Microbial taxa (amplicon sequence variant or ASV) statistical analyses for two seagrass genotypes from wasting disease mesocosm experiments at Bodega Marine Laboratory in July-Sept of 2015

Website: https://www.bco-dmo.org/dataset/883070
Data Type: experimental
Version: 1
Version Date: 2022-10-27

Project
» CAREER: Linking genetic diversity, population density, and disease prevalence in seagrass and oyster ecosystems (Seagrass and Oyster Ecosystems)

Contributors	Affiliation	Role
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Abstract
This dataset includes outputs from statistical analyses of differences in microbial taxa (amplicon sequence variant or ASV) abundance among two groups of seagrass, Zostera marina, genotypes: those that showed reduced Labyrinthula zosterae parasites when warmed vs those that showed increased L. zosterae parasites when warmed; and two seawater temperature treatments: ambient or elevated +3.2oC. Data were collected as part of a mesocosm study at the Bodega Marine Laboratory examining the independent and interactive effects of warming, host genotypic identity, and host genotypic diversity on the prevalence and intensity of infections of seagrass by the wasting disease parasite L. zosterae. These data were published in Schenck et al (2022). Related sequence data from this experiment is accessible from the National Center for Biotechnology Information (NCBI) BioProject PRJNA716355.

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Coverage

Spatial Extent: **Lat**:38.31753 **Lon**:-123.06572
Temporal Extent: 2015-07-01 - 2015-09-14

Methods & Sampling

We used a substitutive design to test the effects of eelgrass (Zostera marina) genotypic identity (eight genotypes), diversity (monocultures of 1 genotype vs. polycultures of 4 genotypes), and temperature (ambient or + 3.2° C) on the prevalence and intensity of *Labyrinthula* over eight weeks in an array of flow-through 120-L mesocosms at the Bodega Marine Laboratory in Bodega Bay, CA. In July 2015, we created ten unique polyculture combinations of four genotypes (4 genotypes per experimental pot) randomly drawn from a pool of eight genotypes; all eight genotypes were also grown in monoculture (1 genotype per pot). We filled pots (8.9 x 8.9 cm) with coarsely sieved sediment collected from Bodega Harbor, and planted 4 shoots per pot, matching the lower range of average field densities reported for Bodega Harbor (Ha and Williams 2018) to allow for growth during the experiment. Plants were originally collected in Bodega Harbor, CA in 2012, confirmed to be unique genotypes using 11 DNA microsatellite loci developed specifically for *Z. marina* (Abbott et al. 2018), and propagated in separate flow through mesocosms at BML.

At the end of the experiment (10 weeks), we collected and preserved the top half of the focal leaf in individual plastic bags sealed with 30 ml of silica (Flower Drying Art Silica Gel; Activa) for subsequent DNA extraction and quantitative PCR to estimate *Labyrinthula zosterae* cells as a proxy for infection (Bergmann et al. 2011, Bockelmann et al. 2013, Groner et al. 2021).

We collected 2 cm of the focal leaf from directly below the midpoint and stored the tissue at -80˚C for later microbiome analyses to assess whether particular leaf microbial taxa changed in association with *Labyrinthula* presence on the focal leaf. From these samples, we selected a subset of 84 leaf microbial samples evenly distributed across temperature treatments and across genotypes in monoculture. We did not assess leaf microbiomes in genotypic polycultures. We extracted the surface community of these leaf segments with a modified protocol for the DNeasy Powersoil Kit (Qiagen) and sequenced the V4-V5 region of the 16S rRNA gene on an Illumina MiSeq to analyze differences in the leaf microbiome between treatments and among genotypes. Specifically, to extract the surface community of leaf segments we used a modified protocol for the DNeasy Powersoil Kit (Qiagen) where we first vortexed leaf samples in 500ul of MilliQ water, and then extracted the supernate of each sample. Extraction success was verified via Qubit dsDNA HS assay kit. We PCR-amplified the V4-V5 region of the 16S rRNA gene and then sequenced pooled barcoded fragments on an Illumina MiSeq through the Integrated Microbiome Resource at Dalhousie University (Halifax, NS; Comeau et al. 2017).

