# Seagrass responses to Labyrinthula zosterae inoculation base on a subpopulation from mesocosm experiments conducted in Nahant, Massachusetts

Website: https://www.bco-dmo.org/dataset/851047 Data Type: Other Field Results Version: 1 Version Date: 2021-05-04

### Project

» <u>CAREER</u>: 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 responses to Labyrinthula zosterae inoculation base on a subpopulation from mesocsm experiments conducted in a greenhouse at Northeastern University Marine Science Center in Nahant, Massachusetts from May to August 2016.

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# Coverage

Spatial Extent: N:42.5971 E:-70.6559 S:42.4206 W:-70.9158 Temporal Extent: 2016-05 - 2016-08

### Methods & Sampling

**Labyrinthula zosterae** *isolation, culturing, and identification:* In May 2016, we isolated *L. zosterae*, the causative agent of *Zostera* wasting disease, from the diseased leaf tissue of live *Zostera* from CB. Briefly, we cut a 2 centimeter section of *Zostera* leaf tissue at the edge of a black or brown lesion, surface sterilized and rinsed the leaf tissue section, and plated the tissue onto a 10 centimeter diameter seawater agar plate (Mckone and Tanner, 2009; Muehlstein, 1988). We confirmed the identity of the culture to be *L. zosterae* by microscopic examination (Mckone and Tanner, 2009). Specifically, we observed culture and cell morphology under 200x and 400x magnification on a Nikon eclipse 50i microscope. We used previously published examples of *L. zosterae* culture and cell morphology as a guide (i.e. branching 'slime' tunnels connecting cells and fusiform cell shape; Muehlstein et al., 1991). We maintained the *L. zosterae* culture by transferring colonized agar plugs to fresh agar plates every two weeks.

**Zostera** collection and preparation: In June 2016, we collected 30 Zostera plants (connected rhizome, stem, and leaves) from the same four subpopulations we surveyed wasting disease: NB (Niles Beach, Gloucester, 42° 35.8268' N, 70° 39.3553' W), WB (West Beach, Beverly, 42° 33.9155' N, 70° 47.1102), LP (Lynch Park, Beverly, 42° 32.6925' N, 70° 51.5057' W), and CB (Curlew Beach, Nahant, 42° 25.2378' N, 70° 54.9474' W).

At each subpopulation location, we collected *Zostera* plants at 1 m intervals along a 30 m transect running parallel to shore 2-5 m from the shoreward extent of the *Zostera* bed at a depth of 1-2 meters MLLW. Plants from each subpopulation were kept in separate flow-through 54 liter seawater holding tanks in a glasshouse at the Northeastern University Marine Science Center (MSC), Nahant, MA. We attached the rhizome of each plant to a weight with a zip-tie so that the plants were oriented naturally in the water column and did not float on the top of the mesocosms (Mckone and Tanner, 2009). We allowed the plants to acclimate to greenhouse conditions for one month before the start of the *L. zosterae* inoculation experiment.

Ten days prior to inoculating *Zostera* with *L. zosterae*, we haphazardly selected 22 plants from each subpopulation and moved them from the flow-through holding tanks into 88 45 centimeter tall and 15 centimeter diameter transparent cylindrical acrylic mesocosms filled with seawater. Immediately prior to isolating each plant in a mesocosm, we used a razor blade to remove all leaf tissue within 3 centimeters of any black and brown lesions characteristic of wasting disease infection. In addition, we clipped the rhizomes of each plant to a length of 5 centimeters from the first node and removed all secondary shoots. We sterilized our workspace and tools with 10% bleach and then rinsed with deionized (DI) water in between handling each plant in order to minimize the risk of cross-contamination.

