The Effect of Differential Flow Speeds and Kelp Morphology on *Membranipora membranacea* Settlement

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Abstract

Kelp provides important habitats for a large variety of organisms such as epiphytic bryozoans. The relationship between the bryozoan and their kelp substrate is influenced by biological, chemical, and physical factors. In this study, I explored how flow regimes and kelp morphology influence settlement patterns of the bryozoan *Membranipora membranacea.* Settlement on two species of kelp, *Costaria costata* and *Alaria marginata,* was studied due to their different morphologies: *C. costata* is heavily corrugated, while *A. marginata* has a relatively smooth blade. Multiple individuals were placed into a location of high flow and a location of low flow to observe differences in the number of newly settled colonies. To accompany this, both kelp species were placed in a flume tank with dye to visualize flow patterns across the blade. I found that flow speed had no effect on the number of newly settled bryozoans over the experiment duration. There was a higher abundance of newly settled bryozoans on the heavily corrugated kelp blade versus the smooth kelp blade. Flow visualizations revealed increased turbulence along *C. costata* blades as well as fluid lingering in blade troughs for a period of time and quickly dissipating along ridges. These data suggests that *M. membranacea* prefer to settle on kelp that increase turbulence along the blade by having corrugations. Troughs provide regions of low turbulence and give planktonic *M. membranacea* sufficient time to select a location for settlement while ridges could provide regions of elevated food availability.

Introduction

Macroalgae are crucial ecosystem engineers for a large diversity of organisms because they are able to alter abiotic factors such as light, nutrients, pH, and flow to produce a variety of habitats and serve as a host to epiphytic organisms such as bryozoans. (Teagle et al., 2017). The bryozoan *Membranipora membranacea* has two life history stages, a planktonic stage and a sessile colonial stage (Seroy & Grünbaum, 2018). Planktonic *M. membranacea* have been found to be highly selective when choosing where to settle, preferring to settle near the meristem of the kelp blade (Matson et al., 2010). Starting in spring and continuing until late autumn, planktonic *M. membranacea* settle on the blades of kelp and begin to bud to produce a colony. The colony will then continue to grow given sufficient space, food, and a lack of predators (Harvell, 1992; Okamura & Partridge, 1999). The relationship between bryozoan colonies and their underlying kelp substrate has been heavily studied and multiple positive and negative interactions have been identified (Førde et al., 2016; Noisette & Hurd, 2018; Oswald et al., 1984). Increased colony size and abundance is associated with decreases in the photosynthetic capabilities of the kelp substrate and may even lead to blade breakage (C. L. Hurd et al., 1994; Oswald et al., 1984). Conversely, colonies have been shown to provide kelp with additional nutrients and CO_2 (C. L. Hurd et al., 1994; Saderne & Wahl, 2012). There is a heavy focus on chemical factors and their influence on bryozoan settlement whereas there is a lack of research on the morphological factors of the kelp blade that may contribute to bryozoan settlement. This study aims to address how kelp blade morphology affects flow along the blade and thus influences bryozoan settlement.

Question 1: How does flow speed affect Membranipora membranacea settlement?

Hypothesis 1: Membranipora settlement densities were predicted to increase in low flow treatments. Planktonic *Membranipora membranacea* carefully select where to settle, taking time to find a location close to the meristem in an attempt to increase their lifespan as the kelp grows and eventually sheds, killing the colony (Matson et al., 2010). Slower flow speeds may provide more time for selective settlement to occur and reduce the energy needed to remain close to the kelp substrate.

Question 2: How does kelp blade morphology affect Membranipora membranacea settlement?

Hypothesis 2: Membranipora membranacea settlement was expected to be greater on corrugated kelp blades of *Costaria costata* over the smooth blades of *Alaria marginata*. Corrugations increase the amount of turbulent flow over a kelp blade. Increased turbulence leads to higher nutrient diffusion to the underlying kelp and an increased supply of oxygen to the bryozoan (Noisette & Hurd, 2018). *M. membranacea* may prefer to settle in a region with higher turbulence to take advantage of the increased oxygen supply and food availability. This preference may change in higher flow regions as sufficiently high flow speeds can become detrimental to settlement success and the preference for corrugations could shift (Okamura & Partridge, 1999).

