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Spatial and Temporal Variability in Processes Regulating Estuarine Fouling Communities

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Spatial and Temporal Variability in Processes Regulating Estuarine Fouling Communities

By

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DISSERTATION

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Dissertation Abstract

Fouling communities are diverse assemblages of sessile, filter feeding invertebrates found in marine and estuarine environments. They are an experimentally tractable study system that is commonly used to test ecological theories, and most research on fouling communities has taken place on artificial structures near docks and marinas. Fouling species are also found in natural habitats within estuaries, such as seagrass beds and cobbles, but few studies have tested ecological theories or processes influencing fouling communities in these natural habitats. Additionally, estuaries are dynamic ecosystems that are highly vulnerable to effects of climate change, and many estuaries contain a large number of non-native fouling species. Since climate change is likely to favor non-native over native species, processes structuring fouling communities may vary over space and time, resulting in biodiversity shifts and the proliferation of invasive species. In this dissertation, I explore how processes influencing fouling communities vary spatially across an estuarine gradient as well as inside and outside of seagrass beds, and vary temporally over three years in Tomales Bay, CA.

In my first chapter, I examined how fouling communities and effects of predators change across the estuarine stress gradient of Tomales Bay, CA. The Environmental Stress Model predicts that effects of predators decrease with increasing stress. In estuaries, this stress gradient occurs from the ocean to freshwater habitats, with increasing stress for marine organisms at greater distances from the ocean. However, this theory might not apply when stress-tolerant non-native species are introduced to ecosystems, such as estuaries. I predicted that predation would decrease with distance into the estuary but that the introduction of non-native species would extend the importance of predation further along this stress gradient than predicted by the Environmental Stress Model. Using a predator exclosure experiment, I evaluated how fouling

communities and effects of predators differed across three sites in the summer of 2019. Fouling community composition differed significantly across sites and predation treatments, but the effects of predators differed significantly across sites. In general, the effect of predation was to reduce abundance, richness, diversity, and the abundance of specific morphotypes. The greatest effect of predation was in the middle of the bay where both native and non-native predators co-occur and was similarly low near the mouth and head of the estuary. This pattern was likely influenced by the abundance of solitary ascidians, which are highly susceptible to predation and were most abundant in the middle of the bay. The results differed slightly from the predictions of the Environmental Stress Model and suggest that ecosystems with large numbers of stress-tolerant introduced species may experience predation at higher levels of stress than predicted by the model.

In my second chapter, I investigated how fouling communities and effects of predators differed inside and outside of seagrass at one site in Tomales Bay, CA. Biogenic habitat, such as seagrass, could directly and indirectly influence fouling communities. Direct effects could occur when the structure associated with seagrass reduces flow or modifies water chemistry, resulting in physiological influences on fouling species. Indirect effects could occur when seagrass provides a habitat for predators, thereby increasing risk of predation for fouling species. To better understand the mechanisms in which seagrass influences fouling communities, I conducted a predator exclosure field experiment in 2018 and a predator exposure field experiment in 2020. Community composition differed significantly inside and outside of seagrass, with abundance, richness, and diversity being higher outside of seagrass than inside, suggesting a strong direct effect of seagrass. Predation differed significantly inside and outside of seagrass with predation being higher outside, though this effect was likely driven by differences in recruitment patterns of specific morphotypes and not differences in predator habitat use. These experiments provided evidence for both direct and indirect effects of seagrass on fouling communities; however, indirect effects of predators could be more variable than what has previously been documented. I caution against the overgeneralization about effects of seagrass on biological communities, and additional research is needed to better understand the mechanisms behind the relationship between biogenic habitat and biodiversity.

Finally, in my third chapter I examined how the effects of predators and seagrass on fouling communities vary over multiple years at Sacramento Landing in Tomales Bay, CA. While fouling communities have been used as a model system to test ecological theories, most previous studies have taken place over short time scales and have not focused on benthic processes in natural habitats. Given that estuaries experience high interannual variability in temperature, salinity, chlorophyll *a*, and other water quality parameters, ecological patterns are likely to vary along with these abiotic changes. This study tested how the importance of predation and seagrass in structuring fouling communities change over three years using results from predator exclosure experiments conducted in 2018, 2019, and 2020. Fouling community composition varied significantly across years, and this was likely due to interannual variability in recruitment, predation, and water quality. While there were some consistent effects of predators (on solitary ascidians), predation had variable effects on community metrics across years, which was likely driven by recruitment variation in specific morphotypes. Seagrass reduced abundance, richness, and diversity in 2018, but there was no significant effect of seagrass when averaging across all years. This pattern is likely due to interannual variability in seagrass bed characteristics.

Overall, processes influencing fouling communities are variable over space and time, and future research should account for this by taking place across greater spatio-temporal scales and should utilize laboratory experiments to isolate mechanisms of change.

CHAPTER 1

Testing the Environmental Stress Hypothesis with Predation on Invertebrate Communities Across an Estuarine Gradient

Abstract

Estuaries represent steep stress gradients for aquatic organisms, with abiotic stress due to temperature and salinity typically increasing with distance into estuary. Invertebrate communities and their predators are strongly influenced by these stress gradients. However, how the effects of predation on invertebrate community composition vary across a range of environmental conditions is unknown. The Environmental Stress Model predicts that the importance of predation in structuring communities decreases with increasing environmental stress. Estuaries are often hotspots of biological invasions, and increased stress-tolerance among non-native species can change the predictions of the Environmental Stress Model. We predict that predation on sessile invertebrate communities will decrease along the estuarine gradient from ocean to river, but effects of predators will remain higher than predicted due to the introduction of stress tolerant non-native species. We investigated this prediction in Tomales Bay, CA using communities of sessile invertebrates on experimental plates distributed at sites along this gradient in the summer of 2019. Our data show that community composition differed across sites, and predation had a significant effect on community composition. However, the effect of predation changed across sites, with mid-bay sites experiencing the greatest effects of predators. This was likely due to patterns in morphotype abundance, as certain morphotypes, such as solitary ascidians, were more susceptible to predation than others. Overall, predation didn’t follow the predictions of the Environmental Stress Model, but rather followed the abundance of particular morphotypes, whose distribution could be mediated by environmental stress gradients. We suggest that this may be a general result and that communities subject to large numbers of stress-tolerant invaders may have high rates of consumption in high stress areas in contrast to predictions by previous models.

Keywords: Environmental Stress Model, Consumer-Stress Relationships, Environmental Gradient, Diversity, Invertebrates, Estuaries

Introduction

While multiple processes structure biotic communities, which processes dominate at the local scale can shift across gradients of environmental stress. Both biotic interactions and environmental stress have been shown to jointly influence community structure (e.g. Menge 2000, Silliman and He 2018). For example, in rocky intertidal ecosystems, tidal elevation drives the importance of biotic or abiotic processes, with environmental stress structuring high tidal elevation communities and predation structuring low tidal elevation communities (Connell 1961, Dayton 1971, Menge 1976). Patterns in variation of stress and biotic interactions along environmental gradients have been documented across tidal gradients (Connell 1961, Dayton 1971, Menge 1976), elevation gradients (Preszler and Boecklen 1996, Callaway et al. 2002) and latitudinal gradients (Pianka 1966).

Estuaries contain distinct stress gradients involving salinity, temperature, pH, flow, dissolved oxygen, turbidity, etc. which often vary predictably as a function of distance from freshwater and ocean sources (Pritchard 1967). The frequency and magnitude of both precipitation and fluvial inputs generally determine salinity in an estuary (Monismith et al.

2002). In low-flow estuaries typical of Mediterranean climates throughout the world, including southern through central California, temperature and salinity are less temporally variable at the mouth of estuaries than at their riverine source (Hearn and Largier 1997, Kimbro et al. 2009b). These longitudinal estuarine gradients in temperature and salinity characteristics, and therefore environmental stress, likely shape the diversity and composition of estuarine communities (Cheng and Hovel 2010) and can be used to identify drivers of differences in consumer-stress relationships.

Abiotic stress that shifts across the estuarine gradient likely is a key factor in the extent to which predation plays a role in shaping estuarine communities. The Environmental Stress Model (ESM) (Menge and Sutherland 1987) suggests that at low levels of stress, predation will have stronger effects on community composition than environmental stress or competition (Menge and Sutherland 1987). This pattern is thought to develop when predators have a greater sensitivity to environmental stress than their prey (Menge and Sutherland 1987). This has been shown to be the case in rocky intertidal ecosystems, where mobile sea star predators suffer greater mortality under tidal elevation stress than their mussel prey (Petes et al. 2008). In an estuary, the abundance of native crab predators declined with distance from the mouth of Tomales Bay (Cheng and Grosholz 2016). This pattern has also translated into differences in predation strength, with predation strength being stronger at the mouth of Mission Bay than near the head (Cheng and Hovel 2010).

Nonetheless, patterns of predation across environmental gradients can be disrupted by species introductions. Non-native species in these systems tend to be more tolerant of environmental stress than their native counterparts (Sorte et al. 2010, Lenz et al. 2011). In Tomales Bay, non-native oyster drill survival in the upper estuary has resulted in greater oyster mortality than predicted by native predators alone (Kimbro et al. 2009a). This pattern was influenced not only by the non-native oyster drill tolerance of stressful abiotic conditions, but also by the reduction in biotic control of the drills by native crab predators in the upper estuary. Hence, the addition of non-native predators could extend the effects of predation further into estuaries than is expected under the ESM (Fig. 1.1a).

Including information on stress-tolerance in non-native predators within the ESM may better predict how sessile invertebrate communities vary along estuarine gradients. Given their stress tolerance and introduction to estuaries worldwide, we believe that non-native predators are altering consumer-stress relationships along estuarine gradients (Kimbro et al. 2009a, Cheng and Grosholz 2016). We hypothesize that when ecosystems contain non-native predators, predation is consistently high under low to medium levels of stress, but eventually decreases as stress gets too high even for mobile stress-tolerant predators (Fig. 1.1a). Effects of predators are likely to be highest in the middle of the bay, where there is the greatest diversity of native and non-native predators, intermediate at the mouth, where stress is low and native predators are abundant, and lowest at the head, where conditions are too stressful for most predators (Fig. 1.1b). We also hypothesize that the effects of predation and environmental stress on prey communities, combined with the greatest abundance of non-native species mid-estuary (Preisler et al. 2009), creates a pattern in which the diversity and abundance of prey species is highest mid-estuary. However, this high prey abundance and diversity is at the same time suppressed somewhat by predation (Fig. 1.1b) since we predict that the effects of predators on prey diversity and abundance will be greatest mid-estuary where predator diversity and abundance is also highest.

In this study, we conducted a field experiment to evaluate how patterns of sessile invertebrate diversity and abundance as well as patterns of consumption change across an estuarine stress gradient. We will determine where along the estuarine gradient the effects of predation are highest and how this interacts with changing patterns of prey abundance and diversity. In doing so we will test the predictions of ESM to see if these fundamental predictions have been changed by the introduction of non-native predator species.

Assuming that invertebrate abundance and diversity are closely linked, we predict that sessile invertebrate diversity and abundance are highest mid-estuary where both native and non-native taxa co-exist and stress is intermediate, intermediate at the mouth where stress is low but non-native species are rare, and lowest towards the head (low salinity) where conditions are the most stressful for marine-derived organisms (Fig. 1.1b). The overall effect of predators is to decrease diversity and abundance, and this effect is greatest mid-estuary where predator diversity is also highest (Fig. 1.1b; B > A > C).

Study System

Tomales Bay is a 20 km long drowned river valley located 60 km northwest of San Francisco, CA. This estuary was the focus of an NSF Land-Margin Ecological Research (LMER) project that detailed its physical and biochemical dynamics (Hearn and Largier 1997, Smith and Hollibaugh 1998). In this Mediterranean climate, low salinity events occur during periods of winter/spring runoff from river inflow at the head of the estuary. However, conditions at the head of the estuary, such as temperature and salinity, are extremely variable across seasons. For example, high residence times and evaporation in the upper bay result in slightly hypersaline conditions in late summer (salinity > 35 ppt). In the summer, temperature and residence time increase with distance into the estuary. In the winter, precipitation events drastically lower the salinity at the head of the estuary (salinity < 20 ppt) (Hearn and Largier 1997). Salinity decreases and variability in salinity increases with distance into the estuary in the winter. While the dominant abiotic characteristics of the stress gradient change across seasons, variability in temperature and salinity increase with distance into the estuary across seasons (Hearn and Largier 1997, Kimbro et al. 2009b, Hollarsmith et al. 2020).

Among the most common invertebrate taxa in intertidal and shallow subtidal habitats across Tomales Bay are sessile filter feeding invertebrates, commonly known as fouling communities. These invertebrate communities provide an excellent, experimentally tractable model system to test ecological theories about community assembly, physiological stress, ecosystem function, and biotic interactions in estuarine as well as fully marine systems (e.g. Osman 1977, Stachowicz et al. 1999, Stachowicz and Byrnes 2006, Altman and Whitlatch 2007, Freestone et al. 2013). Although the species in this community dominate piers and marinas, they also establish on other invertebrates, seagrass blades, cobbles, exposed bedrock, and hard mud, and can greatly affect foundation species and many estuarine functions (Ruiz et al. 1999, Fitridge et al. 2012, Forrest et al. 2013, Aldred and Clare 2014, Long and Grosholz 2015, Carman et al. 2016). Abiotic stressors for fouling communities include changes in salinity, temperature, and residence time. Since fouling species are marine in origin, low salinity, high temperature, and variability in temperature and salinity are stressful to fouling species. Additionally, longer residence times results in lower recruitment and less food availability (Kimbro et al. 2009b), causing more stress to these filter feeding invertebrates.

Predation is an important process regulating fouling community composition. Predation and bulldozing (dislodgement by grazers) of fouling species recruits is a dominant source of mortality in fouling species (Osman and Whitlatch 1995). Additionally, some fouling species adults experience high predation rates. Solitary and colonial ascidians are the most susceptible to consumers such as crabs, sea stars, and chitons (Rogers et al. 2016), while bryozoans (both arborescent and encrusting) tend to be consumed the least (Osman et al. 2010). Predators of fouling species at temperate latitudes are mostly benthic, and include sea stars, rock crabs, shore crabs, hermit crabs, snails, limpets, chitons, caprellid amphipods, flatworms, and nudibranchs (Osman and Whitlatch 2004, Collin and Johnson 2014, Rogers et al. 2016, Kincaid and de Rivera 2020). Intertidal surveys across Tomales Bay have shown that possible predators at the sites used in this study include oyster drills (native and non-native), crabs (native and non-native), turban snails (native), limpets (native), chitons (native), and nudibranchs (native) (Rubinoff, *unpublished data*). Crab predators are most abundant at the mouth of the bay, and shift from native to non-native with distance into the estuary. Additionally, oyster drills are most abundant in the middle of the bay, and shift from native to non-native drills with distance from the mouth (Kimbro et al. 2009a, Cheng and Grosholz 2016).

Methods

To test our hypotheses regarding the influence of environmental stress and predation on fouling communities, we deployed a fully factorial experiment across Tomales Bay, CA from June to October 2019 (Fig. 1.2). We used a standardized substrate (PVC plates, 10.16 cm x 10.16 cm) deployed on the benthos at -0.3 m below MLLW to measure the potential for settlement on hard substrates. PVC plates are commonly used in experiments on fouling communities (Osman and Whitlatch 1995, Stachowicz et al. 2002, Freestone et al. 2011), and while PVC often has a higher species richness of taxa compared to other natural and artificial materials, communities do not differ significantly among substrate types after a year (Brown 2005). Settlement plates were deployed across three sites spanning the estuarine gradient of Tomales Bay (Fig. 1.2): Pelican Point (38.187016˚N, -122.933211˚W), Sacramento Landing (38.151244˚N, -122.906417˚W), and Teachers Beach (l38.113183˚N, -122.868941˚W). At each of the three sites, we deployed a total of 48 replicate plates in one of six blocks (3 m x 3 m, spaced 5m apart) with three blocks inside and three outside of eelgrass beds. Plates were oriented perpendicular to the sediment with the bottom edge of the plate touching the benthos to allow access by benthic predators. Within each block, we randomly assigned plates to one of three caging treatments: 1) open plates as controls, 2) small mesh cages (1 mm opening) to exclude most predators, and 3) partial cages with the same caging on three sides to control for cage artifacts. Previous research has shown that small mesh cages exclude all predators, but still allow for larval recruit access (Freestone et al. 2011). Six replicates of each of the three treatments were randomly assigned locations within each block. We cleaned cages every two weeks to reduce fouling and to maximize water flow into cages. At the end of the experiment, all plates were removed from the field, returned to Bodega Marine Laboratory, and maintained at 15˚C prior to live counts.

At the start of the experiment, one temperature logger (HOBO Pendant, Onset Computer, Bourne, MA) was placed in each block to allow for comparisons between blocks and effects of seagrass. Over the course of the experiment, temperature was highest at Teachers Beach (mean = 21.37˚C, CV = 0.097), intermediate at Sacramento Landing (mean = 19.24˚C, CV = 0.058), and lowest at Pelican Point (mean = 15.84˚C, CV = 0.11). Temperature increased with distance into estuary, and this suggests that the sites sampled during this study encompassed an estuarine gradient in temperature (Appendix S1; Fig. S1). In the summer, residence time and the prevalence of hypersalinity increase with distance into Tomales Bay, and this pattern is captured in the three sites of this study (Hearn and Largier 1997). Therefore, the three sites in this study occur across a stress gradient, with Pelican Point being low stress, Sacramento Landing being intermediate stress, and Teachers Beach being high stress.

The community composition on the plates was quantified within 48 hours of return to the lab using a 49-point count under a dissecting microscope. Organisms were identified to species or morphospecies when the species identity was less certain. We conducted point counts of canopy and understory communities, and given no significant differences in these communities, analyses presented here are on canopies. Given that many of the predators of fouling species in this system are generalists and likely respond more to growth forms than species specific traits, taxa were grouped by morphotype. Morphotypes were based on growth form (e.g. encrusting, solitary, colonial, arborescent, etc.) and broad taxonomic group (Macroalgae, Anthozoa, Ascidia, Bryozoa, Bivalvia, Hydroidea, Porifera, Tubeworm). A list of species identified within each morphotype can be found in the supplementary materials (Appendix S1; Table S1).

Statistical analyses

We conducted analyses using three types of metrics: multivariate community responses, univariate community responses, and univariate morphotype responses (outlined below). All statistical analyses were completed using R version 3.6.3. All plots were created using the package ‘ggplot2’ version 3.3.0 (Wickham et al. 2020).

Multivariate Community Response

Given that standard distance-based measures of community composition often fail to account for the mean-variance relationship of the data (Warton et al. 2012), we analyzed community composition using a multivariate generalized linear model (MGLM) framework (Wang et al. 2012). The MGLM was fitted with the proportional coverage of each morphotype on a plate as the response, and predation treatment, seagrass treatment, site, and their interactions as fixed effect terms. A negative binomial distribution was used after examining the residuals vs. fitted plots to better represent the data. Model fit was determined by comparing AIC values, and when models had similar AIC values, the model with the lowest AIC was always more parsimonious than the other models with similar scores, so the model with the lowest AIC was always used. AIC values and the best model were selected with a backward stepwise selection using the ‘stepAIC’ function in the R package MASS (Ripley et al. 2021). Pit-resampling was used to calculate Wald Test values using the summary.manyglm function in the ‘mvabund’ package version 4.1.3 (Wang et al. 2012). An analysis of deviance was conducted on the best fit model using Wald test values. The inclusion of the random effect of block did not significantly change model fit for any of the statistical analyses; therefore, all reported effects are fixed and pooled across experimental blocks.

Univariate Community Response

We conducted univariate analyses on space occupied, species richness, and Simpson’s diversity index (Simpson 1949) on abundances of species, not morphotypes. Using species instead of morphotype for these analyses allowed for a more nuanced perspective on community metrics, since some morphotypes had >3 species and some only had 1. Species richness and Simpson’s Diversity Index were calculated in R using the package ‘vegan’ version 2.5-6 (Oksanen et al. 2018). Each of these different response variables were fit with generalized linear models using predation, site, and their interaction as fixed effects. We compared model fits with different distributions and selected a negative binomial distribution for space occupied, Poisson for species richness, and Gaussian for diversity. After identifying the correct distribution, we selected the best model using AIC and conducted an analysis of variance on this model to identify the contribution of fixed effects using F values for space occupied and diversity and chi-squared values for richness. Post-hoc pairwise comparisons were conducted across predation x site treatment interactions using Tukey HSD for species richness and Dunnett's Test for diversity and space occupied.

Univariate Morphotype Response

The abundance and response of each morphotype was calculated as part of the MGLM framework outlined above. The univariate p values were adjusted to account for multiple tests and collinearities with morphotype abundance using the p.uni = "adjusted" argument in 'mvabund'. This approach is preferred over SIMPER, as it allows for the variances of each morphotype to be independent and removes the bias of abundant groups (Warton et al. 2012). The Wald test statistic was used given the negative binomial distribution specified in the MGLM. Solitary ascidians are thought to be greatly reduced by predators (Nydam and Stachowicz 2007, Freestone et al. 2011, Rius et al. 2014, Rogers et al. 2016), so we conducted an analysis on solitary ascidian abundance separately from the MGLM framework. Solitary ascidian abundance was square root transformed and fit using a Gaussian family GLM to meet model assumptions. An analysis of variance was conducted on the best fit model using F value test statistics. Post-hoc pairwise comparisons across predation x site treatments were conducted using Dunnett's Test.

