Prevalence and intensity of oyster parasite species following a reef restoration experiment in Quonochontaug Pond, Rhode Island, USA from 2017-2020

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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
Hanley, Torrance C.	Northeastern University	Scientist
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Intraspecific variation in host susceptibility to individual parasite species is common, yet how these effects scale to mediate the structure of diverse parasite communities in nature is not as well understood. To address this knowledge gap, we tested how host genetic identity affects parasite communities on restored reefs seeded with juvenile oysters from different sources – a regional commercial hatchery or one of two wild progenitor lines. We assessed the prevalence and intensity of three micro- and two macro-parasite species for four years following restoration. Despite the spatial proximity of restored reefs, oyster source identity strongly predicted parasite community prevalence across all years, with sources varying in their relative susceptibility to different parasites. Oyster seed source also predicted reef-level parasite intensities across space and through time. Our results highlight that host intraspecific variation can shape parasite community structure in natural systems, and reinforce the importance of considering source identity and diversity in restoration design.

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Coverage

Spatial Extent: **Lat**:41.33961 **Lon**:-71.728111
Temporal Extent: 2017-05-01 - 2020-10-31

Methods & Sampling

Oyster Reef Restoration Experiment
In May 2017, we created nine oyster reefs in Quonochontaug Pond, Rhode Island (RI) (41°20'22.6"N, 71°43'41.2"W), with three separate reefs each located within three distinct regions (West, Northeast, East). Each ~22 square meter (m^2) reef (0.5-0.8 m height) was constructed from a base layer of steam-shucked clam shell topped with clean, recycled oyster shell, and then seeded with remote-set spat on shell (see

Davenport et al. 2022 for a detailed description of reef construction). A goal of this study was to compare the performance of different oyster sources and the consequent structure of associated parasite communities, so one replicate reef per oyster source was constructed in each of the three regions of Quonochontaug Pond (hereafter, blocks). The oyster seed sources included one line from a regional commercial hatchery, as well as two wild progenitor lines spawned from broodstock collected from nearby existing wild populations in Green Hill Pond, RI and Narrow River, RI. All oyster lines were spawned at local hatcheries, transferred to Roger Williams University Shellfish Hatchery and set on oyster shell in June 2016, and then stored in cages on an oyster lease in Quonochontaug Pond until reef construction in May 2017.

Reef Monitoring and Oyster Collection
In the fall of each year for four years post-restoration (2017-2020), we monitored oyster density and size distribution by non-destructively sampling six haphazard 0.25 m^2 quadrats per reef and recording the number of live and dead oysters, as well as shell height of a subsample of up to 50 live and 30 dead oysters (following methods of Griffin et al. 2012 (see Supplemental Files) and guidance of Baggett et al. 2015). Coincident with each fall monitoring event, we harvested ~35 haphazardly-selected live oysters from each reef for analysis of parasite communities; samples were transported to the Northeastern University Marine Science Center on ice and then stored at -80 degrees Celsius prior to processing.

Parasite Prevalence and Intensity
Crassostrea virginica is commonly infected by a variety of micro- and macro-parasites simultaneously. We assessed the prevalence (proportion of sampled oysters infected per oyster reef) and intensity (parasite concentration per infected host) of five common parasite species (microparasites: *Perkinsus marinus* (Dermo disease), *Haplosporidium nelsoni* (MSX disease), and *Haplosporidium costale* (SSO disease); macroparasites: *Cliona* spp. and *Polydora*spp.).

To assess microparasite prevalence and intensity, DNA was extracted from up to 32 oysters per reef and then amplified using both a polymerase chain reaction (PCR) assay modified from Stokes & Burreson (2001) SSO protocol, and a multiplex quantitative polymerase chain reaction (qPCR) assay modified from De Faveri et al. (2009) Dermo protocol and Wilbur et al. (2012) MSX protocol. DNA was extracted using 20-40 milligrams (wet weight) of gill and mantle tissue with the Omega Bio-Tek E-Z 96® Tissue DNA Kit.

We used a modified version of the De Faveri et al. (2009) Dermo protocol and the Wilbur et al. (2012) MSX protocol to analyze the samples on a Bio-Rad CFX96TM Real-Time System with Bio-Rad CFX Manager Software (version 3.1). Each reaction consisted of 1 microliter (µl) template DNA, 3 µl water, 5 µl TaqMan Multiplex Master Mix, and 0.5 µl of each 20X primer/probe master mix, which contained 18 µM of each *P. marinus* primer and 5 µM of TaqManTM QSY probe (De Faveri *et al.* 2009) for Dermo, and 18 µM of each *H. nelsoni* primer and 5 µM of TaqManTM MGB probe (Wilbur et al. 2012) for MSX. qPCR cycling conditions included initial denaturation at 95°C for 30 sec, followed by 40 cycles of 95°C for 10 sec and 60°C for 30 sec. We used gBlocks® (gene fragments containing the target regions from *P. marinus* and *H. nelsoni*; Integrated DNA Technologies) to develop standard curves, and extracted DNA from cultures of *P. marinus* and *H. nelsoni* with known cell quantities to use as positive controls. All standards, samples, and positive and negative controls were run in duplicate; if samples differed by >1 Cq, they were rerun to confirm presence/absence and/or parasite concentration.

