UNIVERSITY OF CALIFORNIA SAN DIEGO

Influence of Eelgrasses on the Microbial Community Structure in San Diego Bay Waters

A Thesis submitted in partial satisfaction of the requirements for the degree

Master of Science

in

Marine Biology

by

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Committee in charge:

Professor Jeff S. Bowman, Chair
Professor Eric Allen
Professor Lihini Aluwihare

2018

The Thesis of Sahra Jasmine Webb is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California San Diego

2018

DEDICATION

In recognition of those who have faced discrimination and adversity while trailblazing a path for their successors, this thesis is dedicated to women and minorities in science.

In recognition of their unending support throughout my academic career, this thesis is dedicated to my family: Miguel Velez, Norma Velez, Rebecca Velez, Bret Webb, Olivia Velez, Violet Webb, and Taylor Webb.

EPIGRAPH

The role of the infinitely small in nature is infinitely large.

Louis Pasteur

TABLE OF CONTENTS

LIST OF SUPPLEMENTAL FILES

Table 1: Sample Data Sample data includes sample ID, lab ID, collection depth, flow cytometry data, latitude, longitude, water conditions during sampling, and eelgrass presence. (.CSV)

LIST OF FIGURES

LIST OF TABLES

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ABSTRACT OF THE THESIS

Influence of Eelgrasses on the Microbial Community Structure in San Diego Bay Waters

by

Sahra Jasmine Webb

Master of Science in Marine Biology

University of California San Diego, 2018

Professor Jeff S. Bowman, Chair

In marine ecosystems, eelgrasses have proven to be influential to their surrounding environments through their many ecosystem functions, ranging from the provisioning of food and shelter to serving as a natural defense against pollution and pathogenic bacteria. In the marine waters of San Diego, CA, USA, eelgrass beds comprised of *Zostera* spp. are an integral part of the coastal ecosystem. To evaluate the impact of eelgrass on bacterial and archaeal community structure we sequenced the 16S rRNA gene from paired eelgrass-present and eelgrass-absent sites. We applied mixed effects models to the gene sequencing results and bacterial abundance data derived by flow cytometry to test the hypothesis that microbial community structure is influenced by the presence of eelgrass. This approach allowed us to

identify specific microbial taxa that were differentially present at eelgrass-present and eelgrass-absent sites. Principal component analysis (PCA) revealed that PC1 and PC2 accounted for a 93.1 % of the variance in microbial community structure among the samples. Microbial taxa that were differentially present between eelgrass present and eelgrass absent sites were identified, along with microbial taxa that were differentially present between samples from the inner and outer San Diego Bay sampling sites. Differentially present bacterial taxa included potential pathogens of order Rickettsiales, family *Flavobacteriaceae*, order Pseudomonadales, and *Tenacibaculum maritimum*.

Introduction

Contributions of Seagrasses to Marine Health

Dwelling in salt and brackish waters across the globe are seagrasses- a unique collection of marine angiosperm species, which have roots, stems, leaves, and may produce flowers or seeds (Smithsonian, 2018). Seagrasses provide several critical marine ecosystem functions including serving as a source of food and habitat, and by acting as an indicator for the overall health of the ecosystems in which they are present (Florida Fish and Wildlife Conservation Commission, 2018). Seagrasses provide a nursery habitat that shelters juvenile and smaller marine organisms like crabs, shrimp, various fish species, sponges, and sea anemones. Larger animals like sharks are also attracted to seagrass beds for feeding on smaller organisms, while grazing creatures like sea turtles, manatees, and dugongs feed on the seagrass directly (Smithsonian, 2018). On a microscopic scale, seagrasses harbor microalgae *e.g.* diatoms and bacteria that live in an epiphytic manner directly on the surface of their grass-like blades (Smithsonian, 2018).

Seagrasses facilitate ecosystem health by trapping fine sediments and bacteria, some of which may be potentially harmful to humans or marine organisms, that are introduced by storm runoff or that are resuspended from the seafloor. By trapping sediments, seagrasses have a direct impact on pollutants, aiding in increased water clarity and purity (Ugarelli *et al.*, 2017). Seagrasses also contribute to health of marine habitats and coasts by oxygen production via photosynthesis and by acting as a buffer against erosion and storms (Smithsonian, 2018).

Genus Zostera

Seagrasses are categorized into the following taxonomic families - *Zosteraceae*, *Hydrocharitaceae*, *Posidoniaceae* and *Cymodoceaceae*. Of particular interest in this research are members of the *Zosteracea* family, specifically the *Zostera marina* and *Zostera pacifica* strains, which are commonly referred to as marine eelgrasses. Family *Zosteracea* is widely distributed across the globe with our strains of interest naturally occurring in the National Marine Fisheries Service (NMFS) West Coast Region. Included in this region are California, Washington, and Oregon (NOAA, 2018). Within California, these eelgrasses may be found along the entire coast from Imperial Beach in Southern California to Humboldt County in the North - specifically within the San Francisco, Humboldt, and San Diego Bays (Merkel & Associates, Inc., 2014).

Aside from their contributions to marine ecosystem maintenance and health, seagrasses have been studied for antimicrobial potential both within their ecosystems and in laboratories for potential use in medicines *i.e.* antibiotic development. The antibacterial potential of *Z. marina* has been specifically highlighted in previous work. Lamb *et al.* (2017) concluded that *Z. marina* eelgrass meadows found in the intertidal regions of islands in the Spermonde Archipelago, Indonesia, significantly reduced overall bacterial and pathogenic load in the surrounding waters to the benefit of both humans and marine organisms. They performed *Enterococcus* assays to test whether *Z. marina* had a significant antibacterial effect in the eelgrass meadow ecosystems, using the reduction of viable *Enterococcus* as an indicator (Ortega *et al*., 2009). Lamb *et al.* (2017) also utilized 16S rRNA high-throughput amplicon sequencing to determine the microbial makeup of the surrounding waters at sites near Bonetambung Island, Indonesia, and found that the presence of eelgrass beds aided in the reduction of animal and human pathogen abundance in the surrounding areas by 50 %. They also detected that eelgrass beds aided in a twofold reduction in coral disease in adjacent reefs in comparison to reefs that were not adjacent to any

eelgrass beds. Along with antibacterial potential, extracts form *Z. marina* have also demonstrated antioxidant properties (Choi *et al.*, 2009).

