Acropora cervicornis transcriptomes: nutrient- and disease-exposed from samples collected at Mote Marine Laboratory in situ nursery from June to July 2022

Website: https://www.bco-dmo.org/dataset/924760
Data Type: experimental, Other Field Results
Version: 1
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Project
» Collaborative Research: Tracking the interacting roles of the environment, host genotype, and a novel Rickettsiales in coral disease susceptibility (Coral Rickettsiales)

Contributors	Affiliation	Role
Vega Thurber, Rebecca	Oregon State University (OSU)	Principal Investigator
Muller, Erinn M.	Mote Marine Laboratory (Mote)	Co-Principal Investigator
Klinges, Grace J.	Mote Marine Laboratory (Mote)	Scientist, Contact
Merchant, Lynne M.	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager
Soenen, Karen	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
We confirmed data from previous studies showing that genotypes of Acropora cervicornis dominated by the coral parasite Aquarickettsia are more susceptible to White Band Disease than genotypes with diverse microbiomes. However, in a study with ramets from genotypes exhibiting each microbiome profile, we found that the strongest predictor of disease development was prior exposure to increased thermal stress that occurred independent of the study on Florida's reef during the experiment. While no ramets of the coral genotype with a diverse microbiome developed disease after exposure to nutrient enrichment, seven ramets of this genotype developed disease after exposure to elevated temperatures and subsequent disease challenge. Two ramets exposed to elevated temperatures and not exposed to disease also died, suggesting that temperature alone could have been fatal to this genotype. An equal number of ramets exposed to elevated nutrients and elevated temperatures developed disease in the genotype dominated by Aquarickettsia. To evaluate the effect of Aquarickettsia infection; nutrient enrichment; disease exposure; and the combination of these factors on coral immune function, we conducted RNAseq on 42 samples to an averaging sequencing depth of 22,168,890.6 reads per sample (paired-end).

Table of Contents

Coverage

Location: Mote Marine Laboratory Elizabeth Moore International Center for Coral Reef Research & Restoration. Research conducted in ex situ aquaria
Spatial Extent: **Lat**:24.56257 **Lon**:-81.40009
Temporal Extent: 2022-06-29 - 2022-07-27

Methods & Sampling

Samples of coral tissue, skeleton, and mucus were taken from two genotypes of *Acropora cervicornis* prior to nutrient enrichment (n = 20 per genotype), prior to disease exposure (n = 18 per genotype), and at various stages during disease development. The samples were collected at the Mote Marine Laboratory in situ nursery from June to July 2022. All surviving ramets at one week after disease exposure were sampled. To sample each coral, 6-8 polyps were excised using a flame-sterilized blade and placed in a 1.5mL microcentrifuge tube containing 1mL of DNA/RNA shield (Zymo Research, R1100-250, Irvine, CA, USA). Samples were transferred to a -80℃ freezer for long-term storage. In preparation for RNA extractions, the samples were removed from the -80℃ freezer and thawed on ice. With flame-sterilized tweezers, half of the biomass was transferred to a Disruptor Tube (Omega Bio-Tek, Norcross, GA, USA), the other half was kept as a bioarchive and returned to -80℃. RNA from each sample was isolated utilizing the E.Z.N.A. DNA/RNA Isolation Kit (Omega Bio-Tek, Norcross, GA, USA) with slight modifications to the manufacturer's protocol to increase yield. RNA isolates were stored at -80℃. DNA quantity and quality was assessed utilizing a NanoDrop spectrophotometer (Thermo Fisher Scientific™, Waltham, MA, USA). Samples were shipped on dry ice to the Oklahoma Medical Research Foundation NGS Core, where RNA cleanup, precipitation, and polyA selection was performed. Libraries were prepared using the IDT xGen RNA Library kit. Final QC was performed using KAPA qPCR and Agilent Tapestation to confirm rRNA content, and libraries were sequenced on a NovaSeq 6000 using S4 chemistry.

