Structural effects of seagrass on fouling communities involve more direct than indirect effects

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ABSTRACT: Biogenic habitats influence biodiversity through both direct and indirect mechanisms. Direct influences result in changes to abiotic conditions or trophic support, while indirect influences result in changes in associated communities and species interactions. Seagrass forms a marine biogenic habitat that slows water flow, modifies water chemistry, provides food to grazers, and offers refuge. In this study, we examined how seagrass directly and indirectly influences invertebrate communities. Using sessile filter feeding invertebrate communities, also known as fouling communities, as a study system, we deployed experiments with bare settlement plates and predator exclosures inside and outside of seagrass over the summer of 2018. We also conducted a predator exposure experiment in 2020 by removing exclosures following community development to better understand predator effects and how they change temporally. We found that seagrass reduced abundance, diversity, and richness of fouling species in a high recruitment year (2018). Predators influenced communities by reducing the abundance of competitively dominant solitary ascidians, allowing for an increase in the abundance of other morphotypes. The effect of predation appeared to be greater outside of seagrass than inside in 2018 for some community responses, although this statistical interaction was small relative to the main predator effect and was likely influenced by variable recruitment of fouling species. While we caution against the overgeneralization of effects of biogenic habitat on biodiversity, our study provides evidence that seagrass ecosystems alter fouling communities through direct effects on abundance and that indirect effects of seagrass on fouling communities are less important than predicted.

KEY WORDS: Seagrass · Invertebrates · Predation · Habitat structure · Biogenic habitats · Biodiversity · Fouling communities · *Zostera marina*

1. INTRODUCTION

Biogenic habitats play an important role in fostering global biodiversity. The structural complexity associated with biogenic habitats has been shown to increase the diversity of bird species in tropical rainforests (MacArthur & 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 (Kovalenko et al. 2012). Biogenic habitat increases diversity through direct and indirect mechanisms. Direct mechanisms occur through amelioration of stressful environmental conditions or provisioning of resources, allowing for higher growth and survival, and thus diversity, inside complex habitats (Bruno et al. 2003). Indirect mechanisms occur when biogenic habitat increases niche availability and provides refuge for predators, allowing for a greater diversity of species, especially

at lower trophic levels (MacArthur & MacArthur 1961, Witman 1985). While many studies have examined these processes on their own, the relative importance of direct and indirect effects of biogenic habitats on diversity are likely to vary across habitats and trophic levels.

One biogenic habitat of ecological importance in marine systems is seagrass. Seagrass beds can be found in temperate and tropical habitats globally, with *Zostera marina* being the most abundant species along the western coast of North America from Baja California to Alaska (Short et al. 2007). Seagrass is a foundation species, and it performs a variety of ecosystem services such as water quality improvement, storm protection, food provision, and habitat provision (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 by which seagrass influences patterns of biodiversity could differ in their direction and relative importance across different taxa.

Seagrass beds provide 3-dimensional structure in habitats often lacking in structure, and therefore could increase niche availability for both benthic and mobile epifaunal organisms. For example, seagrass provides habitat for ascidians that 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 and supply subsidies to other habitats, which has provided additional justification for restoration efforts (Heck et al. 2008, 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 & Grosholz 2015). Additionally, the structure associated with seagrass limits the movement of larger mobile species and could select for smaller predators (Yeager & Hovel 2017). Therefore, the effect of seagrass structure on niche availability might not be as clear as previous complexity–diversity predictions have indicated.

Seagrass beds modify the flow environment around them by reducing water flow, decreasing light penetration, and increasing sedimentation through the structure associated with blades (Short & 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 dislodgment 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 growth or even kill sessile filter feeding invertebrates (Eckman & Duggins 1991). Additionally, the reduction in flow associated with structure could result in decreased larval transport into seagrass beds, resulting in lower recruitment.

Seagrass can also alter the chemical environment. Respiration and photosynthesis cycles inside of seagrass could increase the variability of pH inside beds (Koweek et al. 2018); however, there is some evidence that seagrass raises ambient pH for longer periods of time than it lowers it, 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 is not 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 investigated.

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 from an infaunal life history outside of seagrass to an epifaunal strategy inside of seagrass (Bouma et al. 2009). Patch size and distance from the edge of the bed can influence this pattern, where predation risk decreased with distance into eelgrass beds for bay scallops (Carroll & 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 & Hovel 2006) and oysters (Lowe et al. 2019).

As a biogenic habitat that influences both the environment and biota, seagrass is likely to increase biodiversity. However, seagrass could have different effects on patterns of biodiversity among different taxa, and there are 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 & 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. 2019). 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 fouling communities are especially likely given that they are taxonomically diverse and consist of diverse assemblage of tunicates, bryozoans, bivalves, hydroids, barnacles, and sponges. Fouling species are most susceptible to predators as post-recruitment juveniles (Osman & Whitlatch 1995). With lower flow and recruitment in eelgrass beds and habitat provisioning for some predators, seagrass will likely increase the chances of mortality for post-recruit juveniles until they grow large enough to reach a size refuge in predation risk and stress-tolerance.

