Seagrass metrics from from seagrass wasting disease mesocosm experiments conducted at Bodega Marine Laboratory from July-September 2015

Website: https://www.bco-dmo.org/dataset/879749
Data Type: experimental
Version: 1
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Project
» CAREER: Linking genetic diversity, population density, and disease prevalence in seagrass and oyster ecosystems (Seagrass and Oyster Ecosystems)

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Abstract
This dataset includes seagrass metrics (diversity, biomass, growth, and wasting disease) from mesocosm experiments at Bodega Marine Laboratory in July-September 2015. 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 Labyrinthula zosterae. These data were published in Schenck et al (2022).

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Coverage

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

Methods & Sampling

Mesocosm experiment.
We used a substitutive design to test the effects of eelgrass 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 the wasting disease parasite *Labyrinthula* over eight weeks in an array of flow-through 120-Liter 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 of eelgrass 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 *Zostera marina* (Abbott et al. 2018), and propagated in separate flow through mesocosms at Bodega Marine Lab. We previously characterized traits of each genotype relating to growth rate, morphology, nutrient content, and chemical defense in common garden experiments at ambient temperature from July 2013 to August 2014 (Abbott et al., 2018). We selected the 8 genotypes used in this experiment to encompass the range of trait values determined for this population of eelgrass measured when the common garden was experiencing marine heatwave conditions (DuBois et al., 2019).

We assigned ten pots -- two unique polyculture combinations and each of the eight monocultures -- to each of ten mesocosms, with five mesocosms per temperature treatment (see DuBois et al. 2020 for a diagram of the experimental set up). All mesocosms received sand-filtered flow-through seawater at a rate of approximately 0.8-1.0 liters per minute (L min^{-1}). We allowed the plants to acclimate for one month prior to initiating the temperature treatments. We maintained an ambient temperature treatment by cooling flow-through seawater in a head tank by approximately 1˚C using an Aqua Logic Delta Star in-line titanium chiller. Seawater in the elevated temperature treatment was raised approximately 3˚C above the ambient treatment in a separate header tank using Process Technologies titanium immersion heaters. This level of warming mimicked the 2014 and 2015 extreme warming events in the Northern Pacific, where the mass of unusually warm water called “The Blob” raised summer ocean temperatures three standard deviations above the long-term average (Sanford et al., 2019).

At the end of the experiment (10 weeks), we estimated lesion percent cover of the third rank leaf of the terminal shoot of each transplant (i.e., focal leaf) to measure the signs of wasting disease (Burdick et al., 1993). We recorded lesions as either absent, <1%, <10%, or ≥10% cover. When lesions were ≥10% cover, we also recorded a numerical estimate of lesion percent cover. We then collected and preserved the top half of the focal leaf in individual plastic bags sealed with 30 milliliters 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).

At one-month intervals over the course of the experiment, we measured leaf growth rate of the terminal shoot of each transplant using the “hole-punch” method (Williams and Ruckelshaus, 1993). *Labyrinthula zosterae* infection can be affected by plant defenses (Steele et al., 2005; Trevathan-Tackett et al., 2015) and these defenses may trade off with plant growth rate, resulting in a positive relationship between growth rate and infection. Alternatively, *L. zosterae* infection can result in reduced leaf growth rate (Graham et al., 2021), leading to a negative relationship. *L. zosterae* prevalence can also be affected by plant size (Groner et al., 2016), due to greater leaf-to-leaf contact and resulting increased parasite transmission (Muehlstein, 1992), so we measured the length of the focal leaf of each transplant at the end of the experiment.

Labyrinthula zosterae DNA extraction and quantitative PCR Assay

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 microliter of 500 ng per microliter (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 SsoAdvanced™ 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 H_2O (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 R^2 0.996 ± 0.004. To convert Ct values to to *L. zosterae* cell numbers, four equations were used. The **equations and details are captured in the Supplemental Files section** of this metadata within the file titled, "Equations for conversion of Ct values to *Labyrinthula zosterae* cell numbers."