We assigned the 88 mesocosms to eleven 54 liter tanks in the MSC glasshouse such that each tank held two *Zostera* from each subpopulation. We randomized the location of the mesocosms within each tank. We then supplied each tank with flow-through seawater to a depth of 3-5 centimeters below the top of the mesocosms. The flow-through seawater acted as a water bath, keeping the temperature of the water in the mesocosms equivalent to ambient conditions. Finally, we added air-stones to each mesocosm to circulate the water and prevent temperature gradients from forming.

**Vector preparation:** One week prior to inoculating *Zostera* with *L. zosterae*, we collected lesionfree *Zostera* leaves from the CB subpopulation. We gently cleaned epiphytes off of the leaf tissue, rinsed the leaves in DI water for five minutes, and cut the leaves into 144 2-centimeter sections. We then haphazardly distributed the leaf sections into twenty-four 1.85 milliliter (mL) glass drams filled with 1.5 milliliter (mL) seawater (six leaf sections per dram) and autoclaved them at 121°C for 20 minutes to eliminate the possible presence of pathogens and to prevent contamination of inoculation cultures.

Next, we transferred the autoclaved leaf tissue sections onto 24 10-centimeter diameter seawater agar plates (six leaf sections per plate) (Mckone and Tanner, 2009). We arranged the leaf sections in a circle with a radius of 3 centimeters around the center of each agar plate and 1 centimeter distance maintained between each leaf section. We sealed the first twelve plates with parafilm immediately after plating on leaf sections and used these plates as control vectors. After plating leaf sections on the remaining twelve agar plates, we inoculated each plate by placing a  $1 \times 1$  cm agar plug cut from the growing edge of the axenic *L. zosterea* culture isolated in May 2016 and maintained in the lab as described above. We then sealed these plates with parafilm to use as disease vectors. We stored all vectors in ambient light at 22°C in a fume hood.

We visually inspected the control and disease vectors daily for signs of contamination. For the *L. zosterae*inoculated plates, we also monitored the expansion of *L. zosterae* across the surface of the agar. Three days following plating, we observed *L. zosterae* cells beginning to spread over the vectors and by the seventh day, *L. zosterae* cells completely covered the vectors in all twelve of the inoculated plates.

**Zostera** *inoculation:* We inoculated all 88 plants (N=22 per subpopulation) on August 2, 2016 (experiment day 0). We haphazardly selected eleven plants from each subpopulation to inoculate with a control vector and we inoculated the other eleven plants from each subpopulation with a disease vector. We first inoculated all the plants receiving the control treatment and then inoculated all the plants receiving the disease treatment to minimize the risk of contaminating the control plants with *L. zosterae*.

To inoculate each plant, we first gently removed the plant from its mesocosm and placed it on a 2 x 0.25 meter laminated paper surface. We used forceps to remove a single vector from an agar plate and placed it on the surface of the 2nd youngest leaf 5 centimeters above the top of the sheath. We clamped the vector to the leaf with a sterilized, rigid split Tygon tube (1 x 0.5 centimeters in diameter) (Muehlstein, 1988) and returned the inoculated plant back into its respective container. To prevent cross-contamination, we rinsed the working surface and forceps with 10% bleach and then DI water between each plant. **Monitoring and breakdown:** We monitored the progression of wasting disease lesions as well as *Zostera* survival, growth, and morphology eight times over the course of the one-month experiment (days 0, 1, 3, 5, 7, 14, 21, and 28). We visually assessed each plant for the production of secondary shoots (stems and leaves) and noted plant mortality. We then photographed each plant using the camera application on an iPhone 5s. To prevent cross-contamination, we photographed all control plants before plants receiving the disease treatment and disinfected the working surface and all tools as described above between each plant. On day 28, we concluded the experiment by removing all plants from their mesocosms, taking photographs, measuring rhizome length, and recording biomass of above- and below-ground tissues after 48 hours drying at 70  $^{\circ}$ C.