Results

Multivariate Community Response

The best fit model included terms for predation, site, and predation x site (AIC = 2483.9). The addition of seagrass treatment to this model decreased the model fit by a negligible amount (ΔAIC = 0.06); however, we chose the model with the lowest AIC score since it was also the most parsimonious (Appendix S1; Table S2). Community composition varied significantly between caged, partial, and open treatments (Table 1.1; $W_{2,105} = 7.1$, $p = 0.001$). Caged communities were dominated by solitary ascidians, colonial ascidians, and encrusting bryozoans with lower amounts of macroalgal cover (Fig. 1.3a). Partial and open communities had very low abundances of solitary ascidians, and increased coverage of algae and encrusting bryozoans (Fig. 1.3a). While partial and open plates were similar, macroalgal cover was slightly higher on open plates and sponge cover was lower (Fig. 1.3a). Anthozoans, bivalves, and hydroids were low in abundance but were found across all predation treatments.

While predation treatment had a significant effect on community composition, communities differed substantially between sites (Table 1.1; $W_{2,105} = 18.31$, $p = 0.001$). Pelican Point communities were dominated by algae, with some colonial ascidian coverage (Fig. 1.3b). Sacramento Landing had less algae than Pelican Point, and an increase in coverage of solitary ascidians, colonial ascidians, and encrusting bryozoans (Fig. 1.3b). Teachers Beach had the lowest macroalgal cover, with communities dominated by encrusting bryozoans, poriferans, arborescent bryozoans, and some solitary ascidians (Fig. 1.3b).

The effect of predation was not consistent across sites, leading to a significant interaction between site and predation (Table 1.1; $W_{4,102} = 8.19$, $p = 0.001$). While this was significant for the community multivariate response, this effect was likely driven by specific morphotypes. Predation generally had the effect of reducing the cover of solitary and colonial ascidians, and this effect was greatest where ascidians were the most abundant (see *Univariate Community Response*).

Univariate Community Response

Mean space occupied was 50.7% ± 2.27% with a max of 95.9% and a min of 0%. The best fit model included the interaction between predation x site x seagrass ($AIC = 319.63$). However, the removal of seagrass and its interactions resulted in the second-best model ($\Delta AIC =$

2.61). Space occupied was significantly different across sites (Fig. 1.4a, ANOVA; $F_{2,105} = 19.99$, $p < 0.001$), with Sacramento Landing having the highest coverage and Pelican Point having the lowest coverage. There was a significant interaction between predation treatment and site (Fig. 1.4a, ANOVA; $F_{4,102} = 7.49$, $p < 0.001$): open plates had similar coverage across all sites, where caged plates had highest coverage at SL and partial plates had the highest coverage at TB. The difference between predation treatments was largest at Sacramento Landing, with caged plates having significantly higher coverage than partial or open plates (Dunnett's Test, $p < 0.05$). At Teachers Beach, partial caged plates had significantly higher coverage than open plates (Dunnett's Test, $p < 0.05$) while caged and open plates had similar coverage. Space occupied didn't differ significantly among predation treatments at Pelican Point, though there is a trend for open plates to have higher coverage. There were some effects of predation averaged across all sites (ANOVA; $F_{2,105} = 3.18$, $p = 0.046$), though this effect is weak and is likely driven by a strong effect at Sacramento Landing and no effect at the other sites. There was also a significant interaction between site and seagrass (ANOVA; $F_{2,103} = 6.28$, $p = 0.003$).

The best fit model for species richness included predation, site, and predation x site ($AIC = 391.26$). Removing predation and the interaction (site only model) resulted in the second-best model ($\Delta AIC = 2.57$). Species richness significantly differed most by site (ANOVA; $\chi^2_{2,105} = 67.837$, $p < 0.001$) and the interaction between site and predation (ANOVA; $\chi^2_{4,102} = 10.703$, $p = 0.03$). Teachers Beach had the highest mean species richness and Pelican Point the lowest species richness across all sites (Fig. 1.4b). Species richness was similar between predation treatments at Pelican Point and Teachers Beach; however, caged plates at Sacramento Landing were significantly higher than partial or open plates (Tukey HSD, $p < 0.05$).

The best model for Simpson's Diversity Index included the main effect of site only (*AIC* = -80.54). The next best model includes predation, site, and their interaction ($\Delta AIC = 2.84$). Simpson's Diversity Index differed significantly across sites (ANOVA; $F_{2,105} = 64.15$, $p < 0.001$). Diversity was significantly higher at Sacramento Landing and Teachers Beach than Pelican Point (Fig 4c, Dunnett's Test, $p < 0.05$). Additionally, there appears to be a trend for caged plates at Sacramento Landing to have higher diversity values than partial and open plates. At Teachers Beach and Pelican Point, the trend suggests that partial plates have higher diversity than caged or open plates.

Univariate Morphotype Response

Multivariate and community metric responses can best be explained by patterns seen in specific morphotypes. Many morphotypes differed significantly among sites (Table 1.1). These groups include encrusting bryozoans ($W_{2,105} = 11.074$, $p = 0.001$), macroalgae ($W_{2,105} = 7.376$, $p = 0.001$), solitary ascidians ($W_{2,105} = 6.158$ $p = 0.001$), colonial ascidians ($W_{2,105} = 6.26$, $p = 0.001$), arborescent bryozoans ($W_{2,105} = 6.645$, $p = 0.001$), bivalves ($W_{2,105} = 3.002$, $p = 0.008$), and poriferans ($W_{2,105} = 5.237$, $p = 0.001$). Anthozoans and hydroids did not differ significantly across sites (Table 1.1).

Solitary ascidians appear to be driving community metrics: abundance significantly varied among predation treatments ($W_{2,105} = 4.577$, $p = 0.001$), site ($W_{2,105} = 6.158$, $p = 0.001$), and predation x site ($W_{4,102} = 4.877$, $p = 0.003$). While there may be a difference in the abundance of macroalgae across predation x site ($W_{4,102} = 4.265$, $p = 0.013$) and poriferans across predation treatments ($W_{2,105} = 3.034$, $p = 0.046$), solitary ascidians are the only morphotype that showed significant differences in response to both predation and the interaction between predation and site (Table 1.1). When modeling the abundance of solitary ascidian independently, the best fit model included predation, site, and predation x site (*AIC* = 104.35). The second-best model included seagrass and all interactions ($\Delta AIC = 7.72$). Solitary ascidian abundance differed significantly across predation treatments (ANOVA; $F_{2,105} = 20.64$, $p < 0.001$), sites (ANOVA; $F_{2,105} = 23.89$, $p < 0.001$), and their interaction (ANOVA; $F_{4,102} = 18.84$, $p < 0.001$). Solitary ascidian abundance was significantly higher in caged plates at Sacramento Landing (Fig. 1.5) than any other predation treatments at the same site or across all sites (Fig. 1.5, Dunnett's Test, $p < 0.05$). Teachers Beach had some solitary ascidians and Pelican Point had very few solitary ascidians, but abundance didn't differ significantly among predation treatments as it did at Sacramento Landing (Fig. 1.5).

Discussion

This study shows that estuaries act as environmental gradients that modify patterns of sessile invertebrate community composition, but that patterns of predation do not completely follow the simple Environmental Stress Model. Sessile invertebrate community composition differed significantly across sites along the estuarine gradient. This was demonstrated by shifts in morphotype abundance (Fig. 1.3b), with 6 of the 9 morphotypes found driving differences between sites (Table 1.1). Space occupied, species richness, and Simpson diversity index differed significantly among sites (Fig. 1.4) and tended to increase with distance into the estuary— Sacramento Landing and Teachers Beach had significantly higher univariate community indices than Pelican Point. When averaging across all predation treatments, this partially supports the prediction that diversity would be highest mid-bay (Fig 1b). However, diversity, richness, and space occupied remained high at Teachers Beach. Residence time tends to increase with distance into the estuary in Tomales Bay in the summer, with the phytoplankton maximum occurring mid-estuary (Largier et al. 1997). This, in turn, influences spatial patterns of invertebrate growth, where oyster growth is highest in the middle of the bay (Kimbro et al. 2019, Hollarsmith et al. 2020). Mortality is usually high within early post-settlement sessile invertebrate recruits and growing quickly to a larger size can increase survival by providing refuge from predation (Osman and Whitlatch 1995, Hunt and Scheibling 1997). The pattern in phytoplankton abundance in Tomales Bay, and therefore food availability, could explain why percent cover, species richness, and diversity were highest in the middle of the bay. While this experiment took place both inside and outside of seagrass, effects of seagrass on communities and the importance of predation and site in influencing seagrass effects were minimal.

Predation had a significant influence on sessile invertebrate community composition when averaging across all sites. While predation did not influence diversity or space covered when pooled across sites, there was a significant effect of predation on species richness. However, this significant effect was from high predation at Sacramento Landing and low predation at the other sites (Fig. 1.4b). The effect was likely driven by solitary ascidians, which had extremely low abundance when exposed to predators (Fig. 1.5). This result supports previous research showing that solitary ascidians are susceptible to predation at temperate latitudes (Osman and Whitlatch 2004, Nydam and Stachowicz 2007, Freestone et al. 2013, Rogers et al. 2016).

Effects of predation varied across the estuarine gradient, with the greatest effect seen mid-estuary. This result was shown with multivariate (Table 1.1) and univariate approaches (Fig. 1.4) and is best demonstrated by patterns in solitary ascidian abundance across predation treatments and sites (Fig. 1.5). As predicted (Fig. 1.1b), the effects of predation were greatest mid-bay at Sacramento Landing and low at the back of the bay at Teachers Beach. However, contrary to the initial prediction (Fig. 1.1b), the effect of predation was not significant at Pelican

Point. Despite high abundances of subtidal solitary ascidians at Pelican Point (Rubinoff, *personal* observation), recruitment of solitary ascidians was low at Pelican Point, potentially diminishing the effect of predation on other community metrics. These results support the predicted modification of consumer-stress relationships, suggesting that predators may be important further along estuarine stress gradients than is expected by the ESM (Menge and Sutherland 1987). Previous studies have shown that predation strength tends to decrease with distance into estuaries (Cheng and Hovel 2010, Lowe et al. 2018). Intertidal diversity surveys from the same year as this study at sites close by show a decrease in total predator abundance with distance into the estuary (Appendix S1; Fig. S3), but a non-linear shift in predator community composition (Appendix S1; Fig. S4). While patterns in predator abundance matched patterns of predation strength from previous studies (Cheng and Hovel 2010, Lowe et al. 2018), we found that predation strength on fouling communities varied across estuarine gradients in non-linear ways. However, additional research is needed to confirm predator identities to the species-level to be able to determine native or non-native status. Nonetheless, we suggest that the addition of non-native species into Tomales Bay could explain this pattern (Cheng and Grosholz 2016), with estuarine prey species experiencing higher predation than expected mid-estuary due to a mix of native and non-native predators.

We also found that patterns of diversity deviated from our predictions. It has been hypothesized that estuarine species diversity decreases with distance into estuary along a salinity gradient (Attrill 2002). We predicted that in ecosystems with non-native prey, that diversity would be highest in the middle of the estuary (due to a mix of native and non-native species), intermediate at the mouth (since fouling species are marine in origin), and lowest at the head (due to greater variability in conditions and therefore stress) (Fig. 1.1b). As predicted, we found that diversity was highest mid-estuary, but contrary to our prediction, diversity remained high further into the estuary than expected. Seasonal variation could be more important in structuring these patterns than was initially expected (Medeiros et al. 2020). The estuarine salinity gradient in Tomales Bay is weakened during the summer when sessile invertebrates have their highest recruitment (Hearn and Largier 1997, Stachowicz and Byrnes 2006), and this pattern is typical among Mediterranean climates. This study took place over the summer, when Tomales Bay experiences a low inflow circulation pattern that can even lead to the formation of an inverse estuarine gradient (Hearn and Largier 1997). While the salinity gradient in Tomales Bay is weakened in the summer, other properties that co-vary with salinity along this gradient, such as residence time, temperature, and dissolved oxygen, maintain the ecological stress gradient for marine organisms. This means that the importance of species interactions could shift across seasons in Mediterranean climate estuaries depending on the sensitivity of organisms to specific abiotic stressors, resulting in high spatial and temporal variability. Additionally, previous winter conditions have a strong influence on fouling community composition (Chang et al. 2018), which combined with other summer-time environmental stressors, likely drove patterns in community composition and effects of predation across sites. In Mediterranean climate estuaries, which stressors dominate vary by season, and predation is likely to be substantially less intense in the winter when there is a stronger salinity gradient than in the summer. While peak recruitment for native and non-native fouling species occurs in the summer, additional research is needed to understand how spatial differences in predation vary seasonally.

It is critical to understand factors that structure estuarine communities because estuaries are marine productivity powerhouses, important habitats for at least part of the life cycle of many species, and essential for carbon sequestration (Barbier et al. 2011, Grabowski et al. 2012).

Given their shallow depths, low relief shorelines, inputs from oceans, watersheds, and surrounding land, they are among the first ecosystems to experience effects of climate change (IPCC 2007) and they face a number of other anthropogenic stressors (Cloern and Jassby 2012). Estuaries are some of the most invaded ecosystems worldwide (Ruiz et al. 1997), and the rate of biological invasions has been increasing over time (Seebens et al. 2017). The introduction of non-native taxa could shift patterns outlined in previous ecological theories. Examining fouling species native/non-native status, we found that the proportion of non-native species differed among all sites and was highest at Sacramento Landing, intermediate at Teachers Beach, and lowest at Pelican Point (Appendix S1; Fig. S2, *Tukey HSD,* $p < 0.005$). The greater abundance of non-native abundance mid-estuary could explain why we found non-linear patterns of diversity and the highest diversity mid-estuary, which is in contrast to previous linear models (Attrill 2002). Additionally, having a greater diversity of prey items supports more predators, which could explain why the effect of predators was highest where the diversity and proportion non-native species were highest. Introduced species within estuaries could extend the importance of species interactions further along estuarine stress gradients, though additional data on predator identity are necessary to understand how non-native predators influence the predictions of the ESM. Additionally, the low proportion of non-native species toward the back of the bay is contrary to predictions about stress-tolerance and warrants additional research on the physiological limits and thus spatial distribution of non-native species.

While we are unable to fully disentangle the influences of introduced predator species relative to stress gradients, we found that the ESM partially predicted patterns of diversity, abundance, and consumer effects across an estuarine gradient. The results of this experiment partially agreed with our predictions (Fig 6) that diversity, abundance, and effects of predators were high mid-estuary. However, the results did not match the predicted decline in diversity and abundance at the back of the bay as both diversity and abundance remained high, meaning that only part of the theoretical stress gradient (Fig. 1.1, Fig. 1.6) was captured in the summer. This suggests that environmental stress could be less than predicted in the summer, but we expect for this pattern to change across other seasons. Estuarine gradients are likely to change into the future, influencing the distribution of estuarine invertebrates.

We conclude by suggesting that the introduction of stress-tolerant non-native species could increase predation in stressful habitats in other systems as well. As a result, biological invasions could influence species interactions across latitude (Pianka 1966) and elevation gradients (Preszler and Boecklen 1996, Callaway et al. 2002) by increasing consumer effects at higher latitudes and higher elevations than what is predicted by the ESM. Given that climate change will increase stress in these higher stress ecosystems, biological invasions pose an additional threat to biodiversity. By accounting for non-native species in the ESM, we can make more nuanced predictions on the spatial variability in biotic interactions and their effects on biodiversity.

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Tables

	Predation		Site		Predation x Site	
	W	*p*<(*W*)	*W*	*p*<(*W*)	*W*	*p*<(*W*)
Multivariate	7.095	**0.001**	18.312	**0.001**	8.190	**0.001**
Macroalgae	2.3	0.193	7.376	**0.001**	4.265	**0.013**
Anthozoa	0.107	0.748	0.192	0.521	0.043	0.809
Arborescent Bryozoa	1.817	0.292	6.645	**0.001**	3.381	0.079
Bivalvia	1.341	0.444	3.002	**0.008**	0.058	0.809
Colonial Ascidia	2.094	0.277	6.26	**0.001**	2.825	0.229
Encrusting Bryozoa	2.321	0.193	11.074	**0.001**	2.372	0.396
Hydroidea	0.113	0.748	0.738	0.392	0.036	0.809
Porifera	3.034	**0.046**	5.237	**0.001**	0.059	0.809
Solitary Ascidia	4.577	**0.003**	6.158	**0.001**	4.877	**0.003**

Table 1.1 Analysis of variance for most parsimonious best fit model (AIC = 2483.1, Morphotype Abundance ~ Predation + Site + Predation:Site) generalized linear model. Multivariate and adjusted univariate morphotype responses using Wald values as test statistics. Bold values indicate significant effects at $\alpha = 0.05$.

Figure Captions

Fig. 1.1 a) Modification of the Environmental Stress Model (Menge & Sutherland 1987) including the relative importance of predation (red) and environmental stress (blue) in regulating communities. b) Diversity and abundance of sessile invertebrate communities without predators (solid) and with predators (dotted) along an estuarine gradient. This prediction assumes that prey abundance and diversity are closely linked. Effect sizes of predation are indicated for sites near an estuary's mouth (A), mid-estuary sites (B), and sites towards the head (C).

Fig. 1.2 Map of Tomales Bay, California including the three sites with plate deployments: Pelican Point (PP), Sacramento Landing (SL), and Teachers Beach (TB).

Fig. 1.3 Proportional abundance of morphotypes found across a) predation treatments (C = cage, P = partial, O = open), and b) sites (PP = Pelican Point, SL = Sacramento Landing, TB = Teachers Beach). Values indicate the proportional abundance of the community (total of 1.0) occupied by each morphotype (see color key).

Fig. 1.4 Differences in community metrics across predation treatments and sites (PP = Pelican Point, SL = Sacramento Landing, TB = Teachers Beach). Metrics consist of a) space occupied, b) species richness, and c) Simpson's diversity index. Post-hoc tests including Tukey HSD (species richness) and Dunnett's test (space occupied and Simpson's diversity index) revealed significant differences ($p < 0.05$) across treatments indicated via different letters.

Fig. 1.5 Mean solitary ascidian abundance across predation treatments (C = cage, P = partial, O = open) and sites (PP = Pelican Point, SL = Sacramento Landing, TB = Teachers Beach). Values represent points out of a total of 49 possible (i.e. 25 = 50% coverage). Letters represent significant ($p < 0.05$) post-hoc pairwise comparisons using Dunnett's test.

Fig. 1.6 Predicted and measured patterns of mean diversity and mean abundance without predators (solid line) and with predators (dotted line) across the estuarine gradient. Plots represent a) predicted mean abundance, b) measured mean abundance, c) predicted mean diversity, d) measured mean diversity.

