Acropora cervicornis coral disease exposure experiment conducted on Summerland Key, FL from August to September of 2015 (Resilient Acerv project)

Website: https://www.bco-dmo.org/dataset/642860
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Project
» CAREER: Applying phenotypic variability to identify resilient Acropora cervicornis genotypes in the Florida Keys (Resilient Acerv)

Contributors	Affiliation	Role
Muller, Erinn M.	Mote Marine Laboratory (Mote)	Principal Investigator, Contact
Gegg, Stephen R.	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

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Dataset Description

Summer 2015 disease susceptibility tests

Methods & Sampling

Sampling and Analytical Methodology:
Three trials were conducted to test the disease susceptibility of 15 different genotypes of Acropora cervicornis in August 2015. Approximately 10 replicates of each genotype were collected from the Mote offshore coral nursery. Replicates consisted of approximately 5cm branch tips collected from different individuals of the same genotype. Fragments were attached to glass microscope slides using super glue and allowed to acclimate in outdoor raceways for 2 days prior to treatment. One replicate of each genotype was placed within a single five gallon glass tank, holding approximately 10 liters of water. A total of ten tanks were used, five for the disease homogenate applications, and five for healthy homogenate applications. Temperature within the raceways were kept at ~28.5C; pH (8.0), and salinity (34ppt) were measured daily and remained constant throughout the trials.

After the acclimation period, fragments of diseased corals were collected from a natural reef located at 24'31.640N, 81'29.938W (Permit: FKNMS 2015-84). Fragments showing active signs of tissue loss were snipped off of the donor colony, placed in plastic containers and brought back to the boat for transport to Mote TRL. Upon return to the lab, the diseased corals were airbrushed with filtered sterilized seawater and collected in 50 ml falcon tubes. Tissue adjacent to the disease edge, up to 4 cm away was removed through this method and collected to create a disease homogenate. Healthy homogenates were created from nursery coral fragments. Approximately 10, 5 cm long fragments were airbrushed and collected for the healthy homogenate applications for each trial. Each tank, holding 10 L of water, were treated with either 100 ml of disease homogenate or 100 ml of healthy homogenate.

For the next 8 days the presence of tissue loss was identified from each individual replicate. Photographs of each coral were taken daily. The rate of tissue loss was calculated (cm/day) from each replicate using ImageJ

software.

An imaging pulse amplitude fluorometer was used after the 2 day acclimation period to quantify the photochemical efficiency of each replicate coral. The photochemical yield was quantified and a light reaction curve was applied.

Snips, approximately 2 cm in size, were taken from two random replicates within each genotype for Symbiodinium sequencing. Preliminary analyses indicate that all genotypes contain only A3 clade symbiodinium, but they may harbor different genotypes of the symbiont. These samples were flash frozed at -80C and stored until processing. DNA was isolated and extracted from 2 - 3 polyps worth of tissue using the Qiagen DNAeasy DNA isolation kit. Samples will be sent to Dr. Iliana Baums at Penn State University for microsatellite analysis and identification of possible variations in symbiont genotypes within coral genets.

In September of 2015, when corals were visibly bleached from anomalous high water temperatures, a fourth trial was conducted using the same methods described above to test the difference in susceptibility during a thermal stress event. Diseased fragments were collected from the same location as the original trial.

Changes from original proposal: Ideally, all 48 genotypes would have been tested during the summer of 2015. However, even in August 2015 (prior to peak bleaching), several coral genotypes were suffering from thermal stress already. Only the 15 genotypes collected and used for experimentation did not visually appear to be initally stressed during August. Collaborator and nursery manager, Erich Bartels, did not encourage the collection of the other genotypes as they were already thermally stressed; however, Erich agreed to provide the same 15 genotypes in September 2015 even though they were visibly bleached for the sake of a novel research experiment.

