**AN EXPERIMENTAL TEST OF BIOTIC AND ABIOTIC DRIVERS OF SPATIAL VARIATION IN ABUNDANCE OF EPIPHYTE *SMITHORA NAIADUM* ON SEAGRASS *ZOSTERA MARINA***

**Or:**

**Experimental transplant reveals that epiphyte abundance on Zostera marina likely influenced by dispersal and not grazing, bacterial assemblages or environmental conditions**

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[ABSTRACT, < 250 words] Ecological communities vary in space at a variety of scales. In marine communities, dramatic changes in species composition can occur across a small spatial distance. Through an experimental manipulation of seagrass (*Zostera marina*), we examined the processes that determine patterns in the abundance and distribution of *Smithora naiadum*, an epiphytic red alga, in *Zostera marina* meadows on the central coast of British Columbia. We hypothesized that *Smithora* could be controlled by invertebrate grazing or bacterial facilitation on individual eelgrass shoots, and that spatial variation in these factors could explain variation in Smithora abundance. We also hypothesized that Smithora abundance could be controlled by environmental factors that vary spatially, affecting the recruitment and persistence of the epiphyte on seagrass shoots. We used a reciprocal transplant to investigate whether *Smithora* to test these hypotheses. We found that Smithora-free eelgrass blades were colonized when placed in a high *Smithora* environment, while colonized blades did not lose existing *Smithora* when moved to a *Smithora* free environment. Using illumina sequencing of bacterial DNA isolated from surface swabs of seagrass transplants, we found a significant difference between bacterial communities on shoots with and without *Smithora*. Further, we found that shoots with *Smithora* had higher invertebrate grazer abundance and distinct grazer and bacterial communities. Our results suggest that eelgrass associated bacterial and invertebrate communities could be altered by the colonization of epiphytic *Smithora,* or could be subject to the same spatial processes as *Smithora*. Our study is the first reciprocal transplant in seagrass to investigate both bottom-up and top-down drivers of epiphyte abundance on seagrass blades and presents valuable insight for future investigations.

**Highlights**

**Graphical Abstract (anyone?)**

1. **Introduction**

Seagrass meadows host vast biodiversity of algae, invertebrates and fish (Bostrom et al. 2006, Duffy 2006). Variation within meadows in shoot-level species composition (beta diversity) contributes to high biodiversity at the meadow-scale, yet the causes of this variation within meadows is not well understood (Johnson et al. 2005; Lavery and Vanderklifft 2002; Prado et al., 2007; Saunders et al., 2003). This variation has been attributed to a range of biotic and abiotic factors, including grazing pressure (Amundrud et al. 2015; Huang et al. 2015; Duffy et al. 2015; Reynolds et al. 2015; Montfrans et al. 1984), environmental conditions (Casola et al., 1987; Knowles and Bell, 1998; Lavery and Vanderklift, 2002; Madsen et al., 2001; Sand-Jensen, 1977; Kendrick and Burt, 1997; Borum, 1985), or even shoot specific microbial community (Harder, 2008; Silva et al. 2013; Holmström et al., 2002b).

At the base of diverse seagrass-associated communities are the algae living on seagrass blades, or epiphytic algae. Epiphytic algae are an important component of the larger seagrass ecosystem but they can have detrimental impacts on seagrass plants by reducing the level of light and nutrients that seagrass plants receive (Mcroy and Goering 1974; Sand-Jensen 1977; Penhale 1977; Harlin 1973a; Coleman and Burkholder 1994; Lin et al. 1996; Morgan and Kitting 1984, Fry 1984). Invertebrate grazers primarily eat epiphytic algae. Thus, like other foundation species, seagrass and the biodiversity it hosts exists in a complex network of positive and negative interactions (Connolly 1994; Boström and Bonsdorff 1997; Sheridan 1997; Webster, Rowden, and Attrill 1998; Heck and Orth 1980; Heck Jr and Orth 1980; Attrill, Strong, and Rowden 2000; Tolan, Holt, and Onuf 1997; Harlin 1975; Fong, Lee, and Wu 2000). Consequently, factors from top-down control by grazers to bottom-up facilitation by microbes or seagrass hosts (Meja et al. 2016; Ettinger et al. 2017, (Lavery and Vanderklift 2002; Milchakova 2000; Alcoverro, Duarte, and Romero 1997, Kendrick and Burt 1997; Cebrian et al. 1999, Reyes and Sansón 1997; Johnson et al. 2005) may interact to influence the spatial patterns of seagrass associated epiphytes.