Methods

Specimen collection and observations of natural populations

Specimens were collected from the Friday Harbor Labs dock tires in San Juan county, Washington (48.5454° N, 123.0141° W). Two species of Laminariales were selected for study: *Costaria costata* and *Alaria marginata*. *Costaria costata* was selected for its heavily corrugated blade morphology. *Alaria marginata* was selected for its relatively smoother blade morphology. *Membranipora membranacea* has been found to settle on both species (Catriona L. Hurd & Stevens, 1997). Ten randomly selected specimens of *C. costata* and of *A. marginata* from the FHL dock were analyzed to determine the presence of *M. membranacea* in the water column. The location of already established *Membranipora membranacea* was recorded based on whether it was in a trough or on a ridge of the kelp blade. All analysis of natural populations was conducted using a dissecting microscope but not analyzed for statistical significance.

Settlement experiment

An apparatus to deploy multiple kelp specimens at the same depth was constructed. Holes were drilled through each end of two 3m PVC pipes for hanging. Soft polypropylene rope was then fixed to the PVC pipe using zip ties at 15cm intervals. The rope was given extra slack in each section to create a loop to attach the kelp specimens. Each PVC pipe held twenty loops for twenty specimens. Ropes were fixed to the holes drilled at each end of the PVC pipes and weights were attached.

Twenty *Costaria costata* and twenty *Alaria marginata* specimens were collected from various locations around the FHL dock and holes were punched 2cm above the base of the blade to mark the start of new growth. Ten of each species were randomly placed on the PVC bar. The bar was then placed on the inside of the FHL breakwater for the low flow treatment at a depth of 1m. This process was repeated and placed on the outside of the breakwater for the high flow treatment at a depth of 1m. The apparatus was left in place for 7 days after which it was retrieved. The number of new *Membranipora* colonies found in the area of new growth on each plant were counted using a dissecting microscope.

Flow estimation

Flow was estimated for each treatment using plaster of paris clod cards (Doty, 1971; Thompson & Glenn, 1994). Twelve spherical clods were produced using an acrylic mold. Plaster of paris was mixed using a ratio of one cup of water to two cups of plaster. A metal screw was placed into the clod before hardening. The plaster clods hardened for twenty-four hours before deployment. Dry and hardened clods were weighed, and a nylon rope was fastened to the metal screw. Clods were deployed along with the seaweed apparatus in the high and low flow treatments. After three days the clods were retrieved and placed into a drying oven at 50°C for twenty-four hours. Once fully dried, the clods were weighed again. The process was repeated with six new clods for an additional three days to cover the full duration of the seaweed apparatus deployment.

Flow visualization

A flume tank was used to visualize flow patterns over the blades of *Costaria costata* and *Alaria marginata*. The flume tank was calibrated by determining how long a particle in the tube takes to move a known distance. Ten trials were conducting then averaged to determine the flow velocity for three different flow settings: low, medium, and high. An individual *C. costata* specimen was placed into the tank to mimic how it would move in a natural environment. A flow speed was set, and fluorescein dye was injected at the base of the blade. This was repeated for each speed setting and then once again for *A. marginata*.

Data analysis

Settlement analysis – Area of new growth was calculated using imageJ and then doubled to account for both sides of the blade. Density was calculated by dividing the number of colonies found in the region of new growth by the area of new growth. A two-way ANOVA was used to determine the statistical significance of interacting variable over the entire data set. The position of new recruits on *C. costata* blades was determined by calculated the percentage of colonies found in a blade ridge versus the total number of colonies. A two-way ANOVA was used to determine the significance of settlement location between troughs and ridges among the *C. costata* specimens.

Flow estimation – A t-test was used to determine whether dissolution differed significantly between the inside and outside breakwater treatments. Clod dissolution acts a proxy for mass transport and flow speed over a period of time.

Results

Flow estimation

The outside breakwater clods had a significantly higher dissolution rate than the inside breakwater clods with an average dissolution weight of 20.11g versus 14.45g. This difference was significant ($p = 0.042$), indicating that mass transport and thus flow speed is statistically different between the inside and outside breakwater (Figure 1).