This study evaluates how the 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 lower fouling community diversity metrics by serving as refuge for predators of fouling species. We also predicted that the effects of seagrass and predation would be greater in recruits and juveniles than in fully grown adult communities due to prey refuge in size and differences in recruitment. We tested these predictions in 2 experiments, of which the first manipulated predator access during community assembly, and the second manipulated predator access after 30, 60, and 90 d of community assembly. The experiments were carried out in different years, and fouling community cover in the second experiment was much lower than in the first.

2. MATERIALS AND 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, USA: 38.1512° N, 122.9064° W) using the same materials. The details of each experiment are outlined below.

2.1. Predator exclosure experiment

We conducted a fully factorial predator exclosure experiment at Sacramento Landing over the summer of 2018. Replicate standardized substrata (PVC plates, 10 × 10 cm) were deployed to measure growth and recruitment on hard substrates. Settlement plates (total N = 48) were placed across 4 blocks from June to October 2018, with 2 blocks inside separate patches of seagrass and 2 outside of seagrass beds. Blocks were deployed in an alternating orientation along the shoreline such that identical seagrass treatments were never directly next to each other to avoid pseudoreplication. Seagrass beds at this site are patchy, so blocks located outside of seagrass were typically within 5 m of the seagrass bed at a similar depth to those inside the bed. Plates were deployed individually on the benthos using 2 pieces of rebar, vexar mesh, and 1 PVC plate per replicate. 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 1 of 4 caging treatments: (1) uncaged open plates, (2) small-mesh cages (1 mm mesh, excludes most predators, height = 8.9 cm), (3) large-mesh cages (5 mm mesh, excludes large predators, height = 8.9 cm), and (4) partial cages with 3 sides (1 mm mesh, cage control, height = 8.9 cm). 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 m below mean lower low water (MLLW) in a stratified random design with 3 replicates of each of the 4 predation treatments per block (n per block = 12). Blocks consisted of a 4 × 3 grid with each plate 0.75 m apart, and blocks were spaced 6 m apart. Cages were cleaned every 2 wk to maintain water flow through the mesh.

Plates were collected during low tides in October 2018 and community composition was quantified within 48 h of return to the lab using a 49-point count under a dissecting microscope. Organisms were iden-

tified to species, or morphospecies when species identity was less certain. We conducted point counts at the canopy level and understory level; however, as there were no species hidden under canopy-forming species, the analyses presented here are on canopies. All organisms were identified to the lowest taxonomic level (mostly species), and vouchers were collected in instances where species identity was less certain. Since 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) and broad taxonomic group (Anthozoa, Ascidia, Bryozoa, Bivalvia, macroalgae, Porifera, Polychaeta). Photographs of each plate were taken before live counts for verification of methods in species enumeration.

2.2. Statistical approach: predator exclosure experiment

We conducted analyses using 2 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 4.4.2 (R Core Team 2024). All plots were created using the R 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 Akaike's information criterion (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 ANOVA was conducted on the best fit model using Wald test values. The MGLM framework does not currently allow for the inclusion of random effects, and when including block as a random effect in the other models discussed in this study, we found that model fits were reduced. There-

fore, 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 Gini-Simpson diversity index (hereafter simply "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 was 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 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 × seagrass treatment interactions using Tukey HSD.

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 analysis, 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.

2.3. Predator exposure experiment

To better understand how predation influences fouling community composition at different stages of community development, 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 6 blocks, 3 inside and 3 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 3 predator exposures on different subsets of plates at 1, 2, and 3 mo 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 d, after which they were collected, and another photograph was taken and weight was measured. Previous experiments have shown that fouling species are consumed by predators within a 3 d period and sometimes over shorter timescales (Freestone et al. 2013, 2020). Changes in weight were negligible (<5 g) 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 calcareous worms in 2020 but not in 2018.

2.4. Statistical analyses: predator exposure experiment

This experiment used the same metrics (multivariate approach, univariate community metrics) as the predator exclosure experiment. 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 (measured as post-exposure metric/pre-exposure metric) to ensure analyses were always conducted on positive values. Figures indicating statistical differences are on models analyzing the proportional change; however, the y-axes have been converted to percentage change ([post-exposure metric — pre-exposure metric] / pre-exposure metric) for ease of interpretation. The MGLMs were 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. Model comparison and selection were completed using AIC scores, and the most parsimonious model with the lowest AIC score was selected. The best fit model only had seagrass as a fixed predictor (AIC = 13.832) and the second-best model only had predation as a fixed predictor (AIC = 16.015). The best model was then analyzed via ANOVA with Wald test statistics.

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. The full model was chosen to generate statistical results that could help explain trends in the figures presented. Post hoc pairwise comparisons were conducted across predation × exposure time treatment interactions using Tukey HSD.

