# Application of a rapid microbiome characterization pipeline to corals afflicted with Stony Coral Tissue Loss Disease in St. Thomas, US Virgin Islands.

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**Data Type**: Other Field Results
**Version**: 1
**Version Date**: 2020-12-07

**Project**
» RAPID: Collaborative Research: Predicting the Spread of Multi-Species Coral Disease Using Species Immune Traits (Multi-Species Coral Disease)


| Contributors | Affiliation | Role |
|---|---|---|
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**Abstract**
Application of a rapid microbiome characterization pipeline to corals afflicted with Stony Coral Tissue Loss Disease in St. Thomas, United States Virgin Islands.

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## Coverage

**Spatial Extent**: **N**:18.3445 **E**:-64.89833 **S**:18.27883 **W**:-64.98595
**Temporal Extent**: 2020-02-11 - 2020-02-13

## Dataset Description

Application of a rapid microbiome characterization pipeline to corals afflicted with Stony Coral Tissue Loss Disease in St. Thomas, United States Virgin Islands.

### Methods & Sampling

#### Sample collection

Coral colonies showing active Stony Coral Tissue Loss Disease (SCTLD) as well as nearby completely healthy colonies were targeted for sampling on February 11 and 13, 2020 on Buck Island ("Outbreak", 18.27883°, -64.89833°), and Black Point ("Existing", 18.3445°, -64.98595°) reefs, respectively, in St. Thomas, USVI (Fig. 1). Buck Island was considered a recent outbreak site, and will be referred to as "Outbreak." Coral species sampled included Montastraea cavernosa, Colpophyllia natans, Meandrina meandrites, and Orbicella franksi (Table 1). Black Point had been experiencing SCTLD for approximately one year, and will be referred to as "Existing." Coral species sampled at the Existing site were Montastraea cavernosa and Colpophyllia natans (Table 1). SCTLD was identified by single or multiple lesions of bleached or necrotic tissue with epiphytic algae colonizing the recently dead and exposed skeleton (Fig. 2). At both reefs, some paling of colonies was apparent, especially on Orbicella spp., as a result of a recent bleaching event in October 2019. Due to this, on Orbicella spp. it was challenging to distinguish SCTLD from White Plague-type diseases, which generally occur following bleaching events (Miller et al., 2009). As a result, we avoided sampling Orbicella spp., except when it was clear the colony had regained full coloration and the disease lesion was consistent with SCTLD infection.

To investigate if putative pathogens were recoverable from seawater surrounding diseased colonies, near-coral seawater was sampled from 2-5 cm away from each coral colony prior to tissue sampling. This occurred via negative pressure with a 60 ml Luer-lock syringe (BD, Franklin Lakes, NJ, USA). Two seawater samples were collected over each colony displaying SCTLD lesions: one sample was taken directly above healthy tissue approximately 10 cm away from the lesion, when possible, and a second sample over diseased tissue. Syringes were placed in a dive collection bag for the duration of the dive, and then the seawater was filtered through a 0.22 mm filter (25 mm, Supor, Pall, Port Washington, NY, USA) while onboard the boat and the filter with holder was placed in a Whirl-pak bag and kept on ice until returning to the shore. While onshore, filters were placed in sterile 2 ml cryovials (Simport, Beloeil, QC, Canada) and frozen in a liquid nitrogen dry shipper.

After near-coral seawater sampling, a single tissue sample was taken from the healthy colonies and two samples were taken from each diseased colony. Diseased tissue samples were collected at the interface between healthy and newly bleached tissue (Fig. 2), and then visually healthy tissue was targeted approximately 10 cm away from the disease interface (when possible, sometimes diseased colonies had very little healthy tissue remaining). For some colonies, limited healthy tissue remained and tissue approximately 3 to 5 cm away was targeted. Coral tissue and mucus "slurries" were collected by using 10 ml non-Luer lock syringes (BD). The syringe tip was used to agitate and remove tissue from the colony, while suspended tissue, mucus, and seawater were simultaneously aspirated into the syringe. To control for the significant amount of seawater and seawater-associated microbiota introduced into the tissue sample, a total of nine 10 ml syringes were used to capture only ambient reef seawater greater than 1 m off the reef benthos. This would allow us to assess how much seawater-based bacteria were in the slurry samples, and are hereafter referred to as "Syringe Method Control" samples. Immediately after collection, the syringes were placed in a Whirl-pak bag underwater to prevent the loss of tissue or mucus. Once back onboard the boat, samples were transferred to 15 ml sterile conical tubes and placed in a 4°C cooler. Upon returning to the lab, samples were frozen to -20°C until analysis.

