

Bryozoan settlement varies by kelp species

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Abstract

Biofouling on kelp by encrusting bryozoans such as *Membranipora membranacea* can decrease the commercial value of cultivated kelp by restricting blade size and increasing breakage. The presence of *M. membranacea* on three species of Laminarian marine algae – *Saccharina latissima*, *Costaria costata*, and *Alaria marginata* – was studied in temperate coastal waters at Friday Harbor Laboratories in Washington State, USA, in May 2021. The settlement at two depths, over a one-week period, and the abundance of previously settled *M. membranacea* were characterized. We hypothesized that settlement of the bryozoan would differ by kelp species. The results supported our hypothesis. Settlement was highest on *S. latissima*, intermediate on *C. costata*, and lowest on *A. marginata*. No significant difference in settlement was detected at 1 versus 3 m. *A. marginata* is least challenged by issues of biofouling and may produce chemical defenses to deter epifaunal settlement.

Introduction

Seaweed cultivation is the fastest-growing aquaculture sector (NOAA 2020, FAO 2020). Marine algae are used in foods, medicine, cosmetics, bioremediation, and biofuels. However, encrusting epifauna are a major challenge for the seaweed industry. Kelp-encrusting bryozoans may reduce photosynthetic rates of kelp, limit blade size, increase brittleness and breakage of the blade, and decrease the kelp's ability to intake inorganic nitrogen (Oswald et al. 1984; Woolcott and North 1971; Førde et al, 2016; Hurd et al. 1994). *Membranipora membranacea* is a widely distributed kelp encrusting bryozoan species in the Atlantic and Pacific Oceans. In areas where *M. membranacea* is invasive, such as in Nova Scotia, Canada, outbreaks of this bryozoan species have been linked to mass defoliation of the kelp canopy (Denley et al. 2014).

Prior to settlement during early spring to summer, *M. membranacea* can remain in the plankton for weeks to months (Yoshioka 1973, Ryland and Stebbing 1973). Settlement is influenced by many variables, including the species of its algae host, small-scale differences on a single kelp blade, water flow, and temperature (Saunders et al. 2007). *M. membranacea* prefers to settle on *Nereocystis luetkeana* over *Mazaella splendens* (Matson et al. 2010). Preferential settlement occurs at the younger, meristematic ends of Laminarian algae blades, allowing access to the intact, stable substrate for the longest period of time possible (Seed & O'Connor 1981, Ryland & Stebbing 1971, Denley et al. 2014). After settlement, the bryozoan colony size can be influenced by carbon dioxide concentration. More specifically, under cold temperatures and high CO² conditions, a compensatory tradeoff occurs whereby colonies degenerate zooids and redirect energy to the growth and maintenance of new zooids (Swezey et al. 2017).

The objectives of this study were to determine whether a species-specific settlement preference exists for *Membranipora membranacea* on three industrially important species of Laminarian algae, *Saccharina latissima*, *Costaria costata*, and *Alaria marginata*, and to investigate the presence of any interactive effect of depth by (1) quantifying abundance of *M. membranacea* on field samples of the three kelp species, and (2) experimentally quantifying new settlement on the same three kelp species at varying depths.

Methods

Field Observation

Ten individuals of each species (*Costaria costata*, *Saccharina latissima*, and *Alaria marginata*) were collected from the Friday Harbor Laboratories dock and breakwater (48.54479326098024, -123.01312136312485) in May 2021. Each kelp blade was roughly in the same size range (on average, 9 cm width and 21 cm length). Naturally settled *M. membranacea*

colonies on both sides of these blades were counted using a dissection microscope. Maximum length and width of each blade was recorded. *M. membranacea* abundance per kelp individual was calculated. Density was estimated using a proxy for blade size, calculated as length X width. A two-factor ANOVA with replication was used to determine whether species of kelp and blade area proxy had a significant effect on *M. membranacea* settlement.

