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

Website: https://www.bco-dmo.org/dataset/883055
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
Hughes, A. Randall	Northeastern University	Principal Investigator
DuBois, Katherine	University of California-Davis (UC Davis)	Scientist
Kardish, Melissa	University of California-Davis (UC Davis)	Scientist
Schenck, Forest	Northeastern University	Scientist
Stachowicz, Jay	University of California-Davis (UC Davis)	Scientist
York, Amber D.	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
This data set includes quantitative PCR cell count estimates from samples of DNA extracted from seagrass wasting disease parasite, Labyrinthula zosterae, cultures of known cell concentrations run with and without DNA extracted from seagrass, Zostera marina, tissue samples to test for quantitative PCR signal inhibition. Seagrass tissue samples 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 plotted in Schenck et al (2022) as Figure S6.

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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 (July – September) in an array of flow-through 120-L mesocosms at the Bodega Marine Laboratory in Bodega Bay, CA. At the end of the experiment 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 extracted *L. zosterae* DNA from dried leaf tissue using Omega Bio-Tek E.Z. Tissue DNA extraction kits at the Northeastern University Marine Science Center in Nahant, MA. For each sample, we separated the dried leaf tissue into 2-16 mg subsamples and homogenized the tissue in a ball mill (Retsch, Germany) at a frequency of 30 Hz for 5 min (Bockelmann et al. 2013). We lysed ground subsamples individually following the manufacturer's instructions and added 1 uL of 500 ng*uL-1 salmon sperm DNA solution (Invitrogen, USA) to the first subsample of each sample immediately before recombining all subsamples in the spin columns. Salmon sperm DNA was added to enhance extraction efficiency and ensure that even low amounts of target DNA are carried through the filter absorption steps (Bockelmann et al. 2013). We eluted all DNA extractions into 100 uL. Following elution, we used Zymo OneStep-96 PCR Inhibitor Removal kits to clean 50 uL sub-samples of each DNA extraction following the manufacturers instructions. We stored cleaned DNA extractions at -20˚C prior to quantitative PCR.

We used a TaqMan quantitative PCR (qPCR) assay with a forward primer: TTGAACGTAACATT-CGACTTTCGT, reverse primer: ACGCATGAAGCGGTCTTCTT, and MBG probe: TGGACGAGTGTGTTTTG that carries the fluorescence label 6-Fam at the 5' end and the dark quencher FHQ at the 3' end (Bio-Rad, USA) developed specifically for *L. zosterae* (Bockelmann et al. 2013, Bergmann et al. 2011). We made up qPCR reactions to a 10 uL reaction volume using standard conditions recommended by the manufacturer: 5 uL SsoAdvancedTM Universal Probes Supermix 2x (Bio-Rad, USA), 1 uL template DNA, 0.4 uL 4:1 Primer:Probe Mix (final concentrations of 400 nM forward primer, 400 nM reverse primer, 100 nM probe), and 3.6 uL Milli-Q H2O (Thermofisher, USA). Reactions were run on a CFX96 Real-Time System (Bio-Rad, USA) using the following thermo-cycling program: 3 min at 95˚C, followed by 40 cycles of 15 sec at 95˚C and 1 min at 60˚C. We tested all samples in duplicate and if replicates differed by greater than one cycle threshold (Ct) reactions were rerun in triplicate. We only used the data from reactions in analyses when replicates fell within one Ct. Our lowest detection was 1.76 copies per reaction or 0.15 cells per extraction.

We ran each 96-well plate of qPCR reactions with a set of nine standards: a dilution series of gBlock Gene Fragments (Integrated DNA Technologies, USA) designed based on the highly conserved sequence of the 5.8s ribosomal RNA gene of *L. zosterae* known as internal transcribed spacer 1 (ITS) targeted by the TaqMan qPCR assay; an *L. zosterae* cell standard consisting of a sample of DNA extracted from a know quantity of pathogenic *L. zosterae* cells; and an inhibition control consisting of a half volume of *L. zosterae* cell standard and a half volume of a haphazardly selected sample. We ran a total of 31 96-well plates of qPCR reactions with a mean efficiency of 97.4% ± 4.3 and R2 0.996 ± 0.004.

