature has been identified as the main abiotic factor controlling seaweed geographic boundaries (van den Hoek et al. 1995). Temperature and light together are important factors in controlling adult *Ulva* growth (i.e. biomass) (Hurd et al., 2014). These factors can interact with respect to photosynthetic capabilities. Seasonal differences in photosynthetic output and efficiency have been documented for *Ulva rigida* in the Mediterranean. Fillet (1995) found that maximum photosynthetic output (i.e. P_{max}) was decreased by half in fall (15°C) and winter (7°C) temperatures compared to spring (18°C) and summer (25°C) treatments. This temperature effect was dependent on light intensity with saturation occurring at lower light intensities in seasonally colder temperatures (7°C and 15°C when compared to 18°C and 25°C) (Fillit, 1995). Their results corroborated other studies that also found seasonal variability in photosynthetic activity corresponded with biomass development (Brinkhuis, 1977; King and Schramm, 1976; Levavasseur and Giraud, 1982; Littler et al., 1979). This decrease in photosynthetic output at these colder temperatures could explain the slow growth in the winter treatment and facilitate survival at a microscopic size through an overwintering period (12-week dark incubation at 11°C). In addition to slowed growth potential at colder temperatures, propagules also exhibit lower adhesion densities at lower temperatures. Although we were unable to detect a decrease in adhesion densities in this study, lower adhesion densities combined with a decrease in growth could facilitate accumulation of microscopic forms in sediments and subsequent recruitment onto nearby rocky surfaces when temperatures increase in spring.

Interestingly, above average summer temperatures also negatively affected recruit growth. This effect, however, was not observed until week 6 of culture. This temperature treatment was selected as representative of extreme heat wave events and temperatures of this magnitude are likely to become more frequent as climate change continues to progress. Recruits in this summer high treatment were on average three times larger (2.87 mm ± 1.14 SD) than those in the winter low treatment by week 4. Average lengths increased by less than 1 mm between weeks 4 and 6 indicating that the larger recruits present in this treatment became increasingly limited after 4 weeks in this warmer environment. These larger recruits can be expected to be physiologically more similar to adult thalli than unicells. While photosynthetic output of adult thalli is greatest at spring and summer mean temperatures, photosynthetic efficiency decreases at 25°C (Fillit, 1995). Decreased photosynthetic efficiency in the 25°C treatment could explain the decreased mean length of recruits after 6 weeks in culture. Similarly, Steffensen (1976) found decreases in *Ulva lactuca* growth at these warmer temperatures. As the temperature in which discs excised from attached and drift thalli were grown increased, growth initially increased, but at temperatures exceeding 20°C growth declined (Steffensen, 1976). There may be a high temperature induced pressure on growth at later stages of recruitment. This could express itself as a culling of individuals that have

survived herbivory by benthic scrapers during the early settlement stage only to be limited by an abiotic factor, i.e. temperature.

4.4 Spatial variation in propagule banks

The three sites included in this study are characterized by strikingly different patterns of water motion and are a small representation of the range of heterogeneity present in the SF bay. For a benthic propagule bank to become established at a given site two events must occur. First a propagule source must release propagules (i.e. adult *Ulva* individuals reproduce) and second these propagules must either be retained at the site of release or arrive from another site, brought by local patterns of water movement within the bay. Dispersal of waterborne propagules is highly dependent upon water motion in order for propagules to be transported between locations and in order for propagules to accumulate in the benthos. Decreases in water motion lead to depositional environments in which smaller particles are more likely to accumulate. Given their microscopic sizes, ulvoid propagules are more likely to contact the benthos and accumulate in sediments in areas with slower moving water (Taylor et al., 2010). Based on water motion alone, one would expect the more sheltered site, Point Potrero, to have the greatest accumulation of propagules. This however was not the case, Point Isabel had the greatest pre-incubation recruitment. Interestingly, the site resulting in the greatest pre-incubation recruitment, did not result in the greatest post-incubation recruitment. In fact, cultures originating from Point Potrero, the site with the least dense pre-incubation recruitment resulted in the greatest post-incubation recruitment densities. Although, Point Potrero recruits were more numerous, they were significantly smaller than recruits originating from Point Isabel after 4 and 6 weeks in culture. While the relationship between growth of Point Isabel and Point Potrero recruits remained constant over these two time-intervals, recruits from Tiburon grew similarly to those from Point Potrero after 4 weeks but had caught up to the Point Isabel recruits by week 6.

The species composition of waterborne propagules delivered to study sites by currents within the bay could explain spatial differences in recruitment success. As mentioned above, hydrodynamic differences between sites could limit dispersal of propagules and exchange of species between sites. Additionally, species-specific ecophysiological constraints (i.e. optimal temperatures for germination, metabolism, photosynthesis; circadian clocks for reproduction) of local adult thalli and the overwintering abilities of their propagules, could lead to variation in species composition of propagules in intertidal sediments. This could explain the differences in site-specific overwintering abilities along with the differences observed between pre- and post-incubation recruitment. For example, Song et al. (2015) found that waterborne *Ulva* propagules exhibit species-specific (RFLP) germination rates dependent on culture temperature. *Ulva linza* propagules germinated at temperatures <25°C, while *U. prolifera* exhibited high densities (20-80 indL^{-1}) of germinated propagules between 10°C-25°C and performed poorly (<10 indL^{-1}) at 30°C (Song et al., 2015). Song et al. (2015) also collected propagules from an unknown *Ulva* sp. that germinated at its highest rates between 20°C-25°C. While these particular species were not observed in the SF Bay, these results support the idea that not all *Ulva* species are created equally, at least physiologically speaking. Genetic barcoding (*tufA* gene) of adult *Ulva* species occurring at the sediment collection locations in the present study, revealed that species

compositions differ among locations with different species dominating at each location (Chapter 1). A small number of recruits grown from waterborne propagules collected at Point Potrero were identified as *Ulva procera* and adult individuals of this species were collected from Point Isabel and Point Potrero but not from Tiburon (Chapter 1). Given that Tiburon is located across the central bay from the other two sites, these results indicate that the freshwater current at Tiburon acts as a potential barrier to dispersal.

Several of the species known to overwinter and cause green tides globally are present in the SF Bay (Chapter 1). Liu et al. (2011), used multiple markers (ITS + 5.8S rDNA and *rbc*L genes) to establish that benthic overwintering propagule banks of the Yellow Sea, Jiangsu province of China, included *Ulva linza-U. prolifera*, *Ulva* sp., *U. flexuosa*, and *U. compressa*. Of these species only *U. compressa*, *U. flexuosa* and *U. procera* (likely the identity of the LPP complex reported as *U. linza-U. prolifera* by Liu et al. 2011, see Gabrielson et al. 2012 and Chapter 1) were present at the three study sites. *U. procera* was the only one that comprised more than 20% of the adult assemblage and was found at two of the three sites sampled. *U. compressa* (17%) was only found at Point Potrero and *U. flexuosa* was found at both Point Isabel (14%) and Point Potrero (<10%) but not at Tiburon. *Ulva flexuosa* has been identified morphologically as a contributor to overwintering benthic propagule banks in the Wadden and Baltic Seas where benthic propagule banks must survive seasonal ice cover (Lotze et al., 2000; Schories, 1995). Unfortunately, previous studies identifying species composition of ulvoid overwintering propagule banks have not measured physiological performance parameters beyond survival and growth of individual *Ulva* species.

4.5 Overwintering of propagule banks not just ice-covered phenomenon

Moderately cold temperatures are enough to slow growth and retain a microscopic size for an entire winter season. Consistent with previous studies identifying benthic microscopic ulvoid forms as important following seasonal ice cover (Lotze et al., 2000; Schories, 1995), the "seed bank" analogs observed in the present study could also provide a spring recruitment mechanism in more temperate regions that do not develop winter ice cover. As global mean sea surface temperatures increase, it is likely that these propagule banks will continue to contribute to nuisance spring ulvoid blooms in higher latitudes. The relative importance of this mechanism for spring recruitment will however depend on the strength of other interactions such as herbivory and eutrophication.

Microscopic propagule banks can provide an advantage to ulvoid species in biotic interactions. For example, Lotze et al. (2000) observed a strong competitive advantage to *Ulva intestinalis* recruitment (as *Enteromorpha intestinalis*) over other bloom forming algae as a result of the presence and abundance of *Ulva* propagules in overwintering epilithic benthic propagule banks. Following initial spring recruitment, *Ulva* lost this advantage to *Pilayella littoralis*, a second blooming alga, due to greater production of new propagules by *P. littoralis*. Similarly, the NE Pacific subtidal macroalga, *Desmarestia ligulata* forms are dormant for 3-4 months in winter and provide competitive advantage over recruitment of kelp (Reed et al., 1997).

In the western Baltic Sea where urchins and limpets are absent and mesoherbivorous gastropods and crustaceans are dominant, Worm et al. (2001) observed

that epilithic benthic propagule banks not only allow *Ulva* species to outcompete other summer annuals but can also interfere with the abundance of perennial rockweeds, even in the presence of grazers. Further, nutrient enrichment exacerbated this result both when grazers were present and excluded (Worm et al., 2001). In contrast, in the SF bay limpets as well as grapsid crabs and other mesoherbivorous gastropods are abundant, thus, there may also be a benefit to remaining microscopic in size in sediment adjacent to rocky intertidal benches and within boulder fields. Similar to the Baltic sea, herbivores in the SF Bay are least abundant in winter (Chapter 3). Ulvoids, as adults, are known for their fast growth rates, these growth rates can allow for a resistance strategy to herbivory (Rosenthal and Kotanen, 1994). This ability to resist by escaping to a larger size is heavily dependent on the physiological demands of rapid growth being met by environmental conditions necessary for photosynthesis. This type of escape has also been observed for the kelp, *Postelsia palmeformis*, which can be found on wave exposed rocky outcrops surrounded by mussel beds. *P. palmeformis* produces spores with limited dispersal capabilities that form microscopic gametophyte banks underneath surrounding mussel cover. The temporary cover by mussels provides this alga with protection from scraping herbivores through winter until gametophytes produce annual macroscopic sporophytes in spring (Blanchette 1997). The compounded effects of temperature and ontogeny on growth ability, could benefit ulvoids in winter by accumulating a microscopic benthic propagule bank ready to opportunistically respond to spring and summer upwelling pulses. These periods of alternating cold nutrient rich water with warmer temperatures and longer daylengths meet the demands of fast growth enabling recruits to escape at a larger size in a quick transition from microscopic propagule to 1 cm recruit.

4.6 Implications for management

This study confirms the presence of ulvoid benthic propagule banks in intertidal sediments throughout the central SF Bay, their ability to overwinter in these sediments, and demonstrates that seasonal increases in temperature are important for recruitment. These data can be used to guide policy through spatio-temporal tailoring of nutrient total maximum daily loads (TMDLs) during spring when reproduction (propagule release) increases in frequency and recruitment from benthic propagule banks is likely to be most important. Adult ulvoid thalli in the SF Bay reach their lowest abundance from December to March (Chapter 3). During this time period, synchronous releases of propagules occur with decreased frequency and average water temperatures are low enough to delay recruitment from sediment propagule banks (Chapter 3, Lüning et al., 2008; Smith, 1947). Restricting TMDLs during the onset of spring would prevent increased nutrient pulses from coinciding with seasonal increases in temperature that trigger recruitment from benthic propagule banks. These types of management strategies could prevent positive feedback loops identified to reduce cover of perennial macrophyte communities and favor monocultures of ulvoid annuals in embayments with low diversity (Worm and Lotze, 2006). By identifying the circumstances that initiate blooms green tide algae, we will be better able to devise practices that can mitigate their negative impacts on coastal biodiversity and ecosystem functioning.

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TABLE CAPTIONS

Table 1. Effects of location of collection (site) on pre-incubation benthic propagule density at the time of collection, November 2016 (a. One-way ANOVA with site as a fixed factor; b. Tukey HSD planned post hoc multiple comparisons test on site).

Table 2. ANOVA tables describing the effects of temperature and location of collection (site) on post-incubation response variables (a. *Ulva* recruitment density after 2 weeks in culture, b. *Ulva* recruit length after 2 weeks in culture, c. *Ulva* recruitment density after 4 weeks in culture, d. *Ulva* recruit length after 4 weeks in culture, e. *Ulva* recruitment density after 6 weeks in culture, f. *Ulva* recruit length after 6 weeks in culture.). Data transformation used indicated by (1) $Y' = Log10 (Y + 1)$ and (2) $Y' = (Y + 3/8)^{0.5}$.

Table 3. Tukey's HSD evaluation of significant temperature*site interaction on mean density of *Ulva* recruits following two weeks in culture. Data was transformed using the following equation (1) $Y' = Log10 (Y + 1)$.