Life Sciences Identifiers (LSID) for taxonomic names:
Zostera marina (urn:lsid:marinespecies.org:taxname:145795)
Labyrinthula zosterae (urn:lsid:marinespecies.org:taxname:395093)
Labyrinthula (urn:lsid:marinespecies.org:taxname:119090)

Data Processing Description

To examine the potential contribution of the microbiome to host-parasite-warming interactions in this system, we ran 16S sequence data through a dada2 pipeline to de-noise sequence data, estimate error rates, identify amplicon sequence variants (ASVs) and remove chimeric sequences (Callahan et al. 2016). We assigned 16S rRNA gene taxonomy with the SILVA database (v. 128). After removing mitochondrial and chloroplast sequences we had 1,432,852 sequences in 3,195 ASVs with a median of 23,200 reads per sample and a minimum of 12,149 reads in a sample.

Analyses were done on unrarefied data. We compared the microbiome of two groups: genotypes that showed reduced parasites when warmed vs those that showed increased parasites when warmed. To identify differences among groups, we computed centered log ratio transformation on ASV counts and examined differences in Euclidian distance among treatments and genotype groups (i.e., the Aitchison distance; Aitchison et al. 2000, Gloor et al. 2017). We computed a permutational ANOVA on group-level differences with adonis2 in the R package ‘vegan 2.5’ (Oksanen et al. 2019). To identify which bacteria of the 2407 ASVs that we observed varied between genotypes with reduced vs. increased parasites when warmed, we built negative binomial models based on the geometric means of ASV counts in DESeq2 (Love et al. 2014). We separately assessed which ASVs varied (1) between treatments and (2) between genotype_group + temperature_treatment + genotype_group:treatment using a Wald test. We then applied a Benjamini-Hochberg correction to all reported p-values. All data analyses were performed in R (version 3.6.1, www.R-project.org).

Code for microbial analyses associated with the experiment: www.github.com/mkardish/Labyrinthula. See "Supplemental Files" section of this page for a zip package of commit 50d4b3f.

BCO-DMO Processing:

- Imported data from source file "Mesocosm_microbial_analyses_results.csv" into the BCO-DMO data system. Data file imported using missing data identifier "NA".
- Modified parameter (column) names to conform with BCO-DMO naming conventions.
- The repository https://github.com/mkardish/Labyrinthula (commit 50d4b3f) was forked to BCO-DMO Github Organization for preservation purposes. A release of the code was created, and the zip package was attached to this dataset as a supplemental file.

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Data Files

File
mesocosm-analyses.csv (Comma Separated Values (.csv), 51.33 KB) MD5:9b3e60bb8a43bd916f37f33115722489 Primary data file for dataset ID 883070

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Supplemental Files

File
Github respository: Labyrinthula filename: Labyrinthula-883070_1.zip (ZIP Archive (ZIP), 9.38 KB) MD5:c74f12166ebe5baf84c0f1609185d7b5 R code for microbial community analysis accompanying Schenck, DuBois, Kardish, Stachowicz and Hughes 2021. This zip file contains the contents of https://github.com/mkardish/Labyrinthula (commit 50d4b3f). This repository https://github.com/mkardish/Labyrinthula (commit 50d4b3f) was forked to BCO-DMO for preservation purposes.

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Related Publications

Abbott, J. M., DuBois, K., Grosberg, R. K., Williams, S. L., & Stachowicz, J. J. (2018). Genetic distance predicts trait differentiation at the subpopulation but not the individual level in eelgrass, Zostera marina. Ecology and Evolution, 8(15), 7476–7489. Portico. https://doi.org/10.1002/ece3.4260
Methods

Aitchison, J., Barceló-Vidal, C., Martín-Fernández, J. A., & Pawlowsky-Glahn, V. (2000). Mathematical Geology, 32(3), 271–275. https://doi.org/10.1023/a:1007529726302 https://doi.org/10.1023/A:1007529726302
Methods

