Performance traits (e.g., survival, growth, size) for hatchery-produced oyster cohorts

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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
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Coverage

Spatial Extent: **N**:34.6951 **E**:-76.6183 **S**:30.0224 **W**:-81.4199
Temporal Extent: 2012-04 - 2012-07

Methods & Sampling

In April 2012, we collected 100 adult oysters (80-100 mm shell length) from 3-5 separate reefs at each of 6 sites: St. Augustine, FL (FL-1; 30.0224, -81.3287), Jacksonville, FL (FL-2; 30.4446, -81.4199), Sapelo Island, GA (GA/SC-1; 31.4777, -81.2726), Ace Basin, SC (GA/SC-2; 32.4846, -80.6001), Masonboro, NC (NC-1; 34.1510, -77.8551), and Middle Marsh, NC (NC-2; 34.6951, -76.6183). They were held in flowing seawater tanks or suspended in cages from docks in their home region for 2-3 weeks until 30 oysters from each site could be tested and certified as disease free. The remaining 70 oysters were then shipped on ice to a single hatchery facility in Florida (Research Aquaculture Inc., Tequesta, FL; 26.9607, -80.0931) at the end of April.

The adult oysters from each site were used as the broodstock to produce 6 separate site-specific "cohorts" (one cohort per site). From their arrival at the hatchery, the adult oysters were held for 2 weeks until they were ready to spawn under the same conditions in separate flow-through seawater systems to prevent cross-contamination. All families were manually spawned (i.e., strip spawned) on May 7 (see details below). Because the original FL-1 family did not produce many offspring, the remaining broodstock oysters from this site were spawned on June 1 using the same process. Due to variation in ripeness and sex, the number of oysters spawned and the ratio of males to females varied across broodstock (Table 1 of Hughes et al., 2019), though our broodstock numbers for each cohort are comparable to those commonly used in hatchery settings (30-60 individuals; Morvezen et al. 2016).

The broodstock oysters from each source site were strip spawned, sexed, and fertilized on the same day by a team of 7 people, who each had a specific job to perform: shucking the animals, sampling and preparing tissue for microscopic analysis of sex, identifying the sex, stripping the male sperm, stripping the female eggs, mixing the sperm and eggs after all of the animals from a particular source were stripped, overseeing the process and keeping track of broodstock source. We sanitized equipment between individuals and again between broodstock sources. Stripping was done by broodstock source independently and quickly so that the sperm and eggs would remain viable, and all viable sperm and eggs were used. During the gamete mixing process, the eggs from all females and the sperm from all males were first pre-mixed and then combined to ensure equal access of gametes to one another. We allowed 30-60 minutes for fertilization; once 75-90% of the eggs were fertilized, they were moved to larval tanks. All larvae were retained except for minimal numbers of individuals in each cohort that did not grow or had improper development. Larval culture occurred in 60-gallon conical tanks utilizing a flow-through seawater system with Banjo screens that is commercially used in multiple bivalve hatcheries (e.g., Taylor Shellfish in WA; Cherrystones in VA).

Over a period of 3 days the week of May 28, oysters were sieved on a 250-micron sieve and settled on crushed oyster cultch in a recirculating flow-through system. The week of June 11, once they reached 800 microns in size, they were moved into a nursery facility compliant with state regulations, again under flow-through seawater conditions (salinity = 32 ppt, temperature = 30ºC). In the hatchery and nursery stages, the oysters were fed a mixed diet of T. isochrysis, Chaetocerous gracilis, and Tetraselmis via a constantly running peristaltic pump. Although growth was similar during the larval culture phase, some cohorts produced more juvenile oysters ("spat") than others during settlement, despite following the same procedures for all. To maintain consistency in their growing conditions, we selected a random sample of each cohort to yield similar total abundances across cohorts on June 18. At the end of June (June 27) at approximately 4mm in size, the 6 cohorts were transferred to a common flow-through facility at the Whitney Marine Biological Laboratory in St. Augustine, FL. To assess genetic diversity within and between oyster cohorts produced in the hatchery, 50 individuals were haphazardly collected from each juvenile cohort prior to the start of the field experiments and preserved at -80°C for genetic analysis. This sample size is sufficient to estimate allele frequencies accurately (Hale et al. 2012).