Antibacterial Potential of other Seagrasses

Aside from *Z. marina*, other seagrasses have been found to exhibit antibacterial properties both in the ocean and *in vitro* (Alam *et al*., 1994; Choi *et al*., 2009; Mayavu *et al*., 2009; Kannan *et al.,* 2010). For instance, methanol extracts from the seagrass *Enhalus acoroides* exhibited antibacterial behavior on Gram-positive bacteria and the model pathogen *Pseudomonas aeruginosa* in a series of disc diffusion tests (Alam et al, 1994). Using ethanol, methanol, acetone, and dichloroethane extracts of seagrass species *Cymodocea serrulata* and *Syringodium isoetifolium* in a series of standard disc diffusion tests, Mayavu *et al*. found that crude ethanol and methanol extracts of *S. isoetifolium* were able to inhibit biofilm growth by *E. coli, P. aeruginosa,* and *Vibrio parahaemolyticus*; crude ethanol and methanol extracts of *C. serrulata* were able to inhibit the growth of 9 bacteria species (Mayavu *et al*., 2009). Specifically, biofilm growth of *P. aeruginosa*, *Bacillus cereus*, *Proteus vulgaris*, *Proteus mirabilis*, *E. coli*, *Listeria monocytogenes*, *Salmonella enteritidis*, *Staphylococcus aureus*, and *V. parahaemolyticus* were inhibited (Mayavu *et al*., 2009).

Antibacterial activity of seagrass species *Halophila stipulacea*, *C. serrulata*, and *Halodule pinifolia* were tested by Kannan *et al.* (2010) against bacteria including *S. aureus*, *Vibrio cholerae*, *Shigella dysenteriae*, *Salmonella paratyphi*, *Shigella boydii*, *P. aeruginosa*, and *Klebsiella pneumoniae* using disc diffusion tests. They found that methanol and chloroform extracts of each seagrass were successful in inhibiting growth of the human pathogens of interest; using minimum inhibitory concentration (MIC) tests, they were also able to determine

exact concentrations of each seagrass extract to inhibit bacterial growth (Kannan *et al.*, 2010). These experiments suggest that seagrasses may directly influence microbial community structure in the coastal marine environment, including a reduced pathogen load.

Water Pollution in the San Diego Coastal Region

In San Diego and other urban watersheds, untreated sewage and storm runoff can influence microbial community structure and introduce pathogenic bacteria. San Diego coastal waters from Imperial Beach to as far north as Coronado are periodically exposed to sewage from the transboundary Tijuana River. During rain events, the sewage treatment plant in Mexico for the Tijuana River, that would otherwise treat 100% of the river water, shuts down its sewage diverter, resulting in the subsequent transfer and crossing of millions of gallons of stormwater runoff into the United States via the Pacific Ocean (U.S. Department of Justice, 2018). This water contains a variety of sewage and trash, including microbial contaminants, and can result in the closure of public beach access (U.S. Department of Justice, 2018; San Diego County, 2018). San Diego Bay is also periodically exposed to toxic stormwater runoff from Chollas Creek (Schiff *et al*., 2003; US Department of Justice, 2018). Furthermore, the bay has a history of locally sourced industrial pollution. In the decades preceding 1960, San Diego Bay was exposed to a constant influx of raw sewage and untreated discharge from a variety of industries including fish canneries, kelp processing facilities, aircraft manufacturing plants, commercial shipyards, and multiple U.S. Naval installations (Fairey *et al.*, 1998). More recently, heavy urban development in San Diego Bay and cross-border sewage outflow have raised concerns about water quality (San Diego County, 2018).

Project Overview

In this study, we tested whether local eelgrass meadows with species *Z. marina* and *Z. pacifica* had significant influence on the structure of the marine microbial community in San Diego Bay. We also wanted to determine whether this influence includes a reduced pathogen load. We examined the structure of bacterial and archaeal assemblages from water samples collected from paired sites where eelgrass beds are either present or absent. Sample pairs were collected from two areas, within and just outside of the San Diego Bay. To determine the influence of local eelgrasses on microbial community structure, we used 16S rRNA gene sequencing and flow cytometry.

METHODS

Sample collection

Sites likely to contain eelgrass were identified from the 2014 San Diego Bay Eelgrass Inventory (Merkel & Associates, Inc., 2014), the most recent survey available. The presence of eelgrass was confirmed visually. Samples at eelgrass present and eelgrass absent sites were collected from a small boat via a peristaltic pump on July 3, 2017. For DNA samples, water was filtered through a sterile 0.2 μm Supor filter (Pall) and the filter was immediately placed on dry ice with longer-term storage at -80 °C. Flow cytometry samples were collected into sterile 15 ml falcon tubes and immediately placed on ice. Chlorophyll *a* concentration, yield, and phytoplankton photosynthetic efficiency (F_v/F_0) were measured with an AquaFlash handheld active fluorometer (Turner Designs) following the manufacturer's instructions. Temperature, salinity, and turbidity were measured with a YSI ProDss (Xylem).

DNA extraction and sequencing

DNA was extracted with the DNEasy PowerWater DNA extraction kit (Qiagen) within two weeks of sampling. Extracted DNA was quantified using the Qubit HS DNA quantification kit (Invitrogen) and then quality checked by gel electrophoresis and PCR amplification of the 16S rRNA gene using primers 515F and 806R (Walters *et al.*, 2015). High quality extracted DNA was submitted to the Argonne National Lab sequencing center for amplification and library preparation with the same primer set, then paired-end sequenced on the Illumina MiSeq platform. Sequence data was submitted to the NCBI SRA under BioProject PRJNA483963.

Flow cytometry

Samples for flow cytometry were stored in the dark at 4 °C until fixation, and fixed to a 4 % final concentration of formaldehyde within 12 hours of sampling. Fixed samples were stained with SybrGreen I (Molecular Probes), spiked with a known concentration of TrueCount beads, and quantified on a Sysmex CyFlow Space flow cytometer. Bacterial cell counts were determined from populations identified from the forward scatter (FSC) and 488 nm (FL1) parameters using a self-organizing map (SOM) following Bowman *et al.* (2017).

Bioinformatics

Illumina MiSeq reads were demultiplexed using the 'iu-demultiplex' command in IlluminaUtils (Eren *et al.*, 2013). Demultiplexed reads were quality-controlled and denoised using the 'FilterandTrim' and 'dada' commands within the R package dada2 (Callahan *et al.*, 2016), and assembled with the 'mergePairs' command. The final, merged reads all had mean quality scores >= 30. The R package dada2 is designed to denoise 16S rRNA amplicon data such that the final "unique" reads reflect true biological diversity. The non-redundant fasta files of unique reads produced by dada2 were made redundant using a custom Python script, and used as input for the paprica pipeline for microbial community structure and metabolic inference (Bowman and Ducklow, 2015). The paprica method for determining microbial community structure differs from most methods in that it relies on the placement of reads on a phylogenetic tree created from the 16S rRNA gene reads from all completed bacterial and archaeal genomes in Genbank. Because the metabolic potential of each phylogenetic edge on the reference tree is known, a reasonable estimate of gene content can be made for the organism of origin for each read. Edge abundance data were normalized to predict 16S rRNA gene copy number prior to

statistical analysis. Sequence reads and accompanying metadata were uploaded to NCBI SRA under BioProjectPRJNA483963.

Phylogenetic placement of some phylotypes of interest yielded relatively low posterior probabilities. The identify of these phylotypes were further investigated with the Ribosomal Database Project's Bayesian classifier (Wang *et al.*, 2007) and blastn megablast (Altschul *et al.*, 1990) against the NCBI 16S rRNA gene database.