Specifically, we first converted Ct values to copy numbers as our g-block standard curve were in units of copy number. We then used the *L. zosterae* cell standard to determine the copy number per *L. zosterae* cell. Finally, we converted copy number to *L. zosterae* cells * mg dw-1. Copy numbers per cell in our reactions were 1227.58 ± 80.66 (mean ± SE).

We used a pure culture of the pathogenic *L. zosterae* isolate 316b provided by D. Martin in 2015 to make our *L. zosterae* cell standard (Martin et al. 2016; GenBank: KU559372.1). We cultured *L. zosterae* cells on serum seawater agar media (Muehlstein et al. 1991). We scraped cells from an actively growing edge of *L. zosterae* culture into serum seawater liquid media (D. Martin pers. com.). We mixed the liquid media-*L. zosterae* cell slurry vigorously on a bench top vortex for 30 sec and aliquoted immediately into three replicate subsamples for cell counts and extraction. In order to break up cell clumps for ease of counting, we added Tween80 (Sigma-Aldrich, USA) to a final concentration of 1:100 into the two subsamples used for cell counts, and mixed for 30 sec. We counted cells of four replicate aliquots per subsample on a hemocytometer. We calculated cell concentration by averaging over all replicates. Prior to DNA extraction, we centrifuged the third replicate *L. zosterae* cell solution at 6,000 g for 10 min and drew off the supernatant without disturbing the cell pellet. We then added a ~4 mg section of dried healthy *Z. marina* tissue to the cell pellet to account for possible interference of *Z. marina* compounds in the extraction process. To extract *L. zosterae* DNA, we followed the DNA extraction and inhibitor removal protocols outlined above.

We designed the gBlock double stranded DNA fragments (Integrated DNA Technologies, USA) using published sequences of the ITS region of the *L. zosterae* genome (GenBank: JN121409-13).

5'-CTGTGATCTCTGAAAATACTTGTTT (1)TTGAACGTAACATTCGACTTTCGTCGATT TTG

(2)TGGACGAGTGTGTTTTGT AAACCTACCC (3)AAGAAGACCGCTTCATGCGT GTCGCTGACTAATGAAACAAACAAA-3’

The gBlock fragment sequences were a total length of 130 bp, which included target regions for the forward (1) and reverse (3) primers and the MGD probe (2), underlined above, as well as 25 base pairs of additional sequence on both the 5’ and 3’ ends to increase fragment stability. We diluted gBlock fragments in Milli-Q H2O (Thermofisher, USA) to seven concentrations: 2.24e1, 1.12e2, 5.61e2, 2.81e3, 1.40e4, 7.02e4, 7.02e5 copies/µL and included this dilution series in each qPCR run as a standard curve (Bergmann et al. 2011). The range of the gBlock dilution curve: approx. 1-60,000 cells/extraction encompassed the range of most *L. zosterae* values observed in our samples: 0.15-450,000 cells/extraction or 1.84e2-5.52e8 copies/extraction.

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

Code that includes quantitative PCR inhibition analysis associated with this experiment:
All code was written and run in R (version 3.6.1, www.R-project.org). Github repository https://github.com/schenckf/BWE_Experiment V2.0.0 archived at Zenodo (DOI: 10.5281/zenodo.7129500). A general description of the code is included in the repository release in file "Analysis Description.docx."

BCO-DMO Processing:

- Imported data from source file "mesocosm_warming_experiment_quantitativePCR_inhibition_control_data.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.