Table 4. Tukey's HSD evaluation of significant temperature and site effects on mean density and length of *Ulva* recruiting in culture from "overwintered" central San Francisco Bay sediments. See methods section for details of overwintering incubation. Data transformation used indicated by (1) $Y' = Log10 (Y + 1)$ and (2) $Y' = (Y + 3/8)^{0.5}$.

TABLES

Table 1. Effects of location of collection (site) on pre-incubation bentic propagule density at the time of collection, November 2016 (a. One-way ANOVA with site as a fixed factor; b. Tukey HSD planned post hoc multiple comparisons test on site).

Table 1.

a. Pre-incubation *Ulva* recruitment density after 4 weeks in culture (α = 0.05).

Source	SS Type I	df	Mean Square	F	P-Value
Site	2.499	2	1.249	5.203	**0.0489**
Error	0.441	6	0.2402		
Corrected Total	0.940	8			

b. Variation in pre-incubation *Ulva* recruitment density (α = 0.05).

Site(i)	Site(j)	P-Value
Point Isabel	Point Potrero	**0.0417**
Tiburon	Point Potrero	0.2402
Point Isabel	Tiburon	0.4026

Table 2.

a. *Ulva* recruitment density after 2 weeks in culuture[1]

Source	SS Type III	df	Mean Square	F	P-Value
Temperature	2.686	2	1.343	103.0	<0.0001
Site	2.634	2	1.317	141.8	<0.0001
Site*Temperature	1.092	4	0.2731	29.42	<0.0001
Tank[Temperature]	0.117	9	0.0130	1.405	0.2566
Tank*Site[Temperature]	0.167	18	0.0093	0.4519	0.9694
Error	1.455	71	0.0205		
Corrected Total	7.952	106			

b. *Ulva* recruit length after 2 weeks in culture[1]

Source	SS Type III	df	Mean Square	F	P-Value
Temperature	1.346	2	0.6731	29.48	0.0001
Site	0.0367	2	0.0183	2.080	0.1540
Site*Temperature	0.0183	4	0.0046	0.519	0.7230
Tank[Temperature]	0.2057	9	0.2286	2.591	0.0409
Tank*Site[Temperature]	0.1589	18	0.0088	2.080	0.0155
Error	0.3015	71			
Corrected Total		106			

c. *Ulva* recruit density after 4 weeks in culture[1]

Source	SS Type III	df	Mean Square	F	P-Value
Temperature	0.271	2	0.1357	10.73	0.0041
Site	4.0835	2	2.0418	165.3	<0.0001
Site*Temperature	0.0635	4	0.0159	1.847	0.3129
Tank[Temperature]	0.11379	9	0.0126	1.023	0.4585
Tank*Site[Temperature]	0.2224	18	0.0235	0.4411	0.9731
Error		72	0.0280		
Corrected Total		107			

d. *Ulva* recruit length after 4 weeks in culture[1]

Source	SS Type III	df	Mean Square	F	P-Value
Temperature	2.491	2	0.7853	28.70	0.0001
Site	0.4422	2	0.4951	7.343	0.005
Site*Temperature	0.0178	4	0.0161	0.1500	0.962
Tank[Temperature]	0.3906	9	0.0252	1.441	0.243
Tank*Site[Temperature]	0.5420	18	0.0153	1.780	0.044
Error	1.216	72	0.0105		
Corrected Total	5.099	107			

e. *Ulva* recruit density after 6 weeks in culture[2]

Source	SS Type III	df	Mean Square	F	P-Value
Temperature	0.078	2	0.039	2.854	0.1097
Site	5.065	2	2.532	378.5	<0.0001
Site*Temperature	0.0027	4	0.0007	0.1001	0.9810
Tank[Temperature]	0.123	9	0.0137	2.045	0.0939
Tank*Site[Temperature]	0.120	18	0.0067	0.246	0.9992
Error	1.959	72	0.0272		
Corrected Total		107			

f. *Ulva* recruit length after 6 weeks in culture[2]

Source	SS Type III	df	Mean Square	F	P-Value
Temperature	5.943	2	2.971	6.033	0.0218
Site	5.694	2	2.847	6.216	0.0089
Site*Temperature	0.3359	4	0.0840	0.1833	0.9441
Tank[Temperature]	4.433	9	0.4925	1.075	0.4251
Tank*Site[Temperature]	8.244	18	0.4580	1.831	0.0375
Error	18.01	72	0.25018		
Corrected Total	42.66	107			

Table 3. Tukey's HSD evaluation of significant temperature*site interaction on mean density of *Ulva* recruits following two weeks in culture. Data was transformed using the following equation (1) $Y' = Log10\,(Y + 1)$.

Table 3.

Temperature$_{(i)}$ *Site$_{(i)}$	Temperature$_{(j)}$ *Site$_{(j)}$	P-Value	Temperature$_{(i)}$ *Site$_{(i)}$	Temperature$_{(j)}$ *Site$_{(j)}$	P-Value
	Point Potrero *11°C	**<0.0001**		Point Isabel *11°C	0.9983
	Point Potrero *17°C	0.5445	Point Potrero *11°C	Point Isabel *17°C	**0.0001**
	Point Isabel *11°C	**<0.0001**		Point Isabel *25°C	**<0.0001**
Point Potrero *25°C	Point Isabel *17°C	**<0.0001**		Tiburon *11°C	**<0.0001**
	Point Isabel *25°C	**<0.0001**		Tiburon *17°C	**0.0003**
	Tiburon *11°C	**<0.0001**	Point Isabel *25°C	Tiburon *25°C	**0.0007**
	Tiburon *17°C	**<0.0001**		Point Isabel *11°C	**<0.0001**
	Tiburon *25°C	**<0.0001**		Point Isabel *17°C	0.9981
	Point Potrero *11°C	**<0.0001**		Point Isabel *11°C	**<0.0001**
	Point Isabel *11°C	**<0.0001**	Point Isabel *17°C	Tiburon *11°C	**<0.0001**
	Point Isabel *17°C	**<0.0001**		Tiburon *17°C	**0.0014**
Point Potrero *17°C	Point Isabel *25°C	**<0.0001**		Tiburon *25°C	**0.0029**
	Tiburon *11°C	**<0.0001**		Tiburon *11°C	1.000
	Tiburon *17°C	**<0.0001**	Point Isabel *11°C	Tiburon *17°C	0.616
	Tiburon *25°C	**<0.0001**		Tiburon *25°C	0.4043
	Tiburon *11°C	0.9816	Tiburon *25°C	Tiburon *11°C	0.2598
Point Potrero *11°C	Tiburon *17°C	0.9412		Tiburon *17°C	1.000
	Tiburon *25°C	0.8013	Tiburon *17°C	Tiburon *11°C	0.4356

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Table 4. Tukey's HSD evaluation of significant temperature and site effects on mean density and length of *Ulva* recruiting in culture from "overwintered" central San Francisco Bay sediments. See methods section for overwintering incubation Data transformation used indicated by (1) $Y' = Log10 (Y + 1)$ and (2) $Y' = (Y + 3/8)^{0.5}$.

Table 4:

a. *Ulva* mean recruits cm^{-2} following two weeks in culture. Note that these effects are not independent as the interaction of temperature and site was significant (see Tables 2 and 3)[1]

Temperature$_{(i)}$	Temperature$_{(j)}$	P-Value
17°C	11°C	**<0.0001**
25°C	11°C	**<0.0001**
17°C	25°C	0.3264

Site$_{(i)}$	Site$_{(j)}$	P-Value
Point Isabel	Point Potrero	**<0.0001**
Point Isabel	Tiburon	**<0.0001**
Tiburon	Point Potrero	**<0.0001**

b. *Ulva* mean recruit length (mm) following two weeks in culture.[1]

Temperature$_{(i)}$	Temperature$_{(j)}$	P-Value
17°C	11°C	**0.001**
25°C	11°C	**0.0001**
17°C	25°C	0.2087

Site$_{(i)}$	Site$_{(j)}$	P-Value
Point Isabel	Point Potrero	0.2396
Point Isabel	Tiburon	0.1863
Tiburon	Point Potrero	0.9897

c. *Ulva* mean recruits cm^{-2} following four weeks in culture.[1]

Temperature$_{(i)}$	Temperature$_{(j)}$	P-Value
17°C	11°C	**0.0086**
25°C	11°C	**0.0070**
17°C	25°C	0.9890

Site$_{(i)}$	Site$_{(j)}$	P-Value
Point Isabel	Point Potrero	**<0.0001**
Point Isabel	Tiburon	**<0.0001**
Tiburon	Point Potrero	**<0.0001**

d. *Ulva* mean recruit length (mm) following four weeks in culture.[1]

Temperature$_{(i)}$	Temperature$_{(j)}$	P-Value
17°C	11°C	**0.0001**
25°C	11°C	**0.0008**
17°C	25°C	0.3596

Site$_{(i)}$	Site$_{(j)}$	P-Value
Point Isabel	Point Potrero	**0.0040**
Point Isabel	Tiburon	**0.0475**
Tiburon	Point Potrero	0.4872

e. *Ulva* mean recruits cm^{-2} following six weeks in culture.[1]

Temperature$_{(i)}$	Temperature$_{(j)}$	P-Value
17°C	11°C	NS
25°C	11°C	NS
17°C	25°C	NS

Site$_{(i)}$	Site$_{(j)}$	P-Value
Point Isabel	Point Potrero	**<0.0001**
Point Isabel	Tiburon	**<0.0001**
Tiburon	Point Potrero	**<0.0001**

f. *Ulva* mean recruit length (mm) following six weeks in culture.[2]

Temperature$_{(i)}$	Temperature$_{(j)}$	P-Value
17°C	11°C	**0.0352**
25°C	11°C	0.9998
17°C	25°C	**0.0362**

Site$_{(i)}$	Site$_{(j)}$	P-Value
Point Isabel	Point Potrero	**0.0068**
Point Isabel	Tiburon	0.3421
Tiburon	Point Potrero	0.1252

FIGURE CAPTIONS

Figure 1. Map of the study location with locations of sediment sample collection marked labeled in bold (●). Map courtesy of Cassandra J. Hansen 2018.

Figure 2. Common garden experimental design used to test for the importance of seasonal increases in temperature to recruitment from overwintering sediment propagule banks. Each aquarium tank served as a heated water bath used to maintain the temperature treatments (11°C, 17°C, and 25°C) within the individual mason jars (9 jars per tank, each representing a different incubation with three incubations from each site).

Figure 3. Example of a petri dish from the common garden experiment following six weeks in culture at 17°C with the most common recruit morphologies observed indicated; a. branched hollow tube, b. blade with hollow tube near base, c. blade, d. hollow tube growing in a clump, e. blades growing in a clump, f. hollow tube, and g. ambiguous morphology. Each mason jar had a single petri dish at the bottom, upon which incubation aliquots were deposited.

Figure 4. Density of pre-incubation recruits cultured from viable propagules present in intertidal sediments at the time of collection, November 2016. Cultures were maintained at mean summer temperatures of 17°C for 5 weeks. See methods section for detailed culture conditions. Significant differences ($p < 0.05$) in mean density of recruits from different sites are indicated by different letters (Tukey HSD planned post hoc comparisons, $\alpha = 0.05$, see Table 1 for F and p-values).

Figure 5. Density of recruits (mean recruits $\cdot$ cm^{-2} $\pm$ SE) grown in culture from overwintering benthic propagule banks at each census (a. 2 weeks, b. 4 weeks, and c. 6 weeks). These data are not transformed, however statistical analyses were performed on transformed data. Significant differences in the temperature effect are indicated by different letters while significant differences in the site effect are indicated by number of *'s (Tukey HSD planned post hoc comparisons, $\alpha = 0.05$, see Tables 2-3 for F and p-values).

Figure 6. Mean length of recruits (mm $\pm$ SE) grown in culture from overwintering benthic propagule banks at each census (a. 2 weeks, b. 4 weeks, and c. 6 weeks). These data are not transformed, however statistical analyses were performed on transformed data and significant differences (Tukey HSD planned post hoc comparisons, $\alpha = 0.05$, see Tables 1-3 for F and p-values).