Figures

Fig. 1.1

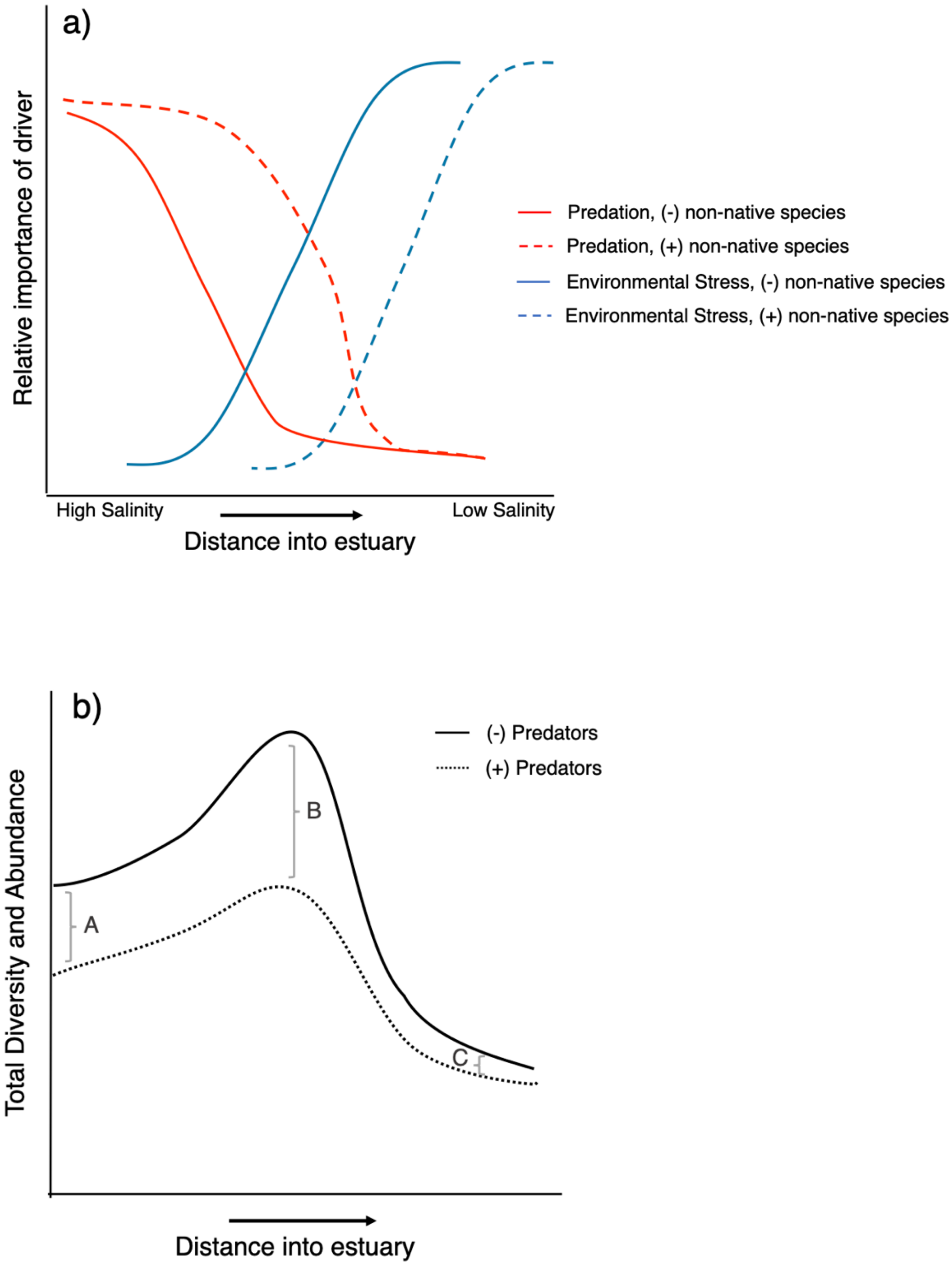


Fig. 1.2

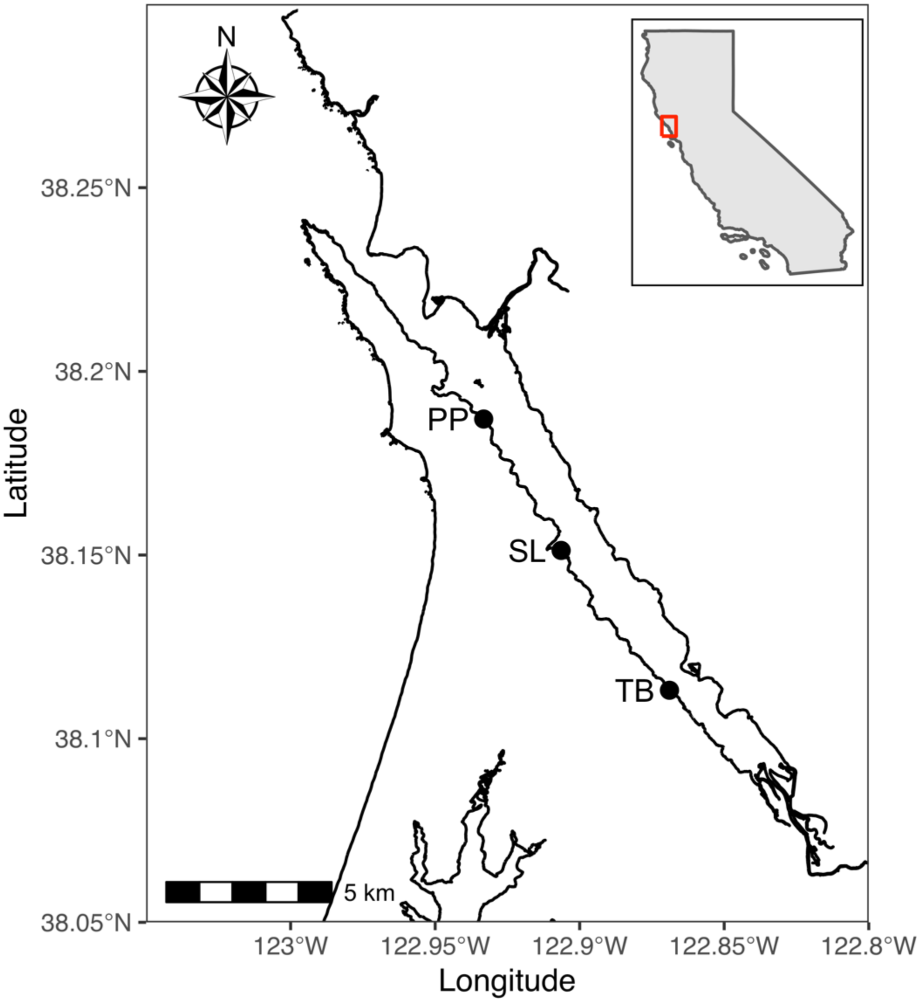
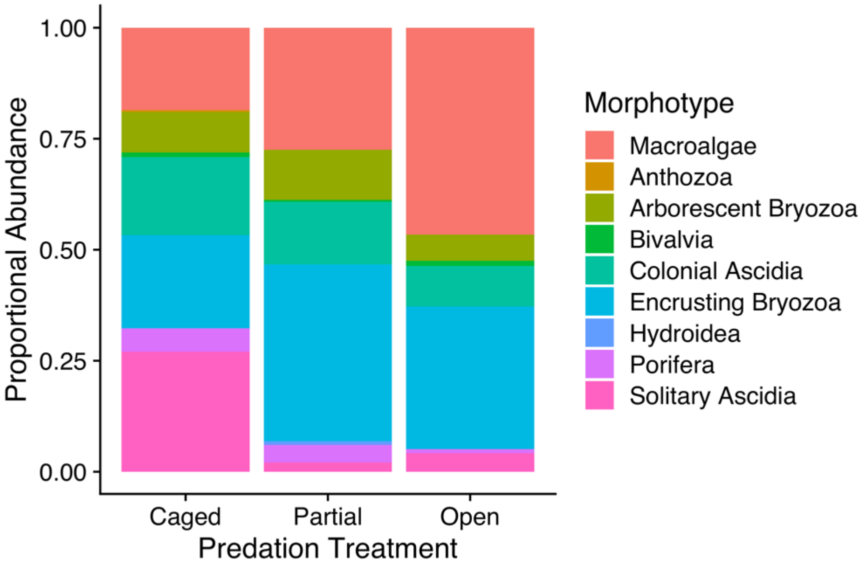

N
38.25°N
38.2°N
38.15°N
38.1°N
38.05°N
Latitude
PP
SL
TB
5 km
123°W
122.95°W
122.9°W
122.85°W
122.8°W
Longitude

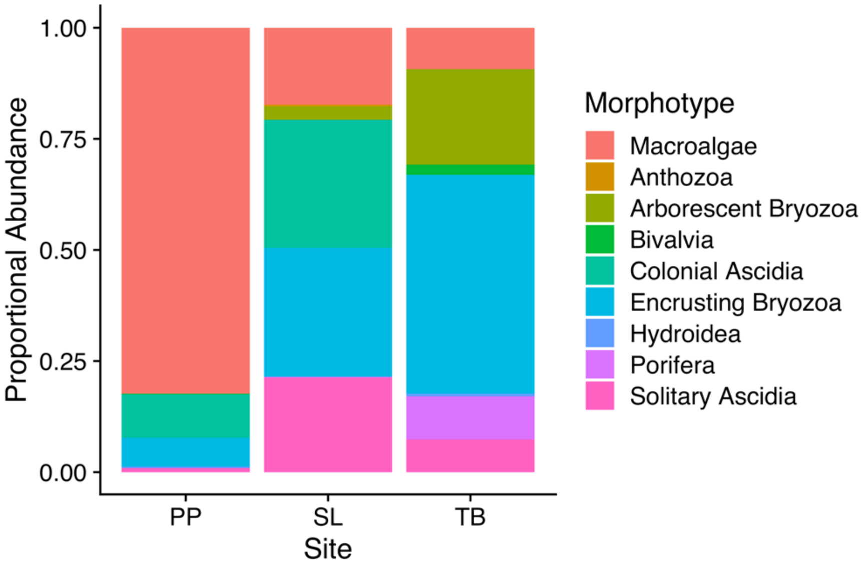
Fig. 1.3

a)



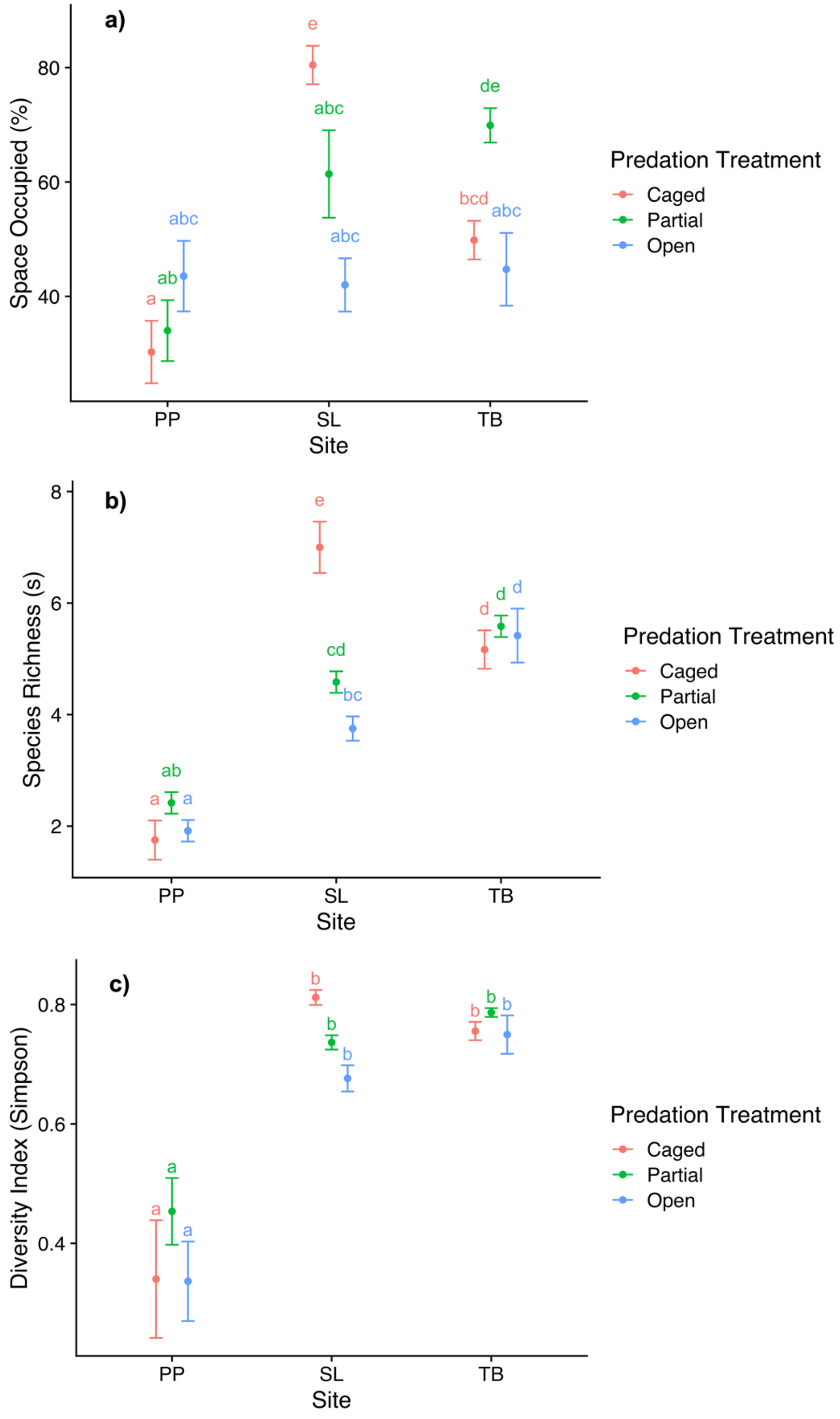
1.00
0.75
0.50
0.25
0.00
Proportional Abundance
Caged
Partial
Open
Predation Treatment
Morphotype
Macroalgae
Anthozoa
Arborescent Bryozoa
Bivalvia
Colonial Ascidia
Encrusting Bryozoa
Hydroidea
Porifera
Solitary Ascidia

b)



1.00
0.75
0.50
0.25
0.00
Proportional Abundance
PP
SL
TB
Site
Morphotype
Macroalgae
Anthozoa
Arborescent Bryozoa
Bivalvia
Colonial Ascidia
Encrusting Bryozoa
Hydroidea
Porifera
Solitary Ascidia

Fig. 1.4



a)
Space Occupied (%)
80
60
40
e
abc
abc
de
bcd
abc
a
ab
abc
PP
SL
TB
Site
Predation Treatment
Caged
Partial
Open
b)
Species Richness (s)
8
6
4
2
e
cd
bc
d
d
d
ab
a
a
PP
SL
TB
Site
Predation Treatment
Caged
Partial
Open
c)
Diversity Index (Simpson)
0.8
0.6
0.4
b
b
b
b
b
b
a
a
a
PP
SL
TB
Site
Predation Treatment
Caged
Partial
Open

Fig. 1.5

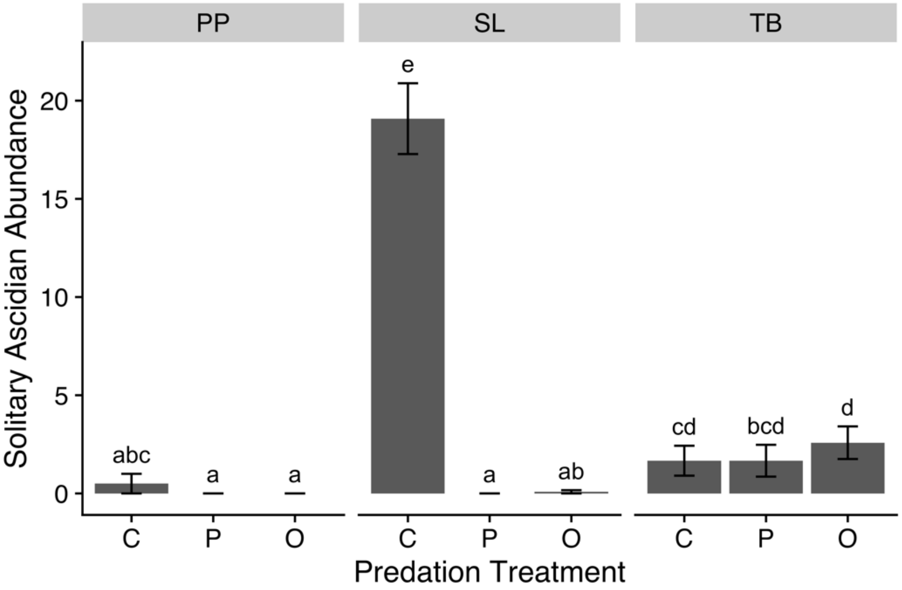
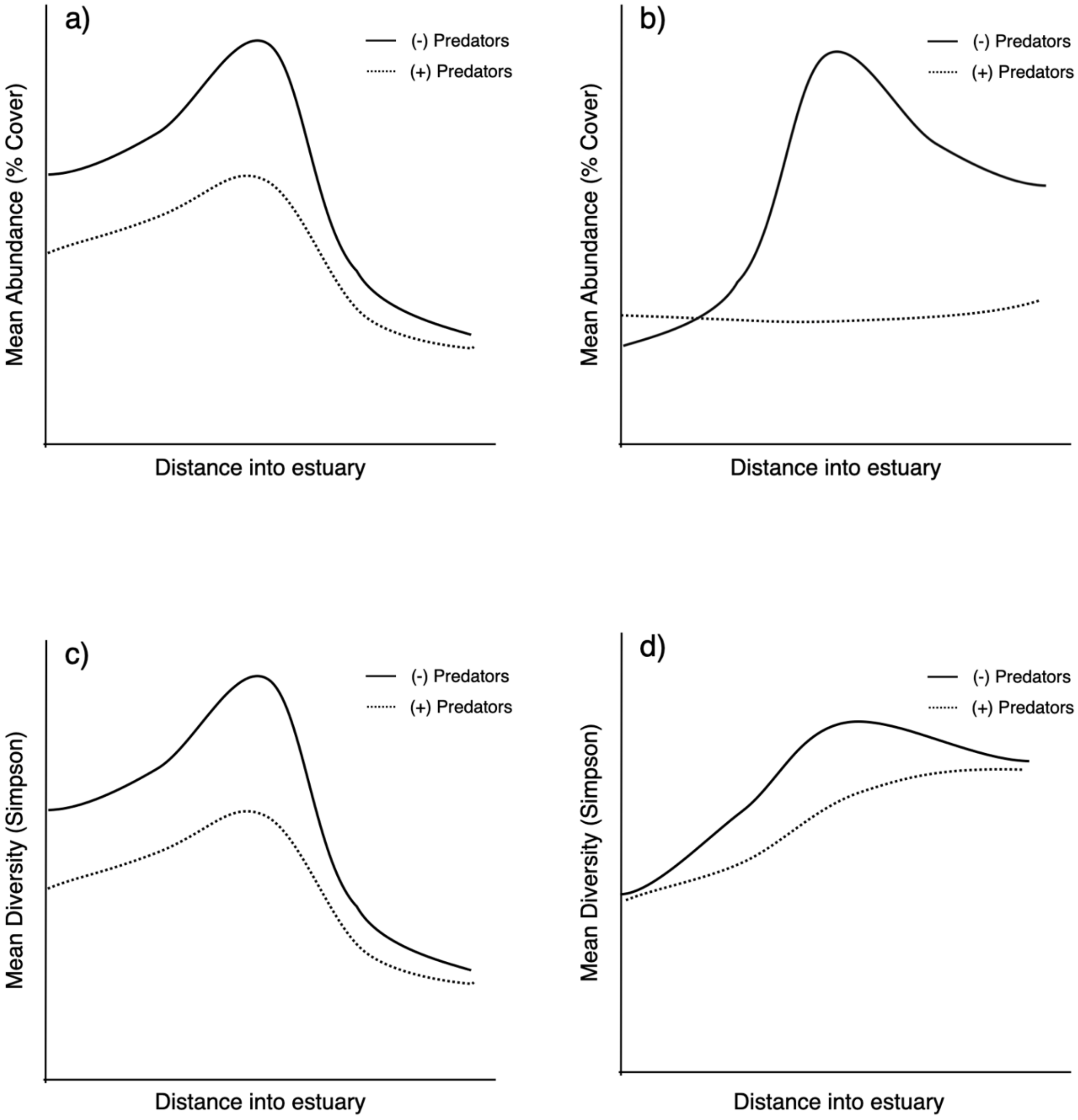

PP
SL
TB
Solitary Ascidian Abundance
0
5
10
15
20
abc
a
a
e
a
ab
cd
bcd
d
C
P
O
C
P
O
C
P
O
Predation Treatment

Fig. 1.6


a)
(-) Predators
(+) Predators
Mean Abundance (% Cover)
Distance into estuary
b)
(-) Predators
(+) Predators
Mean Abundance (% Cover)
Distance into estuary
c)
(-) Predators
(+) Predators
Mean Diversity (Simpson)
Distance into estuary
d)
(-) Predators
(+) Predators
Mean Diversity (Simpson)
Distance into estuary

Appendix

Table S1: Species list of all sessile invertebrate taxa found on settlement plates in 2019. Invasion status (native, non-native, cryptogenic) was verified using the Nemesis Database (Fofonoff et al. 2020).

Morphotype	Species	Invasion Status
Anthozoa	*Diadumene* sp.	Non-native
Arborescent Bryozoa	*Bugula neritina*	Non-native
	Bugulina stolonifera	Non-native
Bivalvia	*Arcuatula senhousia*	Non-native
	Ostrea lurida	Native
Colonial Ascidia	*Botrylloides violaceus*	Non-native
	Didemnum vexillum	Non-native
	Diplosoma listerianum	Native
Encrusting Bryozoa	*Alcyionidium sp.*	Cryptogenic
	Cellaporella hyalina	Non-native
	Conopeum sp.	Cryptogenic
	Cryptosula pallasiana	Non-native
	Schizoporella errata	Non-native
	Schizoporella japonica	Non-native
	Watersipora subtorquata	Non-native
Hydroidea	*Clytia* sp.	Cryptogenic
	Coryne japonica	Non-native
Porifera	*Halichondria* sp.	Cryptogenic
	Hymeniacidon perlevis	Non-native
Solitary Ascidia	*Ascidia ceratodes*	Native
	Ciona robusta	Non-native
	Molgula manhattensis	Non-native

Table S2: Results of the ‘stepAIC’ function in R to determine the best model for multivariate community data. The response variable for all models is the ‘mvabund’ matrix of morphotype abundances. The five best models and their respective AIC scores are presented here.

