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

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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, resulting in communities that vary across seascapes. Through an experimental manipulation of seagrass (*Zostera marina*), we examined the processes that drive the abundance and distribution of *Smithora naiadum*, an epiphytic red alga, in *Zostera marina* meadows on the central coast of British Columbia. At, the shoot level, we hypothesized that *Smithora* could be controlled by invertebrate grazing or bacterial facilitation. Whereas, at the site level, we hypothesized that Smithora could be controlled by environmental factors leading to the recruitment and persistence of the epiphyte on seagrass shoots. We used a reciprocal transplant to investigate whether *Smithora* load was affected by shoot level characteristic, location (and associated environmental characteristics) or an interaction between them. We found that uncolonized blades were colonized when placed in a high *Smithora* environment, while colonized blades did not lose their 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 a higher invertebrate abundance, indicating that *Smithora* is likely not grazer controlled. Our results suggest that seagrass 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*. This research suggests potential drivers of epiphytic community composition in seagrass meadows.

**Highlights**

**Graphical Abstract (anyone?)**

1. **Introduction**

[***spatial variation in host - epiphyte relative abundance***]

What drives community variation across spatial scales continues to puzzle ecologists in a variety of ecosystems (Boström et al., 2006, A. Driscoll, 2008; Levins, 1969). In communities strongly influenced by foundation species, variation the presence and abundance of resident species may reflect spatial patterns in the host species or in the environment (Wahl 2008).

[***seagrass host - epiphyte system***]

Seagrasses are a foundation species that hosts richly diverse animal and algal communities. Algal epiphytes living on the seagrass blades support secondary productivity (Fry 1984) (Figure 1), yet also compete with seagrasses for light and nutrients (Mcroy and Goering 1974), (Sand-Jensen 1977), (Penhale 1977), (Harlin 1973a) (Coleman and Burkholder 1994) (Lin et al. 1996) (Morgan and Kitting 1984). Thus the foundation species and the biodiversity it hosts exist 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). When the interaction network is perturbed, the system may change dramatically and produce extreme or undesirable states including seagrass die-offs (Burkholder, Tomasko, and Touchette 2007)(Best and Stachowicz 2012).

[***shoot-level or abiotic drivers: possibilities and examples. (shoot-level: microbes, other?; location: abiotic, or grazers…)***]

Spatial variation in the types and abundance of epiphytes on a single foundation species such as Zostera can reflect local environmental conditions, such as nutrient concentration or flow rates (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). For example, large changes in epifaunal communities can be associated seagrass meadow edges (Bowden, Rowden, and Attrill 2001; Bell et al. 2001)[*find a better example*]. In general, edge effects affect communities due to changes in physical structure, abiotic conditions or grazing pressure (Soule 1986) (Renhorn et al. 1996; Fagan, Cantrell, and Cosner 1999). Abundances of invertebrates can vary significantly both between and within meadows due to predation by fish (Amundrud, Srivastava, and O’Connor 2015) (Boström and Mattila 1999) (Tanner 2005). Understanding the drivers of this spatial variation helps to understand the dynamics of the eelgrass-based community, supporting better understanding of temporal variation and events such as algal blooms. Epiphytes may also be used as indicators of nutrient pollution, if their abundance is known to reflect water column nutrients (ref).

Another possible driver of variation in algal epiphytes is variation in the host plant’s structure or chemistry. *Z. marina* plants could be changing their blade surface chemistry to discourage epiphyte colonization (Bell, Lang, and Mitchell 1974) (Mejia et al. 2016). Colonization of *Zostera marina* 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.

*can we comment on whether we are aware of any other reciprocal transplant experiments on epiphytes? that makes our study novel.*

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. 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).