3. RESULTS

3.1. Predator exclosure experiment

The multivariate community analysis revealed that the effect/strength of predation differed inside and outside of seagrass (Table 1, ANOVA; $W_{3,40} = 5.041$, $p = 0.017$). However, univariate measures of community composition showed mixed evidence of the interaction between predation and seagrass. The interaction was not significant for space occupied ($p > 0.05$, Fig. 1a) and species richness ($p > 0.05$, Fig. 1b). However, there was a significant interaction between seagrass and predation with the Simpson diversity index (Fig. 1c, ANOVA; $F_{3,40} = 2.951$, $p = 0.044$). Communities across predation treatments inside of seagrass had similar Simpson diversity values (Fig. 1c, Tukey HSD, $p > 0.05$), but communities across predation treatments outside of seagrass had different Simpson diversity index values (Fig. 1c, Tukey HSD, $p < 0.05$), with partial plates having significantly higher diversity values than small- and large-mesh plates, and similar values to open plates. This difference in effects of predation on communities inside and outside of seagrass was driven by colonial ascid-

Table 1. ANOVA for best fit generalized linear model examining community differences in the 2018 exclosure experiment (Akaike's information criterion, AIC = 80.028, Functional Abundance ~ Predation + Seagrass + Predation:Seagrass). Multivariate and adjusted univariate morphotype responses using Wald values as test statistics. **Bold** values indicate significant effects at $\alpha = 0.05$

	Predation		Seagrass		Predation × 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

ians (Table 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). 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).

We detected a main effect of seagrass through the multivariate community analysis (Table 1, ANOVA; $W_{1,46}$ = 4.738, p = 0.008). While the difference in communities inside vs. outside seagrass was not driven by any specific morphotype (Table 1), when looking at small-mesh caged plates only (Fig. 3a), solitary ascidians were higher outside of seagrass (mean space occupied = 32.33%) than inside (mean space occupied = 9.83%). Univariate metrics of community composition (space occupied, species richness, Simpson diversity index) were higher outside of seagrass than inside. Space occupied (Fig. 1a, ANOVA; $F_{1,46}$ = 24.938, p < 0.001), species richness (Fig. 1b, ANOVA; $F_{1,46}$ = 28.169, p < 0.001), and Simpson diversity index (Fig. 1c, $F_{1,46}$ = 27.434, p < 0.001) were 1.58, 1.42, and 1.51 times higher outside of seagrass than inside, respectively, when averaged across predation treatments. When looking at small-mesh caged plates only (Fig. 1a), abundance, richness, and Simpson diversity index were 1.71, 1.11, and 1.26 times higher outside of seagrass than inside.

Predation also significantly altered fouling community composition. The multivariate community analysis indicated a significant difference in observed community composition among the 4 predation treatments (Table 1, ANOVA; $W_{3,44}$ = 11.592, p = 0.001). A few different morphotypes drove this pattern, with encrusting bryozoans contributing the most to the difference

(Table 1, ANOVA; $W_{3,44}$ = 7.459, p = 0.001), followed by solitary ascidians (Table 1, ANOVA; $W_{3,44}$ = 6.669, p = 0.001), and then macroalgae (Table 1, ANOVA; $W_{3,44}$ = 3.993, p = 0.012). Encrusting bryozoans covered less than 10% of caged plates (both small and large mesh) and more than 20% of partial and open plates, with coverage being highest on partial plates (Fig. 3c). Solitary ascidians covered >25% of small-mesh caged plates and <1% of large-mesh, partial, and open plates (Fig. 3c). 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 cages (2% coverage). While space occupied (Fig. 1a) and species richness (Fig. 1b) did not differ significantly between predation treatments, diversity differed significantly among predation treatments (Fig. 1c, 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. 1c, ANOVA, Tukey HSD, p < 0.05).