Statistical analysis

The count matrix generated from the 16S rRNA gene read data was used in combination with phyloxml-format phylogenetic trees generated by Guppy (Matsen *et al.*, 2011) as implemented by paprica, along with the Archaeopteryx visualization software tool (Han and Zmasek, 2009), to identify the phylogeny of observed microbial taxa. All statistical analysis was carried out in R (R Core Team, 2012) and RStudio (RStudio Team, 2016). The R package vegan (Oksanen *et al.*, 2013) was used to examine microbial community ecology by generating a dendrogram from the count matrix via dissimilarity analysis with hierarchical clustering based on Bray-Curtis dissimilarity index. The R package clustsig and the similarity profile analysis (SIMPROF) tool were used to determine the number of significant sample clusters based on gene content using the 'hclust' function, assuming no predetermined clustering (Yoshioka, 2008; Clarke *et al.*, 2008). These results were utilized in conjunction with the gplots package and its 'heatmap.2' function to generate all abundance heatmaps (Warnes *et al.*, 2016).

Principal component analysis (PCA) was performed using the 'prcomp' function. We used the R package DESeq2 (Love *et al*., 2014) to identify phylogenetic edges or clades that

were differentially present between eelgrass present and eelgrass absent sites, and between sites inside and outside of San Diego Bay. DESeq2 performs differential abundance analysis based on the negative binomial/Gamma-Poisson distribution. We applied the default analysis which uses estimation of size factors with the "median ratio method" described in Anders and Huber (2010), followed by estimation of dispersion. Next, the Wald test for generalized linear model coefficients was used to test for significance of coefficients, taking into account the size factors and dispersion estimates that were previously calculated. The DESeq2 'results' ('res') function, with a p-value set to 0.05, allowed for testing of the null hypothesis: sampling site has no effect on which microbial taxa are present based on sampling location or eelgrass presence. This process was repeated for the domain Archaea. After the most abundant, differentially present bacterial taxa were identified, we determined which of these taxa had potential pathogens in their lower-order clades. To determine if specific clades contained bacterial strains of pathogenic bacteria, we utilized the National Institutes of Health (NIH)- National Center for Biotechnology Information (NCBI) Pathogen Detection Isolates Browser, which includes bacterial pathogen genomic sequences sourced from food, hospital patients, and the environment, as well as a literature search. Clades were labeled as potentially pathogenic if the strain itself or members of the taxonomic clade have been previously identified as being pathogenic to animals or humans.

The high nucleic acid (HNA) content, low nucleic acid (LNA) content, and total bacterial abundance were used to test for a correlation between eelgrass presence/absence and marine bacterial abundance in our samples. A mixed-effects modeling approach was applied to evaluate differences in HNA, LNA, and total bacterial abundance between sites. The *lme*4 package (Bates *et al*., 2012) was used to construct linear mixed effects models to determine and understand the interaction between eelgrass presence, bacterial abundance, and HNA and LNA content in the

collected samples. For the mixed effects models, the fixed effect was "eelgrass" (present vs. absent). The random effect was an intercept for sample site location, in conjunction with by-location random slopes for the effect of eelgrass presence. Random slope models were generated to observe how the effect of eelgrass presence differed between locations (*i.e.* inside bay or outside bay). Significance was determined by p-values generated with likelihood ratio tests (ANOVA) against the full mixed effects models.

RESULTS

Differentially Present Bacterial and Archaeal Clades and Potential Pathogenicity

We determined significant differences in the microbial community structure across sample location and seagrass presence or absence through use of 16s rRNA sequencing, flow cytometry, and statistical analysis. SIMPROF clustering identified 9 significant sample clusters, each cluster having similar overall bacterial community composition among its members (Figure 2, Figure 3).

The taxa identified using the 'results' function in DESeq2 as having a p-value < 0.05 were determined to be differentially present between the inner and outer bay samples and between eelgrass present and absent sites. From our list of 416 bacterial clades ids, 127 differentially present bacterial clades were identified between the inner and outer bay sites (Figure 2). From these 127 clades, we focused on the top 30 taxa ranked by abundance (Figure 3, Table 1). In the outer bay, bacterial taxa including *Fluviicola taffensis, Pseudohongiella spirulinae* KCTC 32221*, Sulfitobacter, Marinobacter, Pelagibacter ubique* HTCC 1062, Family *Fimbriimonadaceae*, *Formosa sp.* Hel3 A148*,* Cyanobacteria, *Thioglobus singularis* PS1, and *Planktomarina temperata* RCA23 were found in distinctly higher abundance in comparison to

the inner bay samples (Figure 2, Table 1). In comparison, bacterial taxa found in greater abundance in samples from the inner bay include *Martelella endophytica* YC6887, *Polaribacter vadi*, Genus *Oxynema*, *Pelagibacter* sp. IMCC9063, and Order Rickettsiales (Figure 2, Table 1). Both within and outside of the bay, there were minor but significant differences in taxa identified between paired sites. Using DESeq2, we identified 13 differentially present bacterial clades between the eelgrass-present and eelgrass-absent sites ($p<0.05$) (Table 2). Taxa found in greater abundance in samples collected at eelgrass present sites include Family *Rhodobacteraceae*, Candidatus *Puniceispirillum marinum* IMCC1322, *Halioglobus pacificus* RR3-57, *Teredinibacter turnerae* T7901, Genus *Colwellia, Thioglobus singularis* PS1, Genus *Tenacibaculum*, *Fluviicola taffensis*, *Synechococcus sp.* CC9311, and Phylum Cyanobacteria (Table 2). In comparison, bacterial taxa found in greater abundance at eelgrass absent sites include *Halobacteriovorax marinus* SJ, *Oscillatoria acuminata* PCC 6304, and Candidatus *Promineofilum breve* Cfx-K (Table 2).

To further examine and explain variation in the microbial structure of each sample due to the influence of eelgrass presence and sampling location, we conducted a PCA. The first principal component (PC1) accounted for 78.6 % of the variance between samples, and the second principal component (PC2) accounted for 14.5 % of variance (Figure 4). The distribution of samples in PC1 is strongly dependent on sample location (inside vs. outside of the bay) (Student's t-test, p approaches 0). We therefore interpret PC1 as a good indicator of sample location. PC2 distribution is largely the result of select bacterial taxa including *Rhodoluna lacicola* MWH-Ta8, Phylum Actinobacteria, and *Tropheryma whipplei* - which reach the greatest abundance in sample 16, a clear outlier from an eelgrass-absent site inside the bay.

Of the 63 archaeal clades identified across the 45 samples, we identified 5 as differentially present between inner and outer bay sampling sites (Table 3). None of these differentially present archaeal clades have known pathogenic members (Table 3). We then identified 5 archaeal clades as differentially abundant between eelgrass present vs. absent sites (Table 4). As for the archaea that were differentially abundant between eelgrass present and absent sites, none have known pathogenic members (Table 4).