We quantified *Zostera* growth, morphology, and wasting disease infection intensity by scoring each photograph using the free ImageJ software for Mac OS X developed by the National Institutes of Health (Schneider et al., 2012). Specifically, we quantified leaf loss and leaf production for each plant over the course of the experiment by counting the number of leaves present relative to the position of the inoculated leaf (i.e., decrease in number of older leaves and increase in number of younger leaves). We used the segmented line function to measure the length of each leaf from the top of the sheath to the leaf tip. We calculated leaf growth as the change in length of the youngest leaf present at the time of inoculation, accounting for changes in sheath length. We used the polygon function to quantify leaf area and lesion area for each leaf. We used published descriptions and photographs of lesions associated with wasting disease to identify lesion area (Burdick et al., 1993; Groner et al., 2014; Groner, Burge, et al., 2016). To quantify the severity of wasting disease infection, we divided the sum of lesioned tissue area by the sum of total leaf tissue area for each plant (Burdick et al., 1993).

### **Data Processing Description**

### Statistical analyses:

*Wasting disease:* We chose a monomolecular model commonly used to describe the progression of disease severity in plants to assess the nonlinear change in wasting disease severity on individual *Zostera* as a function of time over the course of the experiment (Gilligan, 1990; Madden et al., 2007).

### y = ai(1 - b\*exp(-ci \* t)) (eqn. 1)

We chose this model to separately capture the initial progression of wasting disease lesion severity (parameter *ci*) and the asymptotic severity levels realized in the final weeks of the experiment (parameter *ai*). We applied simulated annealing (SA), a global optimization procedure for minimizing a cost function, to fit a monomolecular function constructed in R to the experimental lesion severity data from each infected plant that survived the duration of the experiment (Kirkpatrick et al., 1983). We chose SA because it is robust against local minimums and requires no prior constraints on parameter values. Our SA procedure consisted of a cost function, which calculates the root mean square error of the model relative to the data to measure the quality of the model fit, and a 'temperature' schedule, which represents the probability of accepting the proposed parameter values (ai and ci) if they result in a worse fit than the current parameter values (ai-1 and ci-1) at each iteration. We began the procedure by selecting a random set of parameter values, defined the initial temperature Tc as 10^8, the rate of decay Tr as 20, and ran the procedure for 10^4 iterations. From this SA procedure, we determined the values of parameters a and c associated with the best fit of the monomolecular model to the experimental data for each plant. We used a one-way ANOVA to examine the effect of subpopulation on the value of parameter a and in the case of significant effects, we used Tukey HSD post hoc tests to differentiate among subpopulations. We used non-parametric Kruskal-Wallace tests to examine the effect of subpopulation on the value of parameter *c* due to non-normality.

To address whether differences in the progression or asymptote of wasting disease severity (the sum of lesioned tissue area divided by the sum of leaf tissue area) aligned with differences in the progression of wasting disease lesions (sum of lesioned tissue area), we used a function describing the logistic growth of wasting disease lesions over the course of the experiment (Madden et al., 2007).

### dN/dt = riN(1 - N/ki) (eq. 2)

We chose a logistic growth function to model wasting disease lesion progression because we assume that lesion progression is the visual expression of the population growth of *L. zosterae* within the seagrass host (Bergmann et al., 2011; Bockelmann et al., 2013). We used vector area (mean  $\pm$  SE cm<sup>2</sup> = 0.804  $\pm$  0.014) as the initial lesion area value in the logistic growth models. We used the SA procedure described above to fit the logistic growth function, constructed as an ordinary differential equation using the deSolve package in R (Soetaert et al., 2010), to the experimental lesion area data and determine values for the intrinsic rate of increase of lesions area (ri) and the per plant lesion area carrying capacity (ki) for each infected plant that survived the duration of the experiment. We then used a one-way ANOVA to examine the effect of subpopulation on parameter k and a non-parametric Kruskal-Wallace test to examine the effect of subpopulation on parameter r due to non-normality.

