Acropora cervicornis protein measurements: 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/924466
Data Type: experimental
Version: 1
Version Date: 2025-07-23

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
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Abstract
In the summer of 2022, 20 ramets each of the Acropora cervicornis genotypes ML-AC-36 and ML-AC-46 were collected from Mote's in situ coral nursery and subjected to three weeks of elevated nitrate, ammonium, and phosphate in the form of a slow-release fertilizer. The duration and concentration of this exposure were selected based on prior experiments conducted at Mote Marine Laboratory, which were found to alter microbial community profiles and growth rates in A. cervicornis (Klinges et al., 2022, 2023). During experiment, corals were held in 2-gallon aquaria with 5 corals per aquarium. As Mote Marine Laboratory's experimental aquarium system is plumbed into nearshore coastal water and thus has a higher nutrient load than the reef, an additional subset of 18 ramets of each genotype was collected from Mote's in situ coral nursery immediately prior to disease challenge to evaluate the impact of nearshore water on disease response. All ramets in the disease group were subjected to disease challenge in the form of a tissue homogenate produced from diseased fragments of random genotypes of A. cervicornis. Ramets in a comparative unexposed group were exposed to a homogenate produced from healthy fragments of random genotypes of A. cervicornis. To evaluate the effect of Aquarickettsia infection; nutrient enrichment; disease exposure; and the combination of these factors on coral immune function, a number of immune related proteins and antioxidants were measured using microplate assays from a total of 55 samples taken prior to disease exposure and following disease exposure as corals either resisted or developed disease. Total host protein and the antioxidant superoxide dismutase were found to be higher in healthy corals regardless of genotype or nutrient enrichment. Phenoloxidase and prophenoloxidase were higher in concentration in diseased samples compared to healthy samples. Disease-exposed but apparently healthy corals had higher superoxide dismutase, prophenoloxidase, and peroxidase activity than either healthy unexposed corals or diseased corals despite total protein concentrations that were lower than healthy unexposed corals.

Table of Contents

Coverage

Location: Mote Marine Laboratory Elizabeth Moore International Center for Coral Reef Research & Restoration
Spatial Extent: **Lat**:24.661552 **Lon**:-81.45438
Temporal Extent: 2022-07-22 - 2022-07-27

Methods & Sampling

Proteins were extracted over ice using a Paansche airbrush with coral extraction buffer (50 mmol tris buffer, pH 7.8, with 0.05 mmol dithiothreitol). Tissues were then homogenized using a VWR 200 tissue homogenizer with a medium saw tooth generator for 60 seconds on ice. Samples were then left on ice for 10 minutes. From the resulting extract, 1 mL was reserved for melanin analysis. The remaining volume was centrifuged for 5 minutes at 4°C and 3500 RPM in an Eppendorf centrifuge 5810R. The resulting supernatant, or coral extract, was split into two ~2 mL aliquots mL which were frozen and stored at -80°C.

Total host protein (total HP) in each sample was determined using Bradford reagent standardized to BSA. These concentrations were used to standardize all biochemical assays conducted on the samples.

All colorimetric assays were run in triplicate on 96-well plates using a Synergy H1 Hybrid Multi-Mode microplate reader and Gen5 software. Samples were stored at -80°C and thawed immediately prior to processing.

To measure peroxidase (POX), 20 µL of sample were diluted with 20 µL 10 mM phosphate buffer, pH 6.0. Then 25 µL of 25 mM guaiacol in 10 mM phosphate buffer, pH 6.0, was added to each well. The reaction was initiated with 20 µL of 20 mM hydrogen peroxide and optical density was measured every 34 seconds for 15 minutes at 470 nm. Results were calculated as change in absorbance per minute, normalized according to mg of protein.
To measure prophenoloxidase (PPO), 20 µL of sample were diluted with 20 µL 50 mM phosphate buffer, pH 7.0. Next, samples were incubated for 30 minutes in 25 µL of trypsin (0.1 mg/mL). Just prior to the assay, 30 µL of 10 mM L-1,3- dihydroxphenylalanine (L-dopa) was added to each sample. Absorbance was then read every minute for 20 minutes at 490 nm at 26C. Results were calculated as change in absorbance per minute at the steepest point of the curve, normalized according to mg of protein.
To measure phenoloxidase (PO), 20 µL of sample were diluted with 20 µL 50 mM phosphate buffer, pH 7.0. Next, samples were incubated for 30 minutes in 25 µL of molecular grade water. Just prior to the assay, 30 µL of 10 mM L-1,3- dihydroxphenylalanine (L-dopa) was added to each sample. Absorbance was then read every minute for 20 minutes at 490 nm at 26°C. Results were calculated as change in absorbance per minute at the steepest point of the curve, normalized according to mg of protein.