FIGURES

Figure 1.
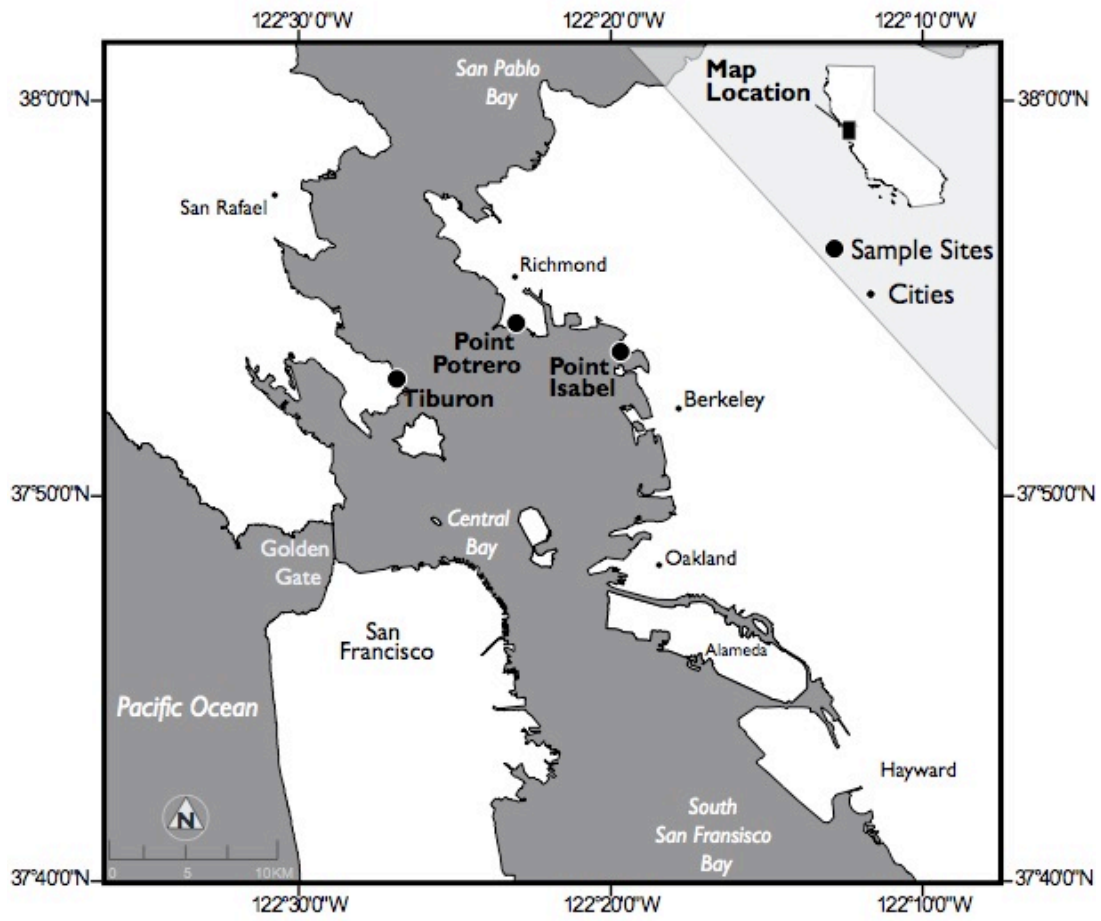
Figure 2.

Figure 3.

Figure 4.

Initial density of *Ulva* propagules recruiting in culture from SF Bay sediments

Figure 5.

Figure 5. *Ulva* recruits at 2-week intervals in culture from SF Bay sediments

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Figure 6.

Lengths of *Ulva* recruits cultured from SF Bay sediments
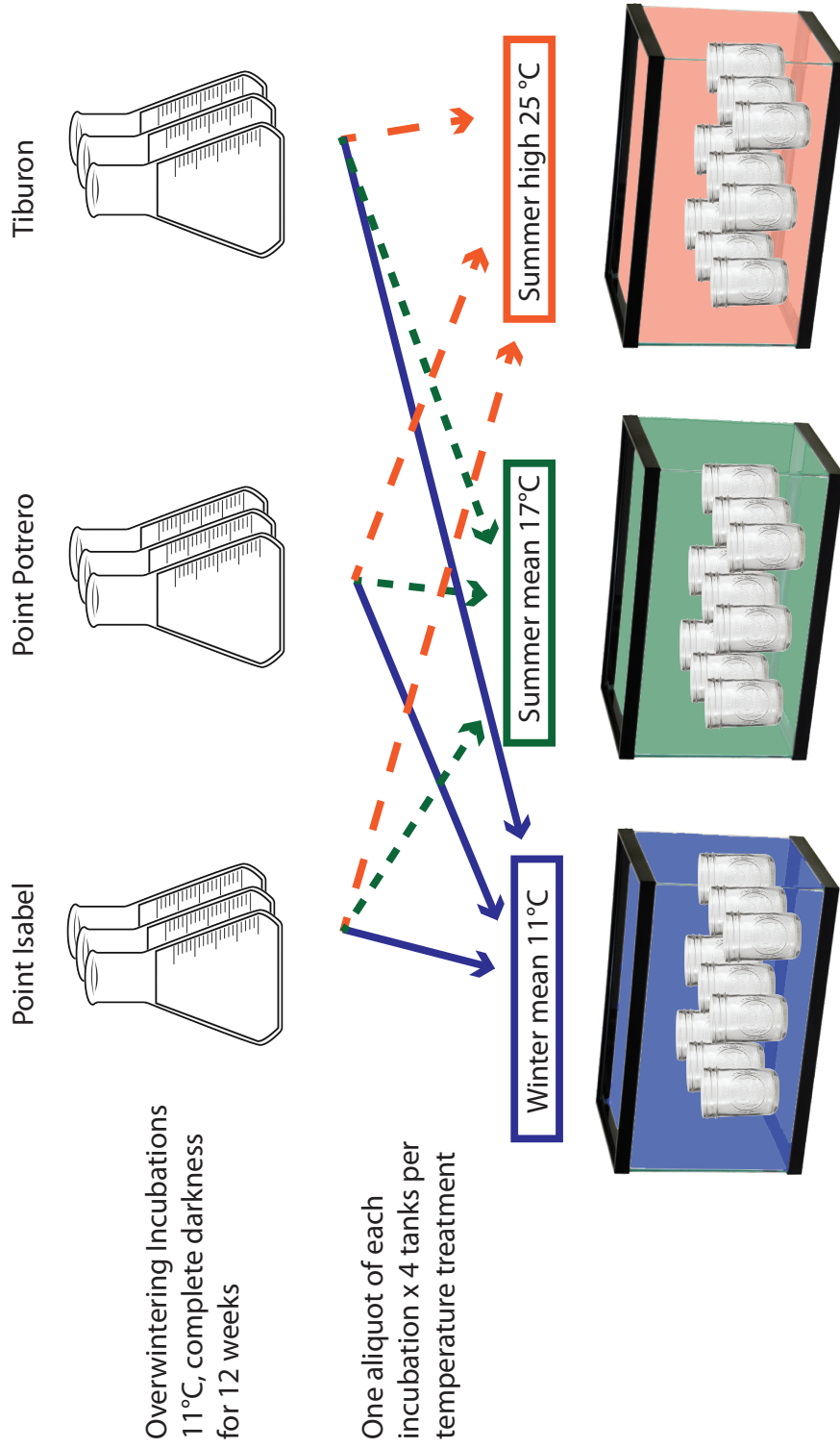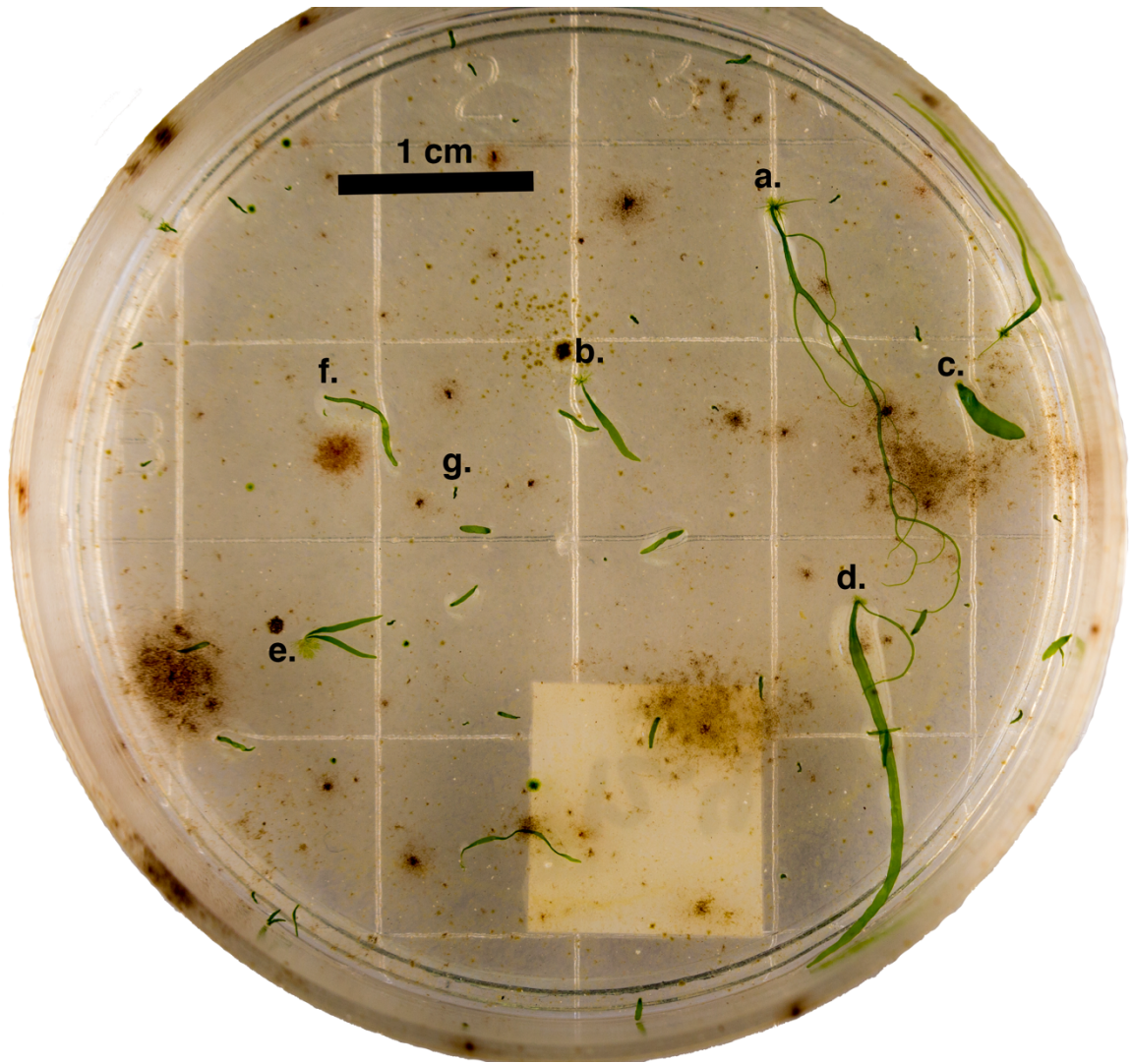
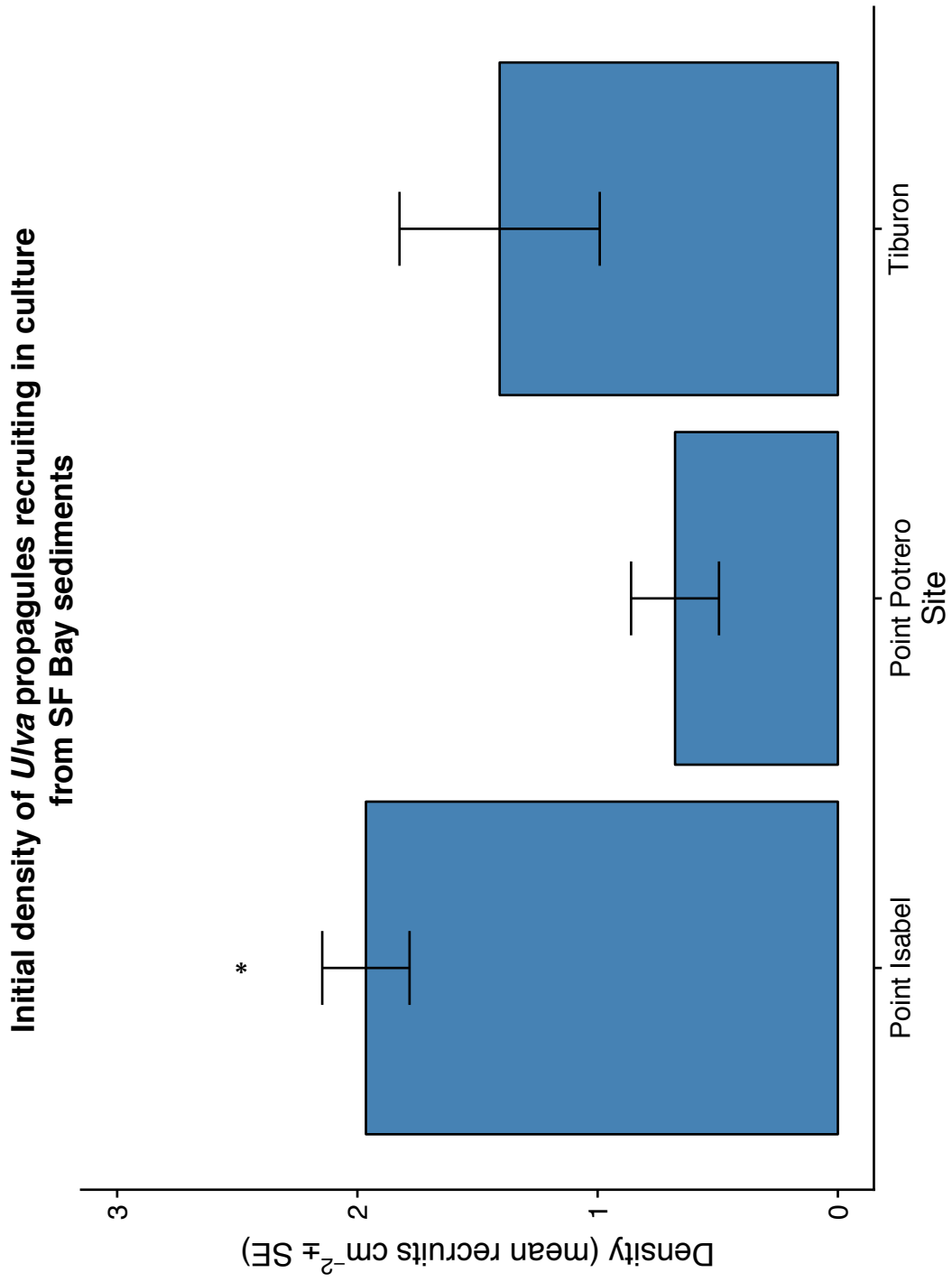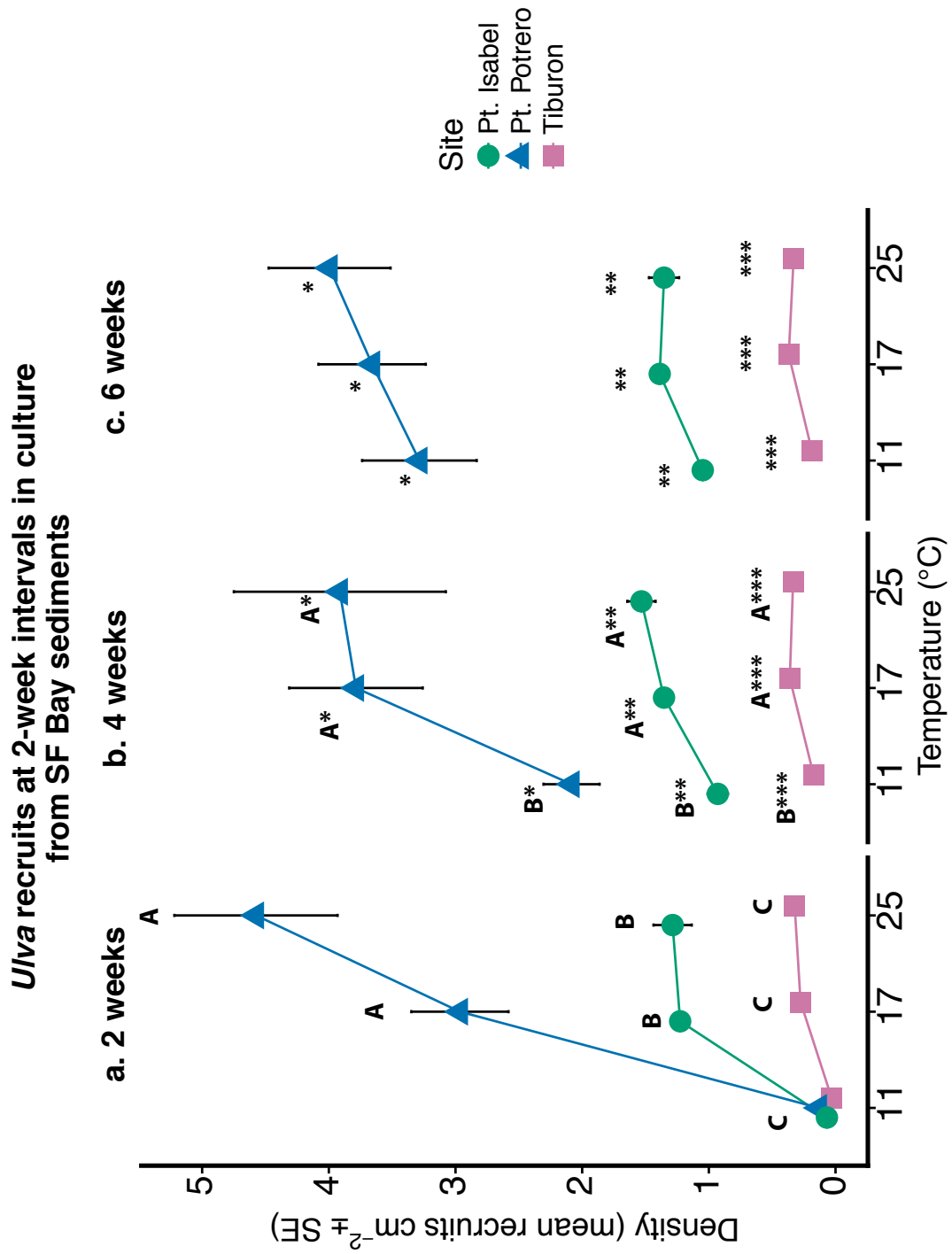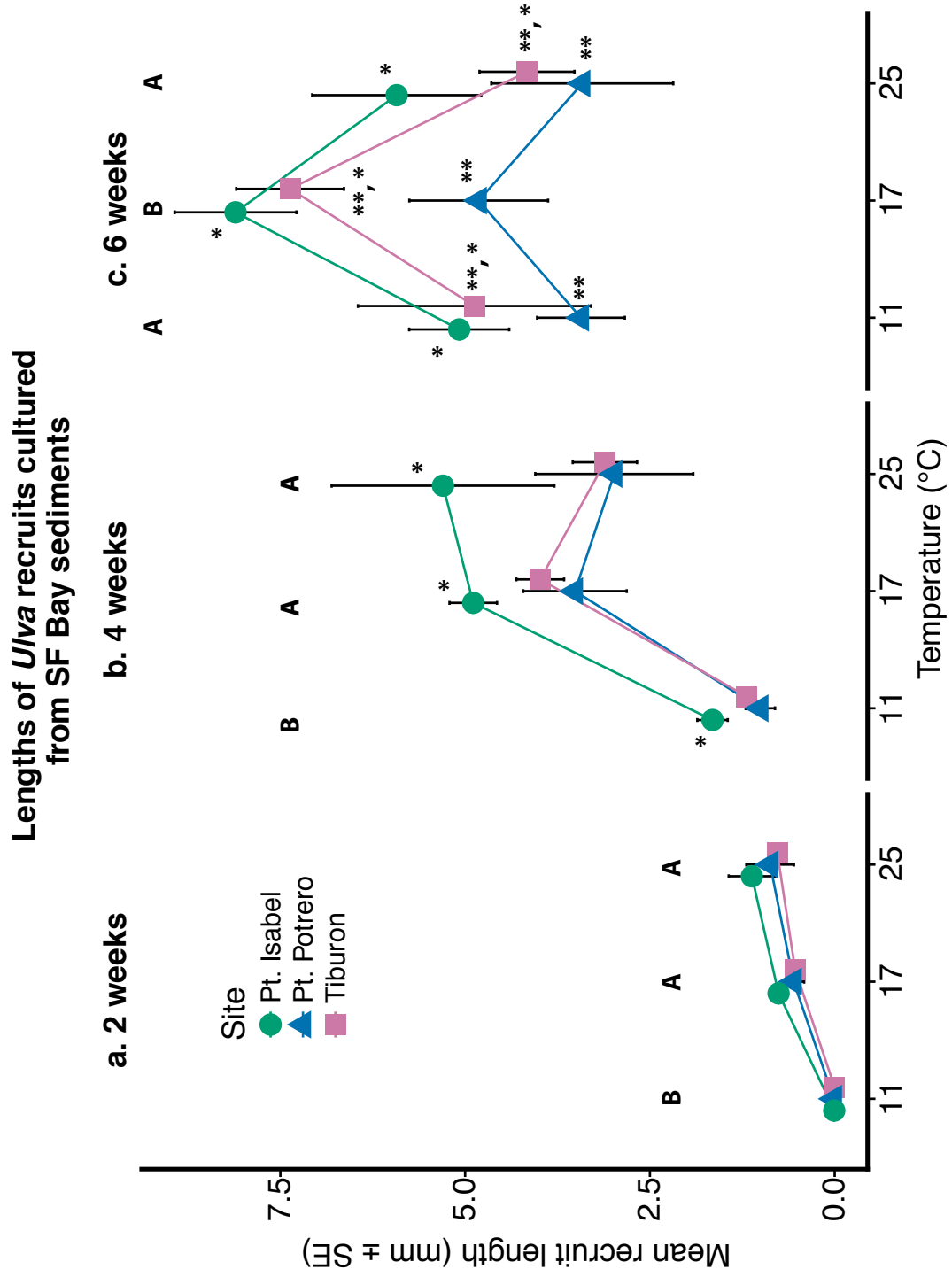## CHAPTER 3 | Is propagule supply a primary driver of fluctuations in benthic *Ulva* assemblages of central San Francisco Bay?