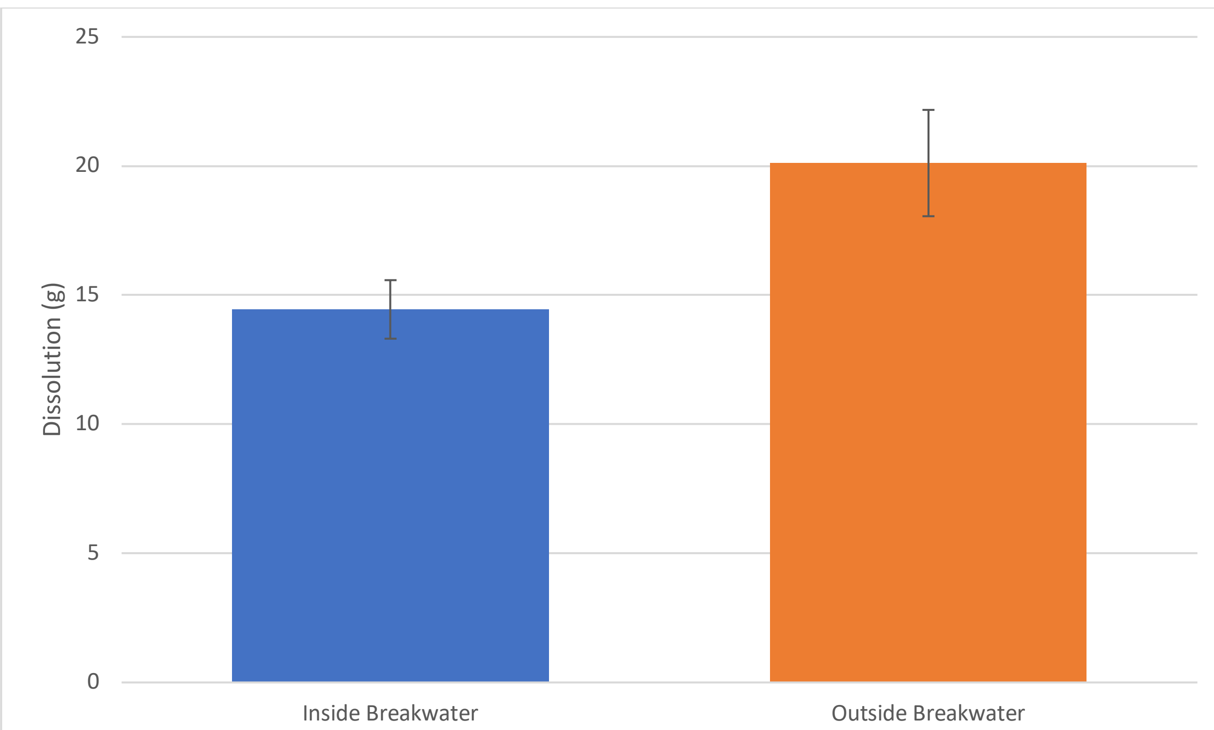

Figure 1: Difference in average weight lost (g) after three days of deployment. Dissolution serves as a proxy for mass transport and flow speed. Standard error bars included. p = 0.042.

Settlement density

The average number of colonies found on *C. costata* among the experimental samples was larger than the that found on *A. marginata* (p = 0.042) (Figure 2). There were significantly greater settlements on *C. costata* than *A. marginata* in the high flow treatment. Out of the twenty *A. marginata* studied only one specimen had newly settled bryozoans, whereas the average number of new colonies found on *C. costata* was 1.1 for both high and low flow.

