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

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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
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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).

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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. 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°C freezer for long-term storage. In preparation for RNA extractions, the samples were removed from the -80°C 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°C. 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°C. 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 files were joined together to create the final dataset.

This is the process to get to the final dataset.

1) The file metadata_rnaseq.xlsx was joined with Accession_numbers_nut_dis_rnaseq.tsv on the columns full_name and a reformatted sample_name.

To join metadata_rnaseq.xlsx with Accession_numbers_nut_dis_rnaseq.tsv, one would logically use the column of "ID" in metadata_rnaseq.xlsx to join on the column of "sample_title" in Accession_numbers_nut_dis_rnaseq.tsv since they are of the same format. But the "ID" value of row 5 of data in metadata_rnaseq.xlsx is "36-C15" but it should be "36-C15-DT1".

The next best columns to join on are "full_name" of format (36.C15.DT1.7.25) in metadata_rnaseq.xlsx with "sample_name" of format (36-C15-DT1_7/25/22) in Accession_numbers_nut_dis_rnaseq.tsv. In order to do that, "sample_name" was duplicated in Accession_numbers_nut_dis_rnaseq.tsv and, reformatted by replacing a dash with a period, an underscore with a period, and a forward slash with a period, and then the last portion of the year was removed with regex in the duplicated "sample_name" column.

2) In the file metadata_rnaseq.xlsx in row 33 of data, there is a value 46.N13.DT1A.7.23 in the column rnaseq_name but the corresponding value in Accession_numbers_nut_dis_rnaseq.tsv is 46-N13-DT1_7/23/22 which looks to be the correct value because the value of the column "ID" in the file metadata_rnaseq.xlsx in row 33 of data is "46-N13-DT1" with no 'A' in the value. So the value of 46.N13.DT1A.7.23 was changed to 46.N13.DT1.7.23 by removing an 'A'.

The joined table was named join_1.

3) The columns "bioproject_accession" and "host" were removed. Duplicated columns were removed and the column "message" was removed because it is specific to an NCBI submission value of "Successfully loaded".

4) Then table join_1 was joined with Nut_Dis_RNAseq_Invertebrate.1.0.xlsx on the column sample_title.

The joined table was named join_2.

5) Duplicate columns sample_name, sample_title, isolate, organism, isolation_source, geo_loc_name, collection_date, Date, tissue, Genotype, Treatment, Exposure, Disease_exposure, Health_status, and group were removed. The column breed was removed because it holds an NCBI specific submission field with the value "not applicable". Finally, the empty columns "bioproject_accession"" and "host" were removed.

6) To prepare table join_2 to be joined with the file SRA_metadata_NutDis_rnaseq.xlsx, a cell value in SRA_metadata_NutDis_rnaseq.xlsx of "46.N13.DT1A.7.23" in the column "library_ID" of data row 33 was changed to "46.N13.DT1.7.23" by removing an 'A'.

This is to match earlier substitution to remove A in file metadata_rnaseq.xlsx which also replaced the value 46.N13.DT1A.7.23 to 46.N13.DT1.7.23 by removing an 'A'. And also because the sample_name value is 46-N13-DT1_7/23/22 which does not contain an 'A'.

7) Then table join_2 was joined with the file SRA_metadata_NutDis_rnaseq.xlsx on the column "full_name" and "library_ID" because they are both unique and of the same format.

The resulting table was named join_3.

8) Empty columns in table join_3 were removed. File information columns were removed that is stored at NCBI and can be retrieved by following accession numbers. Instrument columns were removed because the instrument will be noted on the dataset page. The breed column was removed which was an NCBI specific submission column with the value of "not applicable".

The duplicate column "full_name" was removed.

9) The columns "*sample_name" and "rnaseq_name" look like duplicate columns but in the column "*sample_name" in row 5 of the data, there is the value 36-C15-DT1_7/25/22 and in the column "rnaseq_name" in row 5 of the data, there is the value 36-C15_7/25/22 which is missing the "DT1" portion.

For now, both the "*sample_name" and "rnaseq_name" columns are retained.

The "sample_title" column looks to be a duplicate of the "ID" column. The "ID" column, however, has a value at row 5 of "36-C15" which is missing a "DT1" portion.

But in the columns "full_name" and "sample_title", row 5 of the data contains the "DT1" portion.

For now, the "ID" column and the "sample_title" column are retained even though the remaining values are duplicates.

Is the missing DT1 portion in the rnaseq_name and ID column a mistake?

10) Columns were renamed to the BCO-DMO format without an asterix in the name.

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	Illumina Nova Seq 6000
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	
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.

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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.

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Funding

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