Epiphytic algae can be quantified easily but bottom-up and top-down influences on their abundances can be more difficult to identify. Seagrass blade surface chemistry can discourage epiphyte colonization (Bell, Lang, and Mitchell 1974; Mejia et al. 2016). Colonization of seagrasses by spores of epifaunal organisms is accomplished through the establishment of a suitable bacterial community before the spores attach (Sieburth and Thomas 1973). Unique bacterial groups are associated with a specific blade surface chemistry (Bagwell et al. 2002; Crump and Koch 2008; Hamisi et al. 2009; Weidner et al. 2000; Duarte, Holmer, and Marba 2005), and the growth of unique bacterial communities can discourage the attachment of spores on marine macrophytes. Abundances of invertebrates can vary significantly both between and within meadows with high invertebrate abundances usually leading to a decrease in algal abundances through grazing (Amundrud, Srivastava, and O’Connor 2015; Boström and Mattila 1999; Tanner 2005). Understanding the drivers of epiphyte spatial variation helps to understand the dynamics of the seagrass-based community, because of the effects the multiple blade level communities have on one another.

**Bottom up process investigated by reciprocal transplant**

One way to begin to distinguish among multiple possible explanations for variation in epiphyte abundance is through reciprocal transplant experiments. Reciprocal transplants have been used to test effects of seagrass leaf morphology and growth on epiphyte presence and abundance? (Backman 1991; Kenworthy and Fonseca 1977). Leaf morphology and surface area influence epiphyte assemblages in marine plants and could be an important bottom-up process explaining variation in epiphyte communities across marine-plant foundation species, but these studies indicate that a drastic change in morphology would have to occur to explain epiphytic variation. Typically, on the meadow scale, shoots vary on the scale of mm and a size change that small within a 5m spatial scale would likely not explain dramatic changes in community structure (Parker et al. 2001).

**Top down process investigated by reciprocal transplant**

Both top-down and bottom-up processes likely influence epiphyte abundance, and past research has mainly focused on how top-down processes explain changes in epiphytes. Reynolds et al. used a reciprocal transplant to investigate the potential of grazer control to influence epiphyte abundance and found that epiphyte biomass decreased following *Zostera marina* transplants to areas of high grazer abundance (2017). Reynolds ruled out bottom up processes as being a dominant force affecting epiphyte abundance because the high nutrient site had less epiphytes. If a community exhibited bottom up control you would expect high epiphyte abundance to be correlated with high nutrient load. While evidence for grazer control is substantial, few studies have investigated the potential for bottom up processes influencing algal abundance and no studies have done this using a reciprocal transplant.

**Evidence for investigating microbial community as a bottom up process**

Blade level microbial communities in seagrass have been shown to vary across large spatial scales. However, Ettinger et al. have recently showed that at the meadow level there was no change in leaf microbial community between the edge and interior (2017). This study only looked at microbial communities and did not investigate other communities on the seagrass blades. Epiphytic algae require a microbial film to colonize and it follows that a change in microbial community could be correlated with a change in epiphytic algae abundance. Reciprocal transplants investigating microbial communities on seagrass blades at a small spatial scale have yet to be performed. Further, no studies have measured epiphytic algae abundance with microbial community on seagrass blades.

**Why investigating the two co-currently is important**

Investigating both bottom-up and top down controls simultaneously is essential (Duffy 2006, Parker et al. 2001). Seagrass blade communities vary widely in space and so investigating these community differences at the meadow level can limit the number of processes that could cause dramatic changes in epifaunal community structure. Finally, investigating top-down and bottom-up processes in relation to other communities (grazers for top-down and bacteria for bottom-up) allows us to determine if all three communities vary in a predictable way. We are interested in observing patterns in blade level community changes that may be consistent with other seagrass meadows. Measuring all three levels of seagrass blade communities in the context of a small-scale transplant is helpful for identifying any correlations between microbial, algal, and invertebrate communities.