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Data Files

File	
mesocosm_pcr.csv	(Comma Separated Values (.csv), 1.16 KB) MD5:9a34116d2a6e4306f2de84d9dab6d741
Primary data file for dataset ID 883055	

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Related Publications

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

Bio-Rad Laboratories, Inc. (2022) Bio-Rad CFX Manager Software (version 3.1). Accessed 2022-10-27 from https://www.bio-rad.com/en-us/sku/1845000-cfx-manager-software?ID=1845000
Software

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

Groner, M., Eisenlord, M., Yoshioka, R., Fiorenza, E., Dawkins, P., Graham, O., Winningham, M., Vompe, A.,

Rivlin, N., Yang, B., Burge, C., Rappazzo, B., Gomes, C., & Harvell, C. (2021). Warming sea surface temperatures fuel summer epidemics of eelgrass wasting disease. Marine Ecology Progress Series, 679, 47–58. https://doi.org/10.3354/meps13902
Methods

Martin, D. L., Chiari, Y., Boone, E., Sherman, T. D., Ross, C., Wyllie-Echeverria, S., Gaydos, J. K., & Boettcher, A. A. (2016). Functional, Phylogenetic and Host-Geographic Signatures of Labyrinthula spp. Provide for Putative Species Delimitation and a Global-Scale View of Seagrass Wasting Disease. Estuaries and Coasts, 39(5), 1403–1421. https://doi.org/10.1007/s12237-016-0087-z
Methods

Muehlstein, L. K. (1992). The host – pathogen interaction in the wasting disease of eelgrass, Zostera marina. Canadian Journal of Botany, 70(10), 2081–2088. https://doi.org/10.1139/b92-258
Methods

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. (2022). *schenckf/BWE_Experiment: The effect of warming on seagrass wasting disease depends on host genotypic identity and diversity - Analyses* (Version V2.0.0) [Computer software]. Zenodo. https://doi.org/10.5281/ZENODO.7129500
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) **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.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-10-27 doi:10.26008/1912/bco-dmo.883070.1 [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.

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Parameters

Parameter	Description	Units
trial_no	Unique identifier number assigned to each of the 31 96-well plate quantitative PCR reactions	unitless
inhibition_sample_plate_id	Unique identifier alphanumeric code assigned to each of the nine Zostera marina tissue DNA extraction 96-well plates	unitless
inhibition_sample_well_id	Unique identifier alphanumeric code assigned to each well in each of the nine Zostera marina tissue DNA extraction 96-well plates	unitless
cells_uL_sample	The number of Labyrinthula zosterae cells detected by quantitative-PCR from DNA extracted from a tissue segment of the focal leaf of a Zostera marina plant per uL of DNA extraction solution	L. zosterae cells per microliter (cells/uL)
cells_uL_pos_cntrl	The number of Labyrinthula zosterae cells detected by quantitative-PCR from DNA extracted from a tissue segment of the focal leaf of cultured L. zosterae cells per uL of DNA extraction solution. (L. zosterae cells * uL-1).	L. zosterae cells per microliter (cells/uL)
cells_uL_inhibition	The number of Labyrinthula zosterae cells detected by quantitative-PCR from DNA extracted from tissue of the focal leaf of a Zostera marina plant combined with DNA extracted from cultured L. zosterae cells per uL of DNA extraction solution to test for inhibition. (L. zosterae cells * uL-1).	L. zosterae cells per microliter (cells/uL)
inhibition_cntrl_ID	Unique identifier associated with the culture of Labyrinthula zosterae cells used in each inhibition control trial. (L. zosterae cells * uL-1).	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	Retsch Mixer Mill 400
Generic Instrument Name	Homogenizer
Dataset-specific Description	For each sample, we separated the dried leaf tissue into 2-16 mg subsamples and homogenized the tissue in a ball mill (Retsch, Germany)
Generic Instrument Description	A homogenizer is a piece of laboratory equipment used for the homogenization of various types of material, such as tissue, plant, food, soil, and many others.

Dataset-specific Instrument Name	Bio-Rad CFX96 Real-Time System
Generic Instrument Name	qPCR Thermal Cycler
Dataset-specific Description	The focal leaf was stored for subsequent DNA extraction and quantitative PCR to estimate Labyrinthula zosterae cells as a proxy for infection. Software: Bio-Rad CFX Manager Software (version 3.1)
Generic Instrument Description	An instrument for quantitative polymerase chain reaction (qPCR), also known as real-time polymerase chain reaction (Real-Time PCR).

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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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