Rosemary Romero & Wayne P. Sousa

ABSTRACT

The concept of supply-side ecology, as related to marine intertidal communities, has focused primarily on invertebrate recruitment with much less attention given to the role of propagule supply in the establishment of macroalgal populations. Post-recruitment processes, such as competition and herbivory can only act on organisms if the supply of offspring to an area is sufficient for recruitment. Most of our knowledge of nuisance green macroalgae emphasizes post-recruitment processes, however recent evidence indicates that propagule supply plays an important role in how these algae interact within benthic intertidal communities. To better understand supply-side dynamics of ulvoids in San Francisco Bay, water samples and benthic intertidal assemblages were monitored at two temporal and spatial scales. Water samples were collected monthly from July 2014–January 2016 from the shoreline at Tiburon on the western shore of the bay and cultured to the recruit stage in growth chambers to estimate temporal variability in propagule supply. To assess patterns of propagule supply at a larger spatial scale, water samples were taken seasonally from February 2015–January 2016 at four additional sites spanning the central San Francisco Bay, and cultured in the same manner. Natural patterns of *Ulva* recruitment were monitored at 4-week intervals from November 2013–January 2016 in the intertidal zone at Tiburon. Waterborne *Ulva* propagules were present in the bay every month of the year, with samples collected in spring and summer months yielding the greatest amounts of recruits $\cdot$ cm^{-2}; indicating increased propagule supply at this time of year. Propagule supply was temporally variable both on the scale of months and seasons. Late summer 2014 recruitment peaks follow peaks in propagule supply. Recruitment was positively correlated with propagule supply indicating recruitment is limited by propagule supply in winter and early spring. Fluctuations in attached *Ulva* cover was modeled with site, season, propagule supply and a suite of environmental and biological factors. Candidate models were used to identify factors that had the most influence on attached *Ulva* cover at five San Francisco Bay locations. Several factors were highly important in explaining *Ulva* cover within the central bay: season, site, benthic herbivore abundance and ammonium. Abundance of algal competitors and propagule supply were of moderate importance in explaining *Ulva* cover within the central bay. These findings indicate that for organisms with complex life histories, temporal and spatial variability in nutrient concentrations, propagule supply, and post-settlement herbivory, are all important in explaining population dynamics.

1. INTRODUCTION

Our early understanding of intertidal ecology was largely based on the study of adult organisms on exposed rocky shores. Unlike the widespread nutrient loading from

industrialized agriculture to today's coastal ecosystems, anthropogenic influences to nearshore environments were episodic, e.g., occasional oil spills killing off herbivores and resulting in large algal blooms that would subside once herbivore populations recovered (North et al., 1965). In the absence of these episodic disruptions, the traditional view was that algal abundance was controlled by either physiological stress or biological interactions impacting adults such as interspecific competition with sessile invertebrates or consumption by herbivores (Connell, 1972; Lubchenco and Menge, 1978; Paine, 1966). The planktonic stages in complex life histories of most intertidal organisms were largely overlooked.

Later, researchers challenged this prevailing paradigm, suggesting that larval supply and recruitment could drive community dynamics as much or more than post-settlement interactions (i.e. competition, predation, etc.) (Gaines and Roughgarden, 1985; Underwood and Denley, 1984; 1979; Underwood and Fairweather, 1989). While the study of larval ecology can be traced back to the mid 1800's, the influence of larval supply on adult abundances was popularized as Supply-Side Ecology a century later (Lewin, 1986; Young, 1990). Early studies focused on invertebrate larvae, particularly barnacles (Caffey, 1985; Connell, 1985; Gaines and Roughgarden, 1985; Raimondi, 1988; Underwood and Keough, 2001) and fish (Carr, 1991; Sale et al., 1984). These organisms all have long-lived larvae or larvae with multiple pelagic stages in the water column before metamorphosing into adults in a vastly different habitat. However, little attention was paid to the effects of propagule supply and dispersal-limitation on benthic algal populations (Dayton, 1973; Hruby and Norton, 1979; Reed et al., 1988; Sousa, 1984)5.