We used a modified version of the Stokes & Burreson (2001) SSO protocol to assess *H. costale* prevalence on a Bio-Rad CT100 thermal cycler. PCR cycling conditions included initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 30 sec, 59°C for 30 sec, and 72°C for 60 sec, and final extension at 72°C for 5 min. PCR products were visualized on a 1% agarose gel and photographed for analysis of band intensity using ImageJ, following the methods of DeLong & Hanley (2013). For the SSO assay, we used gBlocks® containing the target region from *H. costale* as positive controls and as standards for estimating parasite concentration based on quantification of band intensity using ImageJ (Abràmoff et al. 2004).

To assess the prevalence and intensity of two common macroparasites, boring sponge (*Cliona* spp.) and mud blister worm (*Polydora* spp.), we photographed the inside (mud blisters) and/or outside (sponge holes) of both top and bottom valves for all samples with holes characteristic of boring sponges and blisters characteristic of mud blister worms to quantify the proportion of affected shell area (i.e., [(total infected area/total shell area)*100]) using ImageJ (Abràmoff et al. 2004), following the methods of Hanley et al. (2019).

Data Processing Description

Data Processing
For oyster density, we calculated the mean number of live oysters per reef as the average density across the

six replicate quadrats sampled. For oyster size, we calculated the mean shell height of up to 50 live oysters per quadrat and then took the average across the six replicates per reef. For each parasite, we calculated reef-level prevalence as [number of infected oysters] / [number of oysters sampled], and reef-level intensity as the mean parasite concentration per infected host (i.e., uninfected hosts were not included).

BCO-DMO Processing
- replaced "NA" with "nd" (no data value);
- renamed fields to comply with BCO-DMO naming conventions.

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Data Files

File
oyster_parasite_data.csv (Comma Separated Values (.csv), 5.12 KB) MD5:9e8ec9523b26d15fd2e1303285ff4a06 Primary data file for dataset ID 883570

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Supplemental Files

File
Griffin et al. 2012 filename: RI-Oyster-Monitoring-Metrics-20120412.pdf (Portable Document Format (.pdf), 1.13 MB) MD5:e01eb06528922f429d475c691efbdcc3 Griffin M, DeAngelis B, Chintala M, Hancock B, Leavitt D, Scott T, Brown DS, Hudson R. 2012 Rhode Island oyster restoration minimum monitoring metrics and assessment protocols. 1–24. Prepared for the Rhode Island Shellfish Technical Working Group. Methods reference for dataset 883570.

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Related Publications

Abràmoff, M.D, Magalhães, P.J., Ram, S.J. 2004. Image processing with ImageJ. Biophotonics International 11(7): 36−42
Software

Baggett, L. P., Powers, S. P., Brumbaugh, R. D., Coen, L. D., DeAngelis, B. M., Greene, J. K., Hancock, B. T., Morlock, S. M., Allen, B. L., Breitburg, D. L., Bushek, D., Grabowski, J. H., Grizzle, R. E., Grosholz, E. D., La Peyre, M. K., Luckenbach, M. W., McGraw, K. A., Piehler, M. F., Westby, S. R., & zu Ermgassen, P. S. E. (2015). Guidelines for evaluating performance of oyster habitat restoration. Restoration Ecology, 23(6), 737–745. Portico. https://doi.org/10.1111/rec.12262
Methods

Davenport, T. M., Grabowski, J. H., & Hughes, A. R. (2022). Edge effects influence the composition and density of reef residents on subtidal restored oyster reefs. Restoration Ecology. Portico. https://doi.org/10.1111/rec.13693
Methods

De Faveri, J., Smolowitz, R. M., & Roberts, S. B. (2009). Development and Validation of a Real-Time Quantitative PCR Assay for the Detection and Quantification of Perkinsus marinus in the Eastern Oyster, Crassostrea virginica. Journal of Shellfish Research, 28(3), 459–464. https://doi.org/10.2983/035.028.0306
Methods

DeLong, J. P., & Hanley, T. C. (2013). The Rate-Size Trade-Off Structures Intraspecific Variation in Daphnia ambigua Life History Parameters. PLoS ONE, 8(12), e81024. https://doi.org/10.1371/journal.pone.0081024
Methods

Hanley, T. C., Grabowski, J. H., Schneider, E. G., Barrett, P. D., Puishys, L. M., Spadafore, R., McManus, G., Helt, W. S. K., Kinney, H., Conor McManus, M., & Randall Hughes, A. (2023). Host genetic identity determines parasite community structure across time and space in oyster restoration. Proceedings of the Royal Society B: Biological Sciences, 290(1995). https://doi.org/10.1098/rspb.2022.2560
Results

Hanley, T., White, J., Stallings, C., & Kimbro, D. (2019). Environmental gradients shape the combined effects of multiple parasites on oyster hosts in the northern Gulf of Mexico. Marine Ecology Progress Series, 612, 111–125. doi:10.3354/meps12849
Methods