We then utilized flow cytometry data and determined whether there was a significant ($p<0.05$) correlation between eelgrass presence and marine bacterial abundance in our samples. Specifically, we conducted random intercepts-random slopes models to examine whether there was a significant correlation between eelgrass presence and bacterial abundance, with random intercepts for the effect of sample site location, in conjunction with by-location random slopes for the effect of eelgrass presence. When comparing the random intercept-random slope HNA model to the null model in a likelihood ratio test (ANOVA), we did not find evidence that eelgrass presence affected HNA abundance ($\chi^2(1)= 2.42$, $p=0.1198$) between seagrass present and absent sites. Similarly, when comparing the random intercept-random slope LNA model to the null model with an ANOVA, we observed that eelgrass presence did not significantly affect LNA abundance ($\chi^2(1)= 2.81$, $p=0.09393$) between seagrass present an absent sites. In terms of overall bacterial abundance, when comparing the random intercept-random slope abundance model with to the null model with an ANOVA, we could not conclude that eelgrass presence significantly affected overall bacterial abundance between eelgrass present vs. absent sites ($\chi^2(1)= 2.91$, $p=0.08799$).

DISCUSSION

In this study, we utilized 16s rRNA sequencing data to examine the microbial structure of sites located within and just outside the San Diego Bay, at sites where eelgrass beds are either present or absent. For these samples, we determined that while eelgrass presence does have some influence on microbial structure of surrounding waters, site location (inside vs. outside of San Diego Bay) exerts a stronger influence on microbial community structure. While pathogenic bacteria were primarily present at collection sites within the bay, there is little variation in pathogen load between eelgrass present vs. absent sites. This suggests that the presence of eelgrass did not significantly reduce pathogen load at this sites, however, caution should be taken in extrapolating these findings to other areas. This study made use of a limited number of samples from two sample locations, and our findings could be influenced by a variety of external factors including sampling depth or the short distance between eelgrass present and absent sites.

Regardless of the presence or absence of eelgrass, potentially pathogenic bacteria were relatively more abundant in samples taken from inside than outside of the bay. Among the 30 most abundant and differentially present bacterial clades (p-value <0.05), Order Rickettsiales (Paddock *et al.*, 2003; Nicholson *et al.*, 2010; Kang *et al*., 2014), Family *Flavobacteriaceae* (Touchon *et al.,* 2011; McBride, 2014; Småge *et al*., 2016), and Order Pseudomonadales were identified as possible pathogenic lineages (Figure 3, Table 1).

Order Rickettsiales was found predominantly in samples collected from within the San Diego Bay, without regard to eelgrass presence or absence. While phylogenetic placement could not identify a specific strain, this order is of interest as it includes both human and animal pathogens such as *Anaplasma phagocytophilum, Ehrlichia canis*, *Ehrlichia chaffeensis, Ehrlichia ewingii, Rickettsia rickettsii, Rickettsia typhi, Rickettsia heilongjiangensis,* etc. - many

of which are transmitted via a tick vector (Schaechter *et al.*, 1993; Paddock *et al.*, 2003; Ando *et al.*, 2010; Nicholson *et al.*, 2010; Kang *et al*., 2014).

A. phagocytophilum is transmitted by the *Ixodes* spp. ticks and sometimes by rodents; infection in dogs *i.e.* anaplasmosis can be recognized by symptoms including fever, depression, myalgia, decreased blood platelet count, and anorexia (Nicholson *et al.*, 2010). *A. phagocytophilum* infection in humans presents itself similarly to dogs with fever, myalgia, decreased blood platelet count, lethargy, and headaches. Disease caused by *E. chaffeensis* is called human monocytic ehrlichiosis (HME) and once transmitted from tick to human, symptoms like fever, back pain, myalgia, headache, and gastrointestinal issues may occur (Paddock *et al.*, 2003). Infection from either *E. canis* or *E. ewingii* causes other forms of ehrlichiosis with similar flu-like symptoms to *E. chaffeensis* (Nicholson *et al.*, 2010). *R. rickettsii*, transmitted from tick to either human or canine, causes Rocky Mountain spotted fever (RMSF). RMSF can cause fatalities and severe disease with fever, severe headache, and maculopapular rash symptoms in humans and fever, lethargy, and anorexia symptoms in dogs (Nicholson *et al.*, 2010). *R. typhi* transmitted to humans from ticks and fleas from bites or feces on human skin causes Murine typhus. This infection can cause headache, fever, and infection of major organs including the brain, lungs, liver, kidney, or heart (Schaechter *et al.*, 1993). *R. heilongjiangensis,* transmitted to humans by tick, can cause spotted fever rickettsiosis (Ando *et al.*. 2010). Fever, rash, and malaise may occur. (Faccini-Martinez *et al*. 2014)

Analysis of Rickettsiales reads by blastn megablast showed close sequence homology with members of the genus *Ehrlichia* (E-value of 2×10^{-28}, 76 % identity across the alignment region). A sharp difference in abundance of the Rickettsiales clades was observed between inner

and outer bay sample collection sites (Figure 3), with minor variation between eelgrass present and absent areas.

Family *Flavobacteriaceae* (map id= 0.87, post-prob = 0.72), was found somewhat evenly in samples collected either within or outside of the bay as well as in eelgrass present or absent sites (Figure 3). Pathogens of this taxonomic family include fish pathogens *Flavobacterium branchiophilum* and *Flavobacterium columnare*, which are associated with gill disease, and human pathogens such as *Elizabethkingia meningoseptica* (McBride, 2014). *E. meningoseptica* is an multi-drug resistant (MDR) opportunistic pathogen of both humans and animals, with a mechanism of transmission that is not completely understood. Infections can range from meningitis, pneumonia, endocarditis, and sepsis to a variety of other tissue and organ infections (Khan, *et al.* 2015).

A phylotype classified as order Pseudomonadales (map id=0.86, post-prob=1.00) was evenly distributed across all sites regardless of condition, suggesting that eelgrass does not influence the presence of this phylotype at local scales. Some pathogenic phylotypes of this order, as determined with the NCBI Pathogen Detection Isolates Browser, include 14 strains of *Acinetobacter baumannii* - PHEA-2, BJAB0868, AB31, 6411, B8300, XH856, AP_882, IEC338SC, YMC2010/8/T346, CA16, SSA3, SSA6, USA15, and HUMV-6483. This MDR pathogen generally can infect mucous membranes or wounds/breaks in the skin and can cause bacteremia potentially leading to sepsis and death (Howard *et al.*, 2012).