[***our objectives***]

The main objective of this work was to investigate potential drivers of changes in *Smithora* abundance within a single *Zostera marina* eelgrass meadow. We performed a reciprocal transplant experiment of seagrass shoots between zones of high and low *Smithora*. We tested the hypotheses that *Smithora* abundance on eelgrass is dictated by a shoot level characteristic rather than by the local environment. If shoot-level attributes dominate, we expected that abundance of *Smithora* would not change with a change in shoot location.

2. Materials and Methods

2.1 Study System

*Zostera marina* is a meadow forming eelgrass common along coastlines in the northern hemisphere (Olsen et al XXX). *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 growing on the seagrass blades rather than the seagrass itself (Valentine and Heck, Edgar and Shaw 1995, Taylor 1998).

*Smithora* *naiadum* is a red alga thought to specialize on *Z. marina. 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.

2.2 Spatial variation in *Smithora*, *Zostera,* and associated organisms

We studied the interaction between *Smithora* and *Z. marina* on the central coast of British Columbia, Canada, in June-August 2015 ([Figure 2](https://docs.google.com/document/d/1fnmB9FjXoJj9Wm9wLuwfoJh-fRqCLzDUWRTvhR-4bhM/edit?usp=sharing)) in Choked Pass, Calvert Island. In a large continuous eelgrass meadow approximately 367,000 square meters in area (Hakai geospatial team), *Smithora* is prevalent along the edges of the meadow, but not in the meadow interior. At the wolf beach study site temperatures range from 6-10 degrees, light, etc.

We surveyed the Choked Pass eelgrass meadow to quantify 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) repeatedly throughout the primary growing season, May to August (Olson 2017; Fig. 2). All transects were in permanently subtidal seagrass, and were separated by at least 100 m. Using SCUBA, we collected one shoot every10m along each transect (n = 5 shoots per site visit; n=15 shoots per site total) by covering shoots with a Ziplock bag and detaching at the rhizome. From each shoot, we measured biomass, leaf area index (LAI), *Smithora* biomass… LAI was calculated for each shoot as the product of the length and width of the longest blade and the number of blades.

We measured the following seagrass and animal attributes: seagrass shoot density and leaf area per X m2, *Smithora* biomass, epiphyte abundance, and grazer abundance and diversity (any abiotic variables??).

In a separate survey, we quantified ambient grazer abundance and diversity at the meadow edge and interior at two adjacent cites: WF and IA. *Z. marina* shoots were collected from 0.25m X 0.25m plots (n = 6) from the interior and edge at the Wolf Beach study site in early June. We used a standardized processing protocol similar to Whippo (2013), the only difference being that we separated (using a Whatman GCF X filter) and weighed periphyton biomass. All invertebrates were removed and preserved with 95% ethanol for diversity analysis. Invertebrate samples were filtered through mesh sizes of (8,5…) and then classified to the closest possible taxonomic grouping. Every invertebrate from the ethanol tube was recorded. Gammaridian amphipods and cnidarians were identified to order. Otherwise, every invertebrate in each quadrat was classified to as specific a grouping as possible, see Appendix 1 for exact groupings.

2.3 Reciprocal Transplant Experiment

We conducted 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 identified two 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 approximately 80% cover in the high *Smithora* zone to 0% cover in the low *Smithora* zone. 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): 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 clipped in the field at the sediment surface, leaving the rhizomes. 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 initial bacterial community analysis. When not being processed they remained submerged in seawater. They were given an ID using flagging tape so that each shoot could be re-sampled at the end of the experiment. Shoots were replaced in the field on July 10th by attaching them by the sheath 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 2 ambient shoots next to each transplant platforms to compare transplanted shoots to unmanipulated shoots. We removed invertebrate grazers were removed from the shoots upon collection.

2.4 Shoot characteristics: morphometrics and microbiota

For all shoots collected for the experiment (N = 12) and environmental surveys (N = 120), we measured the following shoot characteristics: length, 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 *Z. marina* shoots with a microscope slide and grouped taxonomically (*Smithora*, porphyra, and periphyton). Dried biomass of *Z. marina* shoots and their associated *Smithora* epiphytes was obtained by drying samples at 60°C for 48hrs.