Data Processing Description

Data Processing:
Statistical analyses:

To determine variations in disease susceptibility we compared fragments exposed to disease homogenates and those exposed to healthy homogenates using a relative risk analysis. The relative risk of exposed (disease homogenates) individuals was compared with the relative risk of non-exposed (healthy homogenate) individuals. The relative risk, or risk ratio, is the number of individuals with disease after exposure to the risk divided by the number of individuals with disease that have not been exposed to the risk:

Relative risk (RR) = Risk in exposed/Risk in non-exposed ,

where the *risk in exposed* individuals was calculated as the prevalence (diseased/total population) of exposed corals and the *risk in non-exposed* individuals was calculated as the prevalence of disease in non-exposed corals. When RR=1 then there is no association between the risk and disease occurrence. However when RR>1 then there is a positive association and when RR<1 there is a negative association. The relative risk was calculated using a Bayesian approach and estimated using a binomial likelihood distribution and a uniform-Beta prior distribution. To obtain an estimate of relative risk, Markov Chain Monte Carlo simulations were used with Gibbs sampling in OpenBUGS (MRC Biostatistics Unit, Cambridge, UK). Ninety-five percent credible intervals were calculated for each estimate of relative risk. The relative risk was calculated for each genotype. Credible intervals that did not include a value of one were considered significant, with a credible interval above one signifying a higher risk of disease because of damage. A credible interval below one signified a higher risk of disease from the lack of damage. Furthermore, the range of the 95% credible intervals provided a general measure of the confidence in each relative-risk estimate, with a large range signifying low confidence in the estimate.

An additional relative risk analysis was conducted where the disease exposed individuals of the non-thermally stressed corals were compared with the disease exposed individuals of the thermally stressed corals. Here, thermal stress was considered the 'exposure' in the relative risk analysis.

BCO-DMO Processing Notes
- Generated from original file "Disease susceptibility Summer 2015_data submitted.xlsx" contributed by Erinn Muller
- Parameter names edited to conform to BCO-DMO naming convention found at Choosing Parameter Name
- Site, Latitude and Longitude added to data to enable mapping
- "nd" (no data) inserted into blank cells

- "NA" and "n/a" replaced with "nd" (no data)
- "Date_Collected" and "Date_Inoculated" values reformatted to YYYYMMDD

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Parameters

Parameter	Description	Units
Site	Sampling/Work Site	text
Latitude	Latitude (South is negative)	decimal degrees
Longitude	Longitude (West is negative)	decimal degrees
Trial	Trial: characterizes the order of the experimental exposure	dimensionless
Tank	Tank: denotes the exact aquaria that held the fragment	dimensionless
Date_Collected	Date Collected: date that the fragment was collected from the coral nursery	YYYYMMDD
Date_Innoculated	Date Inoculated: date that the disease or healthy homogenate was applied to the tanks	YYYYMMDD
Treatment	Treatment: identifies whether the tank was either treated with the disease homogenate (E); or the healthy homogenate (C)	text
Genotype	Genotype: identifying number of the distinct genotype of the coral fragment	integer
Color	Color - Color represents a certain genotype of coral, which is also denoted within the genotype number as well.	text
Initial_disease_signs	Initial disease signs: the date that first disease associated mortality was observed	M/DD/YYYY HH:MM (AM/PM)
Complete_mortality	Complete mortality: the date that the entire fragment was dead	M/DD/YYYY HH:MM (AM/PM)
Fv2Fm_at_innoculation	Fv/Fm at inoculation: photochemical efficiency on the day of inoculation (prior to exposure)	dimensionless
Fv2Fm_after_innoculation	Fv/Fm after inoculation: photochemical efficiency 3 days after inoculation occurred	dimensionless
Second_Fv2Fm_data_after_innoculation	Second Fv/Fm after inoculation: a repeat measurement of photochemical efficiency 3 days after inolculation occurred	dimensionless