Experimental

Twenty individuals from each of the three species were collected from the Friday Harbor lab dock and breakwater in May 2021. Again, each kelp blade was roughly in the same size range (on average, 9 cm width and 21 cm length). A hole was punched at the base of each meristem so that new growth could be identified between the hole and the stipe. Individuals were collected and then deployed at depths of 1 and 3 m on a frame hung from the breakwater attached to the Friday Harbor lab dock (Figure 1, 2). Collection and frame deployment was done in the evening to minimize desiccation. The deployment location has low wave exposure and is partially shaded. The collection and experimental period took place in spring, an ideal period for *M. membranacea* settlement (Forbord et al. 2012). To control for epiphytes and recruitment caused by attraction to other bryozoans, *M. membranacea* settlement was only analyzed in the area of new growth on the kelp blade.

The frame was made of two parallel, 3m long PVC pipes, one hung at 1m below the water's surface and the other, at 3 m. Thirty individuals were hung from polypropylene rope at each depth, spaced 10 cm apart. After 7 days of deployment, the frame was drawn up, kelp blades were removed from the frame, and *M. membranacea* colonies in the meristematic zone on both sides of each blade were counted using a dissection microscope. A photo of each individual was taken next to a ruler so ImageJ could be used to find the area of new growth on each blade.

Density in number of *M. membranacea* colonies per cm² of kelp blade was calculated for areas of new growth. A two-factor ANOVA with replication was used to determine whether the species of kelp and depth had a significant effect on *M. membranacea* settlement.

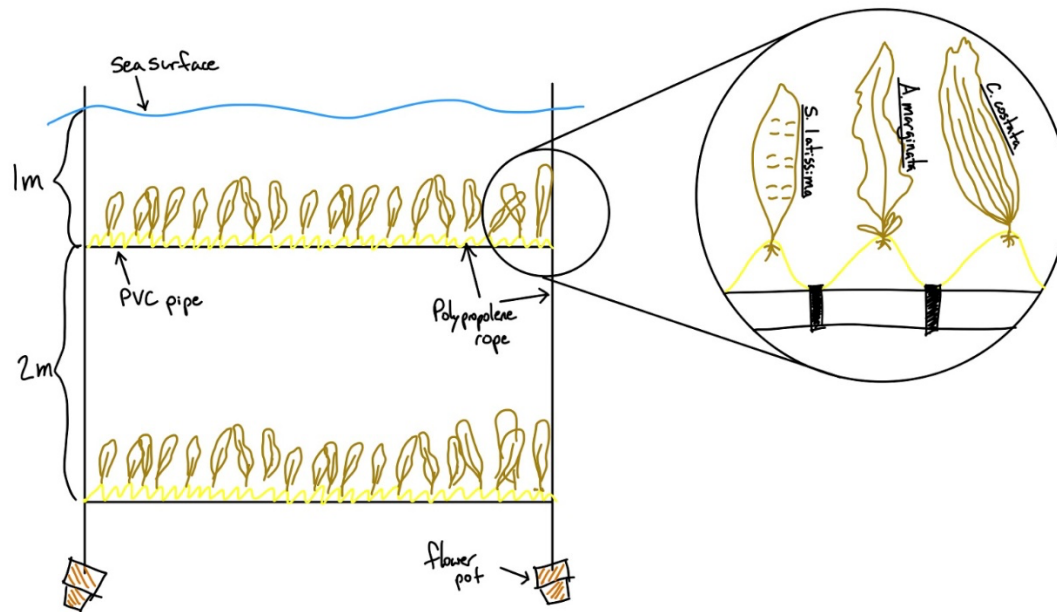


Figure 1. The frame for hanging kelp during the one-week period of *M. membranacea* settlement. Thirty blades were hung at 1m and another 30 were hung at 3m. Kelp blades were arranged on the frame so that species alternated between *S. latissima*, *A. marginata*, and *C. costata*. Flower pots were used as weights. The polypropylene rope was untwisted and the kelp blade was pushed through the hole which resulted from untwisting to hold kelp blades in place.