To quantify the diversity and composition of the shoot’s external microbiota, bacterial samples were taken from each experimental shoot (both before and after the transplant), as well as from ambient shoots collected at the time of transplant retrieval. An area halfway up the shoot that was free of *Smithora* was chosen bacterial sampling. 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 quantify variation in *Smithora* among edge and interior sites in Choked Pass, multiple generalized linear models were fit using an inverse Gaussian distribution, with LAI and site as explanatory variables for *Smithora* biomass. Models were compared using Akaike Information Criterion (AIC). To compare seagrass density and *Smithora* biomass at the edge vs. interior, one-way ANOVA’s were used with R. 325 statistical software. Both *Smithora* biomass and shoot density fit a normal distribution and so a linear model was used to fit the data.

We constructed a dissimilarity matrix on rarefied data (5000 sequences/sample) using the UniFrac metric, which takes phylogenetic distance 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 abundance and diversity between edge and interior plots using ANOVA. We used NMDS plots to visualize invertebrate community dissimilarity using a bray-curtis distance 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 location (edge vs. interior) 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 Spatial variation in *Smithora* and *Zostera* and associated organisms

In choked pass, *Smithora* presence and abundance on eelgrass shoots varied strongly from site to site (Table 1). *Smithora* abundance was not related to leaf size (Table 1). There was a significant difference in *Smithora* abundance on *Z. marina* between meadow edge and interior (P = 0.0021, N = 12, F = 16.92, df = 10). Additionally a one-way ANOVA showed significantly higher shoot density at the edge vs. interior (p = 0.002913, N = 12, F = 15.289, df = 10).

Grazer abundance and diversity also varied substantially among the inner and outer sites. Interior quadrats had significantly lower amphipod abundance compared to edge quadrats (p = 0.0005631, df = 1, F = 99.898) (Figure 5). We are still waiting for grazer data but it appears that there will be a significant difference in invertebrate communities between the edge and interior sites (Figure 5).

Can we say something about initial microbial assemblages too? And mention in the second paragraph that *Smithora* abundance patterns at the local site were like the general patterns.

3.3 Reciprocal transplant

Shoots that were moved from the interior of the meadow to the edge were colonized by *Smithora* while shoots moved from the edge to the interior did not become *Smithora* free like surrounding ambient shoots. Controls showed no change in *Smithora* biomass compared to surrounding unmanipulated shoots. Comparing *Smithora* biomass between shoots moved to the edge vs. shoots moved to the interior showed no significant difference in *Smithora* biomass (n = 6, P= 0.0866, df = 4, F = 5.112) (Figure 6).

3.4 Shoot characteristics:

Bacterial Analysis

A two-way PERMANOVA showed that there was a significant effect of transplant and *Smithora* presence on shoot level bacterial community.

Same pattern on experiments

July edge: Sapro,Methy only consistent ones that edge share

July interior: Flavo, Sapro,Rhodo,Thio,unknown, Rickett

August experiments: colonized by everything

**4. Discussion**

Notes from composition plots:

smithora dominated by: Flavobacteraia,Thiotrichaceae,unknown,rhodobacteria,Hyphomonadacea

* signals of decay on smithora, from microbial perspective they were dying (rhizomes not attached). There were also signals of zostera colonization. Be clear about this, and what we can infer from it, and what are the limitations; shoots grouped together post transplant, and separately from the healthy shoots.

Saprosporacea methylophalacae are probably indicators of healthy shoots, and higher in smithora covered shoots.

- marcus can remake the figures.

ambient obs: ambient shoots from august dominated by: Saprospiracea, one edge dominated by Shewanellaceae,rickettsiacea (3 but not edge 2), Methylophilaceae, Rhodobacteria, Unknown, vibrio also in other edge one

July Edge controls; Saprospiraceae, Methylophilaceae, Flavobacteriacea, Thiotrichacea, Unknown

July Interior controls; More even in appearance: Saprospiracea, Rhodobacteria, Shewanelliacea, Unknown, Verrucomicrobiacea, Cryomorphaceae.