Model Terms	**AIC**
Predation + Site + Predation:Site	2483.9
Predation + Site + Seagrass + Predation:Site	2484.05
Predation + Site + Seagrass + Predation:Site + Predation:Seagrass	2492.41
Predation + Site + Seagrass + Predation: Site + Predation:Seagrass + Site:Seagrass	2508.67
Predation * Site * Seagrass (full model)	2534.73

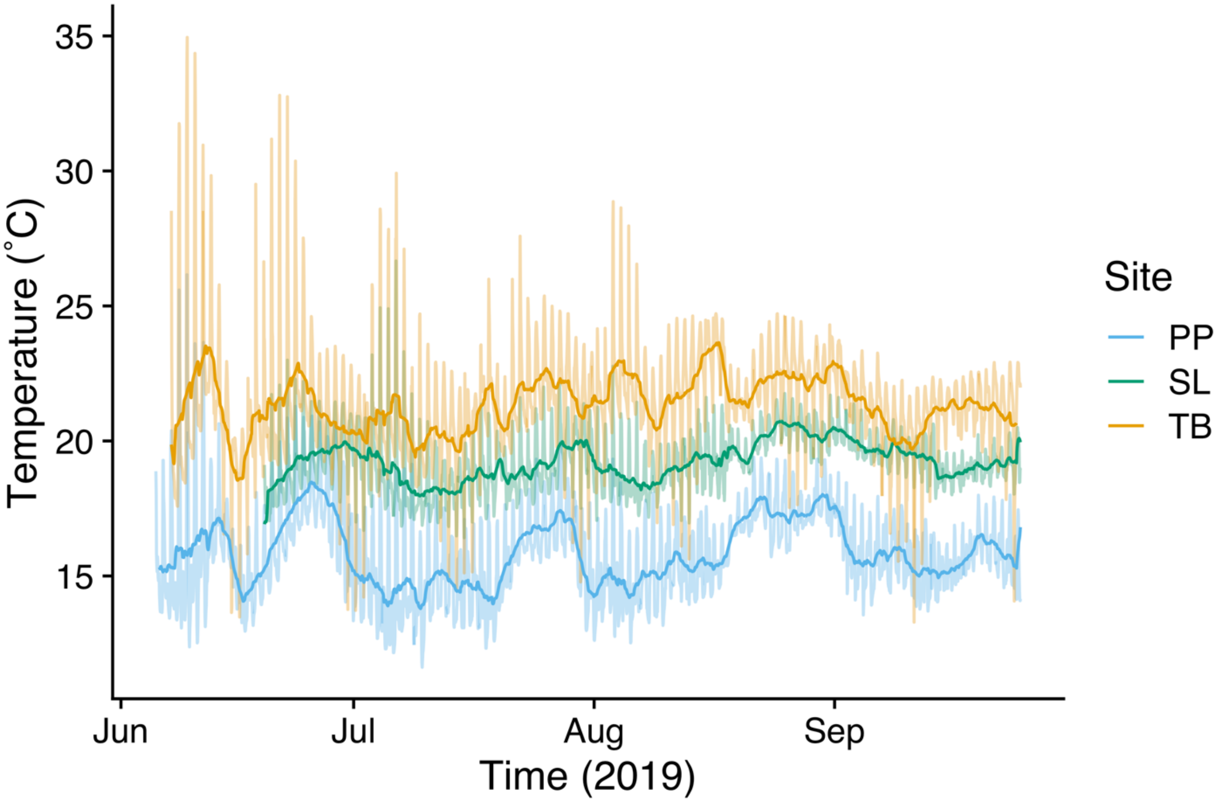


Fig. S1: Mean hourly temperature at Pelican Point (blue), Sacramento Landing (green), and Teachers Beach (yellow) over the course of the field deployment in 2019. Solid lines indicate the rolling daily mean across sites. Over the course of the experiment, temperature was highest at Teachers Beach (mean = 21.37˚C, CV = 0.097), intermediate at Sacramento Landing (mean = 19.24˚C, CV = 0.058), and lowest at Pelican Point (mean = 15.84˚C, CV = 0.11).

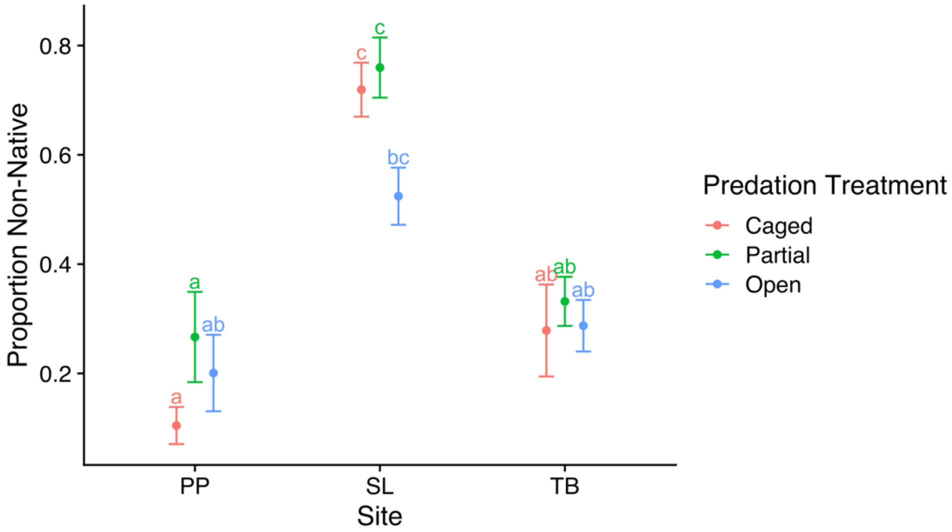


Fig. S2: Mean proportion of non-native across predation treatments (C = cage, P = partial, O = open) and sites (PP = Pelican Point, SL = Sacramento Landing, TB = Teachers' Beach). Proportion value includes the abundance of non-native species divided by the abundance of all species found (including native, non-native, and cryptogenic species). Post-hoc comparisons were completed using Tukey HSD and different letters indicate significant comparisons between treatments ($p < 0.05$)

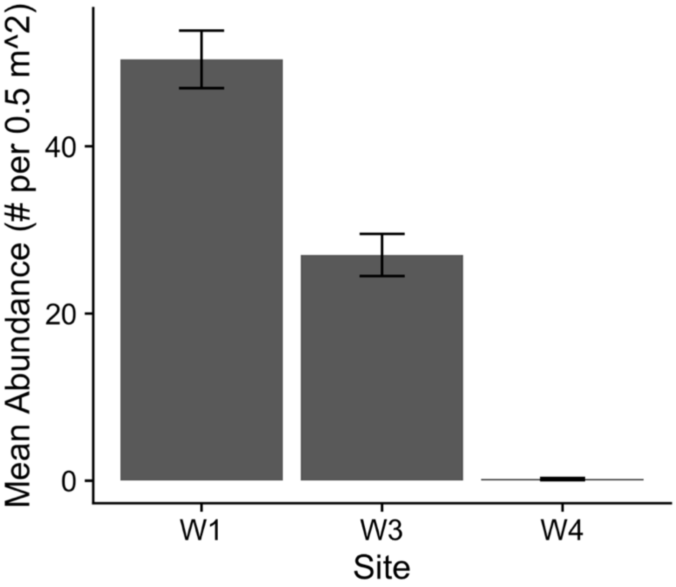


Fig. S3 Abundance of all predators found in intertidal diversity surveys in 2019 across sites. Sites move from the mouth to the head of the estuary from left to right. Surveys were conducted within one kilometer of study sites: W1 ~ Pelican Point, W2 ~ Sacramento Landing, and W4 ~ Teachers Beach. Values represent mean abundance per 0.5 m^2 quadrat (10 per transect, 30 m transect) and error bars representing standard error. Predator communities differed significantly across sites, and differences were driven by turban snails (*Chlorostoma* sp.), limpets, and shore crabs (*Hemigrapsus oregonensis*). Predator abundance decreased with distance into the estuary. Greatest number of limpets was found mid-estuary.

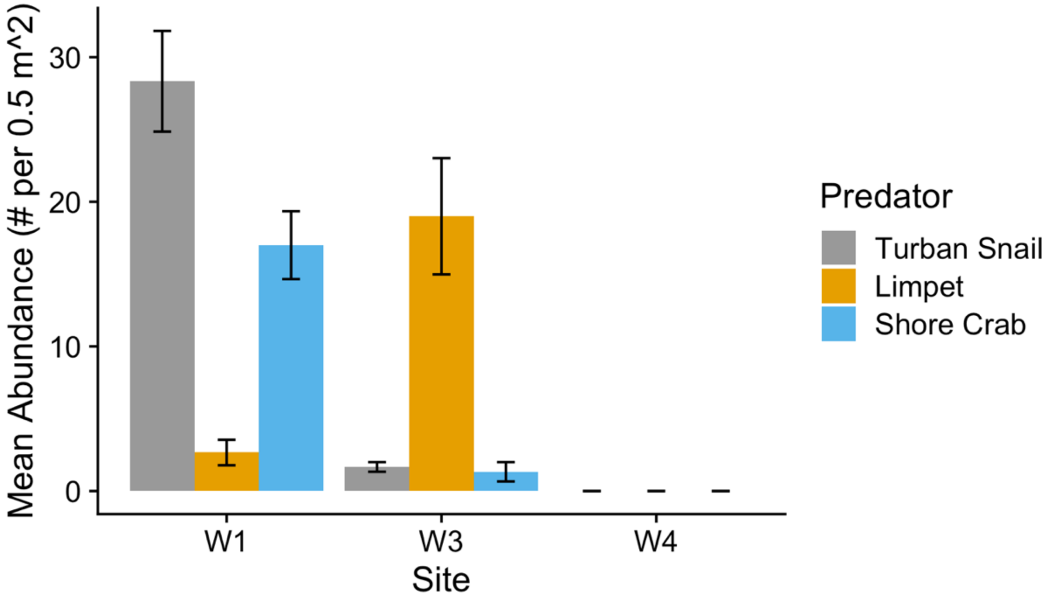


Fig. S4 Abundances of the three most important predators driving community differences across sites. Community composition was modeled across sites using the ManyGLM approach used in this study with a negative binomial distribution ($W_{2,27} = 15.54$, $p = 0.001$). The three predators driving this multivariate difference are turban snails ($W_{2,27} = 9.489$, $p = 0.001$), limpets ($W_{2,27} = 8.819$, $p = 0.001$), and shore crabs ($W_{2,27} = 8.19$, $p = 0.001$). Values plotted are their mean abundance per 0.5 m^2 quadrat and error bars represent their standard error.

References

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CHAPTER 2

Direct and Indirect Effects of Seagrass on Fouling Communities

Abstract

Biogenic habitats have been shown to promote biodiversity through direct and indirect structural mechanisms. Direct influences occur when habitat structure influences abiotic conditions, which affect the performance and survival of an organism. Indirect influences occur through changes in community properties, for example when habitat structure provides refuge for or from predators, which then results in changes in species interactions. One ecologically important biogenic habitat in marine systems is seagrass, which slows down water flow, modifies water chemistry, provides food for grazers, and serves as a structure for organisms to settle on or take refuge in. In this study, we examined how seagrass directly influences biological communities by modifying flow and recruitment and indirectly influences biological communities by modifying patterns of predation. Using sessile filter feeding invertebrate communities, also known as fouling communities, as a study system, we deployed experiments involving settlement plates and predator exclosures inside and outside of seagrass over the summer of 2018. We also conducted a second predator exposure experiment in 2020 to better understand the effect of predators and determine how they change across stages of community development. We found that communities differed significantly inside and outside of seagrass, and that seagrass lowered abundance, diversity, and richness of fouling species. We also found that predators altered community composition by reducing the abundance of solitary ascidians, which allowed for an increase in abundance of other functional groups. The effect of predation appeared to be greater outside of seagrass than inside, though this was likely influenced by differences in recruitment of fouling species and therefore differences in the strength of predation effects. Our study provides evidence that seagrass ecosystems alter fouling communities and that predator effects could be more variable than what is previously noted. We caution against the overgeneralization of effects of biogenic habitat on biodiversity and suggest that additional research is needed to understand the effects of changes in biogenic habitat on patterns of biodiversity.

Keywords: Fouling Communities, Seagrass, *Zostera marina*, Predation, Biogenic Habitats, Habitat Structure, Recruitment, Diversity

Introduction

Biogenic habitats play an important role in fostering biodiversity across the earth. The structural complexity associated with biogenic habitats has been shown to increase the diversity of bird species in tropical rainforests (MacArthur and MacArthur 1961), aquatic invertebrate species in submerged aquatic vegetation (Jeffries 1993), marine invertebrate species within mussel beds (Witman 1985), and across many other terrestrial and marine ecosystems. There are a variety of mechanisms that can explain the positive relationship between habitat complexity and diversity. First, greater habitat complexity increases niche availability, allowing for a greater diversity of species (MacArthur and MacArthur 1961). Second, biogenic habitat can ameliorate stressful environmental conditions and provide food resources, allowing for higher growth and survival, and thus diversity, inside complex habitats (Bruno et al. 2003). Finally, habitat structure can offer refuge from predation, supporting a higher diversity of lower trophic levels (Witman 1985). While many studies have examined these processes on their own, these processes might not act in the same way in some habitats due to ecological tradeoffs and variability in functional traits; therefore, it is important to better understand the relative importance of each mechanism to address complexity in patterns of biodiversity.

One biogenic habitat of ecological importance in marine systems is seagrass. Seagrass beds are abundant along the western coast of North America from Baja California to Alaska (Short et al. 2007), with most beds in California dominated by the eelgrass, *Zostera marina*. Seagrass is a foundation species, and it performs a variety of ecosystem functions such as water quality improvement, storm protection, food provision for endangered species, and habitat provision for economically important species (Orth et al. 2006, Short et al. 2007, Lefcheck et al. 2017). While seagrass ecosystems are often thought to enhance biodiversity (Orth et al. 2006), the mechanisms in which seagrass influences patterns of biodiversity could differ in their direction and relative importance across different taxa.

Seagrass beds provide three-dimensional structure in habitats often lacking in structure, and therefore could increase niche availability. Seagrass blades provide structure for both benthic and mobile epifaunal organisms in soft-sediment areas that normally lack hard structure. For example, seagrass provides habitat for ascidians who require a hard structure for settlement (Carman et al. 2016). Additionally, the structural complexity across seagrass bed height was important in influencing patterns of fish diversity in Thailand, where habitat differentiation occurred among fish species across different heights in the seagrass bed (Hori et al. 2009). Not only does seagrass increase species diversity, but it can also increase functional diversity, which has provided additional justification for restoration efforts (Dolbeth et al. 2013). This habitat provision can also result in negative effects on seagrass, where settlement on seagrass blades by epiphytes and colonial sessile invertebrates like ascidians can decrease eelgrass growth (Burkholder et al. 2007, Long and Grosholz 2015). Additionally, the structure associated with seagrass limits the movement of larger mobile species and could select for smaller predators (Yeager and Hovel 2017). Therefore, the effect of seagrass structure on niche availability might not be as clear as previous complexity-diversity predictions.

Seagrass beds modify the flow environment around them, and therefore could act as habitat ameliorators. Seagrass beds reduce water flow, decrease light penetration, and increase sedimentation through the structure associated with blades (Short and Short 1984). Structure associated with seagrass blades results in beds having longer water residence times than adjacent non-vegetated areas (Borum et al. 2012). This longer residence time could have opposing effects on species with different flow requirements. For example, longer residence times could be beneficial for epifaunal taxa by accumulating more organic matter and decreasing risk of dislodgement but could be detrimental to filter feeding organisms that rely on water flow for reproduction and food acquisition (Peterson et al. 1984). With longer residence times comes an increase in sedimentation, which can decrease the growth of sessile filter feeding invertebrates in marine environments (Eckman and Duggins 1991).

Seagrass is also known to alter the chemical environment around it. Respiration and photosynthesis cycles inside of seagrass could increase the variability of pH inside beds (Koweek et al. 2018); however, there is evidence that seagrass can buffer against ocean acidification by locally raising pH, benefitting calcifying organisms (Ricart et al. 2021). These modifications to the chemical environment could be beneficial to calcifying organisms but might not provide the same habitat amelioration for non-calcifying organisms to which pH variability isn’t as important. Thus, the effects of seagrass on the flow and chemical environment could either be habitat amelioration or environmental stress depending on which taxa are in question.

The biogenic structure associated with seagrass could also serve as either refuge from or habitat for predators. Seagrass beds are thought to provide refuge from predators, resulting in shifts of life history strategies from infaunal outside of seagrass to epifaunal inside of seagrass (Bouma et al. 2009). Patch size and distance from the edge of the bed influence this pattern, where predation risk decreased with distance into eelgrass beds for bay scallops (Carroll and Peterson 2013). However, this gradient in predation risk is complex and can also move in the opposite direction, with predation risk in mesopredators (i.e. small fish, crabs, shrimp) increasing with distance from the edge of the bed (Mahoney et al. 2018, Hovel et al. 2021). Increased predation risk inside of seagrass has also been seen in different filter feeding bivalves including soft sediment mussels (Kushner and Hovel 2006) and oysters (Lowe et al. 2018).

Overall, seagrass acts as a biogenic habitat that influences both the environment and biota around them. Seagrass could have different effects on patterns of biodiversity depending on what taxa are within question, and there could be ecological tradeoffs associated with living within seagrass. For example, seagrass structure could protect some filter feeding invertebrates from predators while also decreasing their ability to acquire food (Carroll and Peterson 2013). However, for different filter feeding invertebrates, seagrass may increase susceptibility to predation and modify food availability depending on location within an estuary (Lowe et al. 2018). Given that the direction of these effects can act in opposite ways, the effects of seagrass on communities are likely to be more complex than single-species studies have found. Complex effects on sessile filter feeding invertebrate communities, also known as fouling communities, are especially likely given that they are taxonomically diverse and consist of diverse assemblage of tunicates, bryozoans, bivalves, hydroids, barnacles, and sponges.

This study evaluates how eelgrass (*Zostera marina*) influences fouling communities via direct (habitat amelioration/stress) and indirect (predator refuge/habitat) mechanisms. We predicted that seagrass would directly lower fouling community diversity metrics due to reduced flow and that seagrass would indirectly negatively affect fouling communities by serving as refuge for predators of fouling species. We also predicted that the effects of seagrass and predation would be greater in younger communities due to prey refuge in size and differences in recruitment.

Methods

To evaluate how seagrass influences fouling community composition directly and indirectly, we conducted a fully factorial predator exclosure experiment in the summer of 2018. The results of the 2018 experiment then inspired additional tests to identify if results were driven by predation or other abiotic factors. In 2020, we conducted a predator exposure experiment to better understand how predation impacts fouling communities at different stages of development and how this differs inside and outside of seagrass. Both experiments took place at the same location (Sacramento Landing, Tomales Bay, CA: 38.151244˚N, -122.906417˚W) using the same materials. The details of each experiment are outlined below.

Predator Exclosure Experiment

We conducted a fully factorial predator exclosure experiment at Sacramento Landing in Tomales Bay, CA over the summer of 2018. Replicate standardized substrata (PVC plates, 10.16 cm x 10.16 cm) were deployed to measure growth and recruitment on hard substrates. Settlement plates (total n = 48) were placed in two blocks both inside and outside of seagrass beds from June to October 2018. Plates were deployed on the benthos using rebar, vexar mesh, and PVC plates. Each plate was oriented perpendicular to the sediment with the bottom of the plate touching the benthos. To assess predator effects, plates were assigned to one of four caging treatments: 1) uncaged open plates, 2) small mesh cages (1 mm mesh, excludes most predators), 3) large mesh cages (5 mm mesh, excludes large predators), and 4) partial cages with three sides (1 mm mesh, cage control). The caging treatments were designed to exclude different predator communities: small mesh treatments excluded both larger predators (crabs, fishes, sea stars) and smaller mesopredators (flatworms, nudibranchs, gastropods, smaller crustaceans) while large mesh cages excluded larger predators but allowed access to mesopredators (Freestone et al. 2011). Plates were deployed at -0.3 below MLLW in a stratified random design with six replicates of each of the four treatments distributed in each of the two blocks both outside and inside of seagrass. Cages were cleaned every two weeks to maintain water flow through the mesh.

Plates were collected during low tides in October 2018 and community composition was quantified within 48 hours of return to the lab using a 49-point count under a dissecting microscope. Organisms were identified to species or morphospecies when the species identity was less certain. We conducted point counts of canopy and understory communities, and given no significant differences in these communities, analyses presented here are on canopies. Given that many of the predators of fouling species in this system are generalists and likely respond more to growth forms than species specific traits, taxa were grouped by morphotype. Morphotypes were based on growth form (e.g. encrusting, solitary, colonial, arborescent, etc.) and broad taxonomic group (Anthozoa, Ascidia, Bryozoa, Bivalvia, Macroalgae, Porifera, Polychaeta). Vouchers were collected in instances where the species identity was less certain. Photographs of each plate were taken before live counts for verification of methods in species enumeration.

Statistical Approach (Predator Exclosure Experiment)

We conducted analyses using two types of metrics: multivariate community responses and univariate community responses. In some cases, univariate morphotype abundances are included to explain community-level effects (outlined below). All statistical analyses were completed using R version 3.6.3. All plots were created using the package ‘ggplot2’ version 3.3.0 (Wickham et al. 2020).

Given that standard distance-based measures of community composition do not account for the mean-variance relationship of abundance data (Warton et al. 2012), we analyzed community composition using a multivariate generalized linear model (MGLM) framework (Wang et al. 2012). The MGLM used percent cover of each morphotype on a plate as the response variable and predation, seagrass, and their interaction as fixed effects. A negative binomial distribution was used after examining the residuals vs. fitted plots to better represent the data. Model fit was determined by comparing AIC values, using the most parsimonious model with the lowest AIC to determine the relative contribution of fixed effects. Pit-resampling was used to calculate Wald Test values using the summary.manyglm function in the ‘mvabund’ package version 4.1.3 (Wang et al. 2012). An analysis of variance (ANOVA) was conducted on the best fit model using Wald test values. The addition of block as a fixed effect or as a random explanatory variable in the model was either insignificant or decreased model fit (increased AIC) for all statistical models in this study; therefore, all analyses presented here are pooled across blocks. We conducted post-hoc pairwise comparisons of multivariate data with the pairwise.comp argument in ‘mvabund’ across predation treatments, seagrass treatments, and their interaction. This method adjusts for multiple comparisons via a free stepdown resampling procedure

We conducted univariate analyses on space occupied, species richness, and Simpson diversity index (Simpson 1949) on abundances of species, not morphotypes. Using species instead of morphotype for these analyses allowed for a more nuanced perspective on community metrics, since some morphotypes had >3 species and some only had 1. Species richness and Simpson diversity index were calculated in R using the package 'vegan' version 2.5-6 (Oksanen et al. 2018). Each of these different response variables were fit with generalized linear models using predation, seagrass, and their interaction as fixed effects. We compared model fits with different distributions and used a Gaussian distribution for space occupied, species richness, and Simpson diversity index. While no transformations were necessary to meet model assumptions for space occupied or species richness, Simpson diversity index was raised to the fourth power to achieve normally distributed model residuals. An analysis of variance was conducted on the full model to identify the contribution of fixed effects using F values. Post-hoc pairwise comparisons were conducted across predation x seagrass treatment interactions using Tukey HSD.