Algal propagules tend to be shorter lived and much smaller in size than invertebrate or fish larvae. Despite these differences, they can be found offshore and throughout the water column (Amsler and Searles, 1980; Zechman and Mathieson, 1985). Algal dispersal distances have been described for several species and can range from small (1-3m) for intertidal kelp to relatively large with viable ulvoid propagules regularly cultured from water samples collected up to 35 km from the nearest population and in ballast water (Amsler and Searles, 1980; Dayton, 1973; Flagella et al., 2007; Reed et al., 2004). This wide range in macroalgal dispersal ability has been used to explain the variation in species composition among disturbance-cleared patches undergoing succession in rocky intertidal assemblages (Sousa, 1984).

In addition to these natural regulators of algal abundance, the influence of anthropogenic pollution is exerting a strong influence on algal productivity in some coastal habitats. Alarmingly large macroalgal blooms have become an increasingly common occurrence in response to terrestrially derived nutrient loading in coastal ecosystems since the 1990's (Smetacek and Zingone, 2013; Valiela et al., 1997; Worm et al., 2000). Excessive nutrients often stimulate high productivity of the green macroalgae, *Ulva*, overwhelming the controlling influence of herbivores, shade other submerged aquatic vegetation and cause anoxic conditions when the algae die and decay (Nielsen et al. 2004). In some instances, these conditions have led to shifts from diverse communities dominated by perennial macrophytes (rockweeds and seagrasses) towards low diversity assemblages dominated by annual algae, effectively raising the stakes on understanding the controls on marine macroalgal dynamics (Worm et al., 1999); (Pang et

al., 2010; Peckol and Rivers, 1996; Sfriso et al., 1992). Here, the early focus was on nutrient pollution, which in some locales (particularly in the tropics) went hand-in-hand with overfishing of large herbivorous fishes (T. P. Hughes, 1994; Lapointe et al., 1996; M. M. Littler and D. S. Littler, 1984). More recent investigations indicate that nutrient pollution is not the whole story (Lotze et al., 1999; Lotze and Worm, 2002). In fact, it often interacts with propagule limitation to shape the onset and intensity of macroalgal bloom dynamics and species interactions. For example, nutrient enrichment and herbivory exacerbate competitive interactions between *Ulva* and *Fucus* as the recruits from ulvoid propagule banks interfere with recruitment of *Fucus* in the Baltic Sea (Worm et al., 2000; 2001).

This study investigates the role of recruitment from waterborne propagules in dictating variation in the abundance of the green alga, *Ulva*, on the rocky shores of San Francisco Bay (SFB); SFB receives considerable nutrient influx from the dense urban environment that surrounds it. At least six species of *Ulva* are found in the central SFB (Chapter 1 this dissertation). These algae are common early colonizers of bare space in the rocky intertidal, can uptake nutrients faster than other macrophytes and thus their presence is used as an indication of both anthropogenic and physical disturbance. As they release numerous propagules into the water column, their recruitment rates at the local scale may be positively correlated to propagule supply. Given that the most conspicuous *Ulva* propagule releases observed in nature coincide with seasonal increases in temperature and following desiccation of reproductive adult thalli (Niesenbaum, 1988), we hypothesized that propagule availability would be greatest in spring and summer. Spring and summer seasons in central California are characterized by annual highs in air temperature, mixed tides that result in low tides occurring midday and upwelling of nutrient rich offshore water. We also hypothesized that if *Ulva* recruitment is limited by propagule supply, increases in attached *Ulva* cover would follow within four weeks of these periods of high propagule availability. With respect to environmental factors, we hypothesized that attached *Ulva* cover would be most influenced by water temperature and salinity as attached macroalgal cover disappears from rocky intertidal in SFB following winter increases in precipitation (Josselyn and West, 1985; Silva, 1977). Lastly, we hypothesized that post-settlement biological interactions such as herbivory and competition would have the strongest effects during seasons in which *Ulva* recruitment was limited by propagule supply. We tested these hypotheses at two spatial and temporal scales. Monthly sampling was conducted at a single site from 2013-2015 while seasonal sampling was conducted at five central SFB sites in 2015.

2. MATERIALS AND METHODS

2.1. Study sites

Field collections were conducted monthly at the San Francisco State University Estuary and Ocean Sciences Center at Tiburon, CA (37° 53' 30.912" N., 122° 26' 49.518" W) in 2013-2015 and seasonally in February, April, July, and November of 2015 at four additional sites on the eastern shore of central SFB, Point Potrero, Richmond, CA (37° 54' 27.0504" N, 122° 22' 26.0724" W), Point Isabel, Richmond, CA (37° 53' 56.418" N, 122° 19' 31.332" W), Berkeley Marina, Berkeley, CA (37° 51' 45.492" N, 122° 18'

53.49" W) and Ballena spit, Alameda, CA (37° 45' 50.04" N, 122° 17' 0.378" W) (Figure 1).

2.1.1 Abiotic factors

Salinity (handheld refractometer part number STX-3, Vee Gee Scientific) and water temperature (Wide Range InfraRed Thermometer model 42515, Extech Instruments) were measured *in situ* at the time of each water sample collection. Additional volumes of water for nitrate, phosphate, and ammonium were collected, immediately filtered (0.7µM GF/F filter) and frozen (-20°C) until processing. Nitrate and phosphate were analyzed using a Lachat QuickChem 3500 Flow Injection Analyst System and Omnion 3.0 software (UCSC, Kudela Lab, Lachat Instruments; Hach Company, Colorado, USA). Environmental parameters observed during field surveys over the course of the study are summarized in Table 1. Meterological and other water quality parameters for the study time period were retrieved from Central and Northern California Ocean Observing System (CenCOOS) for Tiburon and from the NOAA Center for Operational Oceanographic Products and Services (CO-OPS) for Richmond, CA (Point Potrero & Point Isabel) and Alameda, CA. The IR thermometer produced error codes during windy night time temperature measurements. For this reason, water temperature included in the statistical models was mean monthly temperatures calculated from CeNCOOS and CO-OPS data corresponding to each month and site of seasonal field surveys.

2.2. Estimating abundance of benthic intertidal communities

2.2.1 Monthly census at Tiburon

A permanent 42m transect was placed in the high intertidal above the splash zone and oriented parallel to the water line. Four locations along this transect were randomly selected each month and used to orient sampling transects placed perpendicular to the permanent transect and waterline. Benthic intertidal communities were censused at each meter from the permanent transect to the waterline; surveys included photoquadrats, percent cover of all sessile organisms (Dethier et al., 1993), and mobile invertebrate counts for all quadrats (900 cm^2). The four transects perpendicular to the waterline ranged from 6-10m in length depending on the time of year. All field sampling was conducted at full or new moon low tides.

2.2.2. Seasonal east bay sampling

The four additional sites on the eastern shore of central SFB were sampled once seasonally. Seasons were delineated based on daylength using the dates of solstices and equinoxes as boundaries because light quantity and quality has been identified as important in green tide development and the upwelling seasons offshore typically occur within two of these time periods (spring and summer) (Nelson et al., 2003a). In order to sample all 4 sites in the same tide cycle, the methods for estimating percent cover were modified from those used at the Tiburon site. Percent cover estimates were made from 10 photoqudrats (900 cm^2) taken at randomly selected locations of the exposed area. Photos were sampled for percent cover by adding a grid layer equivalent to the 5x5 grid

(photoquadrats were 30x30 cm with each grid square representing 4% of the total 900cm^2 area sampled) used in field estimates at Tiburon but because estimates were observed from photos, only the canopy layer is included in these estimates. Photoquadrats collected during the same low tide series at Tiburon were randomly subsampled (n=10) and percent cover estimates derived from these photos were used for comparison with the east bay estimates.

2.3 Estimating propagule availability

Water samples (2L amber HDPE bottles, Thermofisher) were collected from shore at the Tiburon site during rising full moon tides each month from July 2014–January 2016 (Christie and Evans, 1962). Water samples were collected from the four eastern shore central SFB locations in February, April, July, and November of 2015, and January 2016.

Each water sample was aliquoted into five vented culture flasks, with each flask receiving 125mL of the sample. Culture flasks were spiked with 2.5mL of F/2 enriched seawater media (NCMA, omitting Na$_2$SiO$_3$ 9H$_2$O, adding 1g/L GeO$_2$, 30-32ppt salinity, from here forward "F/2") and maintained at 16-17°C, mean irradiance of 28 µmol m^{-2}s^{-1}, and 12-hour day light regime. Sample water was replaced with 125mL of new media seven days following culture initiation and then weekly for 4 weeks. In week five of culture, *Ulva* recruits were counted, and gross morphology noted.

2.4. Estimating recruitment rates

Resin settlement plates were attached to substrate at the Tiburon Site to estimate recruitment rates, all plates were cast from the same rock mold to minimize any effects of substrate rugosity on recruitment rates (Muth, 2012). Settlement plates were deployed from one full moon tide series to the next full moon tide series; fouled plates were transported to the lab in sterile petri dishes and stored in the dark at 4°C until sampling. Temporal variation in recruitment of all organisms recruiting to plates over 4-week time periods was visually estimated for % cover following methods outlined in (Dethier et al., 1993).

2.5. Statistical Analyses

2.5.1 Spatio-temporal variation in attached Ulva, propagule supply and recruitment

Spatio-temporal differences in attached *Ulva* cover were tested using a type II ANOVA (α = 0.05, JMP Pro 14.0.0) with both site and season as fixed factors. Given the spatial heterogeneity of the SFB and the previously documented seasonal blooming habit of *Ulva* on the central coast, we performed post-hoc planned pairwise comparisons on the transformed data for all levels of season and site (Tukey HSD, α = 0.05, JMP Pro 14.0.0). Similarly, seasonal differences in waterborne propagule supply were compared by testing for differences in mean density of recruits cultured from nearshore water samples collected during rising tides. Both attached *Ulva* percent cover and mean densities of cultured recruits were log transformed when necessary to improve normality and heteroscedasticity (Keough 2002). Planned *post hoc* multiple comparisons of significant

main effects were tested using Tukey HSD ($\alpha = 0.05$, JMP Pro 13.0.0) on the transformed density data.

2.5.2 Identifying abiotic and biotic factors associated with Ulva

Factors potentially associated with *Ulva* population dynamics were evaluated using a general linear model ANOVA with R version 3.5.0 and packages "lm", "multcomp", "arm", "MuMIn", (The R Foundation for Statistical Computing 2018). Percent cover of attached *Ulva* was the response variable and was transformed to improve normality using the following transformation:

$$(1)\ Y' = \sqrt[4]{(Y + \frac{3}{8})} \text{(Zar, 1999)}.$$

Factors were included based on known ecological relationships. Abiotic explanatory variables were scaled and centered; including concentrations of ammonium, nitrogen, and phosphate, salinity, and water temperature . Categorical fixed effects included season and site. Collection methods for these parameters is discussed in detail above. Biotic factors included counts of mobile herbivores, percent cover of algal competitors and sessile invertebrates to estimate available space and conditions favorable to algal growth in general and mean density of waterborne propagules as a measure of propagule supply. Assumptions of normality and variance homogeneity were evaluated by visual inspection of residuals plots. Models were then compared using the Akaike Information Criterion (AIC) with correction for small sample sizes using the MuMIn package. Candidate models within 2 delta AICc of the lowest AICc score (n = 19) were subset and then that subset of models was averaged using the zero method (Grueber et al., 2011; Nakagawa and Freckleton, 2010). Planned post hoc comparisons (Tukey) between seasons and sites were performed on a linear model containing the parameters included in the top candidate model subset.

3. RESULTS

3.1. Spatio-temporal patterns of Ulva in SFB

Monthly monitoring of attached *Ulva*, recruitment and propagule supply at Tiburon revealed seasonal variation in recruitment and propagule supply (Figure 2). Attached *Ulva* cover at Tiburon peaked in late summer and began to decline in October of all years surveyed (2013, 2014, and 2015) (Figure 2a). Attached *Ulva* cover was the least in March of 2014 and January of 2015. Waterborne propagules were detected in each month of the study (Figure 2b). However, propagule supply was highly variable with the greatest amounts of propagules cultured in spring (April–June) and summer (July–August) months and the least in winter months (December–January, Figure 2b, Table 2). *Ulva* recruitment rates peaked in early fall (September) of 2014 and 2015, with high rates of recruitment throughout the summer (July–August) of 2015 (Figure 2a). In 2014, recruitment increased with increasing attached *Ulva* cover, peaked as the attached assemblage began to decline and continued to decline with attached *Ulva* cover until January 2015. *Ulva* cover and recruitment increased in February 2015 and while attached *Ulva* cover continued to increase until summer, recruitment fell in spring (April) and then recovered to 2014 peak levels by August of 2015.