Bergmann, N., Fricke, B., Schmidt, M. C., Tams, V., Beijing, K., Schwitte, H., Boettcher, A. A., Martin, D. L., Bockelmann, A.-L., Reusch, T. B. H., Rauch, G. (2011). A quantitative real-time polymerase chain reaction assay for the seagrass pathogen Labyrinthula zosterae. Molecular Ecology Resources, 11, 1076-1081.
Methods

Bockelmann, A.-C., Tams, V., Ploog, J., Schubert, P. R., Reusch, T. B. H. (2013). Quantitative PCR reveals strong spatial and temporal variation of the wasting disease pathogen, Labyrinthula zosterae in northern European eelgrass (Zostera marina) beds. PLoS ONE, 8(5), e62169.
Methods

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7), 581–583. doi:10.1038/nmeth.3869
Methods

Comeau, A. M., Douglas, G. M., & Langille, M. G. I. (2017). Microbiome Helper: a Custom and Streamlined Workflow for Microbiome Research. MSystems, 2(1). https://doi.org/10.1128/msystems.00127-16 https://doi.org/10.1128/mSystems.00127-16
Methods

Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., & Egozcue, J. J. (2017). Microbiome Datasets Are Compositional: And This Is Not Optional. Frontiers in Microbiology, 8. https://doi.org/10.3389/fmicb.2017.02224
Methods

Groner, M., Burge, C., Kim, C., Rees, E., Van Alstyne, K., Yang, S., Wyllie-Echeverria, S., & Harvell, C. (2016). Plant characteristics associated with widespread variation in eelgrass wasting disease. Diseases of Aquatic Organisms, 118(2), 159–168. https://doi.org/10.3354/dao02962
Methods

Ha, G., & Williams, S. L. (2018). Eelgrass community dominated by native omnivores in Bodega Bay, California, USA. Bulletin of Marine Science, 94(4), 1333–1353. https://doi.org/10.5343/bms.2017.1091
Methods

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology, 15(12). doi:10.1186/s13059-014-0550-8
Methods

Oksanen, J., F. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. Minchin, R. O'Hara, G. Simpson, P. Solymos, M. Stevens, E. Szoecs, H. Wagner (2019). Vegan: Community ecology package. R Package Version 2.5-6. Available from https://CRAN.R-project.org/package=vegan.
Software

R Core Team (2019). R: A language and environment for statistical computing. R v3.6.1. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/
Software

Schenck, F. R., DuBois, K., Kardish, M. R., Stachowicz, J. J., & Hughes, A. R. (2023). The effect of warming on seagrass wasting disease depends on host genotypic identity and diversity. Ecology, 104(3). Portico. https://doi.org/10.1002/ecy.3959
Results

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Related Datasets

IsRelatedTo

Schenck, F., DuBois, K., Kardish, M., Stachowicz, J. J., Hughes, A. R. (2022) **Quantitative PCR cell count estimates from samples of DNA extracted from seagrass wasting disease parasite, Labyrinthula zosterae from wasting disease mesocosm experiments at Bodega Marine Laboratory in July-Sept of 2015.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-10-27 doi:10.26008/1912/bco-dmo.883055.1 [view at BCO-DMO]
Relationship Description: Data collected as part of the same experiment.

Schenck, F., DuBois, K., Kardish, M., Stachowicz, J. J., Hughes, A. R. (2022) **Seagrass metrics from from seagrass wasting disease mesocosm experiments conducted at Bodega Marine Laboratory from July-September 2015.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-10-06 doi:10.26008/1912/bco-dmo.879749.1 [view at BCO-DMO]
Relationship Description: Data collected as part of the same experiment.

Schenck, F., DuBois, K., Kardish, M., Stachowicz, J. J., Hughes, A. R. (2022) **Temperature from seagrass wasting disease mesocosm experiments at Bodega Marine Laboratory in June-July 2015.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-10-27 doi:10.26008/1912/bco-dmo.883037.1 [view at BCO-DMO]
Relationship Description: Data collected as part of the same experiment.