The abundance and response of each functional group was calculated as part of the MGLM framework outlined above. The univariate *p* values were adjusted to account for multiple tests and collinearities with morphotype abundance using the p.uni = "adjusted" argument in 'mvabund'. This approach is preferred over SIMPER, as it allows for the variances of each morphotype to be independent and removes the bias of abundant groups (Warton et al. 2012). Given the negative binomial distribution in the MGLM, we used the Wald test statistic.

Predator Exposure Experiment

To better understand how predation influences fouling community composition, we conducted a predator exposure experiment in the summer of 2020, where predators were initially excluded from plates, but then permitted access at different time points. All plates were deployed randomly across six blocks, three inside and three outside seagrass using only the small mesh cage (1 mm) treatment and the same deployment and cleaning methods as the predator exclosure experiment. To understand the influence of time since initial deployment on predation impacts, we conducted three predator exposures on different subsets of plates at one month, two months, and three months after the initial deployment. Prior to each exposure, we removed cages from all plates and took a photograph for image point counts and a wet mass of the whole plate to measure changes in biomass. Plates were then either redeployed without a cage (exposure treatment) or with a cage (control) for 3 days, after which they were collected, and another photograph and weight was measured. Changes in weight were negligible (< 5 grams) and within the margin of error of the scale used; therefore, biomass data are left out of this analysis.

To analyze community composition, we uploaded photographs of plates to CoralNet (coralnet.ucsd.edu) for 49-point counts distributed across a uniform grid. The uniform grid was chosen to capture any changes in individuals or colonies pre and post exposure. Organisms were identified to species when possible, or morphospecies when lower taxonomic resolution was not possible. Species were grouped into the same morphotype categories as the predator exclosure experiment; however, we did not find any hydroids in 2020 and we did find tubeworms in 2020 but not in 2018.

Statistical Analyses (Predator Exposure Experiment)

This experiment used the same metrics (multivariate approach, univariate community metrics) as the predator exclosure experiment. The MGLM was fit using a negative binomial distribution with proportional change in percent cover pre and post exposure as the response variable and predation treatment, seagrass treatment, exposure time and all interactions as fixed predictors. Since the predator exposure experiment was often measuring a decrease in measured values, all responses across models in this experiment were on the proportional change.

To analyze univariate community metrics, we compared model fits with different distributions and used a Gaussian distribution for space occupied, species richness, and Simpson diversity index. Given that the response variable in all of these was a proportional change, we transformed data to meet model assumptions of normally distributed residuals. We used an arcsine square root transformation for space occupied and Simpson diversity index, and a square root transformation for species richness. ANOVA was conducted on the full model to identify the contribution of fixed effects using F values. Post-hoc pairwise comparisons were conducted across predation x exposure time treatment interactions using Tukey HSD.

Results

Predator Exclosure Experiment

Seagrass influenced fouling community composition in a few ways. The multivariate community analysis indicated that community composition differed significantly inside and outside of seagrass (Table 2.1, ANOVA; $W_{1,46} = 4.738$, $p = 0.008$). While this was not driven by any specific morphotype (Table 2.1, Fig. 2.1a), univariate metrics of community composition (space occupied, species richness, Simpson diversity index) were higher outside of seagrass than inside. Of the three most abundant morphotypes, encrusting bryozoa were most abundant, colonial ascidia intermediate in abundance, and solitary ascidia lowest in abundance (Fig. 2.1a). These patterns were the same inside and outside of seagrass; however, abundance of these morphotypes was higher outside of seagrass than inside. Space occupied (Fig. 2.2a ANOVA; $F_{1,46} = 24.938$, $p < 0.001$), species richness (Fig. 2.2b, ANOVA; $F_{1,46} = 28.169$, $p < 0.001$), and

Simpson diversity index (Fig. 2.2c, $F_{1,46} = 27.434$, $p < 0.001$) were all 1.58, 1.42, and 1.51 times higher outside of seagrass than inside respectively.

Predation also significantly altered fouling community composition. The multivariate community analysis indicated that community composition differed significantly across the four predation treatments (Table 2.1, ANOVA; $W_{3,44} = 11.592$, $p = 0.001$). A few different functional groups drove this pattern, with encrusting bryozoans contributing the most to the difference (Table 2.1, ANOVA; $W_{3,44} = 7.459$, $p = 0.001$), followed by solitary ascidians (Table 2.1, ANOVA; $W_{3,44} = 6.669$, $p = 0.001$), and then macroalgae (Table 2.1, ANOVA; $W_{3,44} = 3.993$, $p = 0.012$). Encrusting bryozoans covered less than 10% of caged plates (SM and LM) and more than 20% of partial and open plates, with coverage being highest on partial plates (Fig. 2.1b). Solitary ascidians covered over 25% of small mesh caged plates, and covered <1% of large mesh, partial, and open plates (Fig. 2.1b). Colonial ascidians were most abundant on large mesh caged plates (>20% coverage), intermediate on open plates (13% coverage), and low on partial (5% coverage) and small mesh (2% coverage). While space occupied (Fig. 2.2a) and species richness (Fig. 2.2b) didn't differ significantly across predation treatments, diversity differed significantly among predation treatments (Fig. 2.2c, ANOVA; $F_{3,44} = 5.485$, $p = 0.002$). Diversity was highest when communities were exposed to all predators (partial and open), intermediate in small mesh cages, and lowest in large mesh cages (Fig. 2.2c, ANOVA, Tukey HSD, $p < 0.05$).

Seagrass modified patterns of predation in a few ways. Multivariate community metrics varied across predation treatments in different ways inside and outside of seagrass (Table 2.1, ANOVA; $W_{3,40} = 5.041$, $p = 0.017$). This difference in effects of predation on communities inside and outside of seagrass was driven by colonial ascidians (Table 2.1, ANOVA; $W_{3,40} =$

4.576, $p = 0.005$), which overall had higher abundance outside of seagrass, and the highest abundance in large mesh cages both inside and outside of seagrass (Fig. 2.3). Colonial ascidians varied most on small mesh and open plates inside and outside of seagrass, where coverage was lower on small mesh plates outside of seagrass than inside, and higher on open plates outside of seagrass than inside (Fig. 2.2). When looking at other univariate measures of community composition, there was no effect of the interaction between predation and seagrass for space occupied ($p > 0.05$, Fig. 2.2a) and species richness ($p > 0.05$, Fig. 2.2b). However, there was a significant effect of the interaction between seagrass and predation with Simpson diversity index (Fig. 2.2c, ANOVA; $F_{3,40} = 2.951$, $p = 0.044$). Communities across predation treatments inside of seagrass had similar Simpson Diversity values (Fig. 2.2c, Tukey HSD, $p > 0.05$), but communities across predation treatments outside of seagrass had different Simpson diversity index values (Fig. 2.2c, Tukey HSD, $p < 0.05$), with partial plates having significantly higher diversity values then small mesh and large mesh plates, and similar values to open plates.

To better understand patterns found between different morphotypes, we conducted a correlation analysis using Pearson's Correlation Coefficient on the most abundant taxa (solitary ascidia, colonial ascidia, encrusting bryozoa). There is a negative relationship between solitary ascidian abundance and colonial ascidian abundance (Fig. 2.4a, $r = -0.361$, n = 48, $p = 0.012$), a negative relationship between solitary ascidian abundance and encrusting bryozoan abundance (Fig. 2.4b, $r = -0.401$, n = 48, $p = 0.005$), and no significant relationship between encrusting bryozoan abundance and colonial ascidian abundance (Fig. 2.4c, $r = -0.142$, n = 48, $p = 0.336$).

Predator Exposure Experiment

The multivariate community analysis showed that the proportional change in fouling community composition did not differ significantly across seagrass, predation, or exposure time treatments (ANOVA; $p < 0.05$). However, some univariate community metrics did change significantly across treatments. Plates that were exposed to predators experienced greater reductions in space occupied (Fig. 2.5a, ANOVA; $F_{1,70} = 14.437$, $p < 0.001$), species richness (Fig. 2.5b, ANOVA; $F_{1,70} = 6.942$, $p = 0.011$), and Simpson diversity index (Fig. 2.5c, ANOVA; $F_{1,70} = 11.604$, $p = 0.001$) than control plates. Additionally, there was a significant difference in the change in species richness across exposure times (Fig. 2.5b, ANOVA; $F_{2,69} = 4.585$, $p = 0.014$), with species richness increasing (more positive/less negative) with exposure time. While not significant, there appears to be a trend toward an interaction between predation treatment and exposure time for all univariate metrics, where the difference between predation treatments seems to increase over time. Seagrass had no statistically significant effect on multivariate or univariate community metrics.

Discussion

In this study, we found that seagrass habitats modify fouling community composition in a variety of ways. The strongest effect of seagrass on fouling community composition was likely from direct effects of eelgrass structure (flow, recruitment, etc.) on community membership. We found that community composition differed significantly inside and outside of seagrass (Table 2.1), but that this difference wasn’t driven by any specific morphotypes and was not interactively influenced by predator presence. Instead, the difference was in univariate community metrics, where space occupied, species richness, and Simpson diversity index were significantly higher outside of seagrass than inside of seagrass (Table 2.1, Fig. 2.1a, Fig. 2.2). Reduced abundance, richness, and diversity inside of seagrass could suggest limited recruitment and/or limited food supply that reduces growth rates. Fouling species recruitment and food acquisition depend heavily on water flow given that they are filter feeders with planktonic larvae. Seagrass is known to reduce the flow of water and mediates patterns of turbulence, which overall reduce suspended particle concentrations inside seagrass beds (Luhar et al. 2008). This acts as a stressor for fouling species and helps to explain why abundance is lower inside of eelgrass beds than outside of eelgrass beds. However, seagrass can provide a hard substrate for fouling species to grow on in areas with limited substrate availability, and therefore could facilitate fouling species survival. Therefore, there is a tradeoff in the facilitative role of seagrass between habitat provision and food limitation.

The strength of the effects of seagrass on fouling abundance depend on the overall amount of recruitment and other environmental conditions. For example, we did not detect any significant effect of seagrass on fouling community composition or the change in community when exposed to predators in the predator exposure experiment in 2020. Recruitment differed between the two experiments, where the space occupied of small mesh cages in 2018 (mean = 63.95%, min = 12.24%, max = 100%) was much higher than the small mesh cages before exposure to predators at three months in 2020 (mean = 12.53%, min = 0%, max = 85.72%). The low recruitment in 2020 decreases the power to detect effects among experimental treatments, including seagrass.

We also found that predation plays a strong role in structuring fouling community composition. Both community composition (Table 2.1, Fig. 2.1b) and Simpson diversity index (Fig. 2.2c) varied among the four caging treatments. Specifically, we found that Simpson diversity index was higher when communities were exposed to predators. This result can best be understood when examining the abundances of specific morphotypes. Solitary ascidians were most abundant when all predators were excluded (small mesh), colonial ascidians were most abundant when only large predators were excluded (large mesh), and encrusting bryozoans were most abundant when predators were allowed access to plates (partial and open, Fig. 2.1b). We found a negative relationship between solitary ascidians and colonial ascidians, and we did not find a significant relationship between encrusting bryozoans and colonial ascidians. Although this remains to be tested, our data are consistent with the idea that solitary ascidians are a competitively dominant species, and that they dominate communities when protected from all predators, resulting in a reduction in diversity (Blum et al. 2007, Nydam and Stachowicz 2007, Rogers et al. 2016). When predators are allowed access to communities, solitary ascidians decrease in abundance, freeing up space for encrusting bryozoans and colonial ascidians to increase in abundance. Colonial ascidians were most abundant in large mesh cages because of reduced solitary ascidian abundance and because colonial ascidians are susceptible to predation by larger predators like crabs and sea stars (Rogers et al. 2016). Therefore, the significant effect of predation was likely driven solely by solitary ascidians, with other differences in caging treatments a result of competitive interactions and/or greater defenses against predators. These results support previous studies that have shown that solitary ascidians are highly susceptible to predation at temperate latitudes (Osman and Whitlatch 1995, Freestone et al. 2013, Rius et al. 2014, Rogers et al. 2016).

While predation also significantly altered community composition in the predator exposure experiment in 2020, the effects on univariate community metrics were in the opposite direction. We found that predation significantly lowered the amount of space occupied, species richness, and Simpson diversity index when pooled across all exposure times and seagrass treatments. Like the effects of seagrass, this result could be explained by reduced recruitment. Space occupied, species richness, and Simpson diversity index were already low from reduced recruitment, so any short-term exposure would drastically impact communities. This short exposure time would allow for the removal of species but would not allow for enough time for significant recolonization or growth. We believe that if we increased the duration of the exposure along with recruitment, the results of this experiment would more closely match those of the predator exclosure experiment.

We also found that the effects of predation did not differ across exposure time. This is in contrast to our prediction that effects of predators would be greatest on young communities. While previous research has shown that predation on recruits and juveniles is a dominant source of mortality in fouling species (Osman and Whitlatch 1995), the low recruitment over the course of the experiment could have reduced the interaction between predation and exposure time. While not significant, there appeared to be a greater effect of predators with increasing exposure time. This is likely due to increases in recruitment near the three-month mark, allowing for a more noticeable change in community metrics (Fig. 2.5).

Initially, we predicted that seagrass would also indirectly impact fouling community composition by increasing predation in seagrass beds, resulting in a significant predation x seagrass interaction. However, the indirect effect (predation x seagrass interaction) was not as strong as the direct effects of seagrass or predation on their own. Colonial ascidians were the only morphotype to contribute significantly to this result (Table 2.1, Fig. 2.3), and it appeared that their abundance in large mesh, partial, and open plates was higher outside of seagrass than inside while their abundance in small mesh cages was lower outside of seagrass than inside. Predators of colonial ascidians include large species like crabs and sea stars (Rogers et al. 2016), who are likely to use seagrass as refuge habitat without the mobility limitations that larger predators experience. Since the differences in colonial ascidia cover across predation treatments were greater inside of seagrass than outside, there could be an indirect effect of seagrass on colonial ascidia by providing habitat for larger predators. However, additional experiments are needed to measure differences in predator community composition and abundance inside and outside of seagrass to confirm this. Nonetheless, the abundance of competitively dominant solitary ascidians was higher outside of seagrass than inside, which could explain the reduction in colonial ascidian coverage. Additionally, we found that the variability in Simpson diversity index was higher outside of seagrass than inside, resulting in a significant interaction (Fig. 2.2c). Since diversity isn't higher when predators are excluded in seagrass, we think that this is from the structural influence of seagrass, with reduced recruitment and food availability impacting all predation treatments inside seagrass similarly. The similar effect of predation inside and outside of seagrass could be an artifact of the low predator abundance found in temperate systems, resulting in ascidian dominated communities both inside and outside of eelgrass (Osman et al. 2010)

Our results provide evidence suggesting that seagrass directly influence biological communities; however, indirect effects may not be as impactful on biological communities at temperate latitudes. Previous studies have shown mixed results with predation sometimes being higher in seagrass (Lowe et al. 2018, Hovel et al. 2021) and sometimes lower in seagrass (Carroll and Peterson 2013). Predators of fouling species (e.g. crabs, shrimps, and fish) experience lower predation risk inside seagrass than at patch edges or outside (Hovel et al. 2021). While the higher predation effects on colonial ascidia inside seagrass aligns with patterns in predator abundance, the indirect effects of seagrass on fouling communities could include mechanisms acting along with predation, where predatory removal of certain species could modify the outcome of competition, and thus influence patterns of diversity. Given that the amount of recruitment can influence the effects of predators (Cheng et al. 2019), direct influences of seagrass on recruitment could also influence the effect of predators in complex ways. Therefore, indirect effects of seagrass could depend on not just predation, but also trophic position, competitive ability, and recruitment. Our study also provides evidence for the importance of predation in shaping fouling communities in seagrass ecosystems at temperate latitudes (Cheng et al. 2019), which is in contrast to previous research downplaying its importance (Freestone et al. 2020).

Biological communities consist of diverse functional groups representing various morphotypes, and we caution against the overgeneralization on effects of biogenic habitats, like seagrass, on patterns of biodiversity. Given their sensitivity to multiple stressors, including climate change and biological invasions, and their importance as foundation species, the status of seagrass is of particular concern (Orth et al. 2006, Lefcheck et al. 2017). The impact of future global changes will alter the abundance and distribution of seagrass ecosystems with complex and sometimes contrary effects on biodiversity, thus, additional research is needed to better predict the outcome of changes in seagrass habitats.

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Tables

	Predation		Seagrass		Predation x Seagrass	
	W	*p*<(*W*)	*W*	*p*<(W)	*W*	*p*<(*W*)
Multivariate	11.592	**0.001**	4.738	**0.008**	5.041	**0.017**
Macroalgae	3.993	**0.012**	1.211	0.436	1.363	0.639
Anthozoa	1.62	0.150	1.961	0.213	0.054	0.702
Arborescent Bryozoa	2.402	0.088	2.724	0.076	0.046	0.804
Bivalvia	0.06	0.925	0.098	0.900	0.026	0.804
Colonial Ascidia	3.069	0.056	1.491	0.436	4.576	**0.005**
Encrusting Bryozoa	7.459	**0.001**	1.466	0.436	1.611	0.639
Porifera	0.06	0.925	0.098	0.900	0.026	0.804
Solitary Ascidia	6.693	**0.001**	2.305	0.165	0.041	0.804
Polychaeta	0.449	0.582	0.116	0.752	0.028	0.804

Table 2.1 Analysis of variance for best fit model (AIC = 80.028, Functional Abundance ~ Predation + Seagrass + Predation:Seagrass) generalized linear model. Multivariate and adjusted univariate functional group responses using Wald values as test statistics. Bold values indicate significant effects at $\alpha = 0.05$.

Figure Captions

Fig. 2.1 Abundance of dominant morphotypes across experimental treatments. Treatments represented a) inside and outside of seagrass beds, and b) predator exclosure treatments (SM = small mesh, LM = large mesh, P = partial cage, O = open). Values represent mean percent cover on settlement plates with error bars representing the standard error. Pairwise comparisons completed with the pairwise.comp argument in ‘mvabund’ between seagrass treatments and predation treatments separately, which adjusts for multiple comparisons via a free stepdown resampling procedure. Different letters indicate significant differences in the multivariate statistics ($p < 0.05$).

Fig. 2.2 Differences in community metrics across predation treatments (SM = small mesh, LM = large mesh, P = partial cage, O = open) and inside and outside of seagrass. Metrics consist of **a)** mean space occupied (% cover on panel surface), **b)** mean species richness, and **c)** mean Simpson diversity index all with error bars representing standard error. Post-hoc tests were conducted using Tukey HSD with significant differences ($p < 0.05$) across treatments indicated by different letters.

Fig. 2.3 Mean abundance of colonial ascidia (% cover on panel surface) across predation treatments (SM = small mesh, LM = large mesh, P = partial cage, O = open) and inside and outside of seagrass. Values represent mean percent cover on settlement plates with error bars representing the standard error. Post-hoc tests were conducted using Tukey HSD with significant differences ($p < 0.05$) across treatments indicated by different letters.

Fig. 2.4 Scatterplots representing the relationships between the abundances (% cover on panel surface) of the three most abundant morphotypes. Relationships include **a)** solitary ascidia abundance vs. colonial ascidia abundance (r = -0.361, n = 48, p = 0.012), **b)** solitary ascidia abundance vs. encrusting bryozoa abundance (r = -0.401, n = 48, p = 0.004), and **c)** encrusting bryozoa abundance vs colonial ascidia abundance (r = -0.142, n = 48, p = 0.336).

Fig. 2.5 Change in community metrics across predation treatments (C = control, E = Exposure) and community age (Exposure time). Metrics consist of **a)** mean change in space occupied (% cover on panel surface), **b)** mean change in species richness, and **c)** mean change in Simpson diversity index all with error bars representing standard error. The dashed line at 0 indicates no change in community metrics, with values above representing increases in community metrics and values below representing decreases in community metrics. Post-hoc tests were conducted using Tukey HSD with significant differences (p < 0.05) across treatments indicated by different letters.