Seasonal surveys of all five SFB sites in 2015 revealed that the effects of season on attached *Ulva* cover was dependent on location within the bay (Figure 3, Table 3). While increased *Ulva* cover was observed in spring and summer compared to fall and winter at Tiburon over a longer time scale these results were not consistent with observations of peak algal cover in fall at the other four central SFB sites in 2015. In fact, focusing in on a single year revealed so much spatial heterogeneity that any effect of season was driven by contrasting patterns at two sites, Berkeley and Alameda. In general, Berkeley had lower amounts of *Ulva* cover when compared to the other four sites and significantly greater cover was observed in fall when compared to the other three seasons. Specifically, attached *Ulva* at Berkeley and Point Potrero was significantly lower in spring ($p < 0.0001$ & $p = 0.0204$), summer ($p < 0.0001$ & $p = 0.0250$), and at Berkeley in winter of 2015 ($p < 0.0001$) compared to Alameda in fall. Alameda had significantly greater attached *Ulva* in fall than spring ($p = 0.0177$), but these observations were not significantly different from those in summer and winter at this site ($p = 0.1494$, $p = 0.1207$). No seasonal effect on cover was detected at any of the other sites (Figure 3 & Table 3).

Cultures of nearshore waters collected at all five SFB sites indicated that seasonal variation in propagule supply observed at Tiburon cannot be generalized for the central SFB, at least for 2015 (Figure 3, Table 2c & 2d). Summer (July) propagule supply was significantly greater than all other seasons sampled. Propagule supply in spring (April) and fall (November) of 2015 yielded a significantly greater number of recruits than winter (February) 2015. No significant differences in density of cultured propagules were detected between winter (February) 2015 and (January) 2016.

3.2. Identifying factors associated with attached Ulva cover in SFB

Given that several abiotic factors exhibit seasonal variation and season and site had a significant effect on attached *Ulva*, we included these spatio-temporal factors in our linear model. Candidate models with the lowest AIC_c values ($\Delta 2AIC_c$) included season, site, ammonium, phosphate, water temperature, nitrogen, algal competitors, herbivore abundance, and propagule density. Variables with equally strong effects, appearing in all of the 19 subset of candidate models included season, site, and herbivore abundance (Tables 4). Consistent with the results of the two-way ANOVA, attached *Ulva* cover was less in all seasons when compared to fall. Similarly, compared to the Alameda site, attached *Ulva* cover was greater at Tiburon, than at Berkeley (Table 5). Herbivore abundance and ammonium both exhibited strong negative relationships with attached *Ulva* cover, ammonium however, was not represented in all of the top weighted models. Algal competitors and propagule density were both moderate effects. The abundance of algal competitors had a negative relationship with *Ulva* cover and propagule supply had a positive relationship with attached *Ulva* cover. While present in the models with the lowest AIC_c scores and weights, the relative strengths of the effects of dissolved phosphate, dissolved nitrates, and water temperature alone were not detectable in the present study (relative importance weight (RIW) < 0.5).

4. DISCUSSION

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Ulva cover was expected to fluctuate as physicochemical and biological environmental parameters changed seasonally. Specifically, given within year variation observed at Tiburon, propagule supply was expected to be an important factor driving attached *Ulva* cover. Surprisingly, propagule supply was only moderately important in explaining attached *Ulva* cover in SFB with general effects of season and site having equally strong effects. Attached *Ulva* assemblage dynamics are dependent upon a suite of ecological factors with no single covariate explaining the majority of the variability. Two extreme weather patterns occurred during this study, influencing seasonal patterns in coastal environments of central California, we discuss these conditions along with important ecological factors in more depth below.

4.1. Temporal variation and supply-side ecology of Ulva

Seasonal variation in cover of the annual macroscopic *Ulva* thalli was expected with regular decreases in winter and peaks in spring and summer. We expected that decreasing propagule supply would become limiting in fall and winter as reproductive output begins to decline. We also expected that lowered salinity from winter rains would increase physiological stress of both unicells released during these seasons and macroscopic thalli. Lowered surface salinity as a result of winter increases in precipitation is thought to drive seasonal declines in total macroalgal cover in SFB. However, *Ulva* spp. have a greater tolerance to low salinity compared to most other marine macroalgae. For this reason, we expected that *Ulva* would persist in benthic communities later into the winter than most algal competitors. Given that our study took place during an extended drought, the amount of seasonal variation in attached *Ulva* cover observed was surprising. So, how important is precipitation-induced low salinity in controlling macroalgal abundances in SFB? *Ulva* cover decreased dramatically each year (2013-2015) in winter at Tiburon even in the absence of typical winter freshwater inputs (discharge from delta, local precipitation, and associated sedimentation) that are often used to explain winter declines in total macroalgae (Josselyn and West, 1985). Algal competitors were equally or more abundant than *Ulva* in most seasons, with the exception of fall 2015 at Alameda Island (Figure 4). Season and location within SFB were the most important factors included in our models while algal competitors and propagule supply were moderately important. Under drought conditions we observed persistent diverse macroalgal cover that often reduced the amount of space available for recruitment in central SFB (Figure 4).

A marine heatwave brought uncharacteristically warm, nutrient-poor water to SFB in the winter of 2014-2015, likely improving conditions for *Ulva* recruitment at Tiburon. Sea surface temperatures in the Pacific Ocean at this time were more than 2.5°C warmer than the long-term mean. Woodhead and Moss (2007) found that zoospore germination rates of *Ulva* (as *Enteromorpha*) at 20°C increased two-fold when compared to rates at 10°C (similar to typical winter mean water temperatures in SFB). We observed a small peak in recruitment in February and March of 2015, months coinciding with low propagule supply. This increase in water temperature may have stimulated growth of settled propagules resulting in the observed short increase in recruitment at that time. Recruitment rates dropped again in April of 2015, coinciding with the return of upwelling and colder water to the region (McCabe et al., 2016).

Yet, this pattern was not consistent across all central SFB sites in 2015. Can decreases in attached *Ulva* abundance be explained by supply-side processes? For the Tiburon site, where all three life stages (adults, recruits and propagule supply) were monitored, we observed evidence of recruitment limitation in 2014 and 2015 along with both inter and intra-annual variation in propagule supply. The magnitude of peaks in observed propagule supply and timing of these peaks in relation to recruitment pulses indicate that *Ulva* is recruitment-limited at Tiburon. This locally observed recruitment limitation in fall and winter could reduce *Ulva's* ability to colonize bare space at these times of year (Hruby and Norton, 1979; Underwood and Denley, 1984).

Ulva propagule supply at several of the four eastern shore sites dropped to the same low levels that led to recruitment limitation at Tiburon. This demonstrates that in the absence of environmental stressors associated with winter rainfall, propagule supply is highly variable within annual timespans. Seasonal variation in propagule supply is inherently linked to the life history and biological rhythms of individual organisms (Hoffmann, 1987). Fecundity, propagule size, and reproductive periodicity are largely responsible for spatial and temporal patchiness in propagule supply. When *Ulva* reproduces, up to 16 zoospores or 128 gametes (male gametophytes) (1-9µm) are released from each cell of the thallus beginning at the blade or tube margins (Smith, 1947). The small size and high motility of ulvoid propagules contribute to large dispersal shadows. For instance, *Ulva* propagules have colonized artificial substrata 35km from the nearest population (Amsler and Searles, 1980) and up to 24 km from the coast (Zechman and Mathieson, 1985). These characteristics have led to the conclusion that *Ulva* is not limited by propagule supply. However, Lüning (2008) found that reproductive intervals shift seasonally from weekly in spring and summer to biweekly in fall and winter. This decrease in reproductive output is tied to an endogenous clock and is supported by observed winter decrease in propagule supply in the present study.

Few have studied algal recruitment limitation at a range of spatial and temporal scales. When considered, the importance of recruitment limitation in structuring intertidal communities often varies across both spatial and temporal scales (Bellgrove et al., 2004; Keough, 1983). We investigated attached *Ulva* abundance and propagule supply at two spatial and temporal scales and found that spatial variation was dependent on temporal variation in attached *Ulva* abundance at the larger spatial scale. Even though propagule supply was also low in both winters, attached *Ulva* peaked in fall at the East Bay sites following the summer peak in propagule supply. Conversely attached *Ulva* was greatest at Tiburon in spring and summer as propagule supply in SFB increased. It is possible that propagule release occurs later in the year at Tiburon than at East Bay sites. Culturing of nearshore water samples more sites on the western shore of the SFB at increased temporal scale would be necessary better understand the intricacies of this contrasting patterns. Attached *Ulva* at Tiburon was numerous in both spring and summer and declined in fall and winter. This pattern was generally delayed for the East Bay sites with fall peaks in attached *Ulva* and a decline in winter. Observations at a single site over multiple years, revealed that recruitment limitation is at work at some locations within the SFB but may not be observable over a single year. SFB *Ulva* populations were recruitment limited at Tiburon, however the results of the spatial models are limited in scope as they do not include information on spatial variation in recruitment within SFB. Together, these observations make it clear that even highly dispersive algal species

can be limited by pre-settlement processes and the extent to which they are limited is dependent on where and when the observations are made.

4.2. Factors associated with Ulva abundance in SFB

As mentioned above, the effects of season were dependent on site within the SFB, and together had the strongest effects on *Ulva* cover as they were present in all of the top candidate models. In addition to site and season, the abundance of benthic herbivores had a strong negative relationship with *Ulva* cover. A negative relationship between attached *Ulva* and abundance of benthic herbivores is consistent with previous studies investigating intertidal succession. Lubchenco and Menge (1978) found that seasonality of ephemeral algae increased with seasonally increasing wave action and decreased abundance of littorinid herbivores. Littorines consume seasonally abundant *Ulva* adult macrothalli that can suppress the colonization and growth of later successional species (Lubchenco and Menge, 1978). In the present study, littorines were only common in summer, surface scraping limpets of the genus *Lottia*, however were common at Point Potrero, Tiburon, and Point Isabel but rare at Berkeley Marina and Alameda Island (Figure 5). Limpets scrape films of algae off of surfaces in the intertidal, potentially having a much greater influence on survival of recently settled propagules compared to littorines. When limpets are excluded from intertidal clearings, *Ulva* is able to rapidly colonize entire clearings regardless of the cleared area's size (Sousa, 1984). While in general benthic herbivores had a negative effect on attached *Ulva* in the present study, the only consistent seasonal pattern was that all sites had low herbivore abundance in winter. Alameda Island and Berkeley Marina both had consistently low numbers of benthic herbivores in all seasons when compared to the other sites.

Nitrogen is the most frequently limiting nutrient to algae followed by phosphorous. General paradigms link macroalgal blooms in eutrophic coastal systems to nitrogen loading derived from terrestrial runoff (Valiela et al., 1997). Several studies, however, have failed to detect significant correlations between rainfall events and *Ulva* biomass. Evidence from temperate upwelling regions suggests that the negative impacts of nuisance blooms are context dependent (Hessing-Lewis et al., 2011; B. B. Hughes et al., 2013; Nelson et al., 2003b). Macroalgae in upwelling systems are not limited by nutrients; runoff derived nutrients are delivered to an already saturated system. Temporal variation of ulvoid biomass in temperate upwelling systems has been strongly correlated to seasonal increases in daylength and negatively correlated to dissolved organic nitrogen (DIN) as a result of collinearity between daylength and DIN (Nelson et al., 2003b). Light indirectly affects nutrient uptake as photosynthesis provides energy for active transport, provides carbon for incorporation of nutrients into amino acids and proteins, and increases growth rates resulting in increased demand for nutrients (Hurd et al., 2014). Ammonium and nitrogen are the most important forms of nitrogen to macroalgal growth and can be impacted differently by photoperiod which could explain the differences in relative importance between these two forms in the model (Hurd et al., 2014). In the SFB localized depletions of nutrients can occur but these depletions are more characteristic of the estuarine north SFB than the central SFB, which maintains a more marine signature (Cloern and Nichols, 1985). While in general ulvoids are known for the ability to rapidly utilize increases in nitrogen, there is evidence to suggest that they are more sensitive to decreases in DIN than slower growing macroalgae such as rockweeds (Pedersen and

Borum, 1997; 1996). The high growth rates of ephemerals species demand higher concentrations of nitrogen availability for saturation than slower-growing perennials. We observed significantly greater concentrations of ammonium in fall and winter seasons than in spring and summer (Figure 6). Thus, in this eutrophic upwelling system where nitrogen levels are typically high, ammonium concentrations were low when daylength was most conducive to growth. Nitrogen levels were lower in spring and fall than summer and winter, these episodic decreases in nitrogen availability coincided with increased daylength, when ephemerals require higher nutrient concentrations for saturation. Together, these relationships between nitrogen forms and light availability could explain the negative relationship observed between ammonium and attached *Ulva* in this study.