Our main objective was to investigate the relative importance of potential drivers of changes in abundance of a dominant epiphyte, the red algae *Smithora naiadum,* on the eelgrass *Zostera marina*. We performed a reciprocal transplant experiment of seagrass shoots between zones of high and low *Smithora* within a single, large *Zostera marina* meadow. We tested the hypotheses that *Smithora* abundance on eelgrass is determined by characteristics of the host plant rather than by the local environment. We investigated the host-plant microbial community as a possible host attribute that could influence settlement of *Smithora*. Our experiment therefore allowed us to test whether epiphyte abundance is clearly determined by the environment or the host plant and its associates. Our experiment also allowed us to compare the microbial communities of *Z. marina* before and after colonization by *Smithora* to determine if there are any identifiable community shifts in microbial community that could be correlated with a decline in shoot health.

2. Materials and Methods

2.1 Study System

*Zostera marina* is a meadow forming eelgrass common along coastlines in the northern hemisphere (Phillips, Macmillan, and Bridges 1983). As a foundation species, *Z. marina* provides habitat for hundreds of invertebrate and fish species that in turn provide food for fish and other large consumers. Thus, eelgrass meadows are highly productive environments, and much of this secondary productivity is derived from epiphytic algae – mostly diatoms and other benthic micro-algae - growing on the seagrass blades rather than the seagrass itself (Valentine and Heck, Edgar and Shaw 1995, Taylor 1998).

In the northeast pacific, the red alga *Smithora naaidum* is one of the more common macroalgal epiphytes on eelgrass. *Smithora* abundance varies substantially among meadows, present on eelgrass primarily in marine environments (not brackish). *Smithora* is one of the highest quality algae for grazers, extremely high in fatty acid content, suggesting an important role in the seagrass-based food chain. *Smithora* also changes the physical structure of the seagrass microenvironment, potentially enhancing protection for invertebrate algal grazers, herin referred to as mesograzers.  *Smithora* abundance and distribution varies widely along the Pacific Northwest coast (Harlin 1975). After colonizing as a microscopic spore, it forms tough basal cushions and then grows into lobed blades (Hansen 1986, Harlin 1973b, Hawkes 1988). *Smithora*’s successful colonization depends on the survival of spores as well as the microenvironment of the *Z. marina* blade. Due to *Smithora*’s large variation over a small distance, *Smithora* on *Zostera marina* is an interesting system in which to investigate the drivers of changes in epiphyte abundance in *Zostera marina* meadows (Kitting, Fry, and Morgan 1984).

2.2 How do *Zostera, Smithora* and associated epifuanal and microbial species assemblages vary between meadow edge and interior?

We studied the interaction between *Smithora* and *Z. marina* on the central coast of British Columbia, Canada, in June-August 2015 in Choked Pass, Calvert Island (Figure 1). In a large continuous eelgrass meadow approximately 367,000 square meters in area (Hakai geospatial team), *Smithora* is prevalent on *Zostera* blades along the edges of the meadow, but not in the meadow interior. The site is primarily ocean influenced, with salinities between 29 and 31 ppt and temperatures between 6 and 10°C in summertime. Within the meadow, depths can get up to 10m, but at our experimental site maximum depth only reached 5m and did not vary between transplant locations. (Should I include more enviro stuff? Conductivity, nitrogen,phosphate,chlorphyll, we have everything from a CTD drop at wolf).

Within the Choked Pass eelgrass meadow, we quantified spatial variation in *Smithora* abundance on eelgrass. We surveyed 8 40-m transects, four in the meadow interior (>200m from the closest edge) and four at the meadow edge (2m from bordering sand habitats) throughout the primary growing season, May to August (Olson 2017; Fig. 1). All transects were in permanently subtidal seagrass, and were separated by at least 100 m. Using SCUBA, we collected one shoot every 10m along each transect (n = 5 shoots per site visit) by covering shoots with a Ziplock bag and detaching at the rhizome. From each shoot, we measured *Z. marina* shoot dry weight and *Smithora* dry weight.

In a second survey, we quantified epifaunal grazer abundance and diversity on *Z. marina* shoots at the meadow edge and interior at two adjacent cites: WF and IA (Figure 2). *Z. marina* shoots were collected from 0.25m X 0.25m quadrats (n = 6) from the interior (IA) and edge (WF) in early June, 2015. Following standard processing protocol (Duffy et a 2015), all invertebrates were removed from shoots and preserved with 95% ethanol. Invertebrates > 500 um in diameter were visually classified to the lowest possible taxonomic group (Appendix 1), usually family but sometimes to species, using a stereo microscope, and invertebrates known to associate with *Z. marina* and graze epiphytic algae were enumerated (Whippo et al in review, Duffy et al 2015).