To capture the surrounding seawater physical and chemical environment an Exo2 multiparameter sonde (YSI, Yellow Springs, OH, USA) was used with probes for temperature, salinity, dissolved oxygen, pH, and turbidity (Table S1). On the day before sampling (February 10, 2020), each sonde probe was calibrated following the manufacturer's protocols, and was not calibrated between sampling dates.

**DNA extraction, PCR, and sequencing**

DNA extraction, PCR, and sequencing preparation protocols were specifically designed for the Illumina iSeq 100 System (Illumina Inc., San Diego, CA, USA), a portable, high-quality sequencing technology. In an approximately 1 cu. ft. size, the Illumina iSeq 100 System produces 4 million paired-end sequence reads of high quality (<1% error rate), making it an attractive technology to adapt for field-based microbiome studies. Sequencing runs took approximately 17 hours, and generated about 2 GB of data, which could easily be offloaded and processed on a standard laptop without the need for Wi-Fi. In the USVI, we brought the iSeq 100 System to a home rental, which was transformed into a remote laboratory where we successfully conducted all DNA extractions, PCR and subsequent sequencing.

DNA extraction on both seawater and tissue slurry samples and associated extraction controls was conducted using the DNeasy PowerBiofilm Kit (Qiagen, Germantown, MD, USA). Modifications at the beginning of the extraction were applied based on the sample type. For seawater filters, the 0.22 mm filter was placed directly into the bead tube, and then manufacturer instructions were followed. Three seawater microbiome DNA extraction controls were included (named D4-D6). For those, an unused 0.22 mm filter was used in the DNA extraction method. For tissue slurry samples and syringe method control samples, the slurries were thawed at room temperature, then immediately transferred to 4°C prior to extraction. Slurries were vortexed for 10 seconds, then 1.8 ml of each slurry was transferred to an emptied bead tube. Samples were vortexed at

12,045 rcf (maximum rcf available on centrifuge) for 10 min to concentrate tissue, mucus, and the associated microorganisms at the bottom of the tube, and supernatant was removed. For samples that were very clear (very little tissue collected via syringe), a second aliquot of 1.8 ml of tissue slurry was centrifuged on top of the existing pellet in order to capture more tissue- and mucus-associated microorganisms. Beads were then returned to the tube now containing the pellet and the extraction continued following manufacturer protocols. Three DNA extraction controls were included for the slurry extractions (named D1-D3), and included DNA extractions proceeding using an empty bead tube.