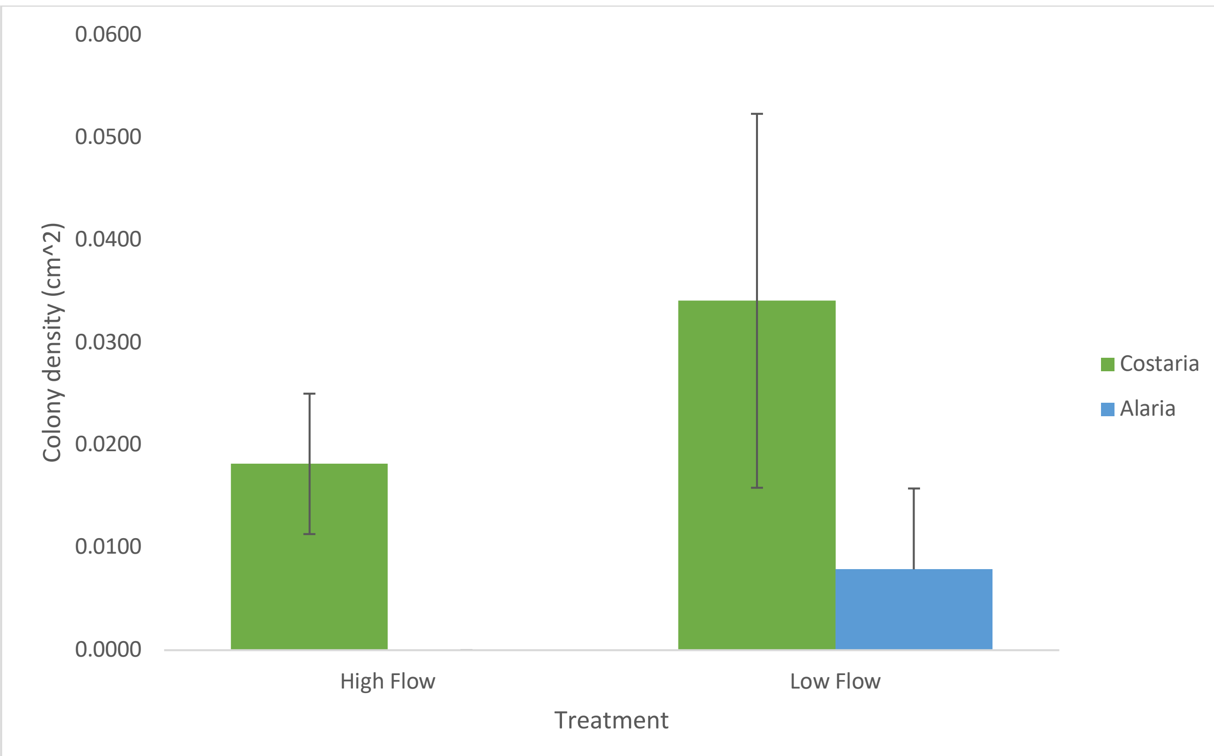

Figure 2: Average settlement +/- one SE in settlement/cm^2. Costaria costata in green and Alaria marginata in blue. Standard error bars included. p = 0.042

Settlement location

There was no significant difference between the number of settlements located in a trough versus a ridge. A two-factor ANOVA revealed a significant interaction between flow and settlement location (p = 0.024). A post-hoc test was not sufficient to parse the interaction between variables and further statistical analysis beyond the scope of this paper is needed to determine the influence of both factors on settlement location.