2.3 Does location and abiotic environment explain variation in epiphyte and microbial communities living on Zostera? A reciprocal transplant experiment.

To test whether *Smithora* abundance on an eelgrass shoot reflects the shoot’s location (environmental conditions) or the shoot itself (defenses, microbiota, age, etc), we conducted a reciprocal transplant experiment. We identified two adjacent source sites within the Choked Pass meadow typical of the high *Smithora* zone (WF) at the meadow edge and the low *Smithora* zone (IA) at the meadow interior. These zones differed in *Smithora* abundance on shoots, from 0.37 + 0.39 *Smithora* / *Z. marina* (g/g dry wt) in the high *Smithora* zone at WF to 0.02 + 0.06 *Smithora* / *Z. marina* (g/g dry wt) in the low *Smithora* zone at IA. Depth and substrate (sandy) were consistent, and the two sites were 5 meters apart and connected by continuous eelgrass habitat.

From each zone, we collected twelve shoots and exposed them to one of two treatments (n = 6 shoots per treatment): transplant and control. Transplanted shoots were collected and moved to the other zone (WF shoots moved to IA zone, and IA shoots moved to WF zone). Control shoots were collected and replaced in their zone of origin to control for the effect of uprooting on *Smithora* abundance and bacterial community. Collection, initial sampling, and replanting procedures consisted of the following steps: Shoots were collected on SCUBA on July 9th. They were collected in the field with a minimum of 6 rhizome nodes. Shoots were placed in a ziploc bag in the field underwater, and transported to the lab immediately. In the lab, shoots were photographed for morphometric analysis and swabbed for bacterial community analysis. When not being processed they remained submerged in seawater. Each shoot was identified and labeled with flagging tape so that it could be re-sampled at the end of the experiment. Shoots were replaced in the field on July 10th by attaching them by the rhizome with zipties to PVC submersible platforms. In the field, platforms were secured to the sediment surface to keep shoots on the sandy substrate and floating upright. On August 10th, all 24 treatment and control shoots were collected and processed and photographed in the lab. We also collected 1 ambient shoot next to each transplant platform to compare transplanted shoots to unmanipulated shoots. We removed mesograzers from the shoots upon collection. Some shoots were lost or torn during the experimental period and sufficient biomass could not be recovered. This lowered the sample size from the initial N=24 (Table 1).

2.4 Sampling shoot characteristics: morphometrics and microbiota

For all shoots collected for the transplant experiment (N = 12 treatment shoots + 2\*2 ambient control shoots) and environmental surveys (N = 120), we measured the following shoot characteristics: leaf length, leaf width, biomass (dry weight, after 48 hours at 60°C), and microbiota. For shoots collected as part of environmental surveys, we also counted the number of blades per shoot. Shoots were brought to the lab, where epiphytes were gently scraped off with a microscope slide and grouped taxonomically (*Smithora*, *Porphyra*, and periphyton). *Z. marina* shoots and associated *Smithora* epiphytes were then dried at 60°C for 48hrs to obtain dry weights.

To quantify the diversity and composition of each shoot’s external microbiota, bacterial samples were taken before and after the transplant, as well as from ambient shoots collected at the time of transplant retrieval. We sampled microbiota from a standard location on each shoot - an area halfway up the third leaf that was free of *Smithora*. This area was rinsed with filtered sterilized seawater for 10 seconds, and then a Puritan® sterile swab was used to swab the area for ten seconds, avoiding any *Smithora* basal thallus cushions. Swabs of *Smithora* blades were taken as above for comparison. The swab was stored in an individual sterile cryovial (VWR) and placed on ice for transport back to the lab, and were transferred to -80˚C for storage within 8 hours.