PCR was used to amplify the V4 region of the small sub-unit ribosomal RNA (SSU rRNA) gene of bacteria and archaea. This amplification occurred in two stages. In stage one, 2 ml of tissue slurry template DNA was added to a 50 ml PCR reaction. One ml template and a 25 ml total reaction volume was used for seawater samples. For negative PCR controls, 1 or 2 ml of sterile PCR-grade water was used. One Human Microbiome Project mock community, Genomic DNA from Microbial Mock Community B (even, low concentration), v5.1L, for 16S rRNA Gene Sequencing, HM-782D was included as a sequencing control, and 1 µl was used in a PCR reaction with seawater samples. Fifty ml PCR reactions contained 0.5 ml polymerase (GoTaq, Promega, Madison, WI, USA), 1 ml each of 10 mM forward and reverse primers, 1 ml of 10 mM dNTPs (Promega), 5 ml MgCl2 (GoTaq), 10 ml 5X colorless buffer (GoTaq), and 29.5 ml UV-sterilized and PCR-grade water. PCR reactions with 25 ml proceeded with the exact proportions of reagents as 50 ml reactions. Earth microbiome project primers, 515F and 806R, targeted bacteria and archaea and were used with Illumina-specific adapters (Apprill et al., 2015; Parada et al., 2016). The PCR reaction continued using two small, portable PCR thermocyclers: mini8 (miniPCR, Cambridge, MA, USA), which contained 8 wells and connected to a laptop for programming and initiation of the run, and the BentoLab (Bento Bioworks Ltd, London, UK), which contained 32 wells and was programmable as a unit. The usage of both machines was ideal because the targeted number of samples per iSeq run was 40. PCR in preparation for iSeq sequencing occurred in two separate stages. Stage one PCR proceeded as follows: 2 min at 95°C, then 35 cycles (coral slurry) or 28 cycles (seawater) of 20 sec at 95°C, 20 sec at 55°C, and 5 min at 72°C, followed by 10 min at 72°C and a final hold at 12°C. The final hold at 12°C was used due to the limitations of the BentoLab thermocycler, and samples were generally removed within an hour of the completed PCR program. Thirty ml of the resulting slurry PCR products were mixed with 6 ml 5X loading dye (Bioline, London, UK), then visualized on a 1.5% agarose gel stained with SYBR Safe DNA gel stain (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Bands from tissue samples that were approximately 350 bp long were excised for purification using the MinElute Gel Extraction Kit (Qiagen) following manufacturer protocols. For seawater PCR products, 5 ml of product was visualized on a 1% agarose gel to verify successful amplification, and the rest of the PCR product was purified with the MinElute PCR Purification Kit (Qiagen).

The stage two PCR procedure attached unique index primers to each sample using the Nextera XT v2 set A kit (Illumina). Purified DNA (5 ml) from stage one PCR products was added to a 50 ml reaction with the following: 5 ml Nextera index primer 1, 5 ml Nextera index primer 2, 5 ml MgCl2 (GoTaq), 10 ml 5X colorless buffer (GoTaq), 0.5 ml Taq polymerase (GoTaq), 1 ml of 10 mM dNTPs (Promega), and 18.5 ml UV-sterilized and PCR-grade water. The PCR stripettes were placed in the BentoLab or mini8 thermocyclers for the following program: 3 min at 95°C, 8 cycles of 30 sec at 95°C, 30 sec at 55°C, 30 sec at 72°C, followed by 5 min at 72°C and a hold at 12°C. A subset of PCR products was visualized on a 1% agarose gel stained with SYBR Safe DNA gel stain (Invitrogen) using 5 ml product with 1 ml 5X loading dye (BioLine) to verify bands of approximately 450 bp, indicating successful attachment of sample-specific indexes. The PCR products were purified with the MinElute PCR purification kit (Qiagen) following manufacturer protocols. Purified products were quantified using the Qubit 2.0 fluorometer dsDNA high sensitivity (HS) assay (Invitrogen) following manufacturer protocols to obtain stock concentrations in ng/ml. Concentrations were then converted to nM assuming an average amplicon length of 450 bp and average nucleotide mass of 660 g/mol. Samples were diluted to 5 nM and pooled. Pooled samples were quantified via Qubit HS assay as before, and diluted to 1 nM, quantified again, and diluted to a loading concentration of 90 pM. A 10% PhiX spike-in was added to the pooled 90 pM library and 20 ml of the resulting library was run on the iSeq 100 System using paired-end 150 bp sequencing with adapter removal. Samples were sequenced over three sequencing runs.

**Data analysis**

Forward reads were exclusively used for the downstream processing and data analysis due to minimal overlap between forward and reverse reads. 515F and 806R primers were removed from all sequence reads, and they were filtered for quality and chimeras using the DADA2 pipeline and amplicon sequence variants (ASVs) were generated for each sample (Callahan et al., 2016). The following parameters were used in the filterAndTrim function: trimLeft = 19, truncLen = 145, maxN = 0, maxEE = 1, rm.phix = TRUE, compress = TRUE, multithread = TRUE. This resulted in the production of 17,190 ASVs of all the same length (126 bp) across all samples. ASVs that classified to mitochondria, chloroplast, eukaryote, or an unknown Kingdom were removed from the analysis. This removed many spurious ASVs, resulting in 7,366 remaining ASVs. We further filtered our dataset based on controls, then conducted a sequence-count-based filtering method. The R package