After shows no real difference between either: Thiotrichaceae common, vibrio became present on inner shoot

July edge: Sapro,Methy only consistent ones that edge share

July interior: Flavo, Sapro,Rhodo,Thio,unknown, Rickett

August experiments: colonized by everything

Based on observations of data: all communities increased in diversity following transplant accept for interior shoots which decreased. Maybe smithora discouraged diatom colonization?

No idea why there is such huge variation in diversity on smithora blades. Could be because they are dominated by one family. High diversity is usually correlated with community health, but all diversity increased indicating that seagrass plants potentially maintain their own bacterial diversity.

Models that just used transplant to explain differences in diversity were the most effective in describing the data.

Discussion outline:

**There appears to be high abundance of smithora at the edge of the seagrass meadow versus the interior.**

-AIC model comparison showed that location was the most imporatnt factor dictating smithora abundance in candidate models.

-On the map we can see that areas with a large amount of smithora are generally at the edge of the meadow.

-High abundances of algae at the edges could be due to a variety of factors: habitat filtering…

-Previous studies have shown that abundances do not vary significantly throughout the meadow and so this effect could be due to a unique characteristic of smithora.

**At the wolf beach study site we found higher biomass at the edge, and a higher density of shoots at the edge.**

**-**At our study site seagrass quadrats at the edge had a higher density of shoots.

-It could be that a certain density of shoots is required to capture smithora spores. Slowing down of the particles through microturbulence between the blades (the same method that allows sediment settlement.

**Smithora did not disappear from plants that were moved to the interior of the meadow.**

-Smithora was capable of surviving in the interior of the meadow for a month

-Smithora looked healthy while seagrass shoots were quite close to dead

-This shows that smithora is capable of surving in the iterior of the meadow. However smithora biomass was lower on these transplanted shoots compared to control edge shoots that were not transplanted.

**Smithora colonized plants that had previously no smithora were colonized when moved to the edge of the meadow.**

**-**Smithora was capable of colonizing shoots that had previously had no smithora.

-This could because smithora was incapable of dispersing to the location.

-Could also be because we essentially killed the plant and so any bacterial/chemical defenses it had were compromised.

**Control shoots at both locations did not change in smithora abundance following detachment.**

**-**Control shoots in the interior were not colonized by smithora even though their health was compromised after detachment

-Likely not shoot health that is the only factor dictating whether shoots are colonized.

-one author showed that time was important in dictating smithora growth. Not because older shoots stop producing phenols but our transplanted shoots had similar biomass levels compared to controls even though they were only exposed to smithora for one month.

**Bacterial composotion plots show unique bacterial communities at the family level for blades of smithora.**

**-**Makes sense algae and seagrass have different properties

-Smithora also had lower diversity at the family level and species level.

-A large proportion of bacterial families on smithora were unknown. It has a very unique community.

**Shoots with and without smithora differed in composition.**

-Composition plots show that before transplant, shoots with and without smithora look very different.

-However following transplant these differences become less striking

-Shoots with smithora were still more similiar to other seagrass shoots compared to smithora blades.

**Shoots before and after transplant differed in composition**

-The increase in diversity after transplant can be seen in the composition graphs

-all shoots communities changed following transplant and 1 month

-There is much less differences between shoots with and without smithora after transplant, indicating that the effect of smithora on seagrass community is stronger when the plant is alive.

**Bacterial diversity is best explained by transplant alone.**

**-**This could be because of the effect of time on bacterial community. Additionally smithoar increased on all shoots following transplant regardless of smithora presence. However control shoots from the interior decreased in diversity following transplant. The only difference between these shoots and others is these shoots were never colnized by smithora. It could be that diversity was higher for these shoots because they weren’t in contact with smithora and when their health was conpromised there were no macroalgae communities to rescue the bacterial surface of the blade.