DNA was extracted from swabs and water filters using the MoBio PowerSoil®-htp 96 well DNA extraction kit (Carlsbad, CA) following the manufacturer’s recommended protocol. The V4 region of 16S rRNA in Bacteria and Archaea was targeted for amplification using redesigned versions of the primers 515f/806r (Caporaso et al. 2012): 515f: 5’–GTGYCAGCMGCCGCGGTAA–3’, 806r: 5’–GGACTACNVGGGTWTCTAAT–3’. Forward primers were tagged with a 12bp Golay barcode to facilitate sample pooling. Each PCR contained 10µl of 5-Prime Master Mix, 1µl of each primer (final concentration = 0.2µM each), 0.5µl of peptide nucleic acid (PNA) chloroplast blocking primer (Lundberg et al. 2013; 0.2µM final concentration, purchased from PNA Bio Inc., Thousand Oaks CA), 2µl of DNA, and PCR grade water to a final volume of 25µl. PCR was carried out with an initial denaturation step at 94˚C for 3 minutes, followed by 25 cycles of denaturation at 94˚C for 45 seconds, PNA clamping at 75˚C for 60 seconds, primer annealing at 50˚C for 60 seconds, and extension at 72˚C for 90 seconds, with a final extension step of 72˚C for 10 minutes. PCR products were quantified using Quant-IT Pico Green® ds DNA Assay Kit (Life Technologies). Equal amounts (25ng) of each sample were pooled and then purified using the MoBio UltaClean® PCR clean-up kit. Pooled library quantitation and paired-end Illumina MiSeq sequencing (2 x 300bp) was carried out at the Integrated Microbiome Resource facility in the Centre for Genomics and Evolutionary Bioinformatics at Dalhousie University (Halifax, Canada).

Raw sequencing reads were demultiplexed using split libraries within the Quantitative Insights into Microbial Ecology (QIIME v.1.9) analysis pipeline (Caporaso et al. 2010b), and then then trimmed to 250 base pairs using FastX Toolkit (<http://hannonlab.cshl.edu/fastx_toolkit/>). Reads were then clustered into “species” level operational taxonomic units (OTUs) using Minimum Entropy Decomposition (MEDs; Eren et al. 2015), with the minimum number of reads per MED node set to 90 (-M parameter). All other parameters were run with default settings; the maximum variation allowed per node (-V) was automatically set at three nucleotides.

Taxonomy was assigned to MED-nodes (hereafter referred to as operational taxonomic units; OTUs) using uclust (Edgar 2010) as implemented in the Assign Taxonomy function of QIIME v.1.9 retrained on the GreenGenes (gg\_13\_8) database (DeSantis et al. 2006). OTUs annotated to either chloroplast or mitochondrial sequences were removed as putative host contamination. Additional OTUs were removed if they occurred in only a single sample. Representative sequences for the remaining OTUs (n = 1984) were aligned with PyNAST v.1.2.2 (Caporaso et al. 2010a) using the GreenGenes 13\_8 alignment as a template, and a tree was constructed using FastTree (Price et al. 2010) as implemented in QIIME v.1.9. Samples with fewer than 1000 reads were removed from the analysis. Sequence data and MiMARKs compliant metadata are deposited at the European Bioinformatics Institute, accession number (XXXXXXXX).

**2.5 Statistical analyses**

To compare *Z. marina* and *Smithora* biomass at the edge vs. interior, one-way ANOVA was used with R. 325 statistical software. *Smithora* biomass and shoot density fit a normal distribution and so a linear model was used to fit the data.

To compare bacterial community composition among treatments, we constructed a dissimilarity matrix on rarefied data (5000 sequences/sample) using the UniFrac metric, which takes phylogenetic distance, but not relative abundance, into account (Lozupone & Knight 2005), to compare microbiota composition among sites and before and after transplanting of shoots. The matrix was constructed in Phyloseq (McMurdie and Holmes 2013) within R. Beta-diversity patterns were visualized with non-metric Multi Dimensional Scaling (NMDS) plots created in Phyloseq. A two-way PERMANOVA (Permutational Analysis of Variance) was used to compare the effect of *Smithora* presence, transplant, and their interaction on bacterial community. We did not include bacterial families with relative abundances lower than 0.02.

We compared epifaunal invertebrate abundance between edge and interior plots with ANOVA fit using a gamma generalized linear model. We used NMDS plots to visualize invertebrate community dissimilarity based on a bray-curtis dissimilarity metric. Invertebrate community data was analyzed in the Vegan 2.3-4 package in R (Okansen et al. 2016). A PERMANOVA was used to test the effect of *Smithora*, Location (edge vs. interior), and month (June vs July) on invertebrate communities. A one-way ANOVA compared amphipod abundance at the edge vs. interior locations. All R analyses used R 3.2.4.

**3. Results**

3.1 How do *Zostera, Smithora* and associated epifuanal and microbial species assemblages vary between meadow edge and interior?