Figures

Fig. 2.1

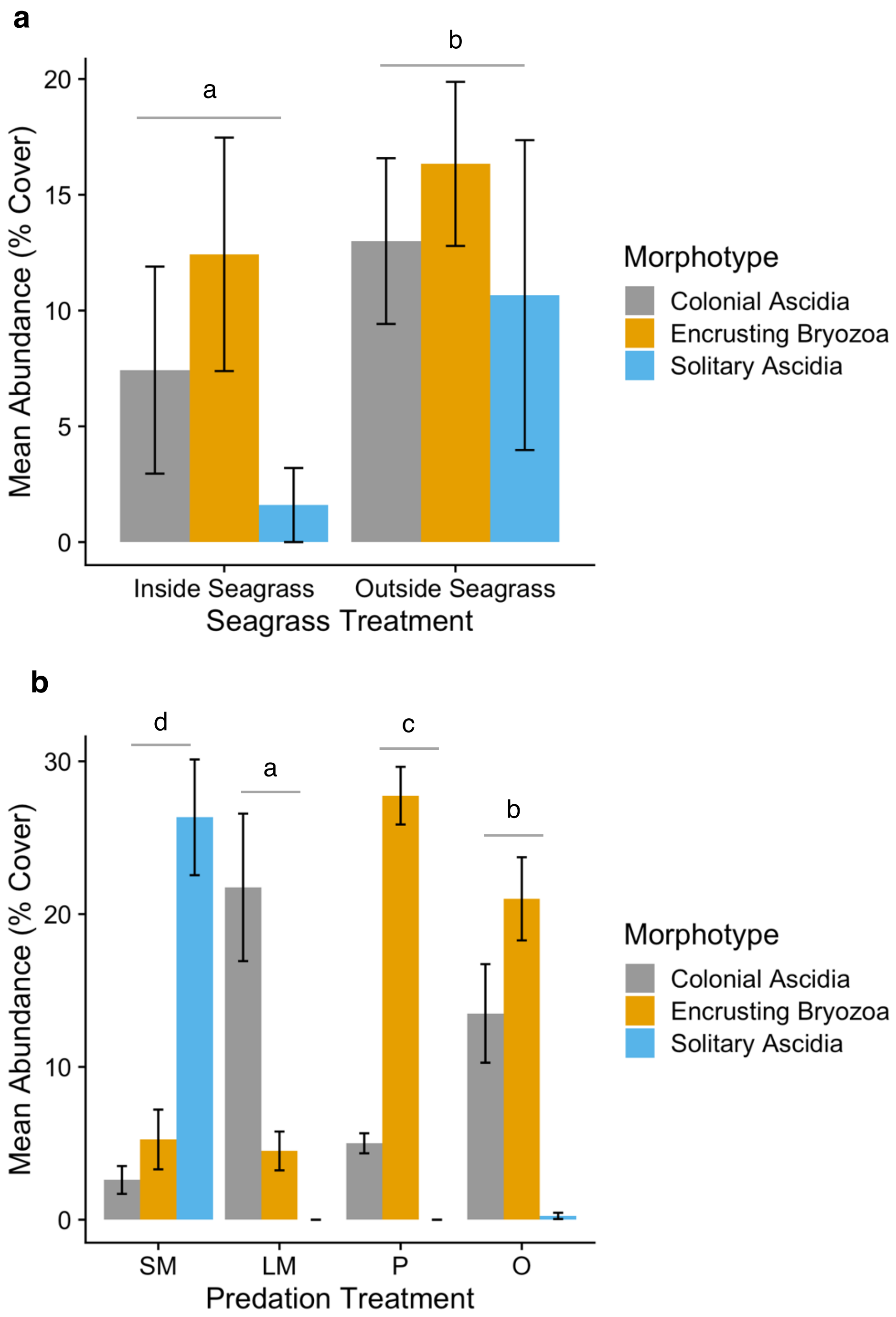
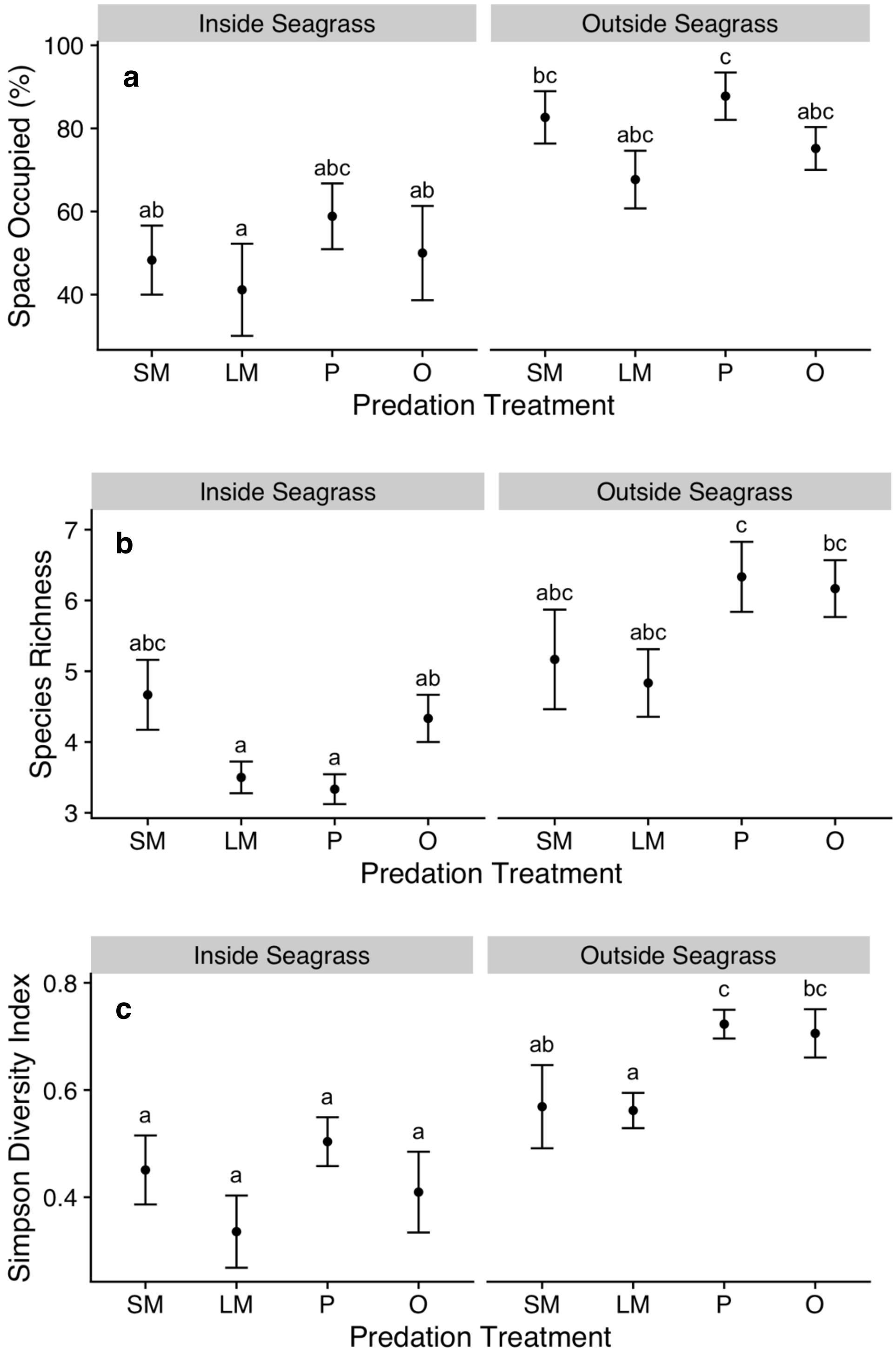


Fig. 2.2



a
Inside Seagrass
Outside Seagrass
Space Occupied (%)
100
80
60
40
ab
a
abc
ab
bc
abc
c
abc
SM
LM
P
O
SM
LM
P
O
Predation Treatment
b
Inside Seagrass
Outside Seagrass
Species Richness
7
6
5
4
3
abc
a
a
ab
abc
abc
c
bc
SM
LM
P
O
SM
LM
P
O
Predation Treatment
c
Inside Seagrass
Outside Seagrass
Simpson Diversity Index
0.8
0.6
0.4
a
a
a
a
ab
a
c
bc
SM
LM
P
O
SM
LM
P
O
Predation Treatment

Fig. 2.3

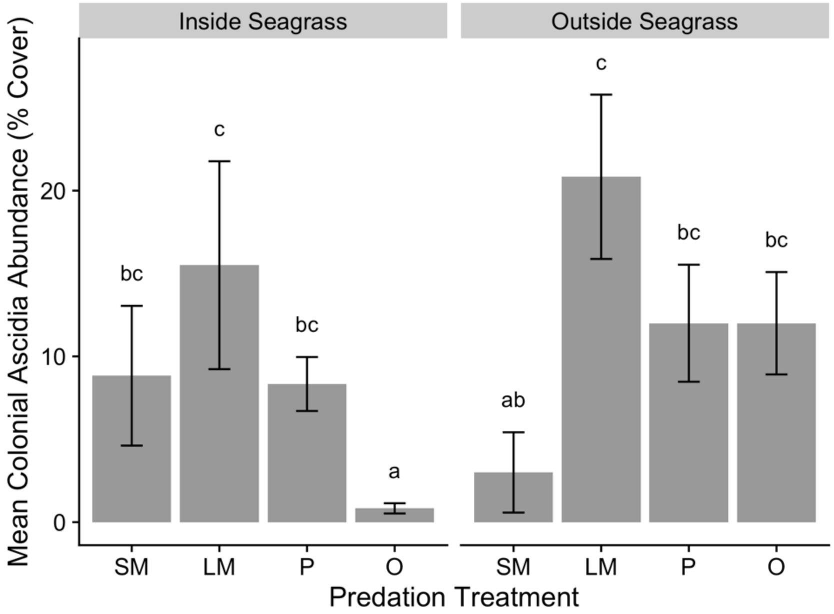

Inside Seagrass
Outside Seagrass
Mean Colonial Ascidia Abundance (% Cover)
0
10
20
bc
c
bc
a
ab
c
bc
bc
SM
LM
P
O
SM
LM
P
O
Predation Treatment

Fig. 2.4

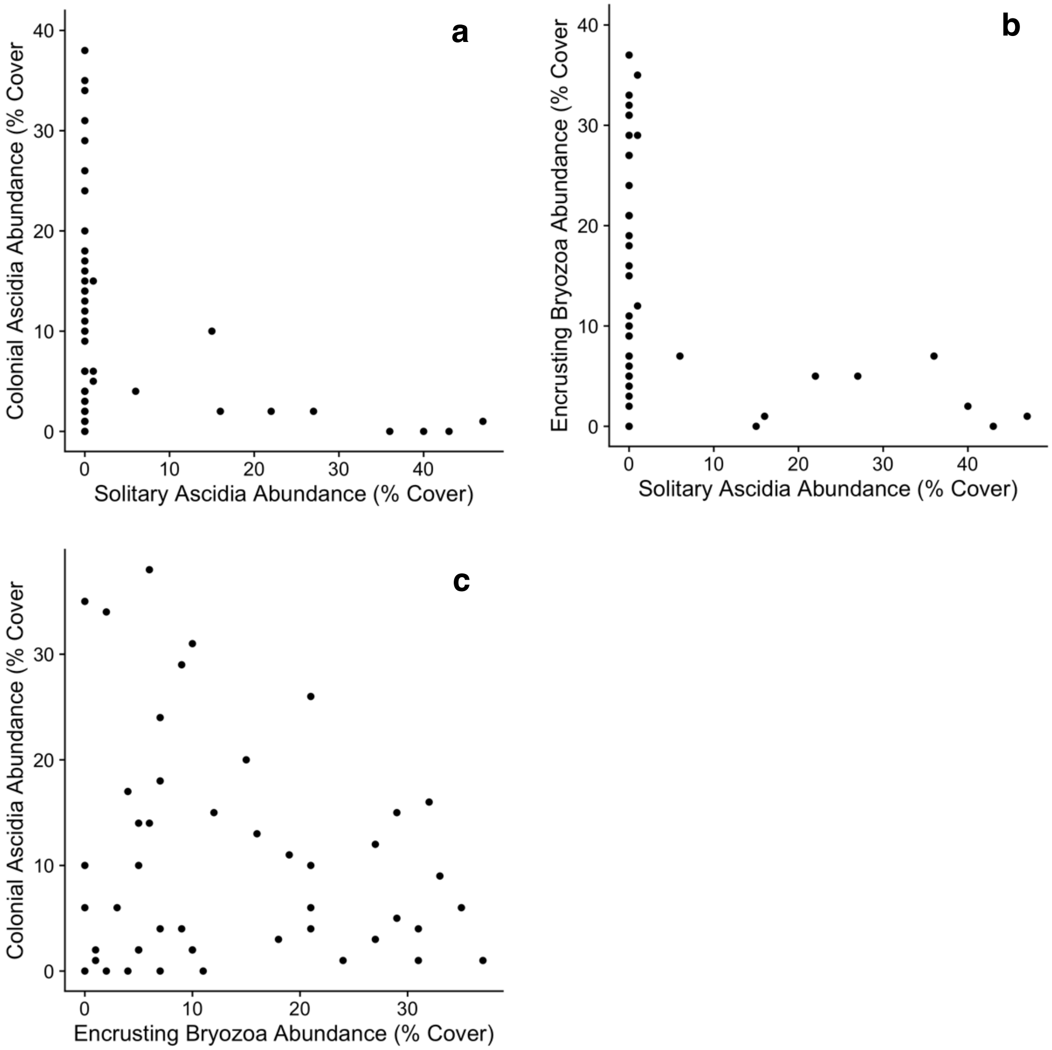
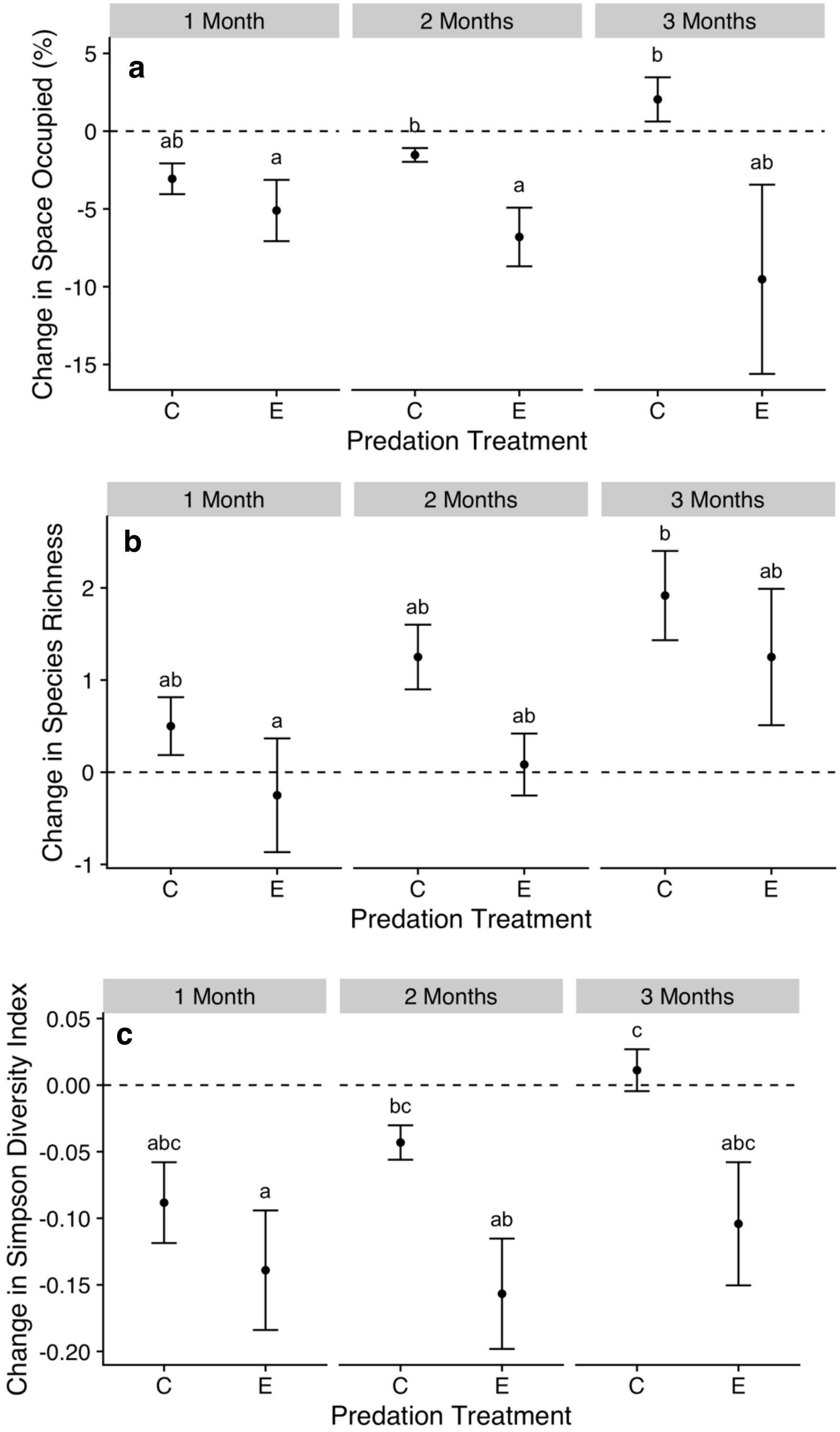

a
Colonial Ascidia Abundance (% Cover
40
30
20
10
0
0
10
20
30
40
Solitary Ascidia Abundance (% Cover)
b
Encrusting Bryozoa Abundance (% Cover
40
30
20
10
0
0
10
20
30
40
Solitary Ascidia Abundance (% Cover)
c
Colonial Ascidia Abundance (% Cover
30
20
10
0
0
10
20
30
Encrusting Bryozoa Abundance (% Cover)

Fig. 2.5


a
1 Month
2 Months
3 Months
Change in Space Occupied (%)
5
0
-5
-10
-15
ab
a
b
a
b
ab
C
E
C
E
C
E
Predation Treatment
b
1 Month
2 Months
3 Months
Change in Species Richness
2
1
0
-1
ab
a
ab
ab
b
ab
C
E
C
E
C
E
Predation Treatment
c
1 Month
2 Months
3 Months
Change in Simpson Diversity Index
0.05
0.00
-0.05
-0.10
-0.15
-0.20
abc
a
bc
ab
c
abc
C
E
C
E
C
E
Predation Treatment

CHAPTER 3

Process-Driven Interannual Variability in Fouling Communities

Abstract

Sessile filter feeding invertebrate communities, commonly known as fouling communities, are ubiquitous on hard substrate in estuarine and marine systems. They also make for an experimentally tractable study system that can productively be used to test ecological theories. Given the high abundance of fouling species associated with the artificial structure, most of the previous research on these communities has taken place at docks and marinas on substrates suspended above the benthos despite fouling species occurring in natural habitats like rocks and cobbles, mangroves, and seagrass. Benthic predators could be an important source of predation on fouling communities across habitats, and previous research has shown varying levels of importance of predation. Most studies that have tested ecological theories using fouling communities have taken place over shorter time scales, and interannual variability in oceanographic conditions within estuaries could result in differences in the importance of processes influencing fouling communities across years. This study examines how fouling community composition and the importance of predation and seagrass in structuring fouling community composition varied from 2018 – 2020 in a California estuary. We found that fouling community composition varied significantly across years, and while there were consistent effects of predators on solitary ascidians, predators had different influences on fouling community composition across years. Differences in effects of predators and fouling community composition were likely driven by variability in oceanographic conditions, predator abundance, and population-level recruitment of fouling species. Additionally, we found differences in effects of seagrass across years, which are likely due to variability in seagrass bed characteristics. Our research shows that processes influencing fouling communities vary significantly across years, and we suggest future research on fouling communities take place across greater spatial scales, temporal scales, and with laboratory experiments to better understand mechanisms of change.

Keywords: Fouling Communities, Invertebrates, Interannual Variability, Predation, Seagrass, Diversity, Climate Change, Estuaries

Introduction

Sessile species are often used as study systems to test ecological theories. Sessile organisms, like plants, corals, and filter feeding invertebrates, are unable to move away from biotic and abiotic stressors, and thus rely on morphological and behavioral adaptations to survive. Additionally, sessile species often exhibit strong competition for limited resources, like space and nutrients. One group of sessile species that is a common study system in marine environments are sessile filter feeding invertebrates which include a taxonomically diverse assemblage of ascidia, bryozoa, bivalvia, hydroidea, cirripedia, polychaeta, and porifera. Known as ‘fouling communities’, these invertebrates provide an excellent, experimentally tractable model system to test ecological theories about community assembly, disturbance, ecosystem function, and biotic interactions in estuarine and marine systems (Osman 1977, Stachowicz et al. 1999, Stachowicz and Byrnes 2006, Altman and Whitlatch 2007, Freestone et al. 2011). While fouling communities have been a focus of considerable research, previous studies have taken place largely on human created structures like docks and pilings in marinas, and studies typically have had a duration of a year or less. These studies have produced contrasting results regarding the importance of predation and have rarely examined how fouling communities are influenced by habitat structure.