**Overall, transplant, smithora, and thier interaction had a signifiacnt effect on bacterial community.**

-From the NMDS you can see that shoots clusters based on before/after and smithora

-The most strking effect on community is the effect of transplant. It is important to note that this transplant was left in the new environment for 1 month. Changes we see with transplant are also confounded with time.

-Ambient shoots collected in august (while still alive and healthy) were more cimilar to dying transplanted shoots collected in august. This indicates that temporal effects are stronger than the seagrass health effects on bacterial community.

-Smithora was also swabbed a week before the initial shoots were swabbed. The extreme difference in community we see in Smithora could also be due to time rather than smithora differences.

**Grazer communities were also significantly different between the edge and interior.**

-High abundance of invertebrates at the edge compared to interior

-Most of this difference was in gammaridian amphipods

-High crustaceans at the edge has been shown before but it could be because of smithora offering food and habitat for these invertebrates.

**There is likely not grazer control of smithora on the location scale.**

-Grazers eat the smithora but its more likely that other factors are dictating it.

-If grazers were controlling the abundance of smithora you would expect to see less smithora where invertebrate abundances are higher.

**Community measurements between the shoot level and location level indicate that neither are sufficient to explain smithora zonation.**

**-**The sexual stage of smithora is unknown

-I think its likely that is dispersing from outside the meadow and then colonizing the first blades it contacts and not continuing further.

-More research into the spatial processes dictating algal spores would be helpful in determing what is driving these patterns in smithora abundance.

**Summary of results for reciprocal transplant**

Since there was no significant loss of *Smithora* when shoots were moved to a *Smithora* free zone I can infer that *Smithora*’s absence in the interior of the meadow is likely not due to grazing pressures by the invertebrates I identified. Shoots did not lose their *Smithora* after one month of being in a *Smithora* free environment. This indicates strongly that there is no change in an environmental variable between the edge and interior that prevents *Smithora* recruitment and settlement. Conditions may or may not be better at the edge for *Smithora*, but it can survive in the interior even though it is not present at that location. The ability of *Smithora* to grow in new locations when it is manually transplanted indicates that it could be experiencing dispersal limitation in Choked Pass.

When shoots were moved to the edge from the interior they were colonized with *Smithora*. This is likely due to *Smithora*’s continual release of spores (Hawkes, 1988b). The blades grow from a basal cushion and produce haploid spores from monostromatic tissue (Hawkes, 1988b). *Smithora* is known as a prolific colonizer and its high growth and colonization in a three-week time frame is not surprising. The fact that clean transplants were colonized indicates that shoots were *Smithora* free because of a lack of spores rather than a shoot level characteristic-and this could mean that *Smithora* is experiencing dispersal limitation.

Many models exist for explaining how currents and wave motions drive dispersal of algal spores (Gaylord et al., 2002). However in general these models show that there is huge variation in dispersal distances of species, and they are difficult to predict (Gaylord et al., 2002). Choked pass is a high current area, it could be that the current is so strong that spores drift at an angle, and are just swept off the meadow entirely. The speed of spreading is also related to the generation time of the species, as species can disperse small distances, grow and then release spores again (Norton 1992). *Smithora* individuals might take a while to develop before they can release spores, and the blades from which the spores release die back in the summer, limiting the dispersal of *Smithora* deeper into the meadow. A combination of life history and wave action could be limiting *Smithora* spread in Choked Pass.