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). We found a negative relationship between solitary and colonial ascidian abundance (Fig. 4a, r = −0.361, n = 48, p = 0.012), a negative relationship between solitary ascidian abundance and encrusting bryozoan abundance (Fig. 4b, r = −0.401, n = 48, p = 0.005), and no significant relationship between encrusting bryozoan abundance and colonial ascidian abundance (Fig. 4c, r = −0.142, n = 48, p = 0.336). The nature of the negative interactions was such that if any solitary ascidians were present,
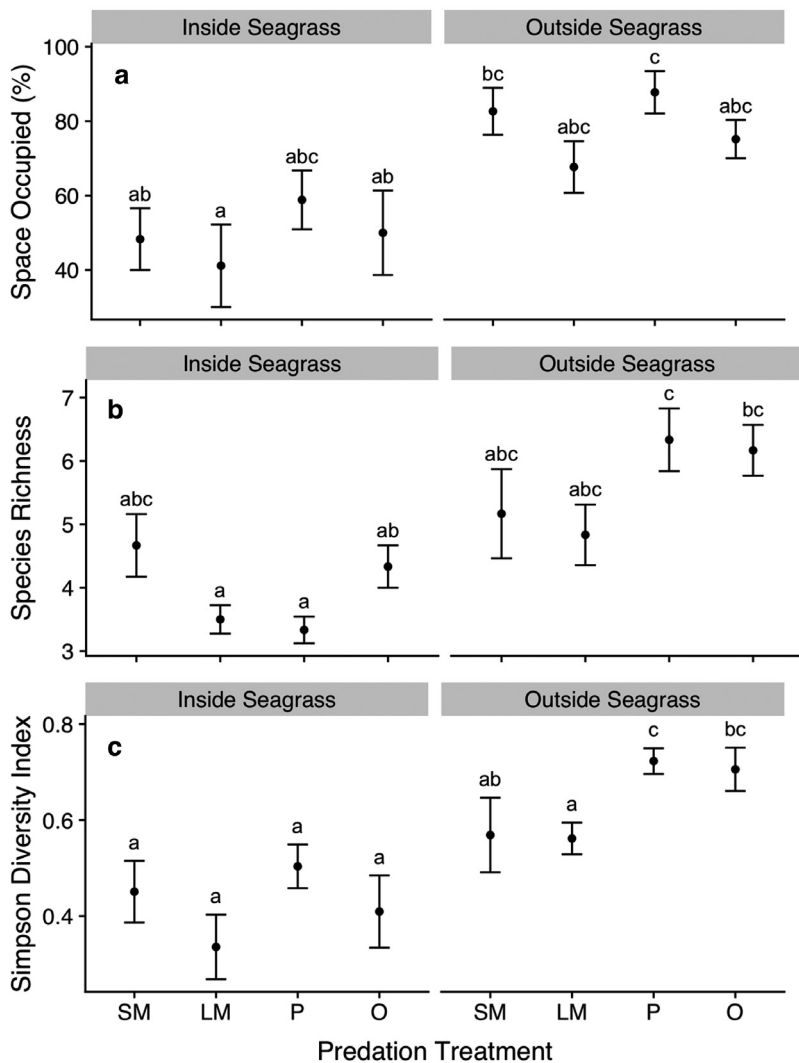
Fig. 1. Differences in community metrics across predation treatments (SM: small mesh; LM: large mesh; P: partial cage; O: open) and inside and outside of seagrass in the 2018 exclosure experiment. Metrics consist of (a) mean space occupied (% cover on panel surface), (b) mean species richness, and (c) 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

there were very few colonial ascidians or encrusting bryozoans (Fig. 4a,b). When very few or no solitary ascidians were present, there was a greater abundance and variability in colonial ascidians and encrusting bryozoans.

3.2. Predator exposure experiment

The multivariate community analysis showed that the proportional change in fouling community composition was not highly influenced by predation or exposure time (as these were not in the best model),

and seagrass did not have any statistical influence on community composition (ANOVA; $W_{1,70} = 9.611$, p = 0.343). When looking at all caged plates pre-exposure at 3 mo (Fig. 3b), it appears that arborescent and encrusting bryozoans were more abundant outside than inside of seagrass, although this difference was not statistically significant (ANOVA; $W_{1,70} = 9.611$, p = 0.343). However, some univariate community metrics did change significantly across treatments. Plates that were exposed to predators experienced greater reductions in space occupied (Fig. 5a, ANOVA; $F_{1, 70} = 14.437$, p < 0.001), species richness (Fig. 5b, ANOVA; $F_{1, 70} = 6.942$, p = 0.011), and Simpson diversity index (Fig. 5c, ANOVA; $F_{1, 70} = 11.604$, p = 0.001) than control plates when pooled across seagrass treatments and exposure time. Additionally, there was a significant difference in the change in species richness between exposure times (Fig. 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 appeared to be a trend toward an interaction between predation treatment and exposure time for all univariate metrics, where the difference between predation treatments seemed to increase over time. Seagrass had no statistically significant effect on multivariate or univariate community metrics.

4. DISCUSSION

In this study, we documented both direct and indirect effects of seagrass on fouling communities. As we hypothesized, seagrass had a direct negative effect on fouling communities, where fouling community abundance, richness, and diversity were lower inside of seagrass than outside of seagrass in the 2018 exclosure study (Fig. 1) when pooled across all predation treatments, but not when looking at small-mesh cages only. However, we did not detect any effect of seagrass on community metrics when looking at small-mesh cages only from the 2018 exclosure ex-
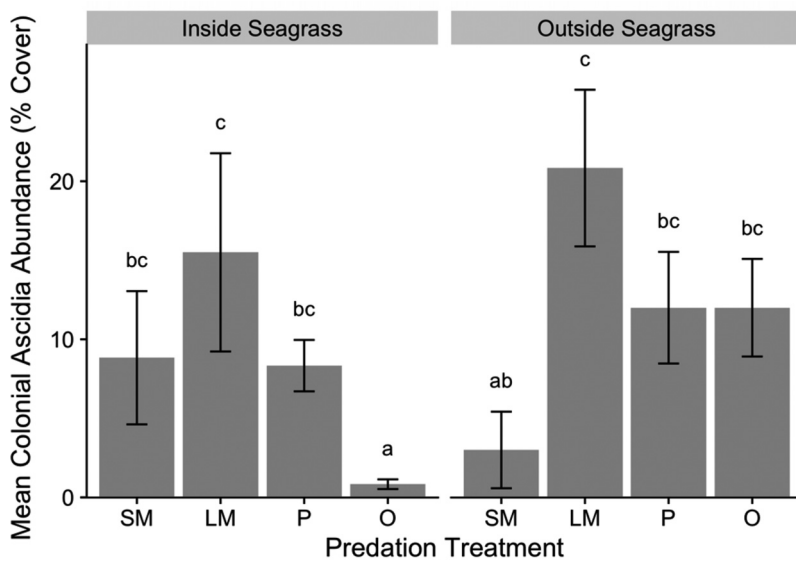
Fig. 2. Mean abundance of colonial Ascidia (% cover on panel surface) across predation treatments (SM: small mesh; LM: large mesh; P: partial cage; O: open) and inside and outside of seagrass. Values represent mean percent cover on settlement plates, with error bars representing the standard error. Post hoc tests were conducted using Tukey HSD with significant differences (p < 0.05) across treatments indicated by different letters