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 *Zostera. marina* tissue to the cell pellet to account for possible interference of *Zostera 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 (base pairs), 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 H_2O (Thermofisher, USA) to seven concentrations: $2.24e^1$, $1.12e^2$, $5.61e^2$, $2.81e^3$, $1.40e^4$, $7.02e^4$, $7.02e^5$ 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 $1.84e^2$-$5.52e^8$ 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

BCO-DMO Processing:
- Imported data from source file "Schenck_etal_mesocosm_warming_data.csv" into the BCO-DMO data system. Data file imported using missing data identifier "NA".
- Added columns for start and end date in year-month-day format
- Added conventional header with dataset name, PI name, version date.
- Modified parameter (column) names to conform with BCO-DMO naming conventions.

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Data Files

File
mesocosm_warming.csv (Comma Separated Values (.csv), 44.21 KB) MD5:9cf115f1b04258d2e93332e6503c7ffb Primary data file for dataset ID 879749

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Supplemental Files

File
Equations for converting Ct values to Labyrinthula zosterae cell numbers filename: Equations_to_convert_Ct_to_cell_numbers.pdf (Portable Document Format (.pdf), 190.01 KB) MD5:360f47a3e61093e90a14d65389c2adad Four equations used to convert Ct values to Labyrinthula zosterae cell numbers

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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

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

Brakel, J., Werner, F. J., Tams, V., Reusch, T. B. H., & Bockelmann, A.-C. (2014). Current European Labyrinthula zosterae Are Not Virulent and Modulate Seagrass (Zostera marina) Defense Gene Expression. PLoS ONE, 9(4), e92448. https://doi.org/10.1371/journal.pone.0092448
Methods

Burdick, D. M., Short, F. T., & Wolf, J. (1993). An index to assess and monitor the progression of wasting disease in eelgrass Zostera marina. Marine Ecology-Progress Series, 94, 83-83.
Methods

Burge, C. A., Kim, C. J. S., Lyles, J. M., & Harvell, C. D. (2013). Special Issue Oceans and Humans Health: The Ecology of Marine Opportunists. Microbial Ecology, 65(4), 869–879. https://doi.org/10.1007/s00248-013-0190-7
Methods

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology Meets Ecology on Its Own Terms: Margolis et al. Revisited. The Journal of Parasitology, 83(4), 575. https://doi.org/10.2307/3284227

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
Software

DuBois, K., Abbott, J., Williams, S., & Stachowicz, J. (2019). Relative performance of eelgrass genotypes shifts during an extreme warming event: disentangling the roles of multiple traits. Marine Ecology Progress Series, 615, 67–77. https://doi.org/10.3354/meps12914
Methods

DuBois, K., Williams, S. L., & Stachowicz, J. J. (2020). Previous exposure mediates the response of eelgrass to future warming via clonal transgenerational plasticity. Ecology, 101(12). Portico. https://doi.org/10.1002/ecy.3169
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

Graham, O. J., Aoki, L. R., Stephens, T., Stokes, J., Dayal, S., Rappazzo, B., Gomes, C. P., & Harvell, C. D. (2021). Effects of Seagrass Wasting Disease on Eelgrass Growth and Belowground Sugar in Natural Meadows. Frontiers in Marine Science, 8. https://doi.org/10.3389/fmars.2021.768668
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

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

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

Johnson, M. T. J., Lajeunesse, M. J., & Agrawal, A. A. (2005). Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. Ecology Letters, 0(0), 051012084514001. https://doi.org/10.1111/j.1461-0248.2005.00833.x
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

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

Muehlstein, L. K., Porter, D., Short, F. T. (1991). Labyrinthula zosterae sp. nov., the causative agent of wasting disease of eelgrass, Zostera marina. Mycologia, 83(2), 180-191.
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

Sanford, E., Sones, J. L., García-Reyes, M., Goddard, J. H. R., & Largier, J. L. (2019). Widespread shifts in the coastal biota of northern California during the 2014–2016 marine heatwaves. Scientific Reports, 9(1).

https://doi.org/10.1038/s41598-019-40784-3
Methods

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

Steele, L., Caldwell, M., Boettcher, A., & Arnold, T. (2005). Seagrasspathogen interactions: pseudo-induction of turtlegrass phenolics near wasting disease lesions. Marine Ecology Progress Series, 303, 123–131. https://doi.org/10.3354/meps303123
Related Research

Sullivan, B. K., Trevathan-Tackett, S. M., Neuhauser, S., & Govers, L. L. (2018). Review: Host-pathogen dynamics of seagrass diseases under future global change. Marine Pollution Bulletin, 134, 75–88. https://doi.org/10.1016/j.marpolbul.2017.09.030
Related Research