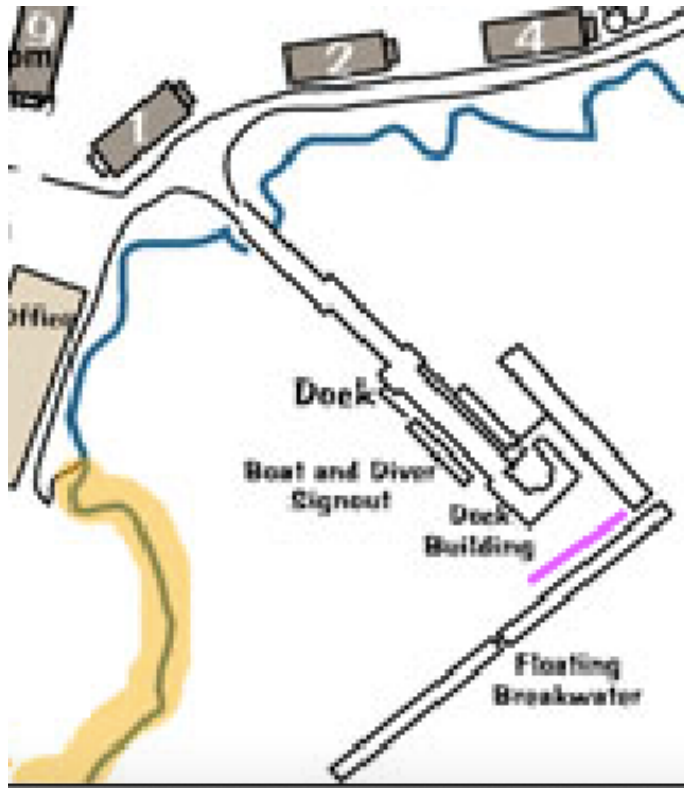


Figure 2. The frame deployment location is highlighted in purple. The frame was tied to the low-exposure side of the floating breakwater.

Results

Field Observation

M. membranacea settlement was highest on *S. latissima*, followed by *C. costata*, and lowest on *A. marginata*. No *M. membranacea* colonies were found on *A. marginata*. All *C. costata* individuals had at least one colony, while three *S. latissima* individuals had zero colonies. The species of kelp had a stronger effect on *M. membranacea* density than blade area (Figure 3). The relationship between species and the number of colonies was significant while blade area was not significant. There was no significant interaction found between the number of colonies and blade area.