Stokes, N.A. & Burreson, E.M. (2001). Differential Diagnosis Of Mixed Haplosporidium Costale And Haplosporidium Nelsoni Infections In The Eastern Oyster, Crassostrea Virginica, Using DNA Probes. Journal Of Shellfish Research, 20(1), 207-213. https://scholarworks.wm.edu/vimsarticles/475
Methods

Wilbur, A., Ford, S., Gauthier, J., & Gomez-Chiarri, M. (2012). Quantitative PCR assay to determine prevalence and intensity of MSX (Haplosporidium nelsoni) in North Carolina and Rhode Island oysters Crassostrea virginica. Diseases of Aquatic Organisms, 102(2), 107–118. https://doi.org/10.3354/dao02540
Methods

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Parameters

Parameter	Description	Units
Site	location of oyster restoration experiment (Quonochontaug Pond, Charlestown, Rhode Island, US)	unitless
Year	year of oyster and parasite sampling (2017-2020); all sampling was conducted in the fall	unitless
Block	replicate block locations in Quonochontaug Pond (1-3)	unitless
Reef_ID	unique ID for each reef in the oyster restoration experiment; number refers to block and letter refers to treatment (seed source or control)	unitless
Seed_Source	source of spat deployed on reef as spat on shell; GHP = Green Hill Pond, NAR = Narrow River, HAT = local hatchery	unitless
Mean_Oyster_Density	mean oyster density (number per m^2) based on sampling of six haphazard quadrats per reef	number per meter-squared (#/m^2)
Mean_Oyster_Size	mean oyster shell height (longest distance from umbo; mm) based on sampling of six haphazard quadrats per reef and measurements of <=50 live and <=30 dead oysters per quadrat	millimeters (mm)

Sample_Count	number of oysters collected and processed from each reef on each sampling date	number of oysters
Boring_Sponge_Prevalence	proportion of oysters with holes characteristic of boring sponge	unitless (proportion)
BS_Count_Infected	count of oysters with holes characteristic of boring sponge	count
BS_Count_Uninfected	count of oysters without holes characteristic of boring sponge	count
Mud_Blister_Worm_Prevalence	proportion of oysters with blisters characteristic of mud blister worm	unitless (proportion)
MBW_Count_Infected	count of oysters with blisters characteristic of mud blister worm	count
MBW_Count_Uninfected	count of oysters without blisters characteristic of mud blister worm	count
SSO_Prevalence	proportion of oysters infected with Haplosporidium costale based on PCR assay	unitless (proportion)
SSO_Count_Infected	count of oysters infected with Haplosporidium costale based on PCR assay	count
SSO_Count_Uninfected	count of oysters not infected with Haplosporidium costale based on PCR assay	count
Dermo_Prevalence	proportion of oysters infected with Perkinsus marinus based on qPCR assay	unitless (proportion)
Dermo_Count_Infected	count of oysters infected with Perkinsus marinus based on qPCR assay	count
Dermo_Count_Uninfected	count of oysters not infected with Perkinsus marinus based on qPCR assay	count
MSX_Prevalence	proportion of oysters infected with Haplosporidium nelsoni based on qPCR assay	unitless (proportion)
MSX_Count_Infected	count of oysters infected with Haplosporidium nelsoni based on qPCR assay	count

MSX_Count_Uninfected	count of oysters not infected with Haplosporidium nelsoni based on qPCR assay	count
Dermo_Intensity_SQ_per_mg	mean concentration Perkinsus marinus (copy number based on gBlocks standard) per wet weight oyster tissue (in milligrams) (calculated for infected oysters only)	copy number Perkinsus marinus per milligram (mg) oyster tissue
SSO_Intensity_pg_per_mg	mean concentration Haplosporidium costale (in picograms) per wet weight oyster tissue (in milligrams) (calculated for infected oysters only)	picogram (pg) Haplosporidium costale per milligram (mg) oyster tissue
BS_Intensity	mean percent of oyster shell (top and bottom valves) with holes characteristic of boring sponge ([(total infected area/total shell area)*100]), calculated for infected oysters only	percent
MBW_Intensity	mean percent of oyster shell (top and bottom valves) with blisters characteristic of mud blister worm ([(total infected area/total shell area)*100]), calculated for infected oysters only	percent

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Instruments

Dataset-specific Instrument Name	iPhone
Generic Instrument Name	Camera
Dataset-specific Description	An iPhone (various models) on a tripod was used to photograph oysters for image analysis.
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	Bio-Rad CFX96
Generic Instrument Name	qPCR Thermal Cycler
Dataset-specific Description	Bio-Rad CFX96TM Real-Time System – used for qPCR analysis of oyster microparasites
Generic Instrument Description	An instrument for quantitative polymerase chain reaction (qPCR), also known as real-time polymerase chain reaction (Real-Time PCR).

Dataset-specific Instrument Name	Bio-Rad CT100 thermal cycler
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	Bio-Rad CT100 thermal cycler – used for PCR analysis of oyster microparasites
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

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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
U.S. Fish & Wildlife Service Wildlife and Sport Fish Restoration Program (USFWS WSFR)	WSFR F20AF00145

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