Trevathan-Tackett, S. M., Lane, A. L., Bishop, N., & Ross, C. (2015). Metabolites derived from the tropical seagrass Thalassia testudinum are bioactive against pathogenic Labyrinthula sp. Aquatic Botany, 122, 1–8. https://doi.org/10.1016/j.aquabot.2014.12.005
Methods

Williams, S. L., & Ruckelshaus, M. H. (1993). Effects of Nitrogen Availability and Herbivory on Eelgrass (Zostera Marina) and Epiphytes. Ecology, 74(3), 904–918. doi:10.2307/1940815
Methods

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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) **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) **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
start_date	Start date of the mesocosm warming experiment	unitless

end_date	End date of the mesocosm warming experiment	unitless
bin	Unique identifier number assigned to each of the ten 120 L mesocosms at the Bodega Marine Lab supplied with flow-through seawater	unitless
pot	Unique identifier number assigned to each of the 100 pots (8.9 x 8.9 cm) planted with Zostera marina	unitless
shoot	Identifier number assigned to shoots from each genotype within each bin; shoots 1-4 of each genotype planted in monoculture and shoot 5 planted in polyculture	unitless
diversity	Descriptor of the genotypic diversity treatment applied to Zostera marina plant communities; mono = one genotype; poly = four genotypes	unitless
temperature	Descriptor of the temperature treatment applied to seawater where ambient = cooled flow-through seawater by approximately 1 degree Celsius; elevated = heated flow-through seawater by approximately 3 degrees Celsius	unitless
genotype	Descriptor of the genotype of each Zostera marina plant	unitless
length	Length of the third rank leaf of the terminal shoot (i.e. focal leaf) of each Zostera marina plant	centimeters (cm)
growth_rate	Growth rate of the focal leaf of each Zostera marina plant	centimeters per day (cm/day)
lesion	Binomial code for the presence of lesions characteristic of wasting disease on focal leaf of each Zostera marina plant. 1 = lesions present; 0 = no lesions present.	unitless
lesion_group	Categorical descriptor of the percent cover of lesion characteristic of wasting disease on the focal leaf. 0; <1%; <10%; or _10% cover	unitless
lesion_cover	Numerical descriptor of the percent cover of lesions characteristic of wasting disease on the focal leaf; for lesions <10% numerical percent covers were converted from categorical estimates using the following method: 0 = 0; <1% = 0.5; <10% = 5; for lesions _10% cover numerical percent cover estimates were recorded during sampling	unitless
mass	The mass of the tissue segment of the focal leaf of each Zostera marina plant from which Labyrinthula zosterae DNA was extracted	milligrams (mg)

cells_per_extraction	The number of Labyrinthula zosterae cells detected by quantitative-PCR from the extracted tissue segment of the focal leaf of each Zostera marina plant	cells
cells_per_mg	The number Labyrinthula zosterae cells detected by quantitative-PCR from the extracted tissue segment of the focal leaf of each Zostera marina plant per milligram of extracted Zostera marina tissue	cells
cells	Binomial code for the presence of *Labyrinthula zosterae* cells detected by quantitative-PCR from the extracted tissue segment of the focal leaf of each *Zostera marina* plant. 1 = cells present; 0 = no cells present.	unitless
latitude	Latitude of Bodega Marine Laboratory (Bodega Bay, CA)	decimal degrees
longitude	Longitude of Bodega Marine Laboratory (Bodega Bay, CA)	decimal degrees

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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	Aqua Logic Delta Star in-line titanium chiller
Generic Instrument Name	Aquarium chiller
Dataset-specific Description	We maintained an ambient temperature treatment by cooling flow-through seawater in a head tank by approximately 1˚C using an Aqua Logic Delta Star in-line titanium chiller.
Generic Instrument Description	Immersible or in-line liquid cooling device, usually with temperature control.

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	Process Technologies titanium immersion heater
Generic Instrument Name	Immersion heater
Dataset-specific Description	Seawater in the elevated temperature treatment was raised approximately 3˚C above the ambient treatment in a separate header tank using Process Technologies titanium immersion heaters
Generic Instrument Description	Submersible heating element for water tanks and aquaria.

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
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