periment or from pre-exposure 3 mo plates in the 2020 exposure study (Fig. 3b). The effect of seagrass that we saw in 2018 can be explained by the structural effects of this habitat-forming species on the movement of water, and therefore, propagule and food availability to filter feeders such as fouling species. The indirect effect of seagrass on fouling communities (habitat-specific predation) was inconsistent across experiments — there was an indirect effect in the 2018 exclosure experiment but not in the 2020 exposure experiment. The indirect effect of seagrass in the 2018 exclosure experiment was in the opposite direction to what we predicted and was less powerful in structuring communities: differences among predation treatments were higher outside of seagrass than inside of seagrass. We believe that this is likely due to differences in recruitment of species and their overall susceptibility to predation. Finally, while we predicted that effects of predation and seagrass would be greatest on younger/earlier-succession fouling communities, we did not find any effects of seagrass on communities, and the effects of predation did not vary across exposure times in the 2020 exposure experiment (Fig. 3b). We think that this was likely due to limited recruitment (Fig. A1 in the Appendix), reducing our ability to detect a statistically significant interaction. Our results from both experiments indicate that direct effects of seagrass were more important in structuring fouling communities than the

indirect effects of seagrass, but the indirect effects of seagrass were highly dependent on the prey fouling species present.

Seagrass habitats modify fouling community composition in a variety of ways. The strongest effect of seagrass on fouling community composition was likely on community membership in 2018. We found that community composition differed statistically inside vs. outside of seagrass (Table 1, Fig. 3a), although the nature of this effect is unclear given that this difference was not driven by any specific morphotypes. It is possible that arborescent bryozoans are influencing the difference in community composition (Table 1, ANOVA, $W_{1,46} = 2.724$, p = 0.076), where arborescent bryozoans were slightly more abundant outside of seagrass than inside of seagrass (Fig. 3a), which was also seen in 2020. When looking at small-mesh caged plates only in 2018, it appeared that solitary ascidians were more abundant outside of seagrass than inside (Fig. 3a), though this only occurred in the absence of predators.

