Seagrass Microbiome Data

Website: https://www.bco-dmo.org/dataset/933635
Data Type: Other Field Results
Version: 1
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Project
» Collaborative Research: The role of a keystone pathogen in the geographic and local-scale ecology of eelgrass decline in the eastern Pacific (Eelgrass disease)

Contributors	Affiliation	Role
Stachowicz, John J.	University of California-Davis (UC Davis)	Principal Investigator, Contact
Newman, Sawyer	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
This dataset includes sample collection information and sequence accessions for 16S rRNA amplicon sequencing of eelgrass leaf and surrounding water column bacteria from 32 eelgrass meadows across latitudes from 55 to 32° N in the Northeastern Pacific during July and August 2019. Sequence Read Archive (SRA) Experiments and BioSamples can be accessed from the NCBI BioProject PRJNA802566 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA802566/) Eelgrass, Zostera marina, is impacted by outbreaks of wasting disease caused by the opportunistic pathogen Labyrinthula zosterae. We investigated how Z. marina phyllosphere microbial communities vary with rising wasting disease lesion prevalence and severity relative to plant and meadow characteristics like shoot density, longest leaf length, and temperature across 23° latitude in the Northeastern Pacific. We sampled 32 eelgrass meadows across latitudes from 55 to 32° N in the Northeastern Pacific during July and August 2019. This range included six regions (AK=Alaska, BC=British Columbia, WA=Washington, OR=Oregon, BB=Bodega Bay Northern California, SD=San Diego Southern California), with 5–6 meadows per region. The location of each region is AK: N 55° 32' 27.124” W 133° 11' 1.0546, BC: N 51° 48' 30.1469” W 128° 13' 27.2182, WA: N 48° 36' 4.9725” W 122° 59' 56.4203, OR: N 44° 69 43.717” W 124° 89 22.7337, BB: N 38° 14' 30.3218” W 122° 58' 32.5723, SD: N 32° 47' 37.5929” W 117° 12' 57.1071”. We selected eelgrass meadows within each region that had consistently high cover of eelgrass in recent years.

Table of Contents

Coverage

Location: Northeast Pacific Region
Temporal Extent: 2019-06-01 - 2019-08-31

Dataset Description

Results summary (Beatty et al., 2022):

We detected effects of geography (11%) and smaller, but distinct, effects of temperature (30-day max sea surface temperature, 4%) and disease (lesion prevalence, 3%) on microbiome composition.

Declines in alpha diversity on asymptomatic tissue occurred with rising wasting disease prevalence within

meadows. However, no change in microbiome variability (dispersion) was detected between asymptomatic and symptomatic tissues. Further, we identified members of Cellvibrionaceae, Colwelliaceae, and Granulosicoccaceae on asymptomatic tissue that are predictive of wasting disease prevalence across the geographic range (3,100 kilometers). Functional roles of Colwelliaceae and Granulosicoccaceae are not known. Cellvibrionaceae, degraders of plant cellulose, were also enriched in lesions and adjacent green tissue relative to nonlesioned leaves. Cellvibrionaceae may play important roles in disease progression by degrading host tissues or overwhelming plant immune responses. Thus, inclusion of microbiomes in wasting disease studies may improve our ability to understand variable rates of infection, disease progression, and plant survival.

Methods & Sampling

We collected Z. marina tissue from the 3rd-youngest leaf of individual shoots exhibiting wasting disease lesions and, from nearby (within 1 m), a 3rd-youngest leaf free of lesions from a different shoot at haphazard locations along each transect. We selected tissue samples from Z. marina as described below. L. zosterae is an opportunistic endophyte that forms an ectoplasmic net, moving through host tissue by degrading cell walls, with the greatest pathogen density at the leading edge of the infection before tissue browning (17, 61). Thus, we sampled lesion tissue as well as green tissue at the leading edge of lesions to determine how microbiomes may change with lesion development (i.e., effects of “tissue type” on the same leaf). Sampling the leading edge of an infection may allow us to determine early microbial interactors in eelgrass wasting disease versus opportunistic microbes that colonize or increase in abundance following pathogen degradation of host tissue. We also sampled green tissue from a different shoot nearby whose third youngest leaf did not exhibit lesions (lesion-free leaves) to compare these to green tissue at the leading edge of infection from lesioned leaves (effects of “lesion status”). Lesion status allows us to test how wasting disease, identified by the presence of characteristic lesions on young (nonsenescing) leaves, affects the green phyllosphere microbiome adjacent to lesions. To minimize cross-contamination, we wore nitrile or similar gloves and cleaned metal tweezers and scissors with 70% ethanol wipes between each tissue sample. We immediately placed samples into 1.5 mL microcentrifuge tubes containing DNA/RNA shield (Zymo Research Cat. R1100) upon collection.