In contrast to Lamb *et al*. (2017), we did not find conclusive evidence the eelgrass beds reduce overall microbial contamination in coastal waters. More diverse sampling sites, variation in sampling depth, sites with more stark differences in eelgrass presence, and paired sites that are further apart would also contribute to better determination of eelgrass presence on microbial

structure of surrounding waters. However, we did identify 13 bacterial taxa that were differentially present between eelgrass-present vs. eelgrass-absent sites. While none of these taxa are known human pathogens, some are of interest. *Teredinibacter turnerae* is of use in the medical field as it has exhibited antibiotic activity against both Gram-positive and Gram-negative bacteria including *Bacillus cereus* and *Staphylococcus sciuri* (Trindade-Silva *et al.*, 2009). *Halobacteriovorax marinus* SJ and other members of Genus *Halobacteriovorax* are predatory bacteria that attack Gram-negative bacteria including those of the *Vibrio, E.coli,* and *Pseudomonas* variety (Enos *et al*., 2018). Genus *Tenacibaculum* contains a pathogenic phylotype, *Tenacibaculum maritimum*, which is a known fish pathogen that infects the kidneys and causes symptoms like skin lesions or rotting of the fins (Avendaño-Herrera *et al*., 2006).

While in general eelgrass beds have proven to be fundamental to marine ecosystems in terms of food, shelter, and sediment filtering, their influence on microbial structure of their habitats and pathogen load reduction may be less significant than other factors, including site location, physical processes, and other external factors not accounted for in this experiment. Although we are cautious about extrapolating our findings to other coastal environments, this experiment presents a unique look into the specific microbial structure of the San Diego Bay and its immediately adjacent waters.

Other peripheral contributions of this research include a more current examination of pathogen content in San Diego Bay and the adjacent beach waters, as well as a contrasting view regarding eelgrass influence on the marine microbiome, which may inspire further research to be conducted in this region. Additionally, this research specifically highlights *Z. marina* and *Z. pacifica*'s influence on microbial structure of surrounding waters- although their influence may be smaller in comparison to influence of previously studied eelgrass species. The identification

of taxa present in both the inner and outer bay also introduces the opportunity for further research regarding influence of specific bacterial taxa on marine ecosystem health in the San Diego region.

This thesis has been submitted for publication of the material as it may appear in *Elementa: Science of the Anthropocene*, 2018, Webb, Sahra J.; Rabsatt, Tia; Erazo, Natalia; Bowman, Jeff S., UC Press, 2018. The thesis author was the primary investigator and author of this paper.

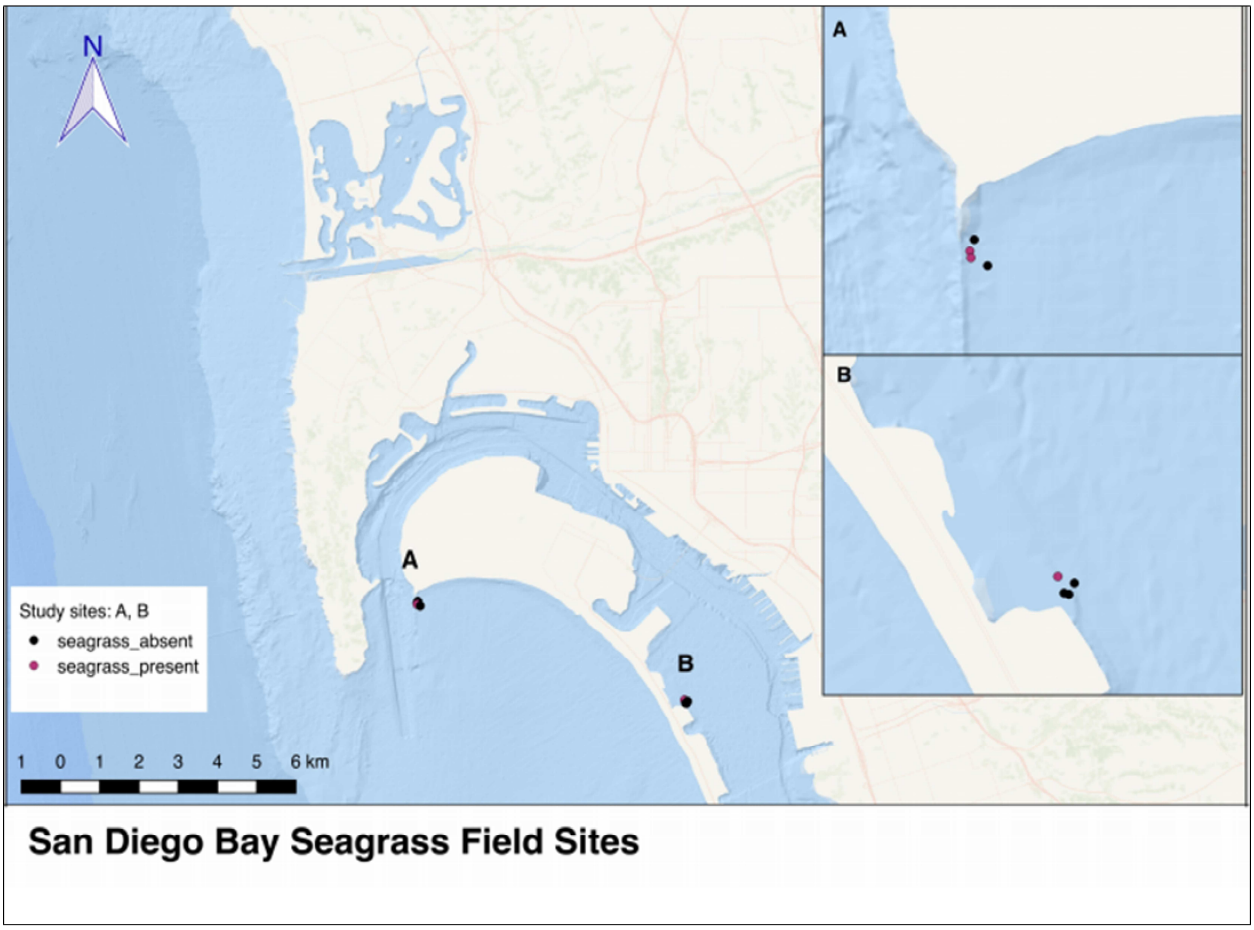
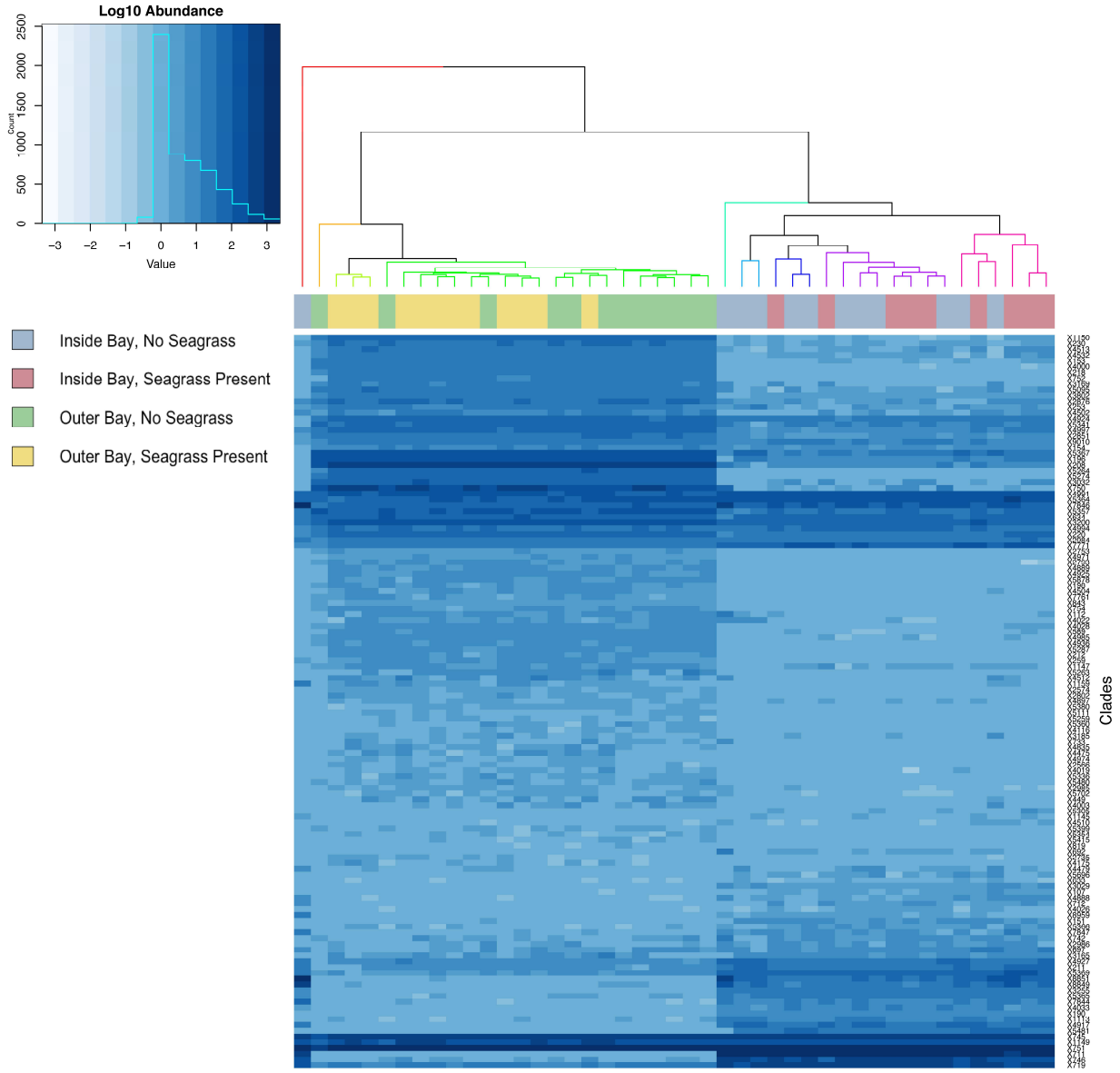