To extract DNA, we ground each tissue sample with a pestle, and used the tissue centrifugation protocol from the Omega Bio-Tek E-Z 96 Tissue DNA Kit. We determined genetic diversity and population structure using 12 highly variable microsatellite loci developed for C. virginica: Cvi9, Cvi11, and Cvi13 from Brown et al. (2000); Cvi1i24b, Cvi2i23, Cvi2j24, and Cvi2k14 from Reece et al. (2004); Cvi4313E-VIMS from Carlsson and Reece (2007); and RUCV1, RUCV66, RUCV73, and RUCV74 from Wang and Guo (2007). We amplified four loci in each multiplexed polymerase chain reaction (PCR) using the Qiagen Type-It Microsatellite PCR Kit. Each 10 l reaction consisted of 1 l DNA template, 5 l 2X type-it multiplex master mix (Qiagen), 2.4 l water, and 0.2 l each 10 M primer. Using a T100 thermal cycler (Bio-Rad), PCR cycling conditions included initial activation/denaturation at 95°C for 5 min, followed by 28 cycles of 95°C for 30 sec, 60°C for 90 sec, and 72°C for 30 sec, and final extension at 60°C for 30 min. PCR products were separated on a 3730xl Genetic Analyzer (Applied Biosystems) with the internal size standard GeneScan 500 LIZ (Applied Biosystems), and fragment analysis was performed using GeneMarker version 2.6 (SoftGenetics).

We created panels for each multiplexed reaction in GeneMarker, which included bins that were assigned manually for all alleles; the same panels were used to score all samples, and the alignment of the panels was checked prior to each analysis to account for any run-to-run variation and to identify any new alleles. We used these panels to do a preliminary first assignment of alleles based on peak position and bin position, but every sample was then scored manually for all loci to examine signal intensity, to confirm the presence/absence of alleles, and to identify any reruns. A subset of samples was then rerun (at least 15% per multiplex PCR reaction) and manually scored again to confirm any uncertain allele calls and account for any genotyping error.

We experimentally evaluated the performance (size, growth, survivorship) of each 2012 juvenile oyster cohort in the field as a function of within-cohort effective allelic diversity. These same oysters were analyzed for different response variables as part of two other studies (Hanley et al. 2016, Hughes et al 2017; see Appendix S1 of Hughes et al., 2019 for additional information). These studies used the same experimental design. Namely, in each experiment, 12 spat from a single cohort were affixed to 10*10 cm experimental tiles using the marine adhesive Z-spar. Tiles were held in flow-through seawater tables for less than 48 hours until being deployed to the field. Prior to deployment, we measured shell height of each spat and photographed all tiles. At the end of each experiment, live oysters were counted and measured.

Oysters at three of the five sites included here have previously been analyzed in a test of genetic by environmental variation across oyster cohorts (Hughes et al. 2017): spat from each cohort were deployed on July 12-14, 2012 across 3 field sites in the South Atlantic Bight that spanned the geographic range of the source populations: FL-EXP (29.6714, -81.2162); GA-EXP (31.9213, -80.9880), or NC-EXP (34.7069, -

76.7631). At each field site, we deployed 18 tiles (6 cohorts * 3 tiles per cohort) to each of 9 natural intertidal oyster reefs. Low spat abundance in the FL-1 cohort limited replication of this cohort to 4 reefs per experimental site (N=147 tiles total). The 3 tiles from each cohort were haphazardly assigned to one of three predation treatments (full cage, with mesh with 6mm*6mm openings; partial cage to control for caging artifacts; no cage) and deployed on the reef in a completely randomized design; only the full cage and no cage treatments are addressed further here. This experiment lasted 6 weeks.