We also found a difference in univariate community metrics, where space occupied, species richness, and Simpson diversity index were significantly higher outside of seagrass than inside (Table 1, Fig. 1) when pooled across predation treatments. We did not detect any significant effects of seagrass when looking at the small-mesh caged plates only in 2018 (Fig. 1), though these small-mesh cages likely reduce flow even more, thus potentially making similar reductions in flow for inside and outside of seagrass. Reduced abundance, richness, and diversity inside of seagrass could suggest limited recruitment and/or limited food supply that reduces growth rates. As filter-feeders with planktonic larvae, fouling species recruitment and food acquisition depend heavily on water flow. 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, growth, and survival, which dictate percent cover, are lower inside 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
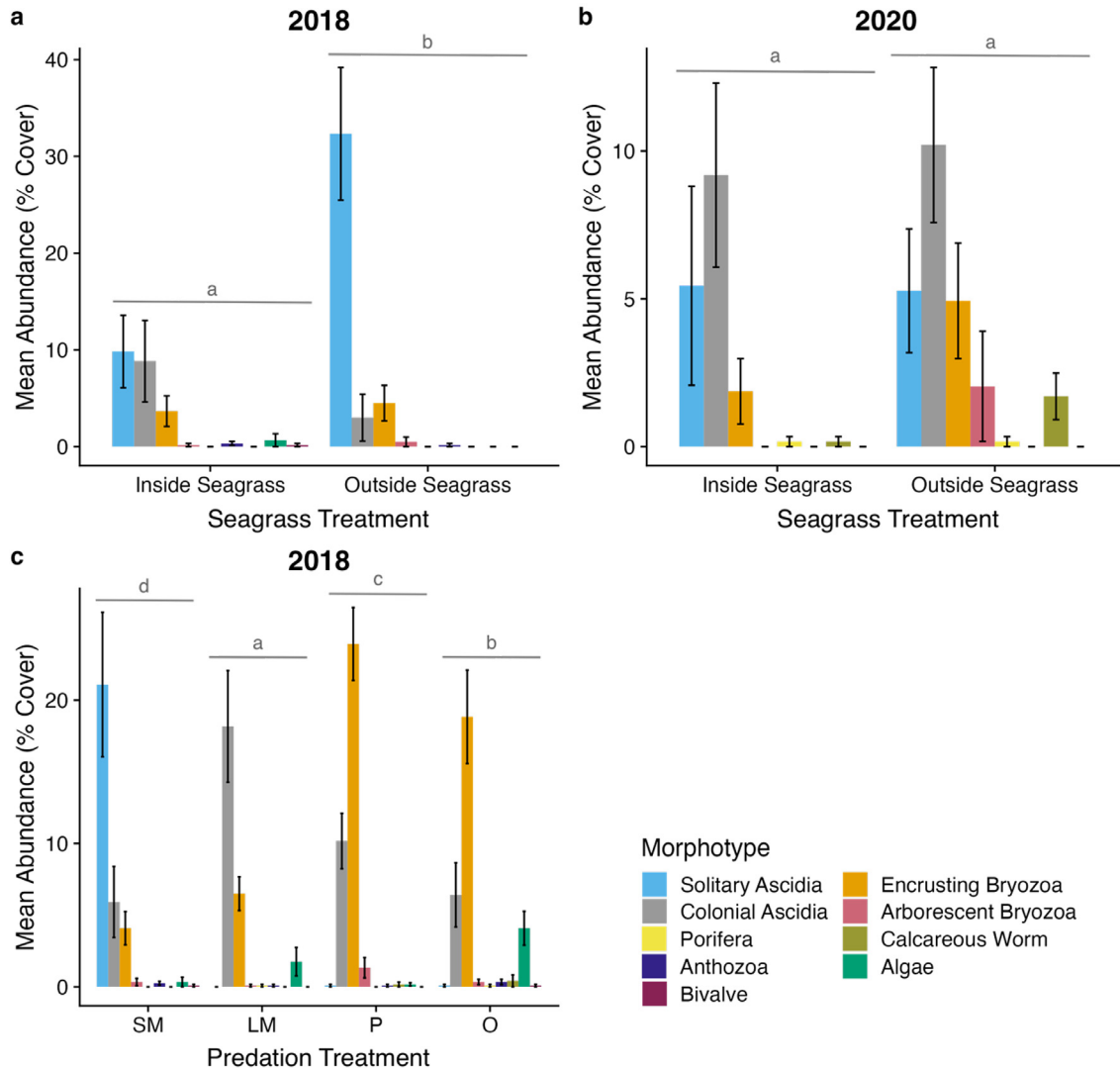
Fig. 3. Community composition across experimental treatments in both experiments. Treatments represented (a) inside and outside of seagrass beds for small-mesh caged plates only from the 2018 exclosure experiment, (b) inside and outside of seagrass beds for all caged plates pre-exposure to predators at the 3 mo mark only from the 2020 predator exposure experiment, and (c) predator exclosure treatments (SM: small mesh; LM: large mesh; P: partial cage; O: open) pooled across seagrass treatments in the 2018 exclosure experiment. Values represent mean percent cover on settlement plates, with error bars representing the standard error. Pairwise comparisons completed with the 'pairwise.comp' argument in the R package '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 ($p < 0.05$)

in the facilitative role of seagrass between habitat provision and food limitation.

The strength of the effects of seagrass on fouling abundance depended 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 2 experiments, where the space occupied in small-mesh cages in 2018 (Fig. A1, mean = 63.95%, min. = 12.24%, max. = 100%) was

much higher than the small-mesh cages before exposure to predators at 3 mo in 2020 (Fig. A1, mean = 12.53%, min. = 0%, max. = 85.72%). The low recruitment in 2020 decreased the power to detect effects among experimental treatments, including seagrass.