Across eight sites at the landward side of the Choked Pass eelgrass meadow (Figure 1), *Smithora* presence and abundance on eelgrass shoots varied strongly from site to site, and there was a significant difference in *Smithora* abundance on *Z. marina* between meadow edge and interior sites (Figure 1C, two-way ANOVA: site type (interior vs edge): F = 63.46, df = 1, p = < 0.001; Site: F = 8.06, df = 6, p = < 0.001, residuals: df = 108).

Additional, plot-scale sampling at the Wolf Beach site, where the experiment was conducted, revealed similar patterns of high *Smithora* abundance at the meadow edge and less in the interior in June-July 2015 (Figure 2). *Zostera* shoot density was higher at the edge vs the interior (one-way ANOVA: F = 15.29, df = 1, 10, p = 0.003; Appendix A1), and so was *Zostera* and *Smithora* biomass per *Zostera* shoot (one-way anova: F = 6.57, df = 1, 10, p = 0.028) (Figure 2A and B). Grazers were more than twice as abundant on *Zostera* shoots at the edge of the meadow compared to the interior (t=4.294, p=0.00776*,* Figure 2C). Grazer density on *Zostera* increased significantly with shoot density (t=2.522, p=0.0268), and over the course of the experiment, between June and July (t=2.754, p=0.0079). Epifaunal invertebrate community composition also varied over time (PERMANOVA F=4.3221, df=1, p=0.065) and with *Smithora* abundance (PERMANOVA F = 4.7201, df = 1, p=0.048) (Appendix A2 and A3).

*Initial microbial assemblages.* Blades with *Smithora* from the meadow edge harbor significantly different microbial communities than blades from the interior without (p=0.027, pseudo-F=2.03,df=1,Figure 2C). Microbial community composition shifted from July to August (NMDS\_before\_after; PERMANOVA for date p=0.001, pseudo-F=4.818, df=1).

3.3 Does location and abiotic environment explain variation in epiphyte and microbial communities living on Zostera?

At the end of the reciprocal transplant experiment, shoots at the meadow edge had high *Smithora* load regardless of source location (Figure 3; two-way anova with interaction term: Source (interior vs edge): F = 32.04, df = 1, p = < 0.001; Treatment (control vs unmanipulated: F = 0.28, df = 1, p = 0.61, Source X Treatment: F = 4.67, df = 1, p = 0.05; residuals: df = 11). Shoots transplanted from the edge to the interior site retained *Smithora,* while interior shoots that stayed in the interior were not colonized. Controls (uprooted but locally planted) and unmanipulated shoots did not differ in *Smithora* load at the time of the end of the experiment (two-way anova: Source (interior vs edge): F = 26.34, df = 1, p = < 0.001; Treatment (control vs unmanpulated: F = 1.59, df = 1, p = 0.27, residuals: df = 10).

*Bacterial results on transplanted shoots.* There was a significant effect of initial *Smithora* presence, but not transplant treatment, on shoot level bacterial community. Following the transplant *Smithora* presence continued to be correlated with different bacterial communities (p=0.027, pseudo-F=2.03,df=1), but dispersion was not different (PERMDISP p=0.441). There was no significant difference in blade bacterial communities associated with their original location (edge vs. interior) (PERMANOVA for start location p=0.583,pseudo-F=0.800,df=1), which also correlated with *Smithora* presence, or by the location they were moved to (edge vs. interior) (PERMANOVA for destination p=0.573,pseudo-F=0.94, df=1). While community composition was different overall, microbial taxonomic richness at the end of the experiment was not significantly different between blades with or without *Smithora* (t-test p=0.59) or between shoots with different final locations (t-test p=0.60), or starting locations (t-test p = 0.664).

**4. Discussion**

We tested the question / hypothesis that a host’s associated floral and faunal communities can be controlled by host specific characteristics or by their surrounding environment or both.We found that *Smithora* abundance on eelgrass shoots did not change to match *Smithora* on neighboring shoots when transplanted in one direction (from meadow edge to interior) but it did change in the other direction (interior to edge). Thus, we reject the hypothesis that in this case, local environmental factors entirely determine *Smithora* abundance on *Zostera* shoots. Our findings indicate that something unrelated to present local environmental conditions limits *Smithora* dispersal into the interior of the meadow.