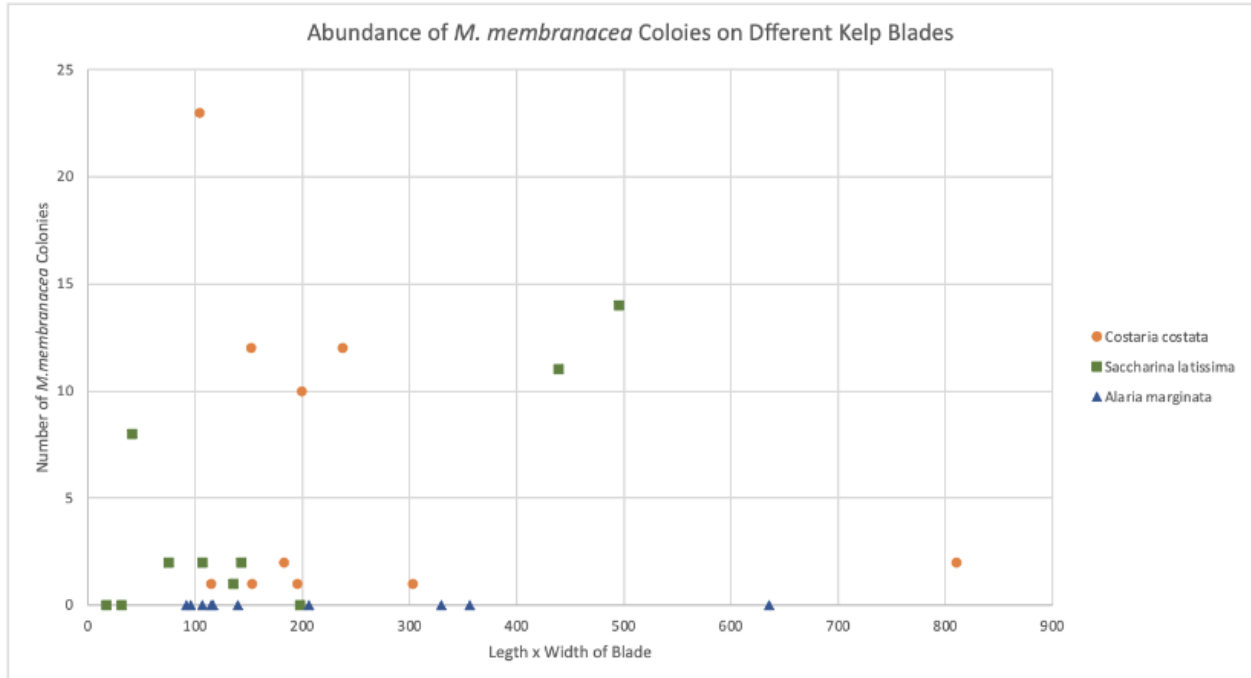


Figure 3. The abundance of *M. membranacea* colonies on *Costaria costata* (orange circles), *Saccharina latissima* (green squares), and *Alaria marginata* (blue triangles) compared to a proxy for blade area (length x width of blade). Kelp for the field observation component of this study were collected at the breakwater and docks at Friday Harbor Laboratories.

Experimental

M. membranacea settlement was significantly higher on *S. latissima*, intermediate on *C. costata*, and lowest on *A. marginata* (Figure 4, 5). *S. latissima* blades averaged 0.98 colonies per blade on the new growth. *C. costata* blades averaged 0.07 colonies per blade on the new growth, and no bryozoans were present on any of the 20 *A. marginata* blades.

Our experiment revealed that depth had little effect on settlement. Individuals at 1 m showed no significant difference in settlement density compared with individuals at 3 m (Figure 6). Additionally, there was no significant interaction found between species and depth (Figure 6).

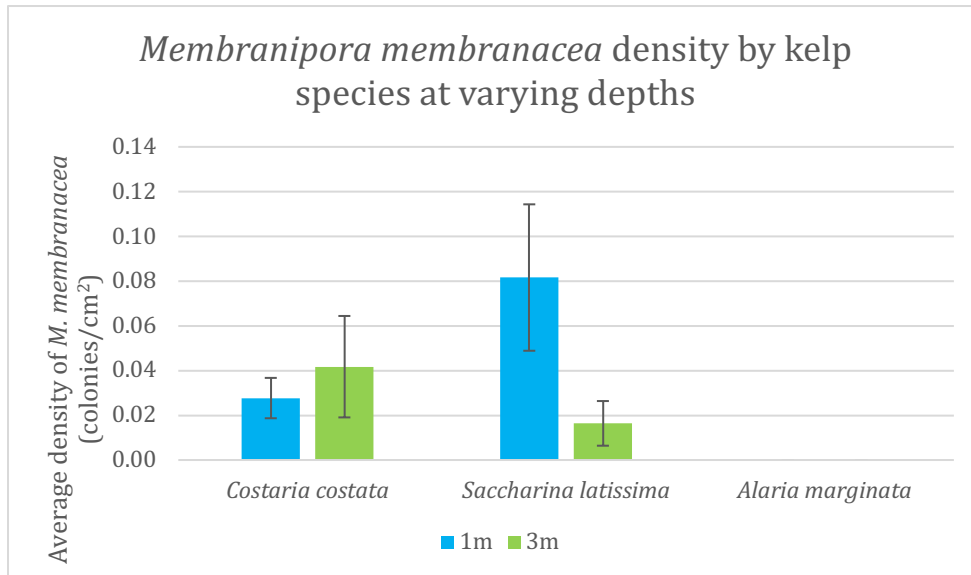


Figure 4. The average density (in colonies per cm²) of *Membranipora membranacea* on three species of kelp – *Saccharina latissima*, *Costaria costata*, and *Alaria marginata* – at two depths. *M. membranacea* settlement is highest on *S. latissima*, followed by *C. costata*, and lowest on *A. marginata*, which had no settlement. Vertical bars represent standard error. Kelp collection and bryozoan settlement took place at the breakwater and docks at Friday Harbor Laboratories.