Fouling species are commonly associated with docks and marinas and many fouling species are often more abundant on artificial structures over natural structures (Ruiz et al. 2009), and many fouling species are non-native and introduced through ballast water and on the hulls of ships (Ruiz et al. 1997). Given the high abundance of fouling species at docks and marinas, much of the research on these communities has been conducted on artificial structures suspended from docks (Sutherland and Karlson 1977, Stachowicz et al. 1999). Although fouling communities dominate piers and marinas, they are able to reach surrounding natural areas in their planktonic larval phase or through rafting (Worcester 1994). As a result, fouling species also can establish on natural substrates including other invertebrates, seagrass blades, cobbles and exposed bedrock, and hard mud, and can greatly affect foundation species and many estuarine functions (Ruiz et al. 1999, Fitridge et al. 2012, Forrest et al. 2013, Aldred and Clare 2014, Long and Grosholz 2015, Carman et al. 2016). Processes influencing fouling communities on the benthos in natural habitats differ from processes influencing them near docks and marinas off the benthos due to greater access by benthic predators, differences in water quality, and differences in flow. However, few studies have examined the importance of different processes influencing benthic fouling communities in these natural habitats.

While many studies on fouling communities occur over a season or a year, interannual variability may play an important role in structuring communities. Fouling species have the highest recruitment and growth from May to October at temperate latitudes (Osman 1977, Stachowicz and Byrnes 2006), and much of the existing research on fouling communities takes place during this season or continuously for a year (Osman 1977, Sutherland and Karlson 1977, Stachowicz et al. 1999, Stachowicz and Byrnes 2006). However, climate change is changing oceanographic conditions, and this change is expected to be large in estuaries where fouling species are common. Changes in precipitation can influence fouling community composition, where drought, moderate, and wet years had drastically different fouling community composition in San Francisco Bay, CA (Chang et al. 2018). These drought years tend to favor non-native fouling species (Chang et al. 2018), and non-native fouling species are predicted to outperform their native counterparts under the higher temperatures projected under climate change scenarios (Stachowicz et al. 2002, Sorte et al. 2010). Given that interannual variability in estuaries, like Tomales Bay, California, is projected to increase for temperature, salinity, and other abiotic parameters (Monismith et al. 2002, Largier et al. 2010), it is important to conduct experiments and monitor communities over multiple years to make predictions about fouling community ecology in the future.

The importance of predation in structuring fouling communities at temperate latitudes has also shown contrasting results in previous studies. Predation is known to be an important source of mortality in fouling species recruits (Osman and Whitlatch 1995) but fouling species recruits can also be dislodged or bulldozed by herbivorous grazers. In addition to high predation on recruits, adults of some species are also susceptible to predation. Solitary and colonial ascidians are the most susceptible to consumers such as crabs, sea stars, and chitons (Rogers et al. 2016), while bryozoans (both arborescent and encrusting) tend to be consumed the least (Osman et al. 2010). While solitary ascidians are consumed by predators at temperate latitudes, other studies have found no effect of predators on diversity and community composition in seagrass beds at temperate latitudes (Freestone et al. 2011, 2013, 2020). Additionally, predation is known to vary across estuarine gradients (see Chapter 1) and given the influence of climate change on estuarine gradients, effects of predators may not be consistent across years.

Fouling species can spread and survive in various habitats with hard substrate; however, most studies have looked at communities within single habitats. The amount of structure that different marine habitats provide can influence both the abiotic environment and the biota living within it. For example, seagrass blades can reduce flow and provide habitat for predators, both of which can significantly alter fouling community structure (see Chapter 2). While some studies have made latitudinal comparisons of fouling communities inside of seagrass beds (Cheng et al. 2019, Freestone et al. 2020), few studies have made comparisons across adjacent habitats within the same estuary or field site. Seagrass blade growth and shoot density are influenced by changes in temperature, salinity, light, and nutrients (Kentula and DeWitt 2003, Kaldy and Lee 2007, Reynolds et al. 2016), and differences in seagrass physical properties will result in varying reductions of flow and varying amounts of habitat quality. Given the variability in seagrass condition and water quality within estuaries, effects of seagrass on fouling communities are likely to differ across years.

Knowing that interannual variability in abiotic conditions is high in estuarine ecosystems, processes influencing fouling communities are likely to change in their importance across years. This study evaluates how the effects of abiotic variability, predation, and seagrass on fouling community composition vary across years. We predict that fouling community composition will differ significantly across years as a function of abiotic variability. We also predict that effects of predators on ascidians will be consistent across years, but that effects of predators on community composition will vary across years due to differences in both predator and prey communities. While we predict that there will be differences in fouling community composition inside vs.

outside of seagrass across all years, we also predict that the effect of seagrass will vary across years due to differences in seagrass bed demographics.

Methods

Community Data

We compared results from three experiments that took place over the summers of 2018, 2019, and 2020 at Sacramento Landing (38.151244˚N, -122.906417˚W) in Tomales Bay, California. We used a standardized substrate (PVC plates, 10.16 cm x 10.16 cm) deployed on the benthos at -0.3 m below MLLW to measure the potential for settlement on hard substrates. While the experiments differed slightly in their focus (see Chapters 1 and 2), they included caged (small mesh, 1 mm) and open plates (n = 6 per treatment combination) deployed using the same methodology. Cage controls were used in 2018 and 2019 (see chapters 1 and 2), but not 2020, so they were not analyzed in this comparison. Plates were distributed across three blocks inside seagrass and three blocks outside of seagrass (n = 24 per year, total n = 72). Plates were deployed from June to October in all three years, and 49-point counts were conducted on plates within 48 hours after retrieval using a dissecting microscope or photographs (caged plates in 2020). Taxa were grouped by morphotype in community analyses, and morphotypes found across all three years included Macroalgae, Anthozoa, Arborescent Bryozoa, Bivalvia, Colonial Ascidia, Encrusting Bryozoa, Porifera, Solitary Ascidia, and Polychaeta (Table 3.1).

While we did not quantify predator community composition at this site, we completed intertidal diversity surveys at a nearby site (1 km South) in the summers of 2018, 2019, and 2020. A total of 10 quadrats (0.5 m^2) were placed along a 30 meter transect at MLLW and all mobile epifaunal taxa were counted and identified to species or broader taxonomic groups. Taxa found included turban snails, whelks, limpets, chitons, shore crabs, hermit crabs, and European green crabs (Table 3.2).

Oceanographic Data

To identify how abiotic conditions varied across years, we analyzed oceanographic data from the UC Davis Bodega Marine Lab Bodega Ocean Observing Node buoy located in Marshall, CA. This location is a few kilometers North of Sacramento Landing, and records water quality characteristics hourly at 1 m below surface. We collected hourly data for temperature (˚C), salinity (PSU), and chlorophyll *a* (ug/L), removed outlier values, and averaged parameters to daily values. We report the annual mean and coefficient of variation in addition to plotting monthly and daily averages.

Statistical Approach

We conducted analyses using three types of metrics: multivariate community responses, univariate community responses, and univariate community responses (see Chapters 1 and 2 for similar analyses). All statistical analyses were completed using R version 3.6.3. All plots were created using the package 'ggplot2' version 3.3.0 (Wickham et al. 2020). We analyzed community composition of morphotypes using a multivariate generalized linear model (MGLM) framework (Wang et al. 2012). The best fit model for the multivariate response data included terms for predation, year, and their interaction (AIC = 186.442) using a negative binomial distribution. While next best model was the full model including all main effects and interactions between predation treatment, year, and seagrass (ΔAIC = 1.767), here we present the results of the best fit (and most parsimonious) model. An analysis of variance (ANOVA) was conducted on the best fit multivariate model using the Wald test statistic. Pit-resampling was used to calculate Wald Test values using the summary.manyglm function in the 'mvabund' package version 4.1.3 (Wang et al. 2012). Univariate morphotype responses were calculated as part of the MGLM framework outlined above. The univariate *p* values were adjusted to account for multiple tests and collinearities with functional group abundance using the p.uni = “adjusted” argument in ‘mvabund’. Pairwise comparisons were completed with the pairwise.comp argument in ‘mvabund’, which adjusts for multiple comparisons via a free stepdown resampling procedure.

Univariate community responses were conducted on species, not morphotypes, and included space occupied, species richness, and Simpson diversity index (Simpson 1949). Using species instead of morphotype for these analyses allowed for a more nuanced perspective on community metrics, since some morphotypes had >3 species and some only had 1. Species richness and Simpson’s Diversity Index were calculated in R using the package ‘vegan’ version 2.5-6 (Oksanen et al. 2018). The best generalized linear model was determined using the stepAIC function in the R package ‘MASS’ (Ripley et al. 2021), and an ANOVA was conducted on the best fit models for each using the F test statistic. The best fit model for space occupied included the terms predation, seagrass, year, predation x year, and seagrass x year (AIC = 617.360). The best fit model for species richness included predation, seagrass, year, and predation x year (AIC = 271.097). The best fit model for Simpson diversity index was the full model including terms for predation, year, seagrass, and all interaction combinations (AIC = -33.179). Post-hoc pairwise comparisons were conducted on space occupied and richness using Dunnett’s Test and Simpson diversity index using Tukey HSD.

Results

We found that fouling communities differed across years in all metrics. Fouling community composition, as measured in the multivariate data, varied significantly across years (Table 3.1, ANOVA; $W_{2,69} = 10.557$, $p = 0.001$). When examining univariate community metrics, we found that space occupied (Fig. 3.1a, ANOVA; $F_{2,69} = 43.729$, $p < 0.001$), species richness (Fig. 3.1b, ANOVA; $F_{2,69} = 17.865$, $p < 0.001$), and Simpson diversity index (Fig. 3.1c, ANOVA; $F_{2,69} = 14.899$, $p < 0.001$) also differed significantly across years. Space occupied, richness, and Simpson diversity index were significantly lower in 2020 than in 2019 and 2018 (Dunnett's Test, Tukey HSD, $p < 0.05$). Space occupied was highest in 2018 while species richness and Simpson Diversity index were highest in 2019 (Dunnett's Test, Tukey HSD, $p < 0.05$). The difference in community metrics across years can best be understood with differences in the abundance of specific morphotypes. The differences in community composition were driven by macroalgae (Table 3.1, ANOVA; $W_{2,69} = 3.603$, $p = 0.016$), colonial ascidia (Table 3.1, ANOVA; $W_{2,69} = 3.639$, $p = 0.016$), encrusting bryozoa (Table 3.1, ANOVA; $W_{2,69} = 7.817$, $p = 0.001$), and solitary ascidia (Table 3.1, ANOVA; $W_{2,69} = 4.248$, $p = 0.002$). The abundances of colonial ascidia, encrusting bryozoa, and solitary ascidia were highest in 2018, intermediate in 2019, and lowest in 2020 (Fig. 3.2). Macroalgae abundance followed an opposite trend, and was lowest in 2018, intermediate in 2019, and highest in 2020.

We also found a significant effect of predation across all years. Caged and open plates differed significantly with the multivariate metric (Table 3.1, ANOVA; $W_{1, 70} = 12.340$, $p = 0.001$)). Additionally, there were significant differences among predation treatments for space occupied (Fig. 3.1a, ANOVA; $F_{1,70} = 8.451$, $p = 0.005$), species richness (Fig. 3.1b, ANOVA; $F_{1,70} = 16.112$, $p < 0.001$), and Simpson diversity index (Fig. 3.1c, ANOVA; $F_{1,70} = 15.053$, $p <0.001$), all of which were greater on caged plates than open plates. In addition, predation altered the abundance of specific morphotypes. Macroalgae (Table 3.1, ANOVA; $W_{1, 70} = 8.559$, $p = 0.001$) and encrusting bryozoa (Table 3.1, Fig. 3.2, ANOVA; $W_{1, 70} = 2.869$, $p = 0.021$) were more abundant on open plates, while colonial ascidia (Table 3.1, Fig. 3.2, ANOVA; $W_{1, 70} =$

2.622, $p = 0.024$) and solitary ascidia (Table 3.1, Fig. 3.2, ANOVA; $W_{1,70} = 7.733$, $p = 0.001$) were more abundant on caged plates.

The effect of predation also varied among years. Community composition, as measured in the multivariate data, differed significantly across the interaction between predation and year (Table 3.1, ANOVA; $W_{2,68} = 6.395$, $p = 0.001$). There was a year x predation interaction for space occupied (Fig. 3.1a, ANOVA; $F_{2,68} = 13.038$, $p < 0.001$), species richness (Fig. 3.1b, ANOVA; $F_{2,68} = 8.251$, $p < 0.001$), and Simpson diversity index (Fig. 3.1c, ANOVA; $F_{2,68} = 8.251$, $p < 0.001$). In 2018 there was no effect of predation on any richness, diversity, or cover, whereas in 2019 cover and richness were decreased by predation (Dunnett's Test, $p < 0.05$) and in 2020 predators decreased diversity (Tukey HSD, $p < 0.05$). The difference in effects of predators across years was likely driven by changes in the abundance of specific morphotypes. Specifically, arborescent bryozoa (Fig. 3.2, ANOVA; $W_{2,68} = 3.083$, $p = 0.025$), colonial ascidia (Fig. 3.2, ANOVA; $W_{2,68} = 3.156$, $p = 0.025$), and encrusting bryozoa (Fig. 3.2, ANOVA; $W_{2,68} = 3.984$, $p = 0.004$) each had year x predation interactions. Predators decreased arborescent bryozoa in 2019, decreased colonial ascidia in 2019 and 2020, and increased encrusting bryozoa in 2018 (Fig. 3.2). All other taxa abundances did not differ across the predation by year interaction.

Using the same MGLM framework (negative binomial ManyGLM model), we saw that there was a significant difference in predator communities across years along the transect locations (Table 3.2, $W_{1,29} = 15.71$, $p = 0.001$). The predators driving this difference were turban snails (Table 3.2, Fig. 3.4, $W_{1,29} = 3.583$, $p = 0.009$), limpets (Table 3.2, Fig. 3.4, $W_{1,29} = 8.567$, $p = 0.001$), shore crabs (Table 3.2, Fig. 3.4, $W_{1,29} = 11.852$, $p = 0.001$), and chitons (Table 3.2, Fig. 3.4, $W_{1,29} = 3.592$, $p = 0.009$). Limpets were the most abundant of all potential predator taxa found, and their abundance was significantly higher in 2018 and 2019 than in 2020. Shore crabs were the next most abundant, with their abundance highest in 2020 and low in 2018 and 2019. Chitons and turban snails were more abundant in 2018 and 2019 than 2020 (Fig. 3.4). Combined abundance of the molluscan predators that drove multivariate differences (limpet, chiton, turban snail) was highest in 2019, intermediate in 2020, and low in 2018. Total abundance of all predators was highest in 2020, intermediate in 2019, and lowest in 2018.

There were no consistent effects of seagrass on fouling communities across all years. Instead, the effect of seagrass on space occupied differed across years (Fig. 3.1a, ANOVA; $F_{2,68} = 8.428$, $p < 0.001$). Space occupied was higher outside of seagrass than inside in 2018, but it did not differ inside and outside of seagrass in 2019 or 2020 (Fig. 3.1a, Dunnett's Test, $p < 0.05$). There was also a significant interaction between predation treatment, seagrass treatment, and year for Simpson diversity index (Fig. 3.1c, ANOVA; $F_{2,60} = 4.962$, $p = 0.01$). The effect of predators on diversity differed inside and outside of seagrass in 2020, but not in 2018 or 2019. (Fig. 3.1c). Predation significantly decreased diversity outside of seagrass in 2020 but did not significantly decrease diversity inside seagrass (Tukey HSD, $p < 0.05$). In 2018, there was a trend for predation to increase diversity outside of seagrass and slightly decrease diversity inside seagrass, though these differences were not significant.

Differences in oceanographic conditions could be influencing interannual variability in fouling community composition and predation. We found that the mean and the coefficient of variation of temperature were highest in 2019 (Fig. 3.3a, $\bar{x} = 14.297$ ˚C, CV = 0.172), intermediate in 2020 (Fig. 3.3a, $\bar{x} = 14.289$ ˚C, CV = 0.155), and lowest in 2018 (Fig. 3.3a, $\bar{x} = 14.081$˚C, CV = 0.130). Values for salinity were highest in 2018 (Fig. 3.3b, $\bar{x} = 32.084$ psu, CV = 0.096), intermediate in 2020 (Fig. 3.3b, $\bar{x} = 31.517$ psu, CV = 0.065), and lowest in 2019 (Fig.

3.3b, $\bar{x}$ = 27.732 psu, CV = 0.286). Variability in salinity followed a different trend, being highest in 2019, intermediate in 2018, and lowest in 2020. Mean chlorophyll *a* content was highest in 2018 (Fig. 3.3c, $\bar{x}$ = 6.788 ug/L, CV = 1.056), intermediate in 2020 (Fig. 3.3c, $\bar{x}$ = 6.186 ug/L, CV = 1.067), and lowest in 2019 (Fig. 3.3c, $\bar{x}$ = 6.007 ug/L, CV = 1.062), whereas variation in chlorophyll *a* was highest in 2020, intermediate in 2019, and lowest in 2018.

Discussion

Overall, we found that processes regulating fouling communities experience significant interannual variability. As we predicted, fouling community composition differed across years, which could be the result of changing abiotic conditions. Overall, we saw that abundance (space occupied), richness, and Simpson diversity index were lowest in 2020, and higher in 2018 and 2019 (Fig. 3.1). Species richness and diversity were highest in 2019 while space occupied was highest in 2018. Variability in temperature and salinity was highest in 2019 (Fig. 3.3b, Fig. 3.3c), and if variability in oceanographic conditions acts as a disturbance, the maximum of diversity and richness in 2019 could be the result of disturbance excluding competitive dominants (Connell 1978, Sousa 1979). Both variability in salinity and low salinity events are known to act as disturbances to some fouling species (Chang et al. 2018), though more mechanistic experiments are needed to verify how well the intermediate disturbance hypothesis explains our results.

Macroalgae, colonial ascidia, encrusting bryozoa, and solitary ascidia were likely responsible for the changes in community metrics across years (Table 3.1). In general, invertebrate abundance decreased from 2018 to 2020 while macroalgal abundance increased. Despite many fouling species being sensitive to low salinities, abundance did not have a positive relationship with salinity. The increase in macroalgal abundance is likely driven by reduced invertebrate abundance, allowing for their proliferation on available space. This result aligns with previous research showing that fouling species can competitively exclude macroalgae on settlement plates (Nandakumar 1996). While we did not measure nutrient concentrations, an increase in macroalgal abundance could also be driven by higher nutrient availability or more suitable environmental conditions. However, chlorophyll *a* content monitored at the Tomales Bay Buoy did not follow the same trend with macroalgal abundance (Fig. 3.3c), as it was highest in 2018 when macroalgal abundance was lowest. Chlorophyll *a* content in the water could be an indication of higher turbidity, which could reduce light available to benthic macroalgae explaining the pattern we saw. However, there were many outliers (n = 22,309 for temperature and chlorophyll *a*, n = 3,081 for salinity) in the buoy oceanographic data due to periodic issues with sensors. Additional oceanographic data sources are needed to better understand environmental drivers of biological patterns.

High interannual variability in fouling community composition is common and is influenced by winter temperature and subsequent competitive exclusion depending on which species recruit first (Stachowicz et al. 2002). Estuarine ecosystems experience both temperature and salinity fluctuations, and differences in precipitation patterns strongly influence fouling community composition (Chang et al. 2018). Tomales Bay experiences strong intra-annual variability in temperature and salinity, and salinity regimes are strongly influenced by winter precipitation events (Largier et al. 1997). Our results showing differences in fouling community composition across years support previous reports of high interannual variability. However, the overall abundance and recruitment was lower than 100%, with the lowest coverage in 2020. Fouling communities suspended off of the benthos near marinas very often have over or close to 100% coverage (Osman 1977, Chang et al. 2018). However, we found that mean space occupied in 2018, 2019, and 2020 were 64%, 61%, and 24% respectively (Fig. 3.1a). One major difference between our methods and previous methods is that we deployed plates on the benthos at -0.3 m below MLLW rather than suspending plates off of a dock or examining communities on subtidal rocks (at least 5 m below MLLW) (Osman 1977, Sutherland and Karlson 1977, Osman et al. 2010). Intermittent exposure to air, exposure to benthic predators, and greater variability in temperature and other water characteristics at the tidal elevation we chose could explain the lower recruitment patterns.

While communities varied across years, we found some consistent effects of predators on community composition across all years. Plates that were open to predators had lower abundance (Fig. 3.1a), richness (Fig. 3.1b), and Simpson diversity index (Fig. 3.1c) than plates protected from predators. This predator effect was driven by four morphotypes; macroalgae, colonial ascidia, encrusting bryozoa, and solitary ascidia. Predator effects were greatest on solitary and colonial ascidia, whose abundance was higher on caged than open plates in 2018 and 2019 for solitary ascidia and 2019 and 2020 for colonial ascidia. These results match previous studies that have shown that ascidians are more susceptible to effects of predators than other fouling species (Osman and Whitlatch 1995, Nydam and Stachowicz 2007, Byrnes and Stachowicz 2009, Osman et al. 2010, Freestone et al. 2013). Encrusting bryozoa and macroalgal abundance was higher on open plates than caged plates and is likely a result of either greater resistance to predators for bryozoans (Osman and Whitlatch 1995), or less invertebrate coverage and therefore more space available for macroalgae. While some studies have shown that predation is weak and that its influence is species-specific at temperate latitudes (Freestone et al. 2011, 2013, 2020), we found that predation does have a significant influence on community composition. This aligns with results from experiments conducted on the outer coast (Osman and Whitlatch 1995, Nydam and Stachowicz 2007, Byrnes and Stachowicz 2009, Rius et al. 2014, Rogers et al. 2016). However, most of these previous studies were done at or near docks and marinas on the outer coast using suspended plates. Our study took place far from commercial ports in an estuary with extensive eelgrass habitat and our methods allowed for access by benthic predators, reflecting conditions that fouling species experience on natural substrates. There are differences in effects of predators on fouling species between disturbed marina environments and less-disturbed surrounding natural areas (Rogers et al. 2016), and this study provides additional evidence for the importance of predation in natural benthic habitats found in estuaries.