decontam (v. 1.6.0) was used to filter out DNA extraction contaminants in all seawater and tissue samples by using a combined frequency and prevalence method (Davis et al., 2018). The method identified 26 ASV contaminants, of which only 11 contained enriched frequency in DNA extraction controls, so those 11 ASVs were removed (Appendix 1). Following this, tissue/mucus slurry samples were subset and ASVs associated with the bulk (off-coral) seawater samples were removed using the prevalence method in decontam (v. 1.6.0) by comparing all slurry samples to the nine seawater-only syringe samples that were collected as methodological controls because the syringe method by nature collects a significant portion of seawater. The contamination method identified 184 ASVs most prevalent in the seawater controls (typically oligotrophic bacteria such as SAR11, Prochlorococcus, OM60 clade, Synechococcus, “Candidatus Actinomarina”, AEGEAN-169 clade, etc.), which were removed from the slurry sample ASV table. These ASVs were generally found at low relative abundance in tissue samples and was at most 0.0074 (Appendix 2). Tissue and near-coral collected seawater samples were re-merged into one large dataset for further filtering. Next, only ASVs with a count greater than 0.5 when averaged across all samples were kept for further analysis to remove sparse ASVs (present at a count of 0 in the majority of samples). This left 2,010 ASVs, which were used for all downstream analyses.

Count data were transformed to relative abundance and coral tissue microbial communities were visualized using stacked bar charts. Data were then further log transformed following addition of a pseudo count of one in preparation for beta diversity analyses. Bray-Curtis dissimilarity between samples was calculated using the R package vegan and the resulting dissimilarities were presented in a Principal Coordinates Analysis (PCoA) (Oksanen et al., 2019). PERMANOVA (Permutational Analysis of Variance) tests using 999 permutations were conducted between healthy and diseased corals to test the hypothesis that coral microbiomes are significantly different between healthy and SCTLD-afflicted tissues using the adonis function in the vegan R package (Oksanen et al., 2019). We also tested the hypotheses that species, reef location, and health state nested within species would significantly structure the microbial community. We tested the same hypotheses on the near-coral seawater directly overlying the coral colony. Dispersion of beta diversity within coral tissue samples was calculated by measuring the distance to centroid within the PCoA as grouped by health state (HH & HD compared to DD) by implementing the betadisper function in R package, vegan (Oksanen et al., 2019). Significant difference in dispersion by health state was measured with an independent Mann-Whitney U test. Variability of beta diversity was additionally measured by extracting the Bray-Curtis dissimilarity values calculated within a tissue condition (diseased or healthy).

To detect ASVs enriched in diseased coral tissue compared to healthy tissue, the R package, corncob, was employed, which modeled the relative abundance of each ASVs and tested for differential abundance between healthy and diseased coral tissue (Martin et al., 2019). Following analysis of significantly differentially abundant ASVs in the coral tissue, we hypothesized that disease-associated ASVs would be recoverable in the near-coral seawater and graphed relative abundances of each disease-associated ASV in the near-coral seawater. We additionally employed corncob to test each identified disease-associated ASV to see if it was detectable at significantly higher abundances in seawater over diseased tissue compared to healthy tissue or apparently healthy colonies. Furthermore, we compared the ASV sequences of disease-associated ASVs to existing literature on SCTLD to understand if identical taxa were associated with SCTLD in other studies.