The sexual stage of *Smithora* is unknown. However there is evidence for its existence (Hawkes, 1988b). The *Zostera marina* meadow is adjacent to a rocky intertidal habitat at the Wolf Beach site. *Smithora* is often found in the intertidal on *Phyllospadix spp*. and this plant has drifted into samples with *Smithora* attached. We also found *Smithora* on artificial seagrass units that were placed along the edge of the site. *Smithora* individuals are coming from somewhere, and it could be other haploid individuals or the diploid stage of the algae. Whether *Smithora* is dispersing as spores from a diploid crust or from blades already growing, limited dispersal from the rocky shore could be occurring. This suggests that dispersal could be highly important in dictating epiphyte community structure. Due to *Smithora*’s presence being influenced strongly by a change in location, it seems likely that we do not have *Smithora* communities in the interior of the meadow simply because the spores haven’t made it there yet, and this could be further tested by mapping seasonal spreading distribution.

**Bacterial Community Analysis**

The significant effect of transplant on bacterial diversity hints at a complex relationship between seagrass health, *Smithora* colonization, and temporal changes. *Zostera marina* possesses the ability to produce phenols that act as antioxidants and chemically defends against epiphytic colonizers (Harder, 2008). In response to shading *Zostera marina* has been shown to increase phenol content to deal with oxidative stress (Silva et al., 2013). Bacterial community is then affected by this change in phenol content (Holmström et al., 2002b). Removing a seagrass shoot from its root system is a stressful event for the plant. Moving a shoot could compromise its ability to produce protective phenols and allow for more species of bacteria to colonize the shoot. The fact that bacterial communities changed on each shoot that was transplanted with and without Smithora hints at some interesting links between seagrass health and bacterial community.

However, we also measured bacterial communities on shoots after a month had passed. If you look at the bacterial NMDS you can see that ambient healthy shoots collected in August are more smiliar to transplanted shoots swabbed at the same time than they are to healthy shoots from July. If phenols and seagrass health were the dominant forces shaping the bacterial communities then you would expect healthy shoots to be more similar to each other than transplanted shoots. Since shoots from the two time frames cluster together regardless of their algal presence or health it indicates that the time that the shoot is swabbed has a large effect on the bacterial community that is observed. In fact time has a similar effect on bacterial communities in a reciprocal transplant in sponges (Weigel and Erwin 2017). Abiotic factors proved to result in less bacterial community change compared to the simple passage of time (Weigel and Erwin 2017). However, healthy ambient shoots collected at the same time as damaged transplanted shoots had different bacterial species present on their surfaces, indicating that time alone is not sufficient to explain these community shifts.

A PERMANOVA and NMDS shows that there is still clustering of shoot level communities based on the presence of *Smithora*, Bacterial communities are very responsive to shoot level changes such as spore colonization. I believe that *Smithora* colonization itself could be altering the blade level community, rather than a specific bacterial community existing at the edge of the meadow that allows spores to colonize.

Furthermore, bacterial communities after transplant appear to cluster more closely than communities before transplant. In the before transplant group shoots with and without Smithora are distinctly different. Shoot communities after transplant appear to cluster much more tightly. Perhaps when the health of the shoot is compromised it loses its unique bacterial community and all unhealthy shoots become more similar regardless of algal colonization. From the NMDS It appears that healthy shoots with and without smithora are more different than unhealthy shoots with and without Smithora. Given the sensitivity of the shoot level communities to shoot health and time, it seems unlikely that a unique bacterial community exists on Z. marina that encourages Smithora colonization.

However, there is still a possibility that bacterial community could be promoting *Smithora* colonization. *Ulva* spores (a green algae species) have been shown to respond to chemical cues produced by a specific bacterial community (Joint et al., 2002). We see a significant correlation between *Smithora* presence and bacterial community composition, and we could be observing chemical communication between prokaryotes and eukaryotes. There is also the possibility that the detrimental impacts of shading caused by *Smithora* are changing the chemical environment of the blade and promoting a different bacterial community. Based on *Smithora*’s ability to colonize various substrates, and the colonization of clean blades that were moved to the high *Smithora* environment, I suggest that bacteria community does not determine *Smithora* colonization, rather *Smithora* colonization alters bacterial community.