Figure 1: Map of Sampling Sites in San Diego Bay.

Site A (Outer Bay) is located just outside of the San Diego Bay and Site B (Inner Bay) is located inside of the San Diego Bay. Collection sites labeled with a red dot signify sampling areas where eelgrass beds were present and sites labeled with black dots lacked eelgrass beds.

Figure 2: Heat map of relative abundance of microbial taxon with hierarchical clustering of samples.

Taxon abundance is shown on a $\log_{10}$ scale for the 127 differentially present taxa between inner and outer bay sites. Darker blue indicates greater relative abundance of specific taxa. Each color in the cluster dendrogram represents a statistically significant cluster of samples with similar microbial structure. Colors below cluster dendrogram indicate location and eelgrass presence for each sample collected.


Log10 Abundance
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0
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1500
2000
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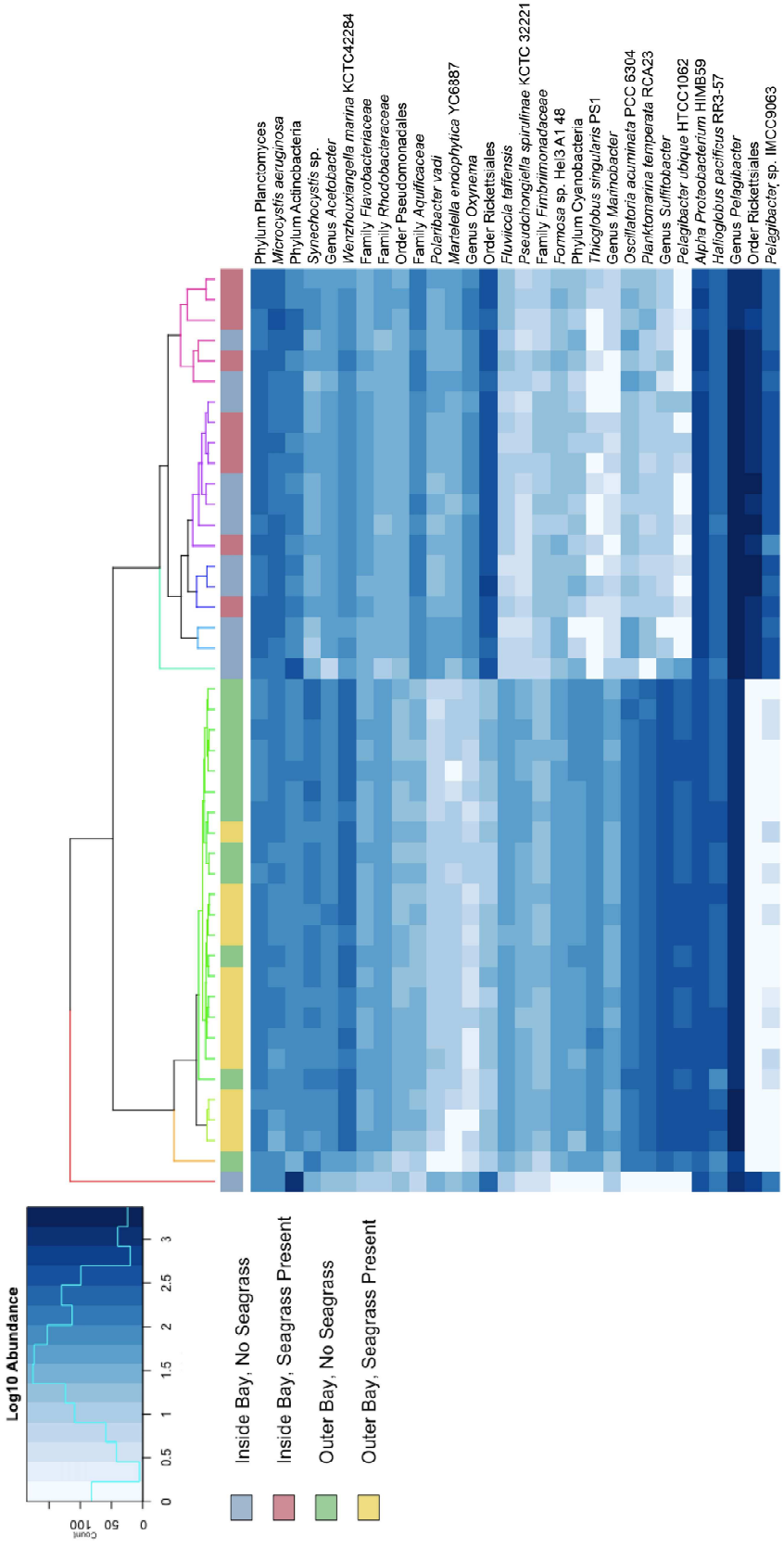
−3
−2
−1
0
1
2
3
Value
Inside Bay, No Seagrass
Inside Bay, Seagrass Present
Outer Bay, No Seagrass
Outer Bay, Seagrass Present
Clades