We also sampled seawater microbial communities to assess whether drivers of eelgrass microbiome structure differ from those of free-living microbial communities in the water column surrounding

eelgrass beds. We collected three bottles of water, using 500 mL sterile bottles (VWR Cat. 76299-562) from 3 haphazardly selected locations within the meadow that were approximately 20

m apart. We kept water samples on ice until filtration within 4–6 h of collection with Nalgene analytical filtration units (Cat. 130-4020), which contained 0.22 mm pore size, 47 mm cellulose nitrate filters. Final volumes of water filtered varied (200–500 mL) due to high turbidity at some sites that reduced the rate of filtration. Upon completion of water filtration, the filter was preserved in 2 mL of DNA/RNA shield (Zymo Research Cat. R1100).

Samples were shipped to the University of California, Davis within 3 weeks of sampling and stored at 280°C until processing. We extracted DNA from our samples with ZymoBIOMICS DNA Microprep Kit (Cat. D4301). Upon thawing, we vortexed the 1.5 mL tubes containing leaf tissue in DNA/RNA shield for 60 s and transferred 500 mL of supernatant to a ZymoBIOMICS bead beating tube. We added 500 mL of ZymoBIOMICS lysis solution to the bead beating tube, vortexed tubes for 20 min on a Vortex-Genie 2 with horizontal microtube holder, and performed DNA extractions for phyllosphere samples according to the manufacturer’s instructions following the bead beating step. To extract DNA from filtered seawater samples, we aseptically cut cellulose nitrate filters into 1–2 mm wide sections, transferred slices plus the 2 mL of DNA/RNA shield used to preserve the filter into two bead beating tubes, and vortexed these on a Vortex-Genie for 20 min. All other DNA extraction steps followed manufacturer instructions, except for final elution of DNA in 40 mL rather than 20 mL of DNase/RNase-free water and further dilution (1 part DNA to 9 parts DNase/RNase-free water) so that DNA concentrations would be similar for both eelgrass tissue and seawater samples, commonly falling between 1 and 10 ng/mL. We processed six negative controls similarly. Four negative controls originated from ZymoBIOMICS DNA/RNA shield and two from sterile cellulose nitrate filters following 100 mL filtration with molecular grade water (Sigma-Aldrich Cat. W4502) preserved in ZymoBIOMICS DNA/RNA shield. Following extraction, negative control samples underwent library prep with all biological samples.

We shipped DNA samples to Dalhousie University's IMR facility for library prep and Illumina MiSeq sequencing according to (92). Briefly, Nextera fusion primers F515 and R926 (93) amplified the V4–V5 region of the 16S rRNA gene with high-fidelity polymerase and 2 mL of template DNA in 25 mL PCR volumes. PCR products from two technical reactions per biological sample were verified with Invitrogen 96-well E-gels. Pooled technical replicates were cleaned and normalized with Invitrogen Sequal-Prep plates. Cleaned and normalized PCR amplicons underwent paired-end

300 bp sequencing on an Illumina MiSeq.

BCO-DMO Processing Description

- Dates converted from %m-%d-%y to %Y-%m-%d format

[table of contents | back to top]

Related Publications

Beatty, D. S., Aoki, L. R., Rappazzo, B., Bergman, C., Domke, L. K., Duffy, J. E., Dubois, K., Eckert, G. L., Gomes, C., Graham, O. J., Harper, L., Harvell, C. D., Hawthorne, T. L., Hessing-Lewis, M., Hovel, K., Monteith, Z. L., Mueller, R. S., Olson, A. M., Prentice, C., Stachowicz, J. J. (2022). Predictable Changes in Eelgrass Microbiomes with Increasing Wasting Disease Prevalence across 23° Latitude in the Northeastern Pacific. MSystems, 7(4). https://doi.org/10.1128/msystems.00224-22
Results

[table of contents | back to top]