*Smithora* colonization is correlated with community change at the bacterial level and this has implications for the larger seagrass community. Microbial communities in seagrass sediments have been connected to overall shoot health (Milbrandt et al. 2008), however this has yet to be shown conclusively with blade level microbial communities. Bacterial shifts on the surface of the blade could be linked to seagrass degradation, epiphytic colonization, or the presence of wasting disease.

**Grazer Community Analysis**

The increase in amphipod abundance correlated with *Smithora* presence suggests that *Smithora* is likely not grazer controlled. Invertebrate herbivores are widely known to eat macroalgae in seagrass meadows (Heck and Valentine, 2006). Amphipods have also been shown to consume a large amount of microalgae (Cruz-Rivera and Hay, 2000). The high fatty acid content makes *Smithora* very nutritious and epiphytic grazers turn red from eating it (Oregon university,Pers. Obs). I expected to Smithora abundance to be negatively correlated with grazer abundance, because of previous evidence for predator-grazer-epiphyte trophic cascades in *Zostera marina* meadows (Amundrud et al., 2015; Duffy et al., 2015)(Duffy et al., 2015)(Duffy et al., 2015).

Recent studies in the Choked Pass seagrass meadow have shown that juvenile rockfish use the meadow edge frequently as habitat, which is also where there is the highest abundance of Smithora (Olson 2017). Predation by rockfish could be reducing grazer abundance and allowing Smithora to grow at these edge habitats. However, a common food source of juvenile rockfish, gammaridian amphipods, had a dramatic increase in abundance where *Smithora* load was high (Cruz-Rivera and Hay, 2000). This suggests that top-down control is not what is causing the dramatic decline in *Smithora* from the edge to the interior of the seagrass meadow. *Smithora* could be providing a food source to amphipods and also sheltering them from predation, which would be influencing the community structure from the bottom up.

However there are many more epifaunal grazers than amphipods. It could be that grazer control of Smithora is occurring at the microscopic scale. Perhaps micrograzers such as copepods are consuming Smithora spores as they disperse before they have a chance to settle and grow. More information on how which grazing species are found in edge and interior habitats would be useful in determining the importance of consumer control in this system.

**5. Conclusion**

In conclusion three layers of the epifaunal community of *Zostera marina* were found to vary in space. *Smithora* varies in abundance from the edge to the interior of the seagrass meadow, possibly through dispersal limitation. Bacterial and invertebrate communities vary significantly on shoots with and without *Smithora*. Whether these variations in communities with *Smithora* are a result of species interactions or spatial processes remains unknown. Edge effects in seagrass meadows are likely playing a crucial role that needs to be further investigated.

Epibiotic communities on seagrass blades represent an intriguing system to use small scale processes to explain large scale patterns. Species interactions on a single blade when multiplied over every shoot in a meadow can have dramatic effects. Understanding the drivers of changes in seagrass epiphytic community structure will help predict large scale changes in the seagrass ecosystem.

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APPENDIX

**Table A1**. Sample sizes for the experiments and treatment levels. Edge and Interior refer to high and low *Smithora* load respectively, as well as their position in the seagrass meadow.

**Test and Treatments Measurement level Sample Size**

***Smithora* Biomass & Microbial Community**

Edge Experiment After Shoot 3

Interior Experiment After Shoot 3

Edge Control After Shoot 5

Interior Control After Shoot 4

Edge Ambient After Shoot 2

Interior Ambient After Shoot 2

**Just Microbial Community**

Edge Experiment Before Shoot 3

Interior Experiment Before Shoot 3

Edge Control Before Shoot 5

Interior Control Before Shoot 4

*Smithora* blades Blades 4

**Grazer Community**

Interior June Quadrat 6

Edge June Quadrat 6

**FIGURE A1**. Reciprocal transplant study design. Arrows show the directions that 12 shoots from each location were moved. A total of 24 shoots were removed from the substrate.