A Superoxide Dismutase (SOD) Activity Assay Kit from Sigma Aldrich was used to measure superoxide dismutase activity following manufacturer's protocols. Briefly, 20 µL of coral extract was incubated with WST dye and 20 µL xanthine oxidase for 30 minutes at 25°C. A standard curve was performed using the provided SOD enzyme. Percent inhibition of absorbance was then measured at 450 nm by comparing the absorbance of the samples to that of the control wells. Activity is reported as superoxide dismutase activity standardized by mg protein.

Data Processing Description

Peroxidase: Results were calculated as change in absorbance per minute at the steepest point of the curve, normalized according to mg of protein.

Prophenoloxidase: Results were calculated as change in absorbance per minute at the steepest point of the curve, normalized according to mg of protein.

Phenoloxidase: Results were calculated as change in absorbance per minute at the steepest point of the curve, normalized according to mg of protein.

Superoxide dismutase: Percent inhibition of absorbance was then measured at 450 nm by comparing the absorbance of the samples to that of the control wells. Activity is reported as superoxide dismutase activity standardized by mg protein.

Problem Description

Coefficient of variation and standard deviation of mean were used to evaluate whether samples performed in triplicate should be rerun. A coefficient of variation less than 30 was considered to be acceptable. In triplicate measures with an obvious outlier, samples were rerun in triplicate and averages were made across both runs, omitting the outlier.

[table of contents | back to top]

Data Files

File	
924466_v1_muller_nutrientdisease.csv	(Comma Separated Values (.csv), 11.38 KB) MD5:20d786e13a3e00195360b1f61a3aa098
Primary data file for dataset ID 924466, version 1	

[table of contents | back to top]

Supplemental Files

File	
Raw PO Data.zip	(ZIP Archive (ZIP), 1.37 MB) MD5:bd56e3b6cee3c31ae1ecc0c1e4e215c0
Raw PO Data	
Raw POX Data.zip	(ZIP Archive (ZIP), 956.63 KB) MD5:d4ecf168745d5bbc25fa7535fc372a57
Raw POX Data	
Raw PPO Data.zip	(ZIP Archive (ZIP), 731.52 KB) MD5:c4fe63b984a4b3f4fed8d3490c043150
Raw PPO Data	
Raw SOD Data.zip	(ZIP Archive (ZIP), 503.98 KB) MD5:ff924cb766901ef262652af94a6516ba
Raw SOD Data	
Raw Total Host Protein Data.zip	(ZIP Archive (ZIP), 849.70 KB) MD5:6dbaff81d3adeef42df87f9f10d8ad8e
Raw Total Host Protein Data	
SummaryStatistics.xlsx	(Microsoft Excel, 19.30 KB) MD5:2eac22e72ca0b276ef5d61461b38a993
Summary statistics by metadata categories.	

[table of contents | back to top]

Related Publications

Fuess, L. E., Pinzón C., J. H., Weil, E., & Mydlarz, L. D. (2016). Associations between transcriptional changes and protein phenotypes provide insights into immune regulation in corals. Developmental & Comparative Immunology, 62, 17–28. https://doi.org/10.1016/j.dci.2016.04.017
Methods

Klinges, J. G., Patel, S. H., Duke, W. C., Muller, E. M., & Vega Thurber, R. L. (2023). Microbiomes of a disease-resistant genotype of Acropora cervicornis are resistant to acute, but not chronic, nutrient enrichment.