We also found that predation played a strong role in structuring fouling community composition. Both community composition (Table 1, Fig. 3c) and Simpson diversity index (Fig. 1c) varied among the 4 caging treatments. Specifically, we found that the Simpson diversity index was higher when communities were exposed to predators. This result can best be
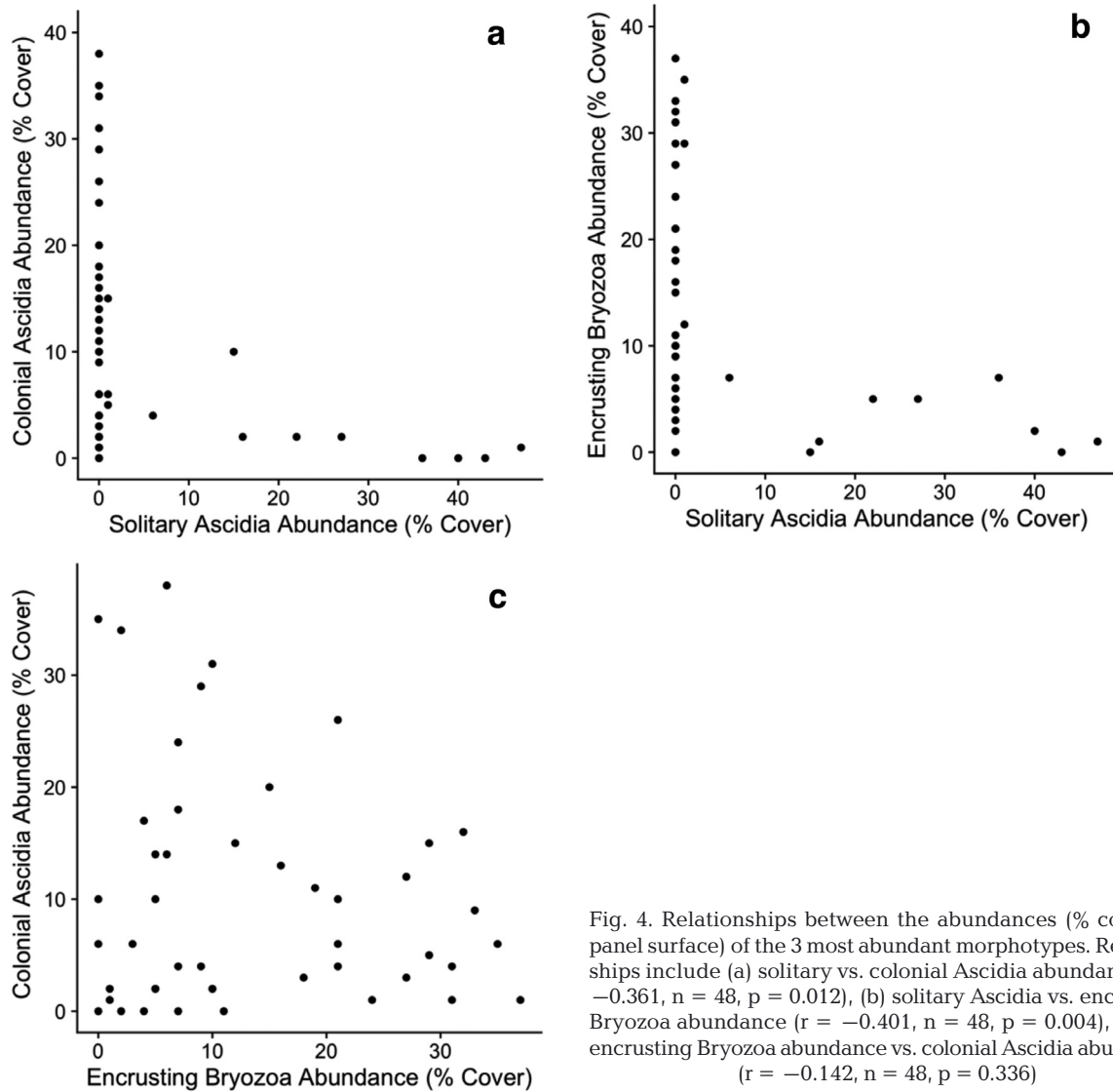
Fig. 4. Relationships between the abundances (% cover on panel surface) of the 3 most abundant morphotypes. Relationships include (a) solitary vs. colonial Ascidia abundance (r = −0.361, n = 48, p = 0.012), (b) solitary Ascidia vs. encrusting Bryozoa abundance (r = −0.401, n = 48, p = 0.004), and (c) encrusting Bryozoa abundance vs. colonial Ascidia abundance (r = −0.142, n = 48, p = 0.336)

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. 3c). 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 & Stachowicz 2007, Rogers et al. 2016). When pred-