Data collected on oysters deployed at the other two field sites used in the present study come from a concurrent longer-term experiment focused on the effects of oyster cohort diversity that included additional treatments not analyzed here (Hanley et al. 2016). In this study, 36 tiles were deployed (6 cohorts * 6 tiles per cohort) at each of two sites in the Matanzas River estuary, FL (FL-North: 29.75177, -81.25578; FL-South: 29.65838, -81.22193) on July 24-25, 2012. The 6 tiles from each cohort were split across the same three predation treatments as above and deployed in a completely randomized design. This experiment lasted 6 months.

Data Processing Description

Our juvenile oyster cohorts differed from each other in several measures of genetic diversity, so we tested whether genetic variation predicted ecological trait variation across cohorts in our experiments. We focused on effective allelic diversity, a metric similar to allelic richness (i.e., number of alleles) that weights the number of alleles by their frequencies to determine the effective number of alleles in the population (Meirmans and Van Tienderen 2004). Thus, effective allelic diversity integrates information about the number of alleles and their distribution (Meirmans and Van Tienderen 2004). This metric is sensitive to variation in sample size (Meirmans 2013), so we held sample sizes consistent across groups of juvenile and adult oysters (Table 1 of Hughes et al., 2019). Effective allelic diversity was highly correlated with both allelic richness (positive; $R^2 = 0.96$; $y = 1.71x + 1.49$) and genetic relatedness (negative; $R^2 = 0.98$; $y = -0.08x + 0.52$), so we focused only on effective allelic diversity here. Results of analyses including allelic richness rather than effective allelic diversity were similar (Appendix S3 of Hughes et al., 2019). Our response variables included: initial size (average shell height per tile before being deployed in the field); survival in the absence of predation (number of live oysters in cages at the end of the experiment, modeled with a binomial generalized linear model (GLM) with logit link); survival in the presence of predation (number of live oysters on open tiles at the end of the experiment, modeled with a binomial GLM with logit link); final size (average shell height per tile at the end of the experiment); and oyster growth (standardized as (final oyster shell height - initial oyster shell height) / initial oyster shell height for each individual per tile). Because we hypothesized that differences in initial oyster size may affect oyster performance, we included initial size as a covariate in our analyses. In all analyses, we tested linear models including a fixed effect of effective allelic diversity with experimental site as a random factor to account for differences in experimental duration or other unmeasured variables. Analyses were run in R software (version 3.0.2) using the packages lme4 and lmerTest (which calculates F and P-values using the Sattterthwaite approximation for degrees of freedom).

BCO-DMO Processing: replaced spaces with underscores in parameter names.

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Data Files

File
oyster_cohort_traits.csv (Comma Separated Values (.csv), 11.40 KB) MD5:160004e8b45c5cf53e35bbc267017145 Primary data file for dataset ID 770157

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Related Publications

Bates, D., Maechler, M., & Bolker, B. (2013). lme4: Linear mixed-effects models using S4 classes. R package version 0.999999-2.

Software

Brown, B. L., Franklin, D. E., Gaffney, P. M., Hong, M., Dendanto, D., & Kornfield, I. (2000). Characterization of microsatellite loci in the eastern oyster, Crassostrea virginica. Molecular Ecology, 9(12), 2216–2218. doi:10.1046/j.1365-294x.2000.105333.x https://doi.org/10.1046/j.1365-294X.2000.105333.x
Methods

CARLSSON, J., & REECE, K. S. (2006). Eight PCR primers to amplify EST-linked microsatellites in the Eastern oyster,Crassostrea virginicagenome. Molecular Ecology Notes, 7(2), 257–259. doi:10.1111/j.1471-8286.2006.01573.x
Methods

Garza, J. C., & Williamson, E. G. (2001). Detection of reduction in population size using data from microsatellite loci. Molecular Ecology, 10(2), 305–318. doi:10.1046/j.1365-294x.2001.01190.x https://doi.org/10.1046/j.1365-294X.2001.01190.x
Methods

Hale, M. L., Burg, T. M., & Steeves, T. E. (2012). Sampling for Microsatellite-Based Population Genetic Studies: 25 to 30 Individuals per Population Is Enough to Accurately Estimate Allele Frequencies. PLoS ONE, 7(9), e45170. doi:10.1371/journal.pone.0045170
Methods