Data Processing Description

A total of 42 samples were successfully sequenced on an Illumina NovaSeq 6000 with S4 chemistry and PE 150 bp reads. Prior to quality filtering, an average single-end read depth of 11,084,445.3 +/- 970,330.08 was produced from sequencing. After filtration, an average of 10,954,237.2 +/- 982,482.11 reads remained per read direction. From quality filtered sequences, 72.54% of single end reads mapped to the *A. cervicornis* transcriptome using STAR. Quantification using Salmon resulted in 24,875 genes having at least one count across all samples, with subsequent filtering (less than 1 count in >10 samples) reducing this to 12,913 genes for downstream analysis. Of reads not aligning to the *A. cervicornis* transcriptome, an average of 22.54% aligned to the Symbiodinium (Clade A) reference transcriptome using STAR. Quantification using Salmon yielded counts for 73,112 transcripts, with 26,225 of these retained for downstream analysis after filtering (less than 1 count in greater than 10 samples). Analysis of differentially expressed transcripts is ongoing.

BCO-DMO Processing Description

Four submitted files (SRA_metadata_NutDis_rnaseq.xlsx, Nut_Dis_RNAseq_Invertebrate.1.0.xlsx, Accession_numbers_nut_dis_rnaseq.tsv, and metadata_rnaseq.xlsx) were joined together to create the primary dataset file.
This is the process to create the final dataset using the BCO-DMO data processor Laminar.
1) The file SRA_metadata_NutDis_rnaseq.xlsx was loaded into Laminar and the following empty columns were removed: filename3, filename4, assembly, fasta_file.

2) The file Nut_Dis_RNAseq_Invertebrate.1.0.xlsx was loaded into Laminar, and the * prefix on variable names was removed to follow the BCO-DMO parameter naming convention.

3) The dataset tables of SRA_metadata_NutDis_rnaseq.xlsx and Nut_Dis_RNAseq_Invertebrate.1.0.xlsx were joined on the common unique column sample_name. The joined dataset is named join_1.

4) The dataset table of Accession_numbers_nut_dis_rnaseq.tsv is joined with the table join_1 on the column sample_name. The joined dataset is named join_2.

5) Duplicate columns were removed in join_2.

6) The column message was removed in join_2. The column message contained entries of "successfully loaded" and it was removed because it is NCBI submission metadata and not related to the dataset.

7) The column breed was removed because it has a constant value of "not applicable" which is NCBI submission metadata and not related to the dataset.

8) The column accession was renamed to BioSample to clarify what accession values the column holds.

9) The file SRA_metadata_NutDis_rnaseq.xlsx was loaded into Laminar and the column full_name was duplicated and then the date portion was removed and the column named full_name_id. This is to create a common unique column to join onto table join_2. For example, the entry 36.C16.DT1.7.24 became 36.C16.DT1 with the date portion of July 24th (.7.24) removed. And then the periods were replaced with hyphens (36-C16-DT1).

10) The column full_name_id in the dataset table of SRA_metadata_NutDis_rnaseq.xlsx nearly matches the column sample_title in table join_2. The value in row 33, not including the header row, of full_name_id, 46-N13-DT1A, is the only mismatch between sample_title in table join_2 and full_name_id in metadata_rnaseq.xlsx. In order to perform the join, the ending 'A' was removed from the row 33 value of full_name_id, but not removed from full_name.

11) The table join_2 was joined with SRA_metadata_NutDis_rnaseq.xlsx on the common unique columns of sample_title in join_2 and full_name_id in SRA_metadata_NutDis_rnaseq.xlsx. The resulting dataset table was named join_3.

12) The temporary column full_name_id was removed from the table join_3.

13) Metadata of latitude and longitude of the Mote laboratory where samples were collected was added to join_3.

14) The column full_name was renamed Library_name from looking on NCBI and seeing the column full_name contains the values of Library name for the BioSamples.

15) The BioProject accession value of PRJNA1047741 was added to table join_3.

16) Duplicate columns and empty columns were removed.

17) The collection date was reformatted from %m-%d-%y to the ISO 8601 format of %Y-%m-%d.