4.3. Implications for monitoring Ulva blooms in SFB

Monitoring an extremely fecund and opportunistic species such as *Ulva*, is highly dependent on spatial and temporal scales. The contrast between the results of the spatial study performed over a single year and the multi-year temporal treatment at a single site emphasize this. Similar work focusing on detached *Ulva* blooms have detected interannual variation in suspended biomass. This entire study was conducted under drought conditions, yet during these anomalous conditions, seasonal variation was still one of the most important factors explaining variation in attached *Ulva*. Josselyn and West (1985) and Silva (1979) have proposed that seasonal increases in rainfall and decreases in salinity of surface waters are responsible for decreased algal cover in winter. The drought conditions during 2015, particularly low rainfall in winter, could explain the temporal lag in attached *Ulva* and persistence in algal competitors when comparing Tiburon to the East Bay sites.

Seasonal decreases in salinity as a result of increases in rainfall and runoff into the SFB in winter and spring are thought to be an important factor limiting macroalgae throughout the bay. Along with decreases in salinity, rainfall and runoff lead to increases in turbidity resulting in sedimentation and reduced light penetration through the water column. However, no effect of salinity on attached *Ulva* was detectable by the present study. There are two explanations for this result, the amount of freshwater inflow into the SFB has declined from 1956-2010 and the 2013-2016 drought. All years included in this study coincided with a one of the worst droughts recorded on record for the U.S. west coast (NOAA 2015). Given these conditions, it was not surprising that no effect of salinity on attached *Ulva* was detected. The results of this study provide an estimate of *Ulva* bloom source populations during drought, or potentially "benign" winter conditions. Under these conditions macroalgae are relieved of the potential stress associated with rainfall including physiological stress due to decreased salinity, light limitation from turbidity, and wave exposure that can lead to detachment.

Climatic variation can alter supply-side dynamics of marine organisms. For example, increases in phytoplankton have been positively correlated to cooler water associated with ocean gyre-scale circulation and in turn with increases in mussel recruitment rates (Menge et al., 2009). An anomalously warm body of water, known as "The Blob", was observed in the NE Pacific in the winter of 2013 (Bond et al., 2015). The region of warm water brought anomalously warm SST temperatures to coastal

regions by May of 2014 that persisted through March of 2015. This heatwave coincided with the long-term drought (2013-2015) in California that resulted in low precipitation and higher than average winter salinities in the central SFB compared to wet years (Cloern et al., 2017). As drought and upwelling periods become more prolonged, conditions of increased nutrient delivery and absence of salinity stress will favor green tide development in coastal ecosystems. Little variation in salinity combined with warming ocean temperatures could shift the *Ulva* recruitment season earlier in the year. Similar patterns have been observed for recruitment of invasive sessile invertebrates under warmer winter temperatures(Stachowicz et al., 2002). Non-native sessile invertebrates are more resistant to heat waves and extreme low salinity events than native species (Chang et al., 2018; Sorte et al., 2010). Die-offs of native species that result from these events provide bare space and recruitment opportunities for species such as *Ulva*.

In conclusion, our study shows that propagule supply can limit *Ulva* recruitment and should not be overlooked when predicting *Ulva* blooms. We also highlight the need for *Ulva* recruitment datasets that span long (greater than 3 years) temporal scales. Changes in temperature due to climatic disturbances, changes in salinity (especially in estuaries), and nutrient content or periodic desiccation due to tidal oscillations are thought to be the most frequent causes of severe changes in algal propagule production (Hoffmann, 1987). Of these factors, those most likely to impact SFB include warming winter temperatures (IPCC, 2014)and salinity resulting from increased diversion of riverine inflow (Cloern and Jassby, 2012). Decadal-scale extremes in freshwater flow can produce among-year variation in sessile invertebrate communities within SFB (Chang et al., 2018). We did not observe these types of salinity driven patterns in our study as a result of drought. Increasing the temporal scale to include data from wet years would be necessary to test the effects of salinity on attached *Ulva* cover. However, we were able to demonstrate that during anomalously warm winter and drought conditions, propagule supply is important in predicting attached *Ulva* cover. Seasonal changes in nutrient supply, abundance of benthic herbivores, algal competitors, and propagule supply must be considered together when trying to predict how *Ulva* assemblages will impact estuarine and coastal ecosystems in the face of global change.

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TABLE CAPTIONS

Table 1. Survey data for environmental variables from five intertidal sites in the central San Francisco Bay in 2014-2015.

Table 2. Effects of season on propagule supply as log transformed density of recruits cultured from water samples collected at two spatial scales within the central San Francisco Bay: a. Interannual variability in propagule supply to Tiburon, b. Multiple comparisons test of season effect on propagule supply arriving to Tiburon , c. Intra-annual variation in propagule supply to all five SFB sites and d. Multiple comparisons test of season effect on propagule supply to all SFB sites.

Table 3. Effects of season and site on log transformed attached *Ulva* cover in central San Francisco Bay.

Table 4. Model averaged coefficients (unconditional) for the top ranked models (by AICc) within Δ2AIC averaged attached *Ulva* percent cover model with variables in order by rank of relative variable importance weights.

Table 5. Tukey's multiple comparison evaluation of significant a. season and b. site fixed effects on percent cover of *Ulva* in the central San Francisco Bay. Response variable transformed ($Y' = (Y + 3/8)^{0.25}$) to improve normality. Model with only the parameters that ended up in the top models when model averaged.

TABLES

Table 1.

Location	Variable	OBS	Range	Mean	SD
Tiburon	Ammonium	18	1.42- 31.20	9.48	8.24
	Nitrate	18	1.18 - 34.54	15.95	7.71
	Phosphate	18	1.19 - 3.92	1.86	0.60
	Temperature		5 - 26.1	15	6
	Salinity	57	20 - 35	28	3
Point Potrero	Ammonium	9	1.80 -18.04	8.83	5.75
	Nitrate	9	6.56 - 51.09	18.40	14.53
	Phosphate	9	0.96 - 3.22	1.81	0.68
	Temperature		1 - 17.2	9	4
	Salinity	30	17 - 33	28	4
Point Isabel	Ammonium	8	2.94 -11.13	8.35	2.71
	Nitrate	8	4.16 - 61.06	10.68	5.40
	Phosphate	8	1.55 - 4.05	2.17	0.78
	Temperature		1 - 17.8	9	5
	Salinity	27	15 - 33	27	5
Berkeley Marina	Ammonium	8	3.40 - 20.69	12.25	5.13
	Nitrate	8	1.80 - 25.63	10.37	8.87
	Phosphate	8	1.28 - 3.54	2.61	0.75
	Temperature		-1.2 - 20.1	6	9
	Salinity	15	25 - 33	31	3
Alameda Island	Ammonium	8	2.05 - 14.89	8.60	5.22
	Nitrate	8	4.17 - 61.06	20.78	17.60
	Phosphate	8	1.88 - 5.01	3.37	0.98
	Temperature		-2.2 - 27.5	8	10
	Salinity	18	25 - 34	31	2

The measured environmental variables: ammonium (µM), nitrate (µM), phosphate (µM), and temperature (°C) are shown. The range, mean, and standard deviation represented by SD, are also provided.

Table 2. Effects of season on propagule supply as log transformed density of recruits cultured from water samples collected at two spatial scales within the central San Francisco Bay: a. Interannual variability in propagule supply to Tiburon, b. Multiple comparisons test of season effect on propagule supply arriving to Tiburon , c. Intraannual variation in propagule supply to all five SFB sites and d. Multiple comparisons test of season effect on propagule supply to all SFB sites.

a. Seasonal variation in density of *Ulva* propagules cultured monthly from Tiburon 2014-2015 (ANOVA, $\alpha = 0.05$).

Source	SS Type I	df	Mean Square	F	P-Value
Season	9.046	6	1.508	5.405	**0.0053**
Error	3.626	13	0.2789		
Corrected Total	12.671	19			

b. Planned *post hoc* pairwise comparisons of season effect on propagules cultured monthly from Tiburon (Tukey HSD, $\alpha = 0.05$).

Season$_{(i)}$	Season$_{(j)}$	P-Value
Summer 2014	Fall 2014	1.0000
	Winter 2015	0.5071
	Spring 2015	0.4342
	Summer 2015	0.1953
	Fall 2015	1.0000
	Winter 2016	0.7743
Summer 2015	Fall 2014	0.1602
	Winter 2015	**0.0076**
	Spring 2015	0.9964
	Fall 2015	0.1423
	Winter 2016	**0.0282**
Spring 2015	Fall 2014	0.3702
	Winter 2015	**0.0209**
	Fall 2015	0.3354
	Winter 2016	0.069
Fall 2014	Fall 2015	1.0000
	Winter 2015	0.5799
	Winter 2016	0.8304
Fall 2015	Winter 2015	0.6233
	Winter 2016	0.8601
Winter 2016	Winter 2015	1.0000

c. Seasonal variation in density of *Ulva* propagules cultured from all five SFB sites in 2015 (ANOVA, $\alpha = 0.05$).

Source	Type I	df	Square	F	P-Value
Season	6.779	4	1.695	17.662	**<0.0001**
Error	11.419	119	0.0960		
Corrected Total	18.197	123			

d. Planned posthoc pairwise comparisons of season effect on propagules cultured from all five SFB sites (Tukey HSD, $\alpha = 0.05$).

Season$_{(i)}$	Season$_{(j)}$	P-Value
Summer 2015	Winter 2015	**<0.0001**
	Winter 2016	**<0.0001**
	Fall 2015	**0.0008**
	Spring 2015	**0.0059**
Spring 2015	Winter 2015	**0.0001**
	Winter 2016	0.1709
	Fall 2015	0.9798
Fall 2015	Winter 2015	**0.0009**
	Winter 2016	0.4508
Winter 2016	Winter 2015	0.1322

Table 3. Table 3. Effects of season and site on log transformed attached *Ulva* cover in central San Francisco bay.

Source	SS Type I	df	Mean Square	F	P-Value
Season	38.89	3		9.5626	**<0.0001**
Site	40.65	4		7.497	**<0.0001**
Season*Site	32.12	12		1.975	**0.0289**
Error	242.7	179	1.356		
Corrected Total	354.3	198			

Table 4.

Parameter	Estimate	Standard Error	Adjusted Standard Error	z value	p value	Relative Importance
(Intercept)	2.249	0.187	0.188	11.97	**<2e-16**	
Season-Spring	-0.827	0.209	0.210	3.944	**8.00E-05**	
Season-Summer	-0.782	0.332	0.334	2.343	**0.0191**	Season (1.00)
Season-Winter	-0.516	0.258	0.259	1.993	**0.0463**	
Site-BKM	-0.210	0.176	0.177	1.183	0.2368	
Site-PTI	0.181	0.208	0.209	0.866	0.3865	Site (1.00)
Site-PTP	0.075	0.237	0.238	0.317	0.7514	
Site-RTC	0.492	0.249	0.250	1.966	**0.0493**	
Benthic herbivores	-0.0875	0.0413	0.0415	2.106	**0.0352**	1.00
Ammonium	-0.1957	0.1210	0.1215	1.612	0.1071	0.91
Algal competitors	0.0017	0.0016	0.0016	1.036	0.3004	0.69
Propagule supply	0.0582	0.0622	0.0624	0.932	0.3512	0.64
Phosphate	0.0661	0.0930	0.0933	0.709	0.4784	0.47
Nitrogen	0.0354	0.0717	0.0719	0.493	0.6223	0.34
Water temperature	-0.0751	0.1807	0.1812	0.415	0.6784	0.22

Table 5.

a. Season

Linear Hypotheses:	Estimate	Standard Error	t value	Pr(>|t|)
Spring - Fall == 0	-0.8681	0.2057	-4.220	**<0.001**
Summer - Fall == 0	-0.6916	0.4636	-1.492	0.3720
Winter - Fall == 0	-0.6909	0.3223	-2.143	0.1120
Summer - Spring == 0	0.1765	0.5046	0.3500	0.9780
Winter - Spring == 0	0.1772	0.3246	0.5460	0.9250
Winter - Summer == 0	0.0008	0.7100	0.0010	1.000

b. Site

Linear Hypotheses:	Estimate	Standard Error	t value	Pr(>|t|)
BKM - BBA == 0	-0.1614	0.1851	-0.8720	0.8976
PTI - BBA == 0	0.2224	0.2302	0.9660	0.8579
PTP - BBA == 0	0.1027	0.2585	0.3970	0.9940
RTC - BBA == 0	0.5052	0.2587	1.952	0.2753
PTI - BKM == 0	0.3838	0.1710	2.244	0.1560
PTP - BKM == 0	0.2640	0.2158	1.223	0.7187
RTC - BKM == 0	0.6665	0.1922	3.469	**0.0054**
PTP - PTI == 0	-0.1198	0.1563	-0.7660	0.9335
RTC - PTI == 0	0.2827	0.1705	1.658	0.4400
RTC - PTP == 0	0.4025	0.1570	2.564	0.0749

FIGURE CAPTIONS

Figure 1. Map of the study location with locations of sediment sample collection marked labeled in bold (●). Map courtesy of Cassandra J. Hansen 2018.