UC Davis. Interactions of host disease susceptibility, warming, and microbiome response. (2021). In: NCBI:BioProject: PRJNA716355. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; Available from: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA716355. https://www.ncbi.nlm.nih.gov/bioproject/PRJNA716355

References

Max Planck Institute for Marine Microbiology and Jacobs University. (2016). Release information of the SILVA SSU and LSU databases 128 as of Sept 29, 2016. The SILVA ribosomal RNA database project. Available from https://www.arb-silva.de/documentation/release-128/

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Parameters

Parameter	Description	Units
Sequence	Amplicon Sequence Variant (ASV) unique DNA base pair sequence of the V4-V5 region of the 16S rRNA gene; A = adenine; C = cytosine; G = guanine; T = thymine	unitless
Kingdom	ASV kingdom taxa assigned with the SILVA database (v. 128)	unitless
Phylum	ASV Phylum taxa assigned with the SILVA database (v. 128)	unitless
Class	ASV class taxa assigned with the SILVA database (v. 128)	unitless
Order	ASV order taxa assigned with the SILVA database (v. 128)	unitless
Family	ASV family taxa assigned with the SILVA database (v. 128)	unitless
Genus	ASV genus taxa assigned with the SILVA database (v. 128)	unitless
Species	ASV species taxa assigned with the SILVA database (v. 128)	unitless
baseMean	Average of the normalized ASV count values; dividing by size factors; taken over all samples	unitless
log2FoldChange_comp1	Differences in ASV abundance between genotypes that showed different responses of L. zosterae intensity to warming in elevated temperature treatment; positive change indicates greater ASV abundance in genotypes that showed increased L. zosterae intensity under warming; negative change indicates greater ASV abundance in genotypes that showed decreased L. zosterae intensity under warming; reported on a logarithmic scale to base 2	unitless
lfcSE_comp1	The standard error estimate for the log2 fold change estimate of ASV abundance between genotypes that showed increased L. zosterae intensity under warming and genotypes that showed decreased L. zosterae intensity under warming in elevated temperature treatment	unitless
padj_comp1	The P-value for difference in ASV abundance between genotypes that showed increased L. zosterae intensity under warming and genotypes that showed decreased L. zosterae intensity under warming in elevated temperature treatment adjusted for multiple testing using a Benjamini-Hochberg correction	unitless
log2FoldChange_comp2	Differences in ASV abundance between genotypes that showed different responses of L. zosterae intensity to warming in ambient temperature treatment; positive change indicates greater ASV abundance in genotypes that showed increased L. zosterae intensity under warming; negative change indicates greater ASV abundance in genotypes that showed decreased L. zosterae intensity under warming; reported on a logarithmic scale to base 2	unitless
lfcSE_comp2	The standard error estimate for the log2 fold change estimate of ASV abundance between genotypes that showed increased L. zosterae intensity under warming and genotypes that showed decreased L. zosterae intensity under warming in ambient temperature treatment	unitless