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Instruments

Dataset-specific Instrument Name	Glass tanks
Generic Instrument Name	Aquarium
Dataset-specific Description	One replicate of each genotype was placed within a single five gallon glass tank, holding approximately 10 liters of water. A total of ten tanks were used, five for the disease homogenate applications, and five for healthy homogenate applications. Each tank, holding 10 L of water, were treated with either 100 ml of disease homogenate or 100 ml of healthy homogenate.
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	Qiagen DNAeasy DNA isolation kit
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Snips, approximately 2 cm in size, were taken from two random replicates within each genotype for Symbiodinium sequencing. Preliminary analyses indicate that all genotypes contain only A3 clade symbiodinium, but they may harbor different genotypes of the symbiont. These samples were flash frozed at -80C and stored until processing. DNA was isolated and extracted from 2 - 3 polyps worth of tissue using the Qiagen DNAeasy DNA isolation kit. Samples will be sent to Dr. Iliana Baums at Penn State University for microsatellite analysis and identification of possible variations in symbiont genotypes within coral genets.
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	Imaging pulse amplitude fluorometer
Generic Instrument Name	Fluorometer
Dataset-specific Description	An imaging pulse amplitude fluorometer was used after the 2 day acclimation period to quantify the photochemical efficiency of each replicate coral.
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

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Deployments

MOTE_Muller_SummerlandKey-Summer2015

Website	https://www.bco-dmo.org/deployment/642857
Platform	Mote Offshore Coral Nursery
Start Date	2015-08-13
End Date	2015-09-23
Description	Three trials were conducted to test the disease susceptibility of 15 different genotypes of Acropora cervicornis in August 2015. Approximately 10 replicates of each genotype were collected from the Mote offshore coral nursery.

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Project Information

CAREER: Applying phenotypic variability to identify resilient Acropora cervicornis genotypes in the Florida Keys (Resilient Acerv)

Coverage: Florida Keys, Summerland Key, FL 24.563595°, -81.278572°

NSF Award Abstract:
Caribbean staghorn coral was one of the most common corals within reefs of the Florida Keys several decades ago. Over the last 40 years disease, bleaching, overfishing and habitat degradation caused a 95% reduction of the population. Staghorn coral is now listed as threatened under the U.S. Endangered Species Act of 1973. Within the past few years, millions of dollars have been invested for the purpose of restoring the population of staghorn coral within Florida and the U.S. Virgin Islands. Significant effort has been placed on maintaining and propagating corals of known genotypes within coral nurseries for the purpose of outplanting. However, little is known about the individual genotypes that are currently being outplanted from nurseries onto coral reefs. Are the genotypes being used for outplanting resilient enough to survive the three major stressors affecting the population in the Florida Keys: disease, high water temperatures, and ocean acidification? The research within the present study will be the first step in answering this critically important question. The funded project will additionally develop a research-based afterschool program with K-12 students in the Florida Keys and U.S. Virgin Islands that emphasizes an inquiry-based curriculum, STEM research activities, and peer-to-peer mentoring. The information from the present study will help scientists predict the likelihood of species persistence within the lower Florida Keys under future climate-change and ocean-acidification scenarios. Results of this research will also help guide restoration efforts throughout Florida and the Caribbean, and lead to more informative, science-based restoration activities.

Acropora cervicornis dominated shallow-water reefs within the Florida Keys for at least the last half a million years, but the population has recently declined due to multiple stressors. Understanding the current population level of resilience to three major threats - disease outbreaks, high water temperatures, and ocean acidification conditions - is critical for the preservation of this threatened species. Results from the present study will answer the primary research question: will representative genotypes from the lower Florida Keys provide enough phenotypic variation for this threatened species to survive in the future? The present proposal will couple controlled laboratory challenge experiments with field data and modeling applications, and collaborate with local educators to fulfill five objectives: 1) identify *A. cervicornis* genotypes resistant to disease, 2) identify *A. cervicornis* genotypes resilient to high water temperature and ocean acidification conditions, 3) quantify how high water temperature and ocean acidification conditions impact disease dynamics on *A. cervicornis*; 4) determine tradeoffs in life-history traits because of resilience factors; and 5) apply a trait-based model, which will predict genotypic structure of a population under different environmental scenarios.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1452538

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