Figure 3. Temporal dynamics of a. attached *Ulva* assemblage (green squares) and *Ulva* recruitment to settlement plates (orange circles) at Tiburon from August 2013 – January 2016 (top panel) and b. *Ulva* propagule density of monthly water samples collected at Tiburon from July 2014 – January 2016 (bottom panel).

Figure 4. Seasonal variation in (a.) attached *Ulva* cover and (b.) waterborne *Ulva* propagule abundance expressed as mean density of recruits cultured from nearshore water samples collected at five central San Francisco bay sites. (a.) Different shapes and colors represent different seasons. Waterborne propagule collections were not replicated within sites.

Figure 5. Spatio-temporal variation in mean percent cover of *Ulva* and other sessile organisms at central San Francisco bay study sites in spring 2015, summer 2015, fall 2015 and winter 2016.

Figure 6. Spatio-temporal variation in abundance (mean counts ± standard error) of benthic herbivores at central San Francisco Bay sites in spring 2015, summer 2015, fall 2015 and winter 2016.

Figure 7. Temporal variation in nutrient concentrations of nearshore seawater in the central San Francisco Bay. Samples were collected during each algal census and are represented as the mean of all five study sites for each season with standard error bars.
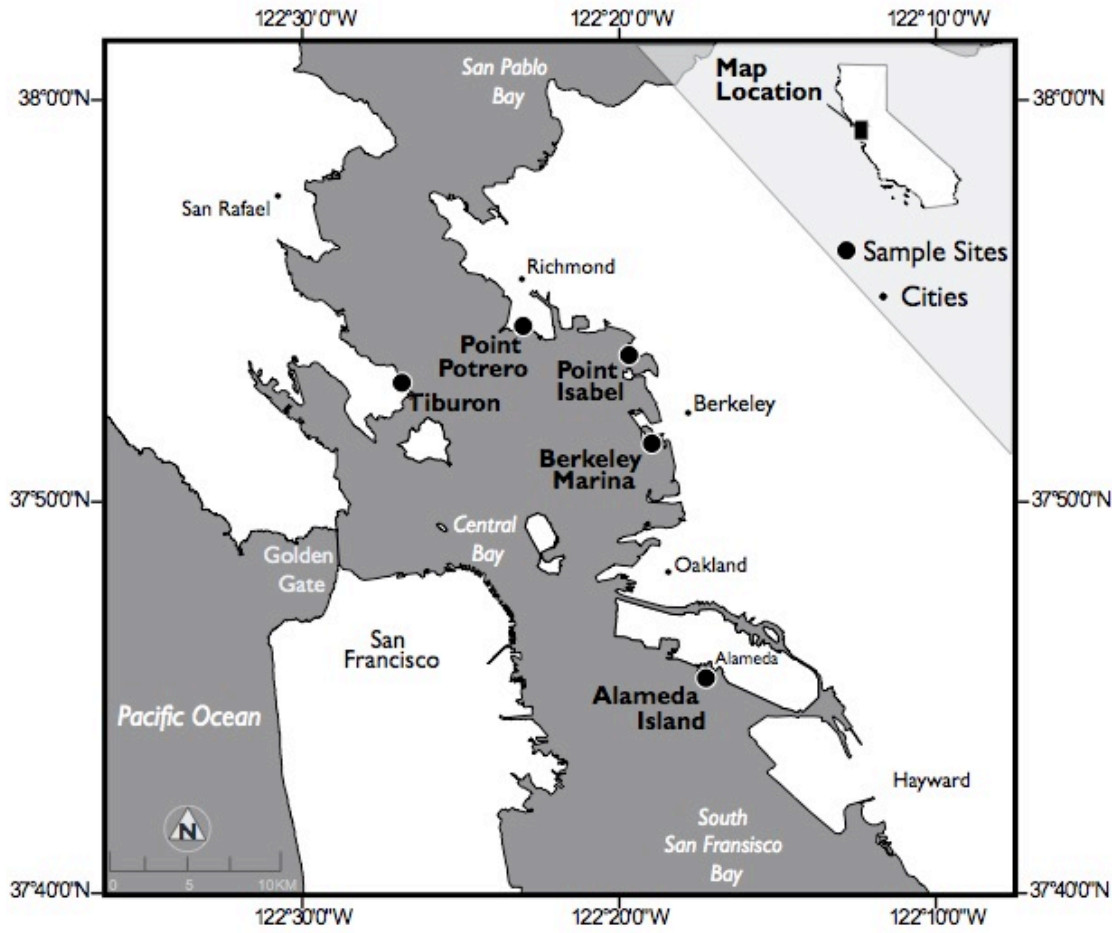
FIGURES

Figure 1.

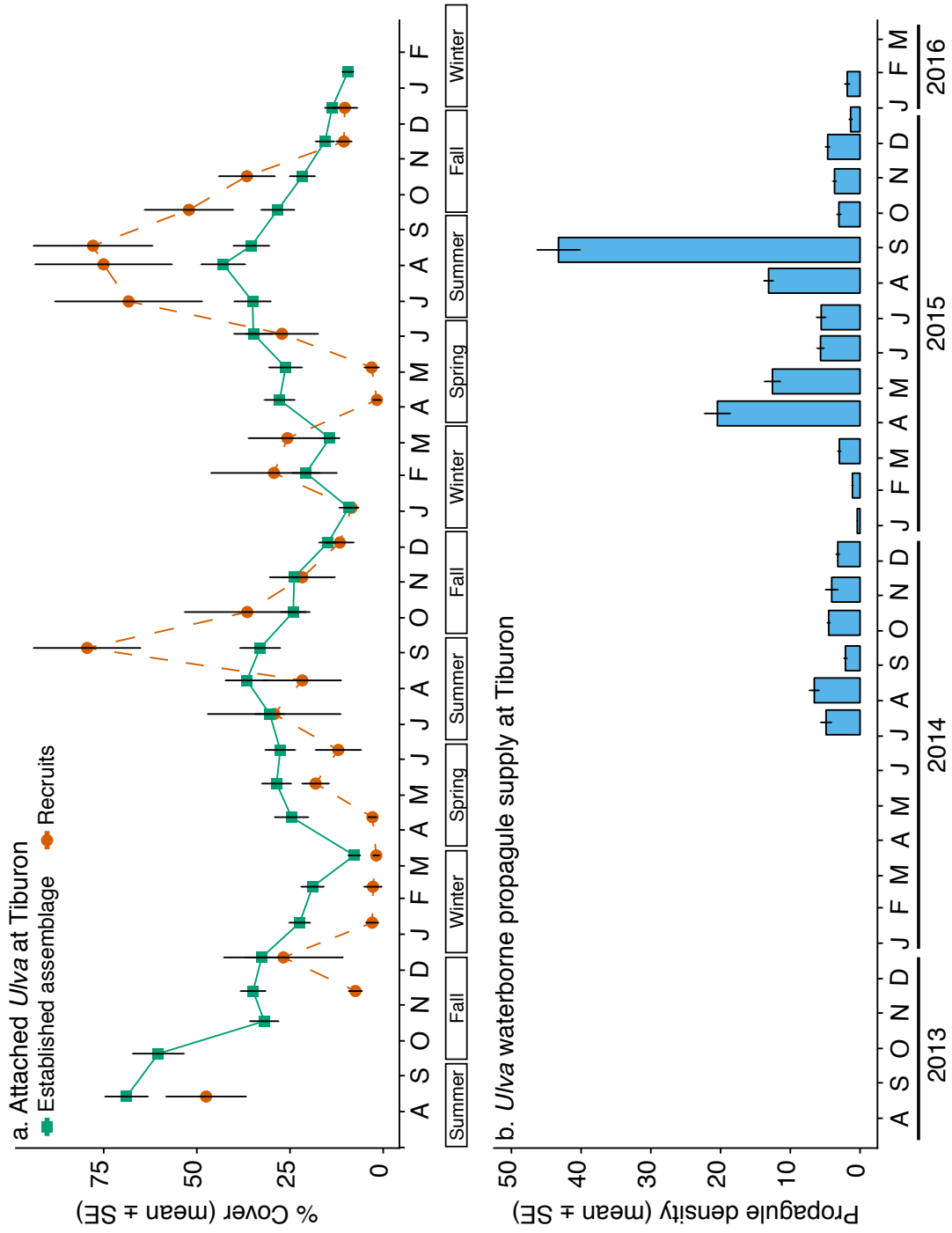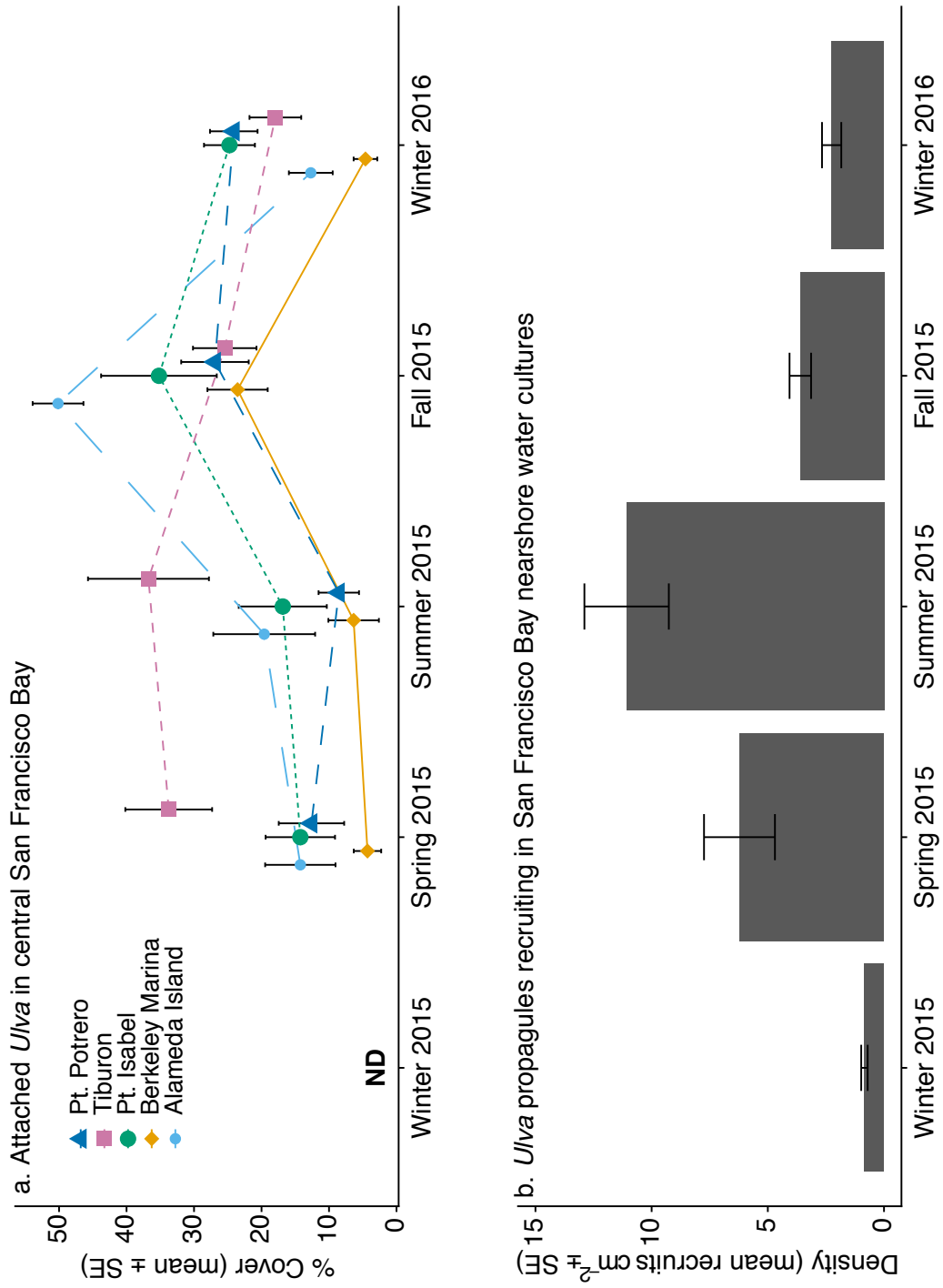**Figure 2.**

Figure 3.

a. Attached *Ulva* in central San Francisco Bay

Pt. Potrero
Tiburon
Pt. Isabel
Berkeley Marina
Alameda Island

b. *Ulva* propagules recruiting in San Francisco Bay nearshore water cultures

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Figure 4.

Figure 5.

Figure 6.

Temporal variation in nutrient concentrations at central SFB sites