Parameters

Parameter	Description	Units
sample_title	Sample number.	unitless
SampleType	Sample type category; eelgrass, water, or negative control.	unitless
RegionName	General region name from which samples were taken.	unitless
SiteCode	Sites labelled A through F within a region. The latitude and longitude values in this dataset correspond with these sites.	unitless
TissueType	If sample type = eelgrass, then can be either green (healthy) or lesioned (diseased). This field does not apply to water and negative control data.	unitless
LesionStatus	If sample type = eelgrass, describes whether leaf from which sample was collected had any lesions on it or not. This field does not apply to water and negative control data.	unitless
collection_date	Date of sample collection.	unitless

LocationName	Local name for each site.	unitless
TidalHeight	U = upper intertidal, L = lower intertidal.	unitless
Transect	Transect number within each site (1 through 6)	unitless
Lat	Latitude of site labelled in SiteCode field in decimal degrees; a positive value indicates a Northern coordinate.	decimal degrees
Long	Longitude of site lablled in the SiteCode field in decimal degrees; a negative value indicates a Western coordinate.	decimal degrees
Run	NCBI Run Number.	unitless
download_path	Weblink for NCBI data access.	unitless
BioProject	NCBI BioProject number.	unitless
Sample	NCBI sample number.	unitless
BioSample	NCBI BioSample number.	unitless
Submission	NCBI submission number.	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	We shipped DNA samples to Dalhousie University's IMR facility for library prep and Illumina MiSeq 300 bp sequencing according to (92).
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

Dataset-specific Instrument Name	ZymoBIOMICS DNA Microprep Kit (Cat. D4301)
Generic Instrument Name	DNA Extractor
Dataset-specific Description	Bacterial DNA was extracted from eelgrass meadow samples with a ZymoBIOMICS DNA Microprep Kit.
Generic Instrument Description	A device that is used to isolate and collect DNA for subsequent molecular analysis.

[table of contents | back to top]

Project Information

Collaborative Research: The role of a keystone pathogen in the geographic and local-scale ecology of eelgrass decline in the eastern Pacific (Eelgrass disease)

Coverage: West coast of North America, from San Diego to Alaska

This project is part of the Marine Global Earth Observatory (MarineGEO), directed by the Smithsonian's Tennenbaum Marine Observatories Network (TMON); a global network of partners focused on understanding how coastal marine ecosystems work—and how to keep them working https://marinegeo.si.edu/

NSF Abstract:

Pathogens may be unrecognized key species in many ecosystems, causing massive impacts on other species and habitats despite the microscopic size of disease-causing organisms. Yet the triggers to disease epidemics likely involve complex interactions among changing environmental conditions and associated biological communities. In the ocean, understanding disease outbreaks has been hindered by inadequate knowledge of how these various influences interact to determine susceptibility and resilience to disease. This project integrates research in community and disease ecology with microbial genomics, geospatial analysis, and state-of-the-art computational approaches toward an unprecedented understanding of the causes and consequences of wasting disease in eelgrass, an important vegetation type supporting coastal and estuarine ecosystems throughout the northern hemisphere. The research advances frontiers in understanding the growing but poorly appreciated threat of marine diseases, how disease ecology interacts with environmental change, and its consequences for the extensive ecosystems and coastal communities that depend on eelgrass, across 23 degrees of latitude along the Pacific coast of North America. The research will inform better management of threatened seagrass ecosystems, which provide important services including fisheries habitat, erosion control, carbon storage, and capture of nutrient runoff. The research will foster integrative approaches in the next generation, including high school students, undergraduates, graduate students, and postdocs working on the project, and each investigator's institution will work to recruit participants from under-represented groups. Best practices developed under this award, including the Eelisa disease app and drone mapping, will be disseminated for broader surveillance of seagrass disease and coastal habitat quality by both professional and citizen scientists in coordination with the Global Ocean Observing System's (GOOS) develpoment of seagrass extent as an Essential Ocean Variable.

The triggers to marine disease epidemics are likely complex, and progress in understanding them has been hindered by a poor understanding of the multifaceted ecological context of the host-disease interaction. This project's overarching goal is to disentangle the web of direct and indirect interactions by which changing climate mediates prevalence of eelgrass wasting disease, and its consequences for threatened but important eelgrass ecosystems. The centerpiece is a comparative, cross-scale survey of eelgrass community composition, microbiome, and disease prevalence along thermal gradients of latitude and exposure to the ocean, providing the first coast-wide picture of disease dynamics in response to environmental change. In situ sampling will be linked to dynamics of eelgrass at landscape scales using unmanned aerial systems (drones) to quantify high-resolution changes in eelgrass extent and habitat quality. Experiments will test how the diverse biological community mediates impacts of the pathogen on eelgrass ecosystems.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1829992

[table of contents | back to top]