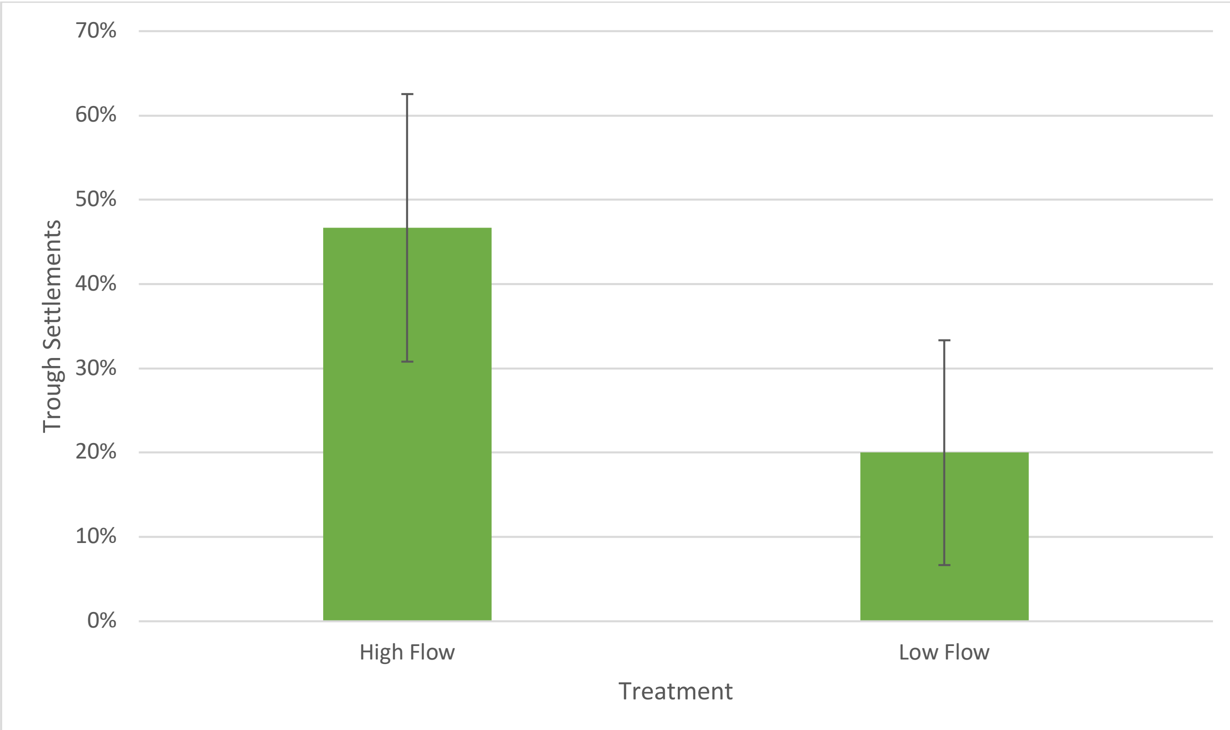

Figure 3: Percentage of colonies found in troughs along the area of new growth on Costaria costata. Standard error bars included.

Flow visualization –

Under low flow (1.59 cm/s) there was minimal turbulence along the blade of *C. costata (Figure 4)*. Fluid would often remain in troughs while flow over ridges would dissipate. Under medium flow (9.44 cm/s), there was increased turbulence and fluid would still remain in troughs, but for a

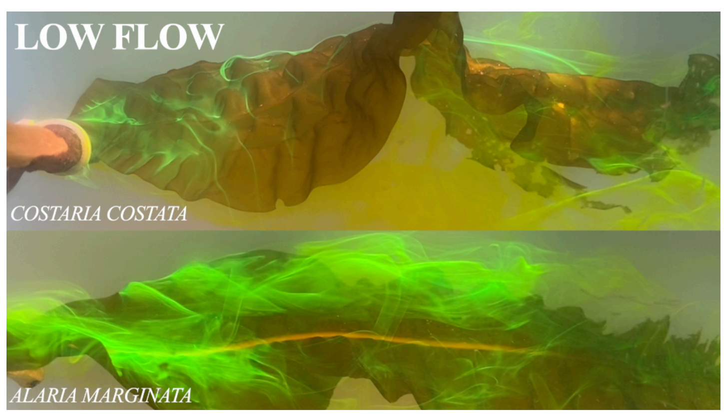

Figure 4: Flow visualization under low flow conditions (1.59 cm/s)

shorter period before it dispersed (Figure 5). Similar results were produced in high flow (18.35 cm/s) where fluid would settle in troughs and be dispersed on ridges despite high turbulence (Figure 6).

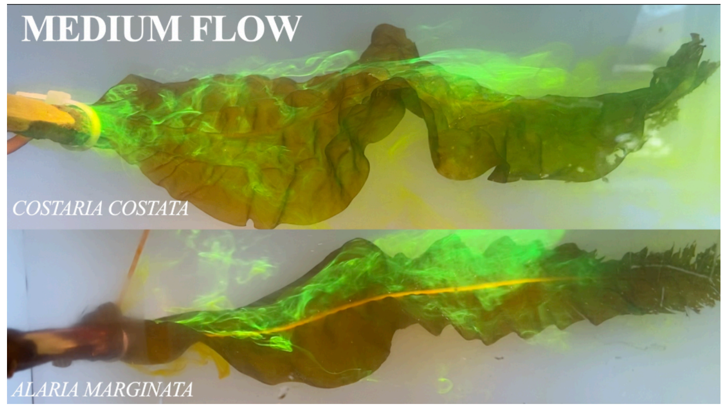

Figure 5: Flow visualization under medium flow (9.44 cm/s)

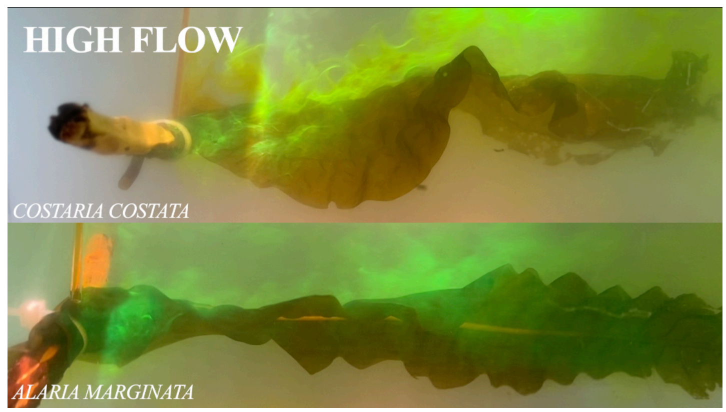

Figure 6: Flow visualization under high flow (18.35 cm/s)

Under low flow (1.59 cm/s) fluid lingered along the entire *A. marginata* blade for a long period before dispersal (Figure 4). In medium flow (9.44 cm/s) there was an increase in turbulence and dissipation over the entire blade (Figure 5). Fluid did not linger as it did under the low flow condition. No fluid lingered along the blade under high flow conditions (18.35 cm/s) (Figure 6). Small eddies formed along the wrinkles of the blade which did not occur at the other speeds.