Scientific Reports, 13(1). https://doi.org/10.1038/s41598-023-30615-x
Methods

Klinges, J. G., Patel, S. H., Duke, W. C., Muller, E. M., & Vega Thurber, R. L. (2022). Phosphate enrichment induces increased dominance of the parasite Aquarickettsia in the coral Acropora cervicornis. FEMS Microbiology Ecology, 98(2). https://doi.org/10.1093/femsec/fiac013
Methods

Mydlarz, L. D., & Palmer, C. V. (2011). The presence of multiple phenoloxidases in Caribbean reef-building corals. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 159(4), 372–378. https://doi.org/10.1016/j.cbpa.2011.03.029
Methods

[table of contents | back to top]

Related Datasets

IsRelatedTo

Klinges, G. J., Muller, E. M., Vega Thurber, R. (2025) **Acropora cervicornis 16S rRNA sequence metadata: nutrient- and disease-exposed from samples collected at Mote Marine Laboratory in situ nursery from June to July 2022.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-07-24 doi:10.26008/1912/bco-dmo.924465.1 [view at BCO-DMO]
Relationship Description: The protein measurements and the 16S rRNA sequence datasets are from the same experiment and same individual animals, but different samples.

[table of contents | back to top]

Parameters

Parameter	Description	Units
Full_ID	genotype and replicate number with sampling timepoint	unitless
Date	Date of sampling in ISO format	unitless
TP	Total host protein	mg
POX	Absorbance read every 34 seconds for 15 minutes at 470 nm at 26C. POX activity as assessed by change in measured absorbance per minute at the steepest point of absorbance curve.	ΔAbs470nm
Normalized_POX	POX activity standardized by mg protein	ΔAbs470nm/mg protein
PO	Absorbance read every minute for 20 minutes at 490 nm at 26C. PO activity as assessed by change in measured absorbance per minute at the steepest point of absorbance curve.	ΔAbs490nm
Normalized_PO	PO activity standardized by mg protein	ΔAbs490nm/mg protein

PPO	Absorbance read every minute for 20 minutes at 490 nm at 26C. PPO activity as assessed by change in measured absorbance per minute at the steepest point of absorbance curve.	ΔAbs490nm
Normalized_PPO	PPO activity standardized by mg protein	ΔAbs490nm/mg protein
SOD	Percent inhibition of absorbance was measured at 450 nm by comparing the absorbance of the samples to that of the standard wells.	One unit of SOD is defined as the amount of enzyme causing half the maximum inhibition of the oxidation of 7.5 mM NADH in the presence of EDTA, manganese ions, and mercaptoethanol at 23°C and pH 7.4 over 15 minutes.
Normalized_SOD	superoxide dismutase activity standardized by mg protein	SOD units/mg protein
Genotype	Coral genotype identifier (AC36 or AC46)	unitless
Replicate	replicate number within genotype	unitless
Coral_ID	genotype and replicate number	unitless
Tank_ID	aquarium identifier	unitless
Treatment	Control or Nutrient	unitless
Trt	C or N	unitless
Timepoint	NT0 = prior to nutrient exposure, NT3/DT0 = after nutrient exposure and prior to disease exposure, DT0 = prior to diease exposure (not nutrient exposed), DT1 = first signs of disease, DT2 = secondary signs of disease, DT1 H = healthy coral sampled contemporaneously with disease timepoint 1, H = healthy, ND = disease exposed but no diease signs, Mortality = non-disease-related mortality	unitless
Exposure	Baseline (before nutrient exposure), Nutrients 3 weeks, or Reef - July Baseline	unitless
Exposure_basic	Disease Exposure or No Disease Exposure	unitless
Group	Disease or Unexposed	unitless

Status	health status at time of sampling	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Paansche
Generic Instrument Name	Airbrush
Generic Instrument Description	Device for spraying liquid by means of compressed air.

Dataset-specific Instrument Name	Eppendorf centrifuge 5810R
Generic Instrument Name	Centrifuge
Generic Instrument Description	A machine with a rapidly rotating container that applies centrifugal force to its contents, typically to separate fluids of different densities (e.g., cream from milk) or liquids from solids.

Dataset-specific Instrument Name	VWR 200
Generic Instrument Name	Homogenizer
Generic Instrument Description	A homogenizer is a piece of laboratory equipment used for the homogenization of various types of material, such as tissue, plant, food, soil, and many others.

Dataset-specific Instrument Name	Synergy H1 Hybrid Multi-Mode microplate reader
Generic Instrument Name	plate reader
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

[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]