Effects of predators were important in structuring fouling communities; however, the strength of these effects differed significantly across years. We found that multivariate data (Table 3.1), space occupied (Fig. 3.1a), richness (Fig. 3.1b), and Simpson diversity index (Fig. 3.1c) all had significant predation x year interactions. Effects of predators were greatest in 2019 for space occupied and species richness but were greatest in 2020 for Simpson diversity index. This pattern was driven by arborescent bryozoa, colonial ascidia, and encrusting bryozoa (Table 3.1). We believe that some of these differences in effects of predators were likely due to differences in population recruitment (Osman et al. 2010). For example, the greatest effect of predation for arborescent and encrusting bryozoa was in 2018, when recruitment was highest (Fig. 3.2). However, the greatest effect of predation on colonial ascidia was in 2019, when recruitment was intermediate. Since the effect of predators directly on colonial ascidia differed across years and not linearly with recruitment (Fig. 3.2), this effect is likely driven by differences in the predator community. The abundance of all molluscan predators/grazers combined (turban snail, limpet, chiton) was highest in 2019 (Fig. 3.4), and limpets and chitons are known to consume colonial ascidia (Nydam and Stachowicz 2007, Byrnes and Stachowicz 2009, Rogers et al. 2016). Higher predator abundance in 2019 could explain the greater effect seen in colonial ascidia. However, additional experiments are needed to verify this. Solitary ascidia were the other morphotype susceptible to predation; nonetheless, the effect of predators on this morphotype were consistent across years. This suggests that predators may prefer solitary or colonial ascidians (Osman and Whitlatch 1995, Nydam and Stachowicz 2007, Freestone et al. 2013, Rius et al. 2014, Rogers et al. 2016), though additional experiments at this estuarine site are needed.

Despite recording significant effects of seagrass on fouling communities in 2018 (see Chapter 2), we did not see any effect of seagrass on community composition in later years. There was a significant seagrass x year interaction for space occupied in 2018 (Fig. 3.1a), where space occupied was higher outside of seagrass than inside in 2018 only. There was also a significant seagrass x predation x year interaction for Simpson diversity index (Fig. 3.1c). While we don't have seagrass measurements for all years, we observed that seagrass density was highest in 2018, intermediate in 2019, and low in 2020 (Rubinoff, *personal observation*). Differences in water temperature, salinity, nutrients, and turbidity likely influence seagrass characteristics, and while we are not quantitatively able to explain what is driving differences in effects of seagrass on fouling communities, we predict that it is due to differences in shoot density and blade height, thereby influencing water flow. Reductions in flow can decrease recruitment, food supply, and modify fouling community composition (see Chapter 2). Therefore, we recommend additional manipulative experiments to determine how changes in oceanographic conditions influence seagrass density and identify how variability in density influences fouling communities and patterns of predation.

Our research shows that processes influencing fouling communities can vary significantly across years. While some predator effects were consistent (such as the reduction of solitary ascidians), differences in environmental conditions and predator communities resulted in different patterns across three years. To yield more generalizable predictions and results in field experiments understanding processing influencing fouling communities, we suggest a few modifications for future research.

First, we suggest conducting research in both degraded and natural habitats. The majority of previous studies on fouling communities have taken place in docks and marinas with plates suspended above the benthos. As a result, some of these studies have shown little effects of predators at temperate latitudes (Freestone et al. 2011, 2013, 2020) as well as high recruitment and coverage (Osman 1977, Stachowicz et al. 1999). This does not reflect conditions that many sessile invertebrate species experience on the benthos attached to natural substrates such as cobbles and seagrass with exposure to benthic predators. Our results showed predator effects and substantial variability in abundance, and additional comparisons that isolate the mechanisms of these differences are necessary.

Second, if possible, field experiments should be replicated over multiple years. This will capture more variation in environmental conditions, which is known to directly influence fouling communities through physiological mechanisms (Chang et al. 2018) as well as indirectly influence them by modifying patterns of predation. Variability in environmental conditions is likely to increase in estuaries as a function of climate change (Largier et al. 2010), so more years of monitoring will allow for a more accurate representation to how biological communities are changing.

Finally, if it is not possible to replicate field experiments across years, we suggest an increase in replication across multiple sites at different spatial scales. We found that communities and effects of predators differed across Tomales Bay (see Chapter 1), and these patterns are likely to vary at larger scales such as between multiple estuaries.

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Tables

	Predation		Year		Predation x Year	
	W	*p*<(*W*)	*W*	*p*<(*W*)	*W*	*p*<(*W*)
Multivariate	12.340	**0.001**	10.557	**0.001**	6.395	**0.001**
Macroalgae	8.559	**0.001**	3.603	**0.016**	1.889	0.121
Anthozoa	0.716	0.691	0.887	0.879	0.049	0.657
Arborescent Bryozoa	1.678	0.248	2.152	0.172	3.083	**0.025**
Bivalvia	0	0.826	0.085	0.879	0	0.998
Colonial Ascidia	2.622	**0.024**	3.639	**0.016**	3.156	**0.025**
Encrusting Bryozoa	2.869	**0.021**	7.817	**0.001**	3.984	**0.004**
Porifera	0.805	0.673	0.42	0.879	0.071	0.657
Solitary Ascidia	7.733	**0.001**	4.248	**0.002**	1.405	0.144
Polychaeta	0.364	0.746	0.693	0.879	0.056	0.657

Table 3.1 Analysis of variance for most parsimonious best fit model (AIC = 186.442, Morphotype Abundance ~ Predation + Year + Predation:Year) generalized linear model. Multivariate and adjusted univariate morphotype responses using Wald values as test statistics. Bold values indicate significant effects at $\alpha = 0.05$.

	Year	
	W	*p*<(*W*)
Multivariate	15.71	**0.001**
Acanthinucella (Whelk)	1.549	0.282
Chiton	3.592	**0.009**
European Green Crab	0.061	0.429
Hermit Crab	1.925	0.120
Limpet	8.567	**0.001**
Ocinebra (Whelk)	0	1.00
Shore Crab	11.852	**0.001**
Turban Snail	3.583	**0.009**
Urosalpinx (Whelk)	1.096	0.344

Table 3.2 Analysis of variance of predator abundance across years using a generalized linear model with a negative binomial distribution. Multivariate and adjusted univariate predator responses using Wald values as test statistics. Bold values indicate significant effects at $\alpha = 0.05$.

Figure Captions

Fig. 3.1 Differences in community metrics between predation treatments (C = caged, O = open) and inside and outside of seagrass. Metrics consist of **a)** mean space occupied (% cover on panel surface), **b)** mean species richness, and **c)** mean Simpson diversity index all with error bars representing standard error. Post-hoc tests were conducted using Dunnett's Test for space occupied and species richness and Tukey HSD for Simpson diversity index. Significant differences ($p < 0.05$) across treatments are indicated by different letters.

Fig. 3.2 Abundance of dominant morphotypes between predation treatments (C = Caged, O = Open) and across years. Values represent mean percent cover on settlement plates with error bars representing the standard error. Pairwise comparisons completed with the pairwise.comp argument in 'mvabund' across the predation x year interaction, which adjusts for multiple comparisons via a free stepdown resampling procedure. Different letters indicate significant differences in the multivariate statistics ($p < 0.05$).

Fig. 3.3 Mean daily water parameters from 2018 – 2020. Parameters consist of **a)** Temperature (˚C, n = 22,309), **b)** Salinity (PSU, n = 3,081), and **c)** Chlorophyll *a* (ug/L, n = 22,309) with different colors representing different years.

Fig. 3.4 Abundance of dominant predators across years. Values represent mean percent count per 0.5 m^2 quadrat with error bars representing the standard error. Pairwise comparisons completed with the pairwise.comp argument in 'mvabund' across years, which adjusts for multiple comparisons via a free stepdown resampling procedure. Different letters indicate significant differences in the multivariate statistics ($p < 0.05$).

Figures

Fig. 3.1

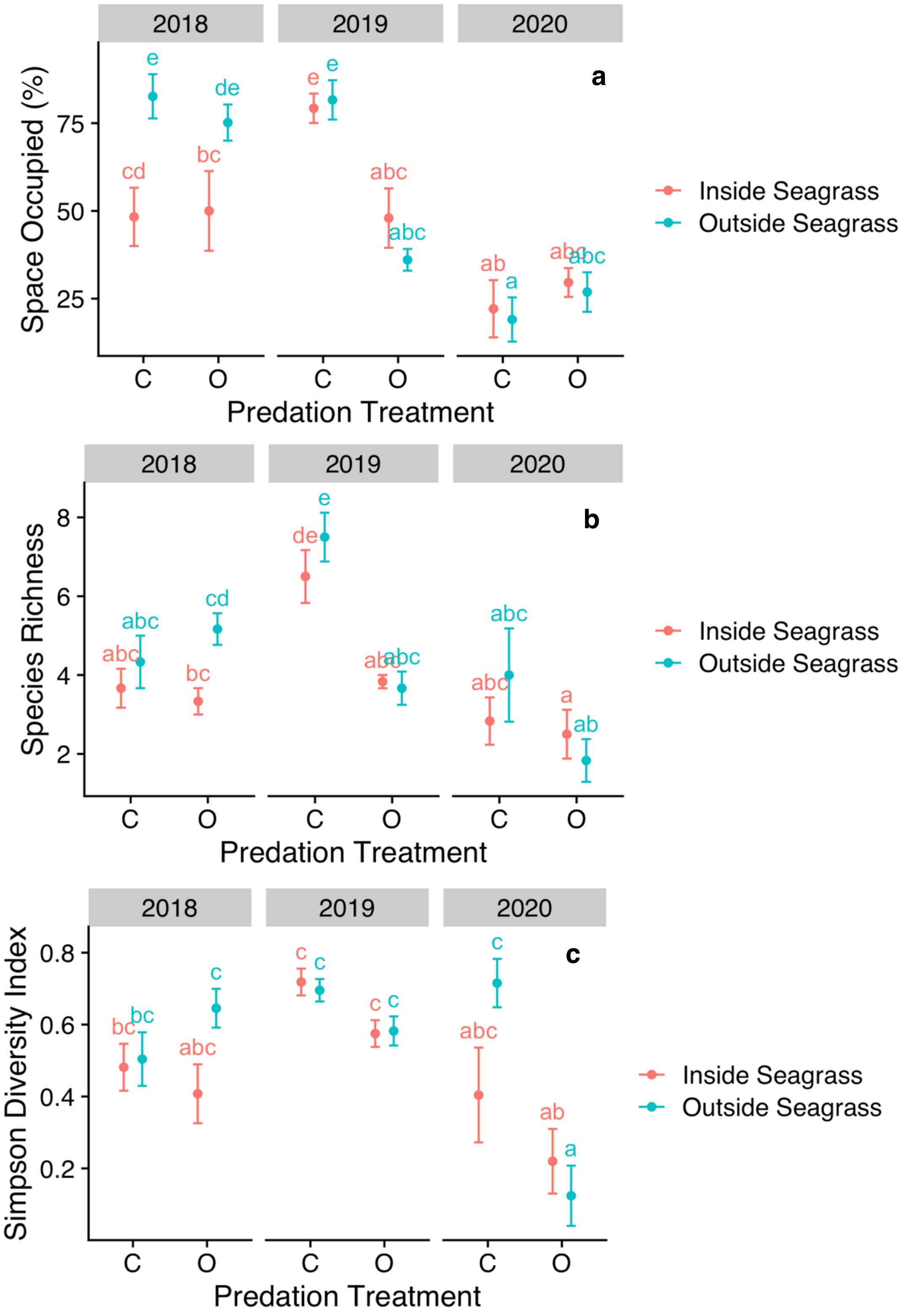


Fig. 3.2

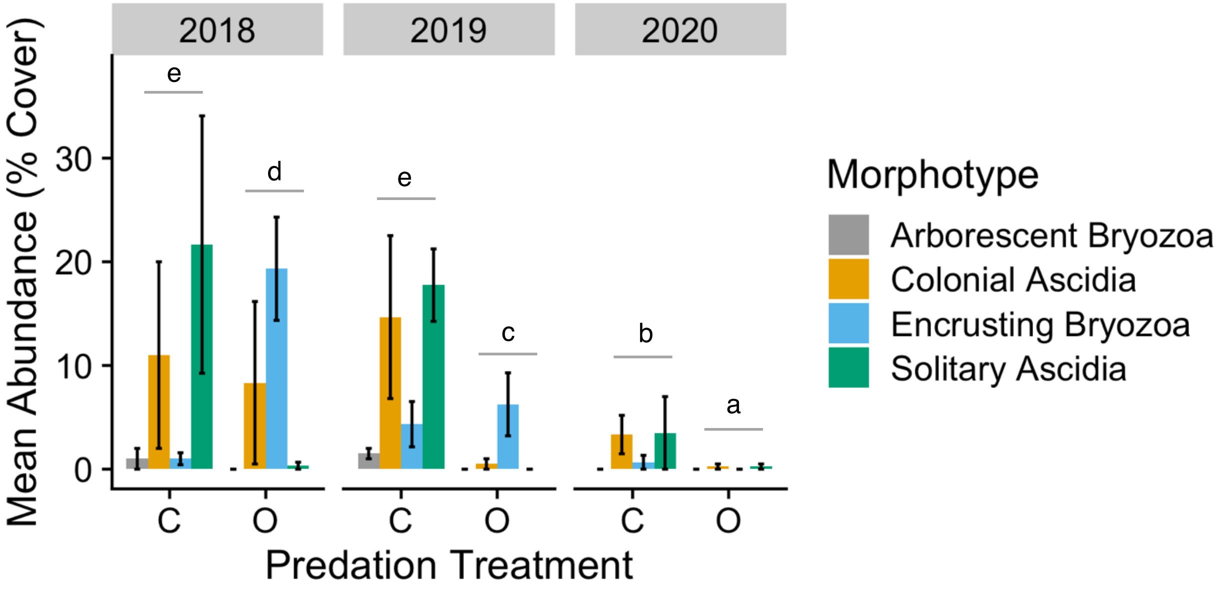

2018
2019
2020
Mean Abundance (% Cover)
0
10
20
30
e
d
e
c
b
a
C
O
C
O
C
O
Predation Treatment
Morphotype
Arborescent Bryozoa
Colonial Ascidia
Encrusting Bryozoa
Solitary Ascidia

Fig. 3.3

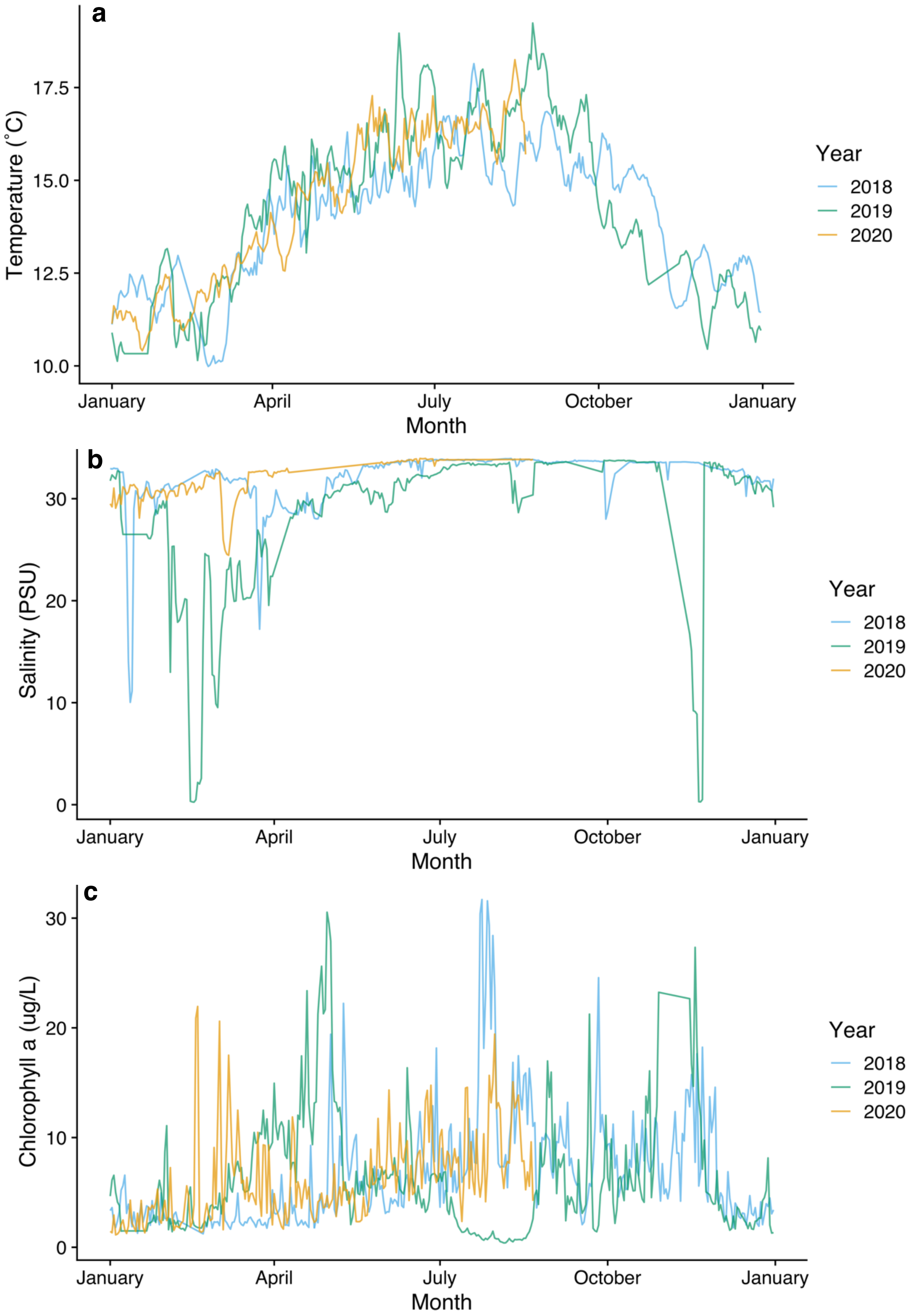
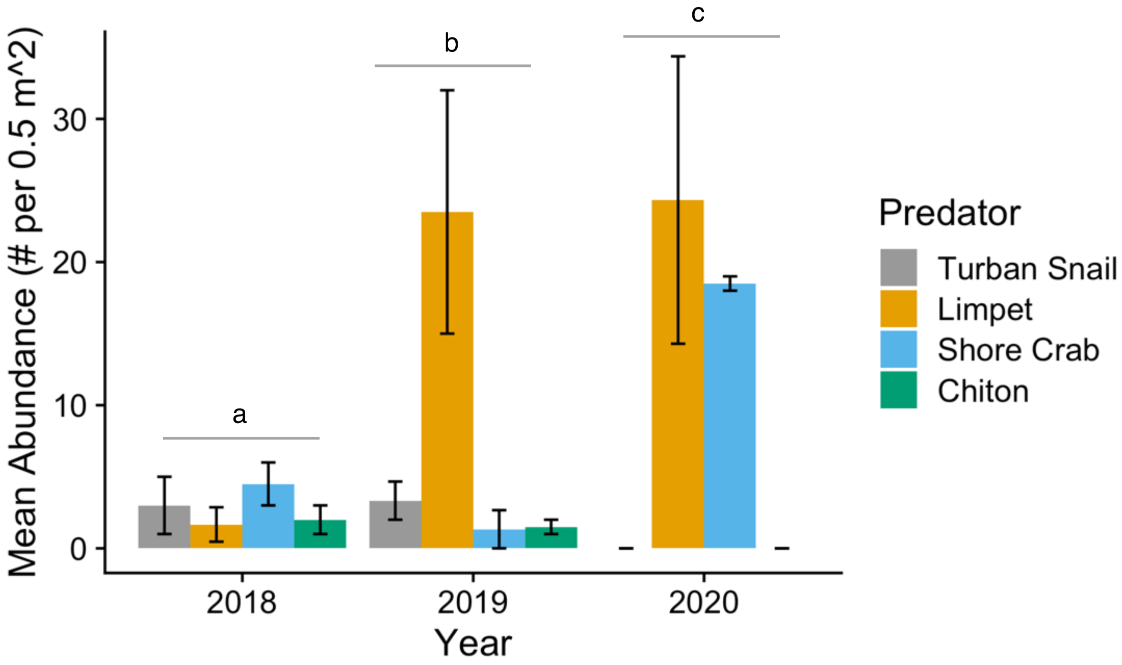

a
Temperature (°C)
17.5
15.0
12.5
10.0
January
April
July
October
January
Month
Year
2018
2019
2020
b
Salinity (PSU)
30
20
10
0
January
April
July
October
January
Month
Year
2018
2019
2020
c
Chlorophyll a (ug/L)
30
20
10
0
January
April
July
October
January
Month
Year
2018
2019
2020

Fig. 3.4



a
b
c
Mean Abundance (# per 0.5 m^2)
0
10
20
30
2018
2019
2020
Year
Predator
Turban Snail
Limpet
Shore Crab
Chiton