Figure 3: Heatmap of top thirty most abundant, differentially present bacterial clades between inner and outer bay sites

Taxon abundance is shown on a $\log_{10}$ scale for 45 samples. Darker blue indicates greater relative abundance of a specific taxa. Each color in the cluster dendrogram represents a statistically significant cluster of samples with similar microbial structure. Colors below cluster dendrogram indicate location and eelgrass presence for each sample collected: blue- inside bay, no eelgrass; pink-inside bay, eelgrass present; green- outer bay, no eelgrass; yellow-outer bay, eelgrass present.


Log10 Abundance
0
0.5
1
1.5
2
2.5
3
Count
0
50
100
Inside Bay, No Seagrass
Inside Bay, Seagrass Present
Outer Bay, No Seagrass
Outer Bay, Seagrass Present
Phylum Planctomyces
Microcystis aeruginosa
Phylum Actinobacteria
Synechocystis sp.
Genus Acetobacter
Wenzhouxiangella marina KCTC42284
Family Flavobacteriaceae
Family Rhodobacteraceae
Order Pseudomonadales
Family Aquificaceae
Polaribacter vadi
Martelella endophytica YC6887
Genus Oxynema
Order Rickettsiales
Fluviicola taffensis
Pseudohongiella spirulinae KCTC 32221
Family Fimbriimonadaceae
Formosa sp. Hel3 A1 48
Phylum Cyanobacteria
Thioglobus singularis PS1
Genus Marinobacter
Oscillatoria acuminata PCC 6304
Planktomarina temperata RCA23
Genus Sulfitobacter
Pelagibacter ubique HTCC1062
Alpha Proteobacterium HIMB59
Haliotoglobus pacificus RR3-57
Genus Pelagibacter
Order Rickettsiales
Pelagibacter sp. IMCC9063

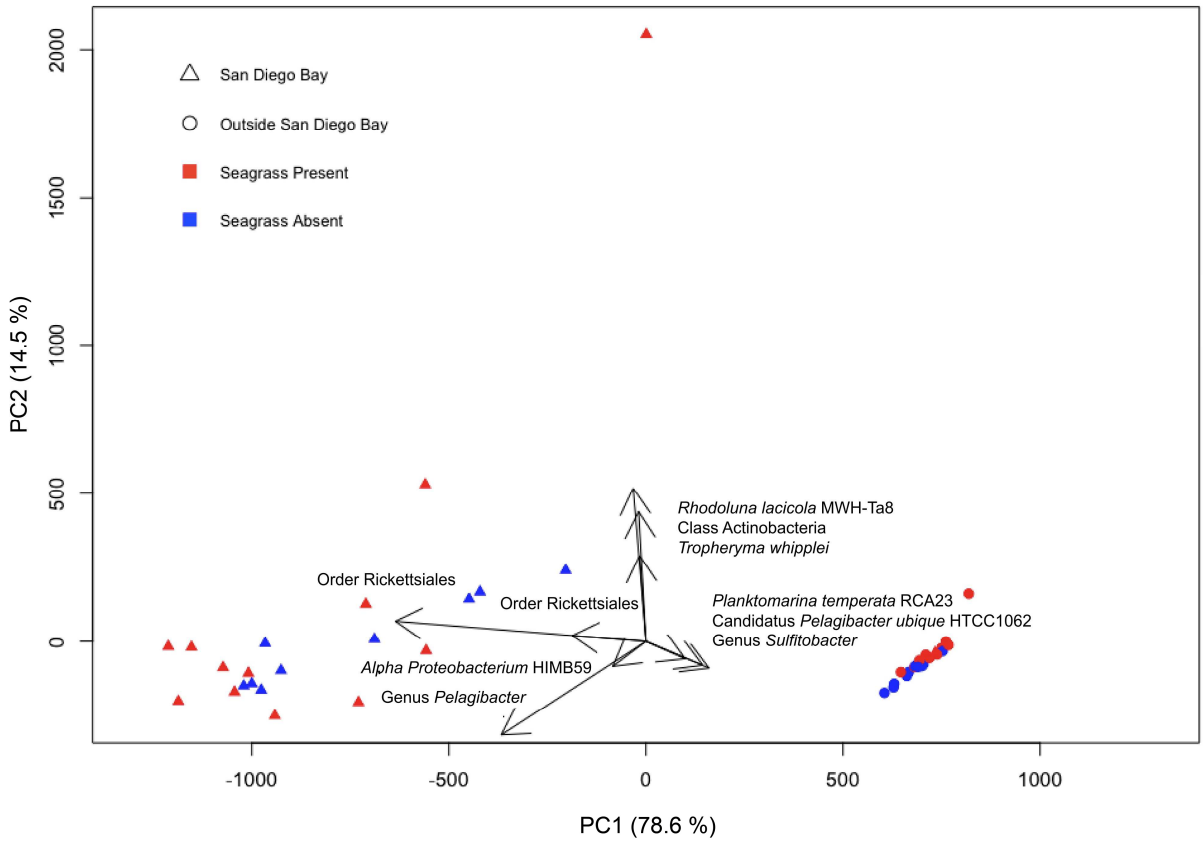


Figure 4: Principal component analysis plot for all bacterial clades.

Distribution of the samples in a space defined by the first two principal components (PC1 and PC2). PC1 accounted for 78.6% of variance, and the samples are distributed in PC1 largely according to sampling location. PC2 accounted for a 14.5% proportion of variance between samples.