Hanley, T. C., Hughes, A. R., Williams, B., Garland, H., & Kimbro, D. L. (2016). Effects of intraspecific diversity on survivorship, growth, and recruitment of the eastern oyster across sites. Ecology, 97(6), 1518–1529. doi:10.1890/15-1710.1
Methods

Hughes, A. R., Hanley, T. C., Byers, J. E., Grabowski, J. H., Malek, J. C., Piehler, M. F., & Kimbro, D. L. (2016). Genetic by environmental variation but no local adaptation in oysters (Crassostrea virginica). Ecology and Evolution, 7(2), 697–709. doi:10.1002/ece3.2614
Methods

Hughes, A. R., Hanley, T. C., Byers, J. E., Grabowski, J. H., McCrudden, T., Piehler, M. F., & Kimbro, D. L. (2019). Genetic diversity and phenotypic variation within hatchery-produced oyster cohorts predict size and success in the field. Ecological Applications. doi:10.1002/eap.1940
Results

MEIRMANS, P. G., & VAN TIENDEREN, P. H. (2004). genotype and genodive: two programs for the analysis of genetic diversity of asexual organisms. Molecular Ecology Notes, 4(4), 792–794. doi:10.1111/j.1471-8286.2004.00770.x
Methods

Meirmans, P. G., & Van Tienderen, P. H. (2012). The effects of inheritance in tetraploids on genetic diversity and population divergence. Heredity, 110(2), 131–137. doi:10.1038/hdy.2012.80
Methods

Morvezen, R., Boudry, P., Laroche, J., & Charrier, G. (2016). Stock enhancement or sea ranching? Insights from monitoring the genetic diversity, relatedness and effective population size in a seeded great scallop population (Pecten maximus). Heredity, 117(3), 142–148. doi:10.1038/hdy.2016.42
Methods

Nei, M. (1987). Molecular evolutionary genetics. New York: Columbia University Press. https://isbnsearch.org/isbn/9780231063210
Methods

Reece, K. S. (2004). Microsatellite Marker Development and Analysis in the Eastern Oyster (Crassostrea virginica): Confirmation of Null Alleles and Non-Mendelian Segregation Ratios. Journal of Heredity, 95(4), 346–352. doi:10.1093/jhered/esh058
Methods

Wang, Y., & Guo, X. (2007). Development and Characterization of EST-SSR Markers in the Eastern Oyster Crassostrea virginica. Marine Biotechnology, 9(4), 500–511. doi:10.1007/s10126-007-9011-7
Methods

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Parameters

Parameter	Description	Units
Exp	Unique identifier for the 2 experiments included in this dataset	unitless
Site	Unique identifier for the site location within each experiment	unitless
Eff_allelic_diversity	Effective allelic diversity for that oyster cohort	unitless
Allelic_richness	Number of alleles for that oyster cohort	Number of alleles
Genetic_relatedness	Metric of genetic relatedness within that oyster cohort calculated using STORM (Frasier 2008)	unitless
Cohort	Unique identifier for one of 6 oyster cohorts used in the experiments	unitless
Final_avg_size	Average shell height of the oysters remaining on that experimental replicate at the end of the experiment	millimeters (mm)
Initial_avg_size	Average shell height of the oysters on that experimental replicate at the start of the experiment	millimeters (mm)
Cage_alive	Number of oysters alive at the end of the experiment in the cage (no predator) treatment	Number of oysters
Cage_dead	Number of oysters that were dead at the end of the experiment in the cage (no predator) treatment	Number of oysters
Open_tile_alive	Number of oysters alive at the end of the experiment in the open tile (control) treatment	Number of oysters
Open_tile_dead	Number of oysters that were dead at the end of the experiment in the open tile (control) treatment	Number of oysters
Growth	Average difference in final shell height and initial shell height standardized by initial shell height for each oyster per experimental replicate	millimeters (mm)

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Instruments

Dataset-specific Instrument Name	3730xl Genetic Analyzer (Applied Biosystems)
Generic Instrument Name	Automated DNA Sequencer
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

Dataset-specific Instrument Name	T100 thermal cycler (Bio-Rad)
Generic Instrument Name	Thermal Cycler
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

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