1m			
	<i>Costaria costata</i>	<i>Saccharina latissima</i>	<i>Alaria marginata</i>
mean density	0.06	0.16	0
standard error (density)	0.01	0.03	0
standard deviation (density)	0.03	0.11	0
3m			
mean density	0.08	0.03	0
standard error (density)	0.01	0.01	0
standard deviation (density)	0.08	0.03	0

Figure 5 The mean, standard error, and standard deviation for density of *Membranipora membranacea* on *Costaria costata*, *Saccharina latissima*, and *Alaria marginata* at 1 m and 3 m depths.

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Sample (depth)	0.017454669	1	0.017454669	1.333880955	0.253201204	4.01954096
Columns (species)	0.101552254	2	0.050776127	3.88029761	0.026633102	3.168245967
Interaction	0.071505107	2	0.035752554	2.732200272	0.074078349	3.168245967
Within	0.706623856	54	0.013085627			
Total	0.897135885	59				

Figure 6. The results from the ANOVA test to determine whether kelp species and/or depth have significant effects on *M. membranacea* settlement. The p-value for columns is significant (0.027), indicating that settlement varies significantly by species tested (*Saccharina latissima*, *Costaria costata*, and *Alaria marginata*). The p-value for sample is not significant (0.25), indicating that settlement does not vary significantly at the depths (1 and 3 m) tested in this study. The p-value for the interaction between species and depth (0.07) is also not significant.

Discussion

We found that when exposed to *S. latissima*, *C. costata*, and *A. marginata*, *M. membranacea* density is significantly different, indicating preferential settlement between some species. This supports our hypothesis that *M. membranacea* settlement varies by kelp species. There is no significant difference between *S. latissima* and *C. costata*, and the field observation found higher abundances of *M. membranacea* on *C. costata* than *A. marginata*. *A. marginata* likely had no *M. membranacea* settlement in both the field and experimental portions of this study because of its slick, mucilaginous surface. Additionally, chemical cues released by marine algae may play a role in attracting *M. membranacea* settlement. *A. marginata* is known to produce phenolics as a chemical defense against herbivores, but the specific chemicals involved in *M. membranacea* settlement are unknown (Kellogg and Lila 2013). However, polyphenolic compounds have also been detected in the meristems of *S. latissima*, which had significantly higher *M. membranacea* settlement than *A. marginata*, so it is possible that the phenolics released by *A. marginata* are more targeted for deterring *M. membranacea* (Johnson & Mann 1986). It is also possible that an entirely different, non-phenolic compound is responsible for the chemical cue which attracts *M. membranacea* to settle.

The results of our experiment suggest that depth has no significant effect on *M. membranipora* settlement. However, this contradicts findings in the published literature in which settler abundance has been shown to be higher at greater depths (Saunders and Metaxas 2007). The contradictory results could be explained by differences in study design. Saunders and Metaxas tested abundance at 12 m, 8 m, and 4 m and found that abundance was high at 12 and 8 m, but significantly lower at 4 m. We tested settlement at 1 m and 3 m, so it is possible that while

settlement does not vary significantly at the depth interval we tested, it does vary significantly at the greater depth intervals tested by Saunders and Metaxas. Additionally, the observed settlement densities are quite low, possible because of the short duration of our experiment.

Bryozoans such as *M. membranacea* can decrease the commercial value of economically and ecologically important kelp. We found that *M. membranacea* settler abundance differs by kelp species. Settlement was highest on *S. latissima*, intermediate on *C. costata*, and no settlement was observed on *A. marginata*. *A. marginata* may produce chemical defenses to deter epifaunal settlement. While no significant difference in settlement was detected at 1 versus 3 m, depth was significant for other studies where a greater depth interval was tested. Kelp species and growing depth are two variables which kelp farmers may want to consider when making management decisions to decrease biofouling on their crop.

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