Table 1: Most abundant and differentially present bacterial clades between inner and outer San Diego Bay sites

Name	P- Value[a]	Map ID[b]	Posterior Probability[c]	Potential Pathogen	Direction of Enrichment[d]
Order Rickettsiales	0.000	0.71	0.11	Yes	Inner Bay
Genus *Sulfitobacter*	0.000	0.94	0.69	No	Outer Bay
Planktomarina Temperata RCA23	4.122×10^{-306}	0.96	0.66	No	Outer Bay
Order Rickettsiales	4.138×10^{-194}	0.74	0.21	Yes	Inner Bay
Pelagibacter sp. IMCC9063	2.383×10^{-155}	0.86	0.80	No	Inner Bay
Genus *Oxynema*	1.047×10^{-117}	0.82	1.00	No	Inner Bay
Thioglobus singularis PS1	5.044×10^{-93}	0.95	0.98	No	Outer Bay
Family *Aquificaceae*	2.586×10^{-72}	0.64	0.88	No	Inner Bay
Genus *Marinobacter*	1.453×10^{-66}	0.91	1.00	No	Outer Bay
Fluviicola taffensis	1.242×10^{-63}	0.85	0.99	No	Outer Bay
Phylum Planctomyces	8.936×10^{-56}	0.90	0.84	No	Inner Bay
Oscillatoria acuminata PCC 6304	1.302×10^{-53}	0.82	0.33	No	Outer Bay
Wenzhouxiangella marina KCTC 42284	1.806×10^{-48}	0.88	0.94	No	Outer Bay
Pseudohongiella spirulinae KCTC 32221	1.081×10^{-44}	0.90	0.99	No	Outer Bay
Polaribacter vadi	3.070×10^{-35}	0.87	0.61	No	Inner Bay
Formosa sp. Hel3 A1 48	1.357×10^{-33}	0.86	0.98	No	Outer Bay
Martelella endophytica YC6887	3.955×10^{-28}	0.94	0.45	No	Inner Bay
Phylum Cyanobacteria	3.287×10^{-23}	0.78	0.66	No	Outer Bay
Genus *Acetobacter*	1.038×10^{-22}	0.48	0.89	No	Outer Bay
Alpha Proteobacterium HIMB59	9.553×10^{-20}	0.86	0.94	No	Inner Bay
Synechocystis sp.	1.841×10^{-19}	0.85	0.36	No	Outer Bay
Candidatus *Pelagibacter ubique* HTCC1062	1.060×10^{-18}	0.93	0.93	No	Outer Bay
Genus *Pelagibacter*	2.432×10^{-18}	0.84	0.38	No	Inner Bay
Microcystis aeruginosa	4.855×10^{-15}	0.81	0.77	No*	Inner Bay
Family *Rhodobacteraceae*	4.496×10^{-10}	0.95	0.66	No	Outer Bay
Order Pseudomonadales	1.933×10^{-9}	0.86	1.00	Yes	Inner Bay
Family *Fimbriimonadaceae*	1.764×10^{-7}	0.78	0.06	No	Outer Bay
Phylum Actinobacteria	1.655×10^{-6}	0.80	0.61	No	Inner Bay
Halioglobus pacificus RR3-57	9.535×10^{-5}	0.88	0.53	No	Outer Bay
Family *Flavobacteriaceae*	0.077×10^{-1}	0.87	0.72	Yes	Inner Bay

[a] P-value ($p<0.05$) indicates strong evidence against null hypothesis that eelgrass presence and sampling location have no influence on the microbial structure at each of the collection sites.

[b] Map ID indicates the fraction of nucleotide bases in the 16S rRNA targeted amplicon reads that matched the reference clade (average for each clade across all samples).

[c] Posterior probability indicates the statistical likelihood that the phylogenetic placement is correct.

[d] Direction of enrichment indicates the sampling condition in which each taxa was found in greater average abundance.

*Known to form harmful algal blooms (Jacoby *et al*., 2000).

Table 2: Differentially present bacterial clades between seagrass present vs. absent San Diego Bay sites

Name	P- Value[a]	Map ID[b]	Posterior Probability[c]	Potential Pathogen	Direction of Enrichment[d]
Family *Rhodobacteraceae*	0.00441	0.95	0.66	No	EG +
Candidatus *Puniceispirillum marinum* IMCC1322	0.0176	0.90	1	No	EG+
Halioglobus pacificus RR3-57	0.0481	0.88	0.53	No	EG+
Teredinibacter turnerae T7901	0.0110	0.92	0.96	No	EG+
Genus *Colwellia*	0.0212	0.95	0.86	No	EG+
Thioglobus singularis PS1	0.0241	0.95	0.98	No	EG+
Halobacteriovorax marinus SJ	0.0255	0.81	0.92	No	EG-
Genus *Tenacibaculum*	0.0303	0.99	0.60	Yes	EG+
Fluviicola taffensis	0.0258	0.85	0.99	No	EG+
Synechococcus sp. CC9311	0.0345	0.98	0.92	No	EG+
Phylum Cyanobacteria	0.0248	0.77	0.65	No	EG+
Oscillatoria acuminata PCC 6304	0.0139	0.82	0.33	No	EG-
Candidatus *Promineofilum breve* Cfx-K	0.0435	0.78	0.35	No	EG-

[a]P-value ($p<0.05$) indicates strong evidence against null hypothesis that eelgrass presence and sampling location have no influence on the microbial structure at each of the collection sites.

[b]Map ID indicates the fraction of nucleotide bases in the 16S rRNA targeted amplicon reads that matched the reference clade (average for each clade across all samples).

[c]Posterior probability indicates the statistical likelihood that the phylogenetic placement is correct.

[d]Direction of enrichment indicates the sampling condition in which each taxa was found in greater average abundance.

Table 3: Differentially present archaeal clades between inner and outer San Diego Bay sites

Name	P-Value[a]	Map ID[b]	Posterior Probability[c]	Potential Pathogen	Direction of Enrichment[d]
Domain Archaea	0.0006	0.150	0.676	No	Outer Bay
Order Methanococcales	0.002	0.626	0.075	No	Outer Bay
Methanospirillum hungatei JF-1	0.037	0.642	0.074	No	Outer Bay
Methanococcus maripaludis S2	0.046	0.645	0.106	No	Outer Bay
Kingdom Proteoarchaeota-TACK	0.047	0.665	0.076	No	Outer Bay

[a] P-value ($p<0.05$) indicates strong evidence against null hypothesis that eelgrass presence and sampling location have no influence on the microbial structure at each of the collection sites.

[b] Map ID indicates the fraction of nucleotide bases in the 16S rRNA targeted amplicon reads that matched the reference clade (average for each clade across all samples).

[c] Posterior probability indicates the statistical likelihood that the phylogenetic placement is correct.

[d] Direction of enrichment indicates the sampling condition in which each taxa was found in greater average abundance.

Table 4: Differentially present archaeal clades between seagrass present vs. absent San Diego Bay sites

Name	P-Value[a]	Map ID[b]	Posterior Probability [c]	Potential Pathogen	Direction of Enrichment
Domain Archaea	0.0006	0.150	0.676	No	SG-
Order Methanococcales	0.002	0.626	0.075	No	SG-
Methanospirillum hungatei JF-1	0.037	0.642	0.074	No	SG+
Genus Ferroplasma	0.046	0.585	0.47	No	SG-
Kingdom Proteoarchaeota-TACK	0.047	0.665	0.076	No	SG-

[a] P-value ($p<0.05$) indicates strong evidence against null hypothesis that eelgrass presence and sampling location have no influence on the microbial structure at each of the collection sites.

[b] Map ID indicates the fraction of nucleotide bases in the 16S rRNA targeted amplicon reads that matched the reference clade (average for each clade across all samples).

[c] Posterior probability indicates the statistical likelihood that the phylogenetic placement is correct.

[d] Direction of enrichment indicates the sampling condition in which each taxa was found in greater average abundance.

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