Discussion

Based on the evidence gathered in this study, the bryozoan *Membranipora membranacea* has higher settlement density on the kelp *Costaria costata* than *Alaria marginata*. Flow speed was found to have no effect on settlement density despite a significant difference in the mass transport. The difference in flow between treatments may have not been extreme enough to influence settlement. There was no difference in the location of settlements between flow regimes as well although significant interaction between the variables was found. These findings do not support the hypothesized higher settlement density in low flow speed treatments. However, the findings do support the expected influence of kelp blade morphology on settlement.

The heavily corrugated blade morphology of *Costaria costata* is associated with an increased density of *M. membranacea* settlement. Between species in Laminariales, those that have corrugated blades are often found in low flow environments whereas those with smooth blades are often found in high flow environments. Within a species, kelp blades have also been shown to be relatively morphologically plastic and increase the amount of corrugation based on ambient flow speeds (Koehl et al., 2008). Blade corrugation promotes turbulent flow and increased mixing. Increased turbulence in low flow environments is advantageous to kelps because it increases the efficiency of nutrient diffusion and disrupts the velocity boundary layer (Hurd & Stevens, 1997). For bryozoans, increased turbulence could produce both positive and negative effects on their settlement success and survival creating a trade off in either settlement location.

One explanation for this result may be that the differences in flow patterns in troughs versus ridges provides the plankton *M. membranacea* additional time to settle. Planktonic *M. membranacea* have been found to have different behaviors when settling on a preferred substrate such as *C. costata* (Matson et al., 2010). In the presence of a preferred substrate, planktonic *M. membranacea* actively search for an ideal location to settle. Flow visualizations revealed that under high, medium, and low flow regimes, fluid would linger in the troughs of *C. costata* for extended periods of time (Figure 4-6). Troughs provide moments of reprieve from ambient flow that presumably allow *M. membranacea* sufficient time to be selective and choose a location to settle. This explanation also could explain the lack of significant difference in settlement seen between flow speeds as fluid was observed to linger in troughs at low, medium, and high speeds in the flow visualizations. However, flow in troughs can become stagnant and lead to O_2 depletion and lack of food. pH can also be highly variable in troughs as the kelp substrate is actively changing the concentration of CO_2 through photosynthesis (Noisette & Hurd, 2018). Variability in the trough habitat can make survival unpredictable for a sessile colonial organism like *Membranipora membranacea.*

The ridge microhabitat has increased food availability, but settlement is more difficult due to higher flow and a decreased boundary layer along the ridge. *M. membranacea* colonies were found to have different morphologies depending on flow conditions (Okamura & Partridge, 1999). In high flow conditions, colonies were squat and had reduced lophophores. The slimmer morphology allowed the colonies to take advantage of feeding in the slower flow of the boundary layer. Feeding in low flow is more efficient for an individual zooid and would lead to preferential settlement in regions of low flow. In addition, zooids would be able to prey on a higher abundance of food flowing past the kelp blade.

The findings from this study provide additional information on physical factors that influence the settlement patterns of *Membranipora membranacea.* Corrugation is an important factor in determining settlement due to its effect on the ambient flow around the blade. A focus of future studies should be how *M. membranacea* colonies affect flow around the kelp. Hurd et al. (1994) studied the influence colonies have on nutrient concentration along kelp blades but did not discuss the influence of flow on those concentrations. There is conflicting information relating to whether the kelp – bryozoan interaction is beneficial or harmful for both individuals (C. L. Hurd et al., 1994; Oswald et al., 1984; Saderne & Wahl, 2012) suggesting that the relationship is more complex and could be context specific. A better understanding of this relationship and of the ridge/trough microhabitats can aid in understanding their response to a globally changing ocean (Seroy & Grünbaum, 2018).

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