Host Response (Multivariate): We used PERMANOVA from the vegan package in R (Oksanen et al., 2019) to assess the independent and interactive effects of subpopulation and inoculation treatment on Zostera response to inoculation in multivariate space. We excluded eight Zostera replicates from the control group that developed lesions characteristic of wasting disease infection during the experiment and seven additional replicates that died over the course of the experiment. (We examine the effects of subpopulation and inoculation treatment on Zostera survival in separate analyses described below.) We used the Euclidean method to calculate pairwise distances among subpopulation and infection treatment combinations. Prior to running the PERMANOVA, we scaled the data and tested eight responses (leaf production, leaf loss, leaf growth, rhizome growth, reduction in sheath length, final aboveground biomass, final belowground biomass, and final total biomass) for collinearity using the corrgram package in R (Wright, 2018). We excluded final aboveground biomass and final belowground biomass from the analysis due to correlations greater than 0.75 with final total biomass. To test whether the effects of subpopulation and inoculation treatment on Zostera responses correlated with initial Zostera traits, we ran a second PERMANOVA analysis where we tested four traits measured at the time of inoculation (initial leaf number, initial shoot length, initial maximum leaf length, and initial sheath length). Initial rhizome length was standardized to 5 cm in all Zostera prior to inoculation and thus was excluded from this analysis. Data were scaled and initial shoot length was excluded due to correlations greater than 0.75 with initial leaf length.

When PERMANOVAs indicated a significant main effect of subpopulation or interactive effect of subpopulation by inoculation, we used multiple paired PERMANOVA post hoc contrasts, adjusted using Bonferroni corrections, to parse out the differences among treatments. Other correction factors (i.e., Benjamini & Hochberg false discovery rate method; Benjamini & Hochberg 1995) yielded similar results. We visualized the results of PERMANOVAs through principal component analyses run using the stats and ggbiplot packages in R (Vu, 2011; R Core Team, 2019).

*Host Response (Univariate):* We examined survival and secondary shoot production in separate analyses due to their binomial distributions. Specifically, we used log-rank tests of Kaplan-Meier curves to examine independent effects of inoculation treatment and subpopulation on *Zostera* survival. Survival analyses were run using the survival and survminer packages in R (Therneau & Grambsch, 2000; Therneau, 2015; Kassambara et al., 2019). *Zostera* from two subpopulations, CB and WB, produced no secondary shoots and thus were excluded from formal analyses due to lack of variance. We used a generalized linear model to examine the independent and interactive effects of inoculation treatment and subpopulation on *Zostera* shoot production for the remaining two subpopulations, LP and NB. In addition, we examined the eight *Zostera* responses and four initial *Zostera* traits considered in the PERMANOVA analyses independently using two-way ANOVAs in order to determine how each was affected by subpopulation and infection treatment. We used Tukey honest significant difference (HSD) post hoc tests to interpret significant effects from ANOVAs as needed.

We conducted all analyses in R (version 3.6.1; R Core Team, 2019) and Rstudio (version 1.1.463; Rstudio Team, 2016).

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### Data Files

 File

 mesocosm\_exp.csv(Comma Separated Values (.csv), 142.47 KB)

 MD5:465128135c108a7f3c4a6c2a7ad9f8ba

Primary data file for dataset ID 851047

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# **Related Publications**

Benjamini, Y., Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B, 57(1), 289-300.

#### Results

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. *Results* 

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. *Results* 

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., Friedman, C. S., Getchell, R., House, M., Lafferty, K. D., Mydlarz, L. D., Prager, K. C., Sutherland, K. P., Renault, T., Kiryu, I., Vega-Thurber, R. (2016). Complementary approaches to diagnosing marine diseases: a union of the modern and the classic. Philosophical Transactions of the Royal Society B: Biological Sciences, 371, 20150207.