padj_comp2	The P-value for difference in ASV abundance between genotypes that showed increased L. zosterae intensity under warming and genotypes that showed decreased L. zosterae intensity under warming in ambient temperature treatment adjusted for multiple testing using a Benjamini-Hochberg correction	unitless
log2FoldChange_comp3	Differences in ASV abundance between temperature treatments in genotypes that showed increased L. zosterae intensity under warming; positive change indicates greater ASV abundance at elevated temperature; negative change indicates greater ASV abundance at ambient temperature; reported on a logarithmic scale to base 2	unitless
lfcSE_comp3	The standard error estimate for the log2 fold change estimate of ASV abundance between temperature treatments in genotypes that showed increased L. zosterae intensity under warming	unitless
padj_comp3	The P-value for difference in ASV abundance between temperature treatments in genotypes that showed increased L. zosterae intensity under warming adjusted for multiple testing using a Benjamini-Hochberg correction	unitless
log2FoldChange_comp4	Differences in ASV abundance between temperature treatments in genotypes that showed decreased L. zosterae intensity under warming; positive change indicates greater ASV abundance at elevated temperature; negative change indicates greater ASV abundance at ambient temperature; reported on a logarithmic scale to base 2	unitless
lfcSE_comp4	The standard error estimate for the log2 fold change estimate of ASV abundance between temperature treatments in genotypes that showed decreased L. zosterae intensity under warming	unitless
padj_comp4	The P-value for difference in ASV abundance between temperature treatments in genotypes that showed decreased L. zosterae intensity under warming adjusted for multiple testing using a Benjamini-Hochberg correction	unitless
log2FoldChange	Differences in ASV abundance between temperature treatments; positive change indicates greater ASV abundance at elevated temperature; negative change indicates greater ASV abundance at ambient temperature; reported on a logarithmic scale to base 2	unitless
lfcSE	The standard error estimate for the log2 fold change estimate of ASV abundance between temperature treatments	unitless
padj	The P-value for difference in ASV abundance between temperature adjusted for multiple testing using a Benjamini-Hochberg correction	unitless

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Instruments

Dataset-specific Instrument Name	flow through tanks
Generic Instrument Name	Aquarium
Generic Instrument Description	Aquarium - a vivarium consisting of at least one transparent side in which water-dwelling plants or animals are kept

Dataset-specific Instrument Name	Illumina MiSeq
Generic Instrument Name	Automated DNA Sequencer
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.

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Project Information

CAREER: Linking genetic diversity, population density, and disease prevalence in seagrass and oyster ecosystems (Seagrass and Oyster Ecosystems)

Coverage: Coastal New England

NSF Award Abstract:
Disease outbreaks in the ocean are increasing, causing losses of ecologically important marine species, but the factors contributing to these outbreaks are not well understood. This 5-year CAREER project will study disease prevalence and intensity in two marine foundation species - the seagrass Zostera marina and the Eastern oyster Crassostrea virginica. More specifically, host-disease relationships will be explored to understand how genetic diversity and population density of the host species impacts disease transmission and risk. This work will pair large-scale experimental restorations and smaller-scale field experiments to examine disease-host relationships across multiple spatial scales. Comparisons of patterns and mechanisms across the two coastal systems will provide an important first step towards identifying generalities in the diversity-density-disease relationship. To enhance the broader impacts and utility of this work, the experiments will be conducted in collaboration with restoration practitioners and guided by knowledge ascertained from key stakeholder groups. The project will support the development of an early career female researcher and multiple graduate and undergraduate students. Students will be trained in state-of-the-art molecular techniques to quantify oyster and seagrass parasites. Key findings from the surveys and experimental work will be incorporated into undergraduate courses focused on Conservation Biology, Marine Biology, and Disease Ecology. Finally, students in these courses will help develop social-ecological surveys and mutual learning games to stimulate knowledge transfer with stakeholders through a series of workshops.

The relationship between host genetic diversity and disease dynamics is complex. In some cases, known as a dilution effect, diversity reduces disease transmission and risk. However, the opposite relationship, known as the amplification effect, can also occur when diversity increases the risk of infection. Even if diversity directly reduces disease risk, simultaneous positive effects of diversity on host density could lead to amplification by increasing disease transmission between infected and uninfected individuals. Large-scale field restorations of seagrasses (Zostera marina) and oysters (Crassostrea virginica) will be utilized to test the effects of host genetic diversity on host population density and disease prevalence/intensity. Additional field experiments independently manipulating host genetic diversity and density will examine the mechanisms leading to dilution or amplification. Conducting similar manipulations in two marine foundation species - one a clonal plant and the other a non-clonal animal - will help identify commonalities in the diversity-density-disease relationship. Further, collaborations among project scientists, students, and stakeholders will enhance interdisciplinary training and help facilitate the exchange of information to improve management and restoration efforts. As part of these efforts, targeted surveys will be used to document the perceptions and attitudes of managers and restoration practitioners regarding genetic diversity and its role in ecological resilience and restoration.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1652320

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