ators 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 such as 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 which have shown that solitary ascidians are highly susceptible to predation at temperate latitudes (Osman & Whitlatch 1995, Freestone et al. 2013, Rius et al. 2014, Rogers et al. 2016).
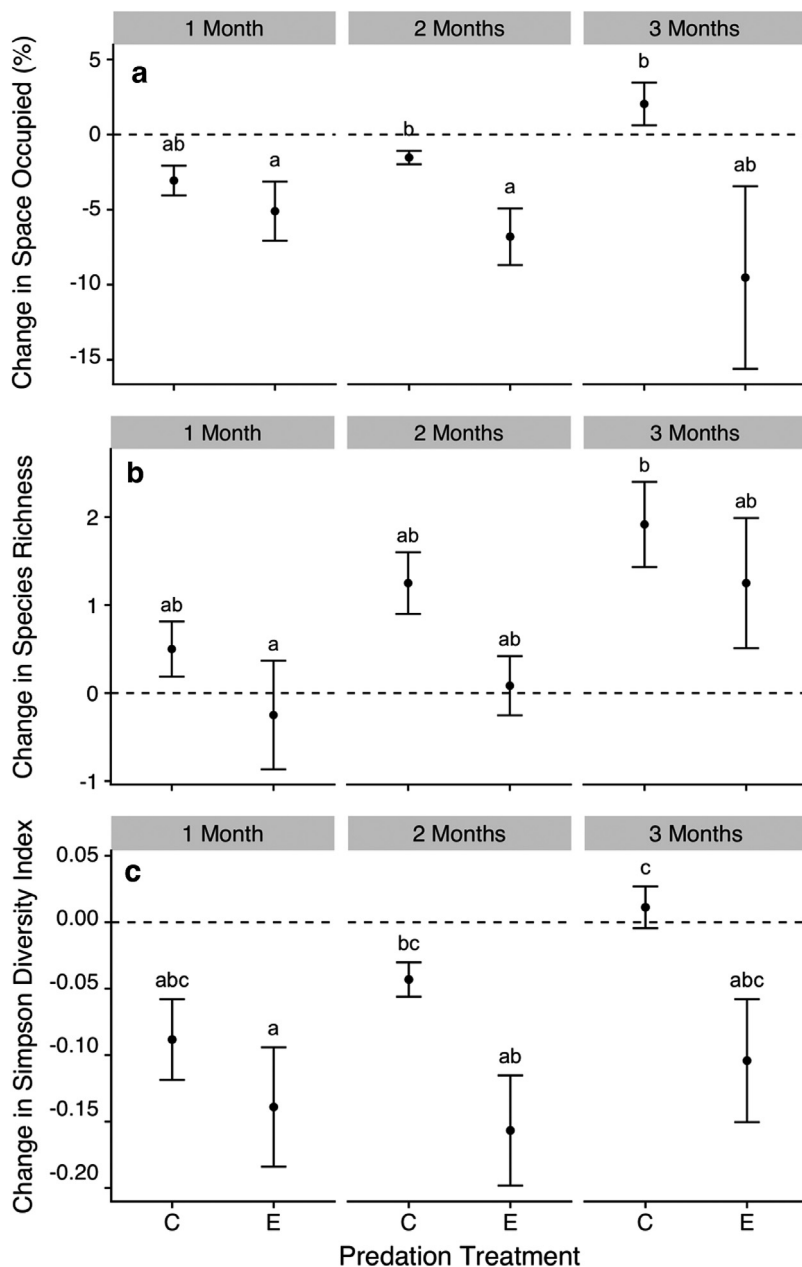
Fig. 5. Change in community metrics across predation treatments (C: control; E: Exposure) and community age (exposure time). Metrics consist of (a) mean change in space occupied (% cover on panel surface), (b) mean change in species richness, and (c) mean change in Simpson diversity index, all with error bars representing standard error. The dashed line at 0 indicates no change in community metrics, with values above (below) representing increases (decreases) in community metrics. Post hoc tests were conducted using Tukey HSD, with significant differences (p < 0.05) across treatments indicated by different letters

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 between exposure times. 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 & Whitlatch 1995), the low recruitment over the course of the experiment could have reduced the interaction between predation and exposure time.

Initially, we predicted that seagrass would also indirectly impact fouling community composition by increasing predation in seagrass beds, resulting in a significant predation × seagrass interaction. However, the indirect effect (predation × 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 1, Fig. 2), 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. Inside of seagrass, their abundance was lower on open plates than on any of the predation treatments. The predation treatments used, including the partial cages, could restrict access by larger fish predators. However, the lack of difference

While predation did not significantly alter community composition in the predator exposure experiment in 2020, the effects on univariate community metrics were in the opposite direction of the effects from the 2018 exclosure experiment. We found that predation significantly lowered the amount of space

between open plates and semi-protected plates outside of seagrass suggest another dynamic is occurring. The abundance of competitively dominant solitary ascidians was higher outside of seagrass than inside, which could explain the reduction in colonial ascidian coverage. The Simpson diversity index was not higher when predators were excluded in seagrass, and we think that this resulted from the structural influence of seagrass. The structural influence of seagrass here likely reduced recruitment and food availability, impacting all predation treatments inside seagrass similarly. The comparable 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 influences biological communities; however, indirect effects from predators 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. 2019, Hovel et al. 2021) and sometimes lower in seagrass (Carroll & 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 a better understanding of predator community differences inside and outside of seagrass is needed to interpret the predation × seagrass interaction for colonial ascidians, 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 not just on predation, but also on trophic position, competitive ability, and recruitment. Our study also provided additional evidence for the importance of predation in altering fouling community composition during community assembly at low tidal elevations in temperate seagrass ecosystems (Cheng et al. 2019).

Biological communities consist of diverse morphotypes representing various functional groups, 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 seagrasses 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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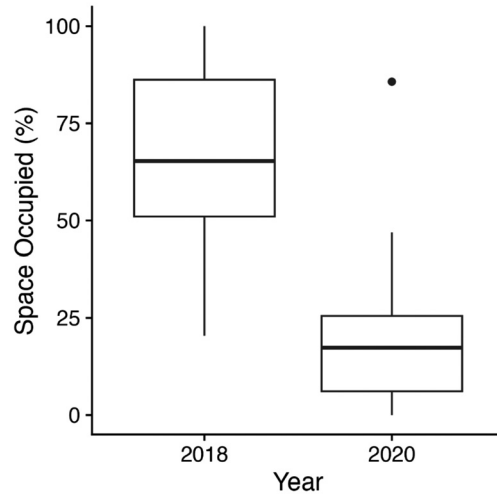
Appendix

Fig. A1. Total space occupied for caged plates from the exclosure experiment in 2018 (n = 12) and 3 mo caged plates pre-exposure in the 2020 exposure experiment (n = 24). Solid lines indicate the median values, upper and lower edges of each box represent the 75th and 25th quartiles, upper and lower whiskers extend to largest and smallest value, respectively, at most 1.5× the interquartile range, and dots represent outliers beyond 1.5× the interquartile range