Methods

Gilligan, C. A. (1990). Comparison of Disease Progress Curves. The New Phytologist, 115(2), 223–242. http://www.jstor.org/stable/2556954 Results

Groner, M. L. Maynard, J.,Breyta, R., Carnegie, R. B., Dobson, A., Friedman, C. S., Froelich, B., Garren, M., Gulland, F. M. D., Heron, S. F., Noble, R. T., Revie, C. W., Shields, J. D., Vanderstichel, R., Weil, E., Wyllie-Echeverria, S., Harvell, C. D. (2016). Managing marine disease emergencies in an era of rapid change. Philosophical transaction of the Royal Society B: Biological Sciences, 371, 1689. *Methods* 

Groner, M. L., Burge, C. A., Couch, C. S., Kim, C. J. S., Siegmund, G.-F., Singhal, S., Smoot, S. C., Jarrell, A., Gaydos, J. K., Harvell, C. D., Wyllie-Echeverria, S. (2014). Host demography influences the prevalence and severity of eelgrass wasting disease. Disease of Aquatic Organisms, 108, 165-175. *Methods* 

Kassambara, A., Kosinski, M., Biecek, P. (2019). survminer: drawing survival curves using 'ggplot2'. R package version 0.4.6. https://CRAN.R-project.org/package=survminer Software

Kirkpatrick, S., Gelatt Jr., C. D., Vecchi, M. P. (1983). Optimization by simulated annealing. Science, 220, 671-680.

Results

Madden, L. V., Hughes, G., Van den Bosch, F. (2007). The study of plant disease epidemics. The American Phytopathological Society. St Paul, Minnesota. *Results* 

McKone, K. L., & Tanner, C. E. (2009). Role of salinity in the susceptibility of eelgrass Zostera marina to the wasting disease pathogen Labyrinthula zosterae. Marine Ecology Progress Series, 377, 123–130. http://www.jstor.org/stable/24873028 Methods

Muehlstein, L. K., Porter, D., & Short, F. T. (1988). Labyrinthula sp., a marine slime mold producing the symptoms of wasting disease in eelgrass, Zostera marina. Marine Biology, 99(4), 465–472. https://doi.org/10.1007/bf00392553 <u>https://doi.org/10.1007/BF00392553</u> *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* 

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens HH, Szoecs E, Wagner H (2019) Vegan: Community Ecology Package. R package version 25-4. https://cran.r-project.org/package=vegan <u>https://cran.r-</u> <u>project.org/src/contrib/Archive/vegan/vegan\_2.5-4.tar.gz</u> Software

R Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical

Computing. Vienna, Austria. https://www.r-project.org 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

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nature Methods, 9(7), 671–675. https://doi.org/<u>10.1038/nmeth.2089</u> *Software* 

Soetaert, K., Petzoldt, T., Setzer, R. W. (2010). Solving differential equations in R: package deSolve. Journal of Statistical Software, 33(9), 1-25. *Results* 

Therneau, T. M. (2015). A package for survival analysis in S. version 2.38. https://CRAN.R-project.org/package=survival Software

Therneau, T. M., Grambsch, P. M. (2000). Modeling survival data: extending the Cox Model. Springer, New York. ISBN 0-387-98784-3. *Results* 

Vu, V. Q. (2011). ggbiplot: A ggplot2 based biplot. R package version 0.55. http://github.com/vqv/ggbiplot *Software* 

Wright, K. (2018). corrgram: plot a correlogram. R package version 1.13. https://CRAN.Rproject.org/package=corrgram *Software* 

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## **Related Datasets**

### IsRelatedTo

Hughes, A. R., Schenck, F. (2022) **Seawater temperature and salinity of mesocosms and a field location collected while conducting experiments on seagrass in Nahant, Massachusetts.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-05-04 doi:10.26008/1912/bco-dmo.851059.1 [view at BCO-DMO]

Hughes, A. R., Schenck, F. (2022) **Wasting disease prevalence and severity and seagrass length and density based on subpopulations of Zostera marina on the North Shore of Massachusetts surveyed in July and September 2016.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-05-04 doi:10.26008/1912/bco-dmo.851122.1 [view at BCO-DMO]