18) Finally, the table join_3 was saved and renamed as the primary dataset file 924760_v1_acropora_cervicornis_transcriptomes_nutrient_and_disease_exposed.csv

[table of contents | back to top]

Parameters

Parameters for this dataset have not yet been identified

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Illumina Nova Seq 6000
Generic Instrument Name	Automated DNA Sequencer
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset-specific Instrument Name	
Generic Instrument Name	Thermo Scientific NanoDrop spectrophotometer
Generic Instrument Description	Thermo Scientific NanoDrop spectrophotometers provide microvolume quantification and purity assessments of DNA, RNA, and protein samples. NanoDrop spectrophotometers work on the principle of ultraviolet-visible spectrum (UV-Vis) absorbance. The range consists of the NanoDrop One/OneC UV-Vis Spectrophotometers, NanoDrop Eight UV-Vis Spectrophotometer and NanoDrop Lite Plus UV Spectrophotometer.

[table of contents | back to top]

Project Information

Collaborative Research: Tracking the interacting roles of the environment, host genotype, and a novel Rickettsiales in coral disease susceptibility (Coral Rickettsiales)

Coverage: at Oregon State University and in the Florida Keys at Mote Marine Laboratory

NSF Award Abstract:
Historically one of the most abundant reef-building corals in Florida and the wider Caribbean, the staghorn coral, *Acropora cervicornis*, is now listed as critically endangered primarily because of previous and reoccurring disease events. Understanding the holistic mechanisms of disease susceptibility in this coral is a top concern of practitioners engaged in conservation and restoration. The investigators recently discovered a group of parasitic bacteria common within the microbial community of *A. cervicornis* that can reduce the growth and health of corals when reefs are exposed to nutrient polluted waters. Determining how interactions among the coral host, this parasitic microbe, and the environment are linked to disease susceptibility provides critical insight and greater success of future restoration efforts. Yet the complexity of animal microbiomes and the contextual nature of disease make it difficult to identify the specific cause of many disease outbreaks. In this project, the investigators conduct experiments to explore the interactions among different genetic strains of coral and these bacteria in various nutrient scenarios to better understand how this bacterium affects the susceptibility of staghorn coral to diseases. This project also characterizes the genomics, host range, and local and global distribution of this bacterial coral parasite to determine how its evolutionary history and physiology drive disease susceptibility in this important coral species. The project trains two postdocs, one technician, and seven students (one graduate, six undergraduates) in integrative sciences that span marine science, physiology, genetics, microbiology, omics, and statistical modeling. A research-based after school program in Florida is expanded to include microbiology and create a new program module called Microbial warriors, with a focus on women in science. The investigators produce documentary style films and outreach materials to broadly communicate the project science and conservation efforts to local and national communities via presentations at Mote Marine Lab and the Oregon Museum of Science and Industry. This project is co-funded by the Biological Oceanography Program in the Division of Ocean Sciences and the Symbiosis, Defense, and Self-recognition Program in the Division of Integrative Organismal Systems.

The investigators recently identified a marine *Rickettsiales* bacterium that, in corals, can be stimulated to grow in the presence of elevated nitrogen and phosphorous species. Based on genomic reconstruction and phylogeography, this bacteria is classified as a novel bacterial genus, *Candidatus Aquarickettsia*, and showed that it is broadly associated with scleractinian corals worldwide. Importantly, using a model system, the endangered *Acropora cervicornis* coral, the team has also shown that the growth of this bacterium in vivo is associated with reduced host growth and increased disease susceptibility. This project aims to more completely evaluate the mechanisms behind and impacts of these inducible infections on coral physiology and host-bacterial symbiosis. The investigators conduct nutrient dosing experiments on different coral genotypes with various *Rickettsiales* abundances. Using a range of omics and microscopy techniques, the team quantifies the resulting effects on holobiont phenotypes. The investigators are also comparing the genomes of these bacteria in the different Acroporid hosts and other coral genera to evaluate facets of the bacterium's evolutionary history, as well as to identify possible mechanisms of its proliferation, virulence, and host

specificity. This interdisciplinary project mechanistically links nutrients to temporal changes in host, algal symbiont, and bacterial parasite physiology and also explain why there is natural variation in these responses by exploring how host and parasite genotypes and growth dynamics combined with environmental contextuality alter holobiont phenotypes.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1923836
NSF Division of Ocean Sciences (NSF OCE)	OCE-1923926

[table of contents | back to top]