We observed that shoots with and without *Smithora* have different microbial communities. This result was unexpected, because we sampled Smithora-free areas of eelgrass shoots. The presence of *Smithora* is correlated with a unique seagrass microbial community. This could be due to a change in shoot phenolics following colonization (Harder 2008, Silva 2013, Holstrom et al. 2002). Secondary metabolites produced by seagrasses deter the attachment of fouling organisms and the differences we observed between shoots with and without Smithora could be due to an underlying difference in phenolic content between the edge and interior that allows Smithora to only colonize edge shoots (de Nys and Steinberg 1999; Butman 1987; Davis and Targett 1989).However, the distinct microbial communities on shoots without *Smithora* did not prevent *Smithora* from colonizing shoots transplanted once moved to a high *Smithora* area. Control shoots were uprooted and replanted in the interior. Uprooting likely lowered shoot health and phenolic content but no *Smithora* colonization occurred in the interior. It is therefore unlikely that *Smithora* colonization at the edge is due to lower seagrass metabolite defenses. The initial differences in bacterial assemblages detected between edge and interior shoots is most likely due to the presence of *Smithora* itself rather than small scale changes in phenolic content.

Shoot-level microbial communities appeared to be unaffected by shoot location. We did not observe any effect of location on shoot microbial community following transplant. Edge shoots that had *Smithora* had similar microbial communities to shoots with *Smithora* in the interior. Our result is consistent with observations that microbial communities do not vary between the edge and interior of a meadow (Ettiner et al. 2017). All microbial communities on uprooted shoots changed following transplant and this could be due to a change in shoot health following uprooting. The blade level microbial community of *Zostera* has been show to vary along an environmental gradient (Meja et al. 2016). We assumed that the small spatial scale of our study removed the possibility of environmental variation causing the blade level microbial differences between edge and interior shoots. We believe that the microbial changes we observed were a reflection of the physiological responses of *Zostera* to algal colonization and uprooting .

In general we found that *Smithora* biomass and *Zostera* biomass and density differed between edge and interior sites. It is unclear why there is consistently more *Smithora* biomass at the edge of the meadow. The adult sexual stage of *Smithora* is unknown and it could be that the diploid stage of Smithora is a conchocelis phase that grows outside the seagrass meadow in shell fragments (Harlin 1973; Hawkes 1988). This could explain why there is higher *Smithora* biomass at edge areas facing the rocky intertidal and why we see high abundances of *Smithora* on *Phyllospadix* spp. in the intertidal. It could be that the dispersal distance of *Smithora* propagules is quite small and so it settles relatively quickly into the seagrass meadow. We could be observing dispersal limitation of *Smithora* as its spores are released from the intertidal and then trapped at the meadow edge without dispersing farther into the meadow.

We also compared grazer community composition between high and low *Smithora* sites. If grazers controlled *Smithora* presence or abundance through consumption, we would have observed a negative correlation between *Smithora* abundance and grazer abundance. But we observed the opposite pattern with higher abundances of invertebrates at the edge where there was also a high abundance of *Smithora*. This could be due to the presence of *Smithora* creating a more structurally complex habitat which in turn provides food and shelter for epifaunal invertebrates (Parket et al. 2006). We also observed that invertebrate communities from quadrats with *Smithora* were significantly different from those without in terms of relative abundances of different species. This indicates that *Smithora* could be providing more than substrate on the blade surface. It could be that *Smithora* provides a specialized habitat to a unique assemblage of invertebrates or is an important food source to certain species. Our results are also consistent with selective predation by juvenile rockfish in the high Smithora areas at the edge of the meadow (Olson 2017).

An important consideration of our study is that our comparisons across shoots with and without Smithora are also comparing between the edge and interior of a seagrass meadow. Large changes in epifaunal communities can be associated with seagrass meadow edges (Bowden, Rowden, and Attrill 2001; Bell et al. 2001, Tanner 2005, Prado et al. 2007). In separate studies edge effects have been shown to effect algal and invertebrate communities. We observed patterns similar to other studies with a strong correlation in edge effects between invertebrate and algal communities and with the added correlation of bacterial community differences, indicating that edge effects could be acting on multiple community levels.

In summary our research highlights some important community forming processes happening at the edges of seagrass meadows. We noted differences in Smithora abundances between the edge and interior of meadows, and we showed that different seagrass microbial communities are correlated with this variation in abundance. We also showed that grazer communities vary with these changes in *Smithora* abundances. It is unclear whether all three communities are influenced by the same edge effects or they are interacting together to form the ecological pattern we are noting. Understanding community forming processes in seagrass epifaunal communities at small spatial scales is important for recognizing large scale patterns in seagrass communities.