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### Parameters

Parameter	Description	Units
tank	Unique identifier number assigned to each of the eleven 54 liter tanks in the MSC glasshouse supplied with flow-through seawater used as water baths for our mesocosms	Unitless
mesocosm	Unique identifier assigned to each of the 88 45 centimeter tall and 15 centimeter diameter transparent cylindrical acrylic mesocosms used to hold a Zostera marina plant	Unitless
infection	Descriptor of the inoculation treatment applied to Zostera marina plants: N (control-inoculation); Y (L. zosterae-inoculation)	Unitless
experiment_day	Timing of sampling following start of inoculation treatment	Days
	·	

subpopulation	Descriptor of the subpopulation source of Zostera marina plants: CB (Curlew Beach, Nahant, 42° 25.2378' N, 70° 54.9474' W); LP (Lynch Park, Beverly, 42° 32.6925' N, 70° 51.5057' W); NB (Niles Beach, Gloucester, , 42° 35.8268' N, 70° 39.3553' W); WB (West Beach, Beverly, 42° 33.9155' N, 70° 47.1102)	Unitless
exp_leaf_no	Descriptor of the relative age of leaves of each Zostera marina plant observed following start of inoculation treatment: 1 (the youngest leaf of each plant at start of inoculation treatment); relative leaf age increases as the value of the integer increases; leaves produced over the course of the experiment were assigned a progressively smaller integer (0, -1, etc.)	Unitless
current_leaf_no	Descriptor of the relative age of leaves of each Zostera marina plant at each sampling point: 1 (the youngest leaf); relative leaf age increases as the value of the integer increases	Unitless
exp_day_leaf_appeared	Timing of first appearance above the sheath of each Zostera marina leaf: 0 (leaf present at start of inoculation treatment)	Days
length	Length of each Zostera marina leaf measured from sheath top to leaf tip	Centimeters
area	Area of each Zostera marina leaf from sheath top to leaf tip	Centimeters squared
wd_lesion_area	Area of lesions characteristic of wasting disease on each Zostera marina leaf from sheath top to leaf tip	Centimeters squared
prior_lesions	Binomial code for the presence of lesions characteristic of wasting disease on leaves prior to isolation of Zostera marina in mesocosms: 1 (lesions present) or 0 (no lesions present)	Unitless
S1_no_leaves	The number of leaves of the primary shoot of Zostera marina plants	Unitless
S2_no_leaves	The number of leaves of the secondary shoot of Zostera marina plants	Unitless
no_leaves_new	Total number of new leaves produced by Zostera marina plants following the start of inoculation treatment	Unitless
no_leaves_lost	Total number of leaves lost by Zostera marina plants following the start of inoculation treatment	Unitless
delta_sl_total	The decrease in sheath length of Zostera marina plants following the start of the inoculation treatment	Centimeters
shoot_no	The number of shoots of Zostera marina plants	Unitless
mortality_day	Timing of the mortality Zostera marina plants	Days
stat	Binomial code for the mortality of Zostera marina plants: 1 (mortality) or 0 (survival)	Unitless
S1_length	Length of the primary Zostera marina shoot measured from the first node to tip of the longest leaf	Centimeters
S2_length	Length of the secondary Zostera marina shoot measured from the first node to the tip of the longest leaf	Centimeters
rhizome_length	Length of the Zostera marina rhizome measured from the first node of the primary shoot to the end of the rhizome	Centimeters
ag_biomass	Above-ground biomass of Zostera marina plants	Grams
bg_biomass	Below-ground biomass of Zostera marina plants	Grams

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# Instruments

Dataset- specific Instrument Name	Nikon eclipse 50i microscope.
Generic Instrument Name	Microscope - Optical
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

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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.

# Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	<u>OCE-1652320</u>

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