Sequences of putative pathogens were identified to the species level, when possible, as part of the DADA2 pipeline. To obtain better genus and species-level identification of putative pathogen ASVs, as well as to relate these ASVs to other studies of coral disease-associated bacteria, we constructed phylogenetic trees for disease-associated ASVs classifying to Vibrio, Arcobacter, Rhizobiaceae, and Rhodobacteraceae. Vibrio and Arcobacter were targeted due to their increased representation in SCTLD-associated ASVs in this study as well as their previous association with SCTLD (Meyer et al., 2019) and coral disease in general (Ben-Haim et al., 2003; Ushijima et al., 2012). Rhizobiaceae and Rhodobacteraceae were targeted for phylogenetic tree analysis given their previous association with SCTLD (Rosales et al., 2020). Phylogenetic trees of coral-associated Vibrio and Rhodobacteraceae bacteria that were previously constructed from the Coral Microbiome Database (Huggett and Apprill, 2019) were used as reference trees for the insertion of SCTLD-associated ASVs that classified as Vibrio or Rhodobacteraceae. Insertion of short SCTLD-associated sequence reads was achieved using the ‘quick add marked’ tool in ARB (version 6.0.6.rev15220). Trees produced from ARB were exported using xFig. Phylogenetic trees for Arcobacter and Rhizobiaceae were constructed de novo using tools from the CIPRES Science Gateway (Miller et al., 2010). For each tree, long-read (~1,200 bp) 16S rRNA gene sequences from closely-related type strains, strains isolated from the marine environment, or clone sequences from corals were identified via BLAST searches and compiled into a .fasta file and used for a sequence alignment in MAFFT (v7.402). This sequence alignment was then used to generate a reference tree using RAxML-HPC (v.8) with the following commands to produce a bootstrapped maximum-likelihood best tree: raxmlHPC-HYBRID -T 4 -f a -N autoMRE -n [output_name] -s [input_alignment] -m GTRGAMMA -p 12345 -x 12345. Next, SCTLD-associated short sequence reads were compiled into a .fasta file and added to the long-read sequence alignment in MAFFT (v7.402) using the “--addfragments” parameter. The sequence alignment with both short

and long reads and the reference tree were then used as inputs for the Evolutionary Placement Algorithm, implemented in RAxML (Berger et al., 2011). RAxML was called as: raxmlHPC-PTHREADS -T 12 -f v -n [output_name] -s [long_and_short_read_alignment] -m GTRGAMMA -p 12345 -t [reference_tree]. The output tree including short read sequences (RAxML_labelledTree.[output_name]) was visualized and saved using the interactive tree of life (iTOL v5.6.3) (Letunic and Bork, 2016).

**Data Processing Description**

**BCO-DMO Processing Notes:**
- data submitted in Excel file "Becker_20201120/BCO-DMO_SCTLD_StThomas.xlsx" sheet "Sheet1" extracted to csv
- added conventional header with dataset name, PI name, version date
- renamed columns to conform with BCO-DMO naming conventions (removed spaces and special characters)
- replace commas with semicolons

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## Data Files

| File |
|---|
| **microbiome.csv** (Comma Separated Values (.csv), 55.28 KB)<br>MD5:f6bb2c29439e262ad49f4e1f6276c3d0<br>Primary data file for dataset ID 833133 |

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## Related Publications

Apprill, A., McNally, S., Parsons, R., & Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquatic Microbial Ecology, 75(2), 129–137. doi:10.3354/ame01753
*Methods*

Ben-Haim, Y., Thompson, F. L., Thompson, C. C., Cnockaert, M. C., Hoste, B., Swings, J., & Rosenberg, E. (2003). Vibrio coralliilyticus sp. nov., a temperature-dependent pathogen of the coral Pocillopora damicornis. International Journal of Systematic and Evolutionary Microbiology, 53(1), 309–315. doi:10.1099/ijs.0.02402-0
*Related Research*

Berger, S. A., Krompass, D., & Stamatakis, A. (2011). Performance, Accuracy, and Web Server for Evolutionary Placement of Short Sequence Reads under Maximum Likelihood. Systematic Biology, 60(3), 291–302. doi:10.1093/sysbio/syr010
*Methods*

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7), 581–583. doi:10.1038/nmeth.3869
*Software*

Davis, N. M., Proctor, D. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome, 6(1). doi:10.1186/s40168-018-0605-2
*Methods*

Huggett, M. J., & Apprill, A. (2018). Coral microbiome database: Integration of sequences reveals high diversity and relatedness of coral-associated microbes. Environmental Microbiology Reports, 11(3), 372–385. doi:10.1111/1758-2229.12686
*Methods*

Letunic, I., & Bork, P. (2016). Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Research, 44(W1), W242–W245. doi:10.1093/nar/gkw290
*Methods*

M. A. Miller, W. Pfeiffer and T. Schwartz, "Creating the CIPRES Science Gateway for inference of large phylogenetic trees," 2010 Gateway Computing Environments Workshop (GCE), New Orleans, LA, 2010, pp. 1-8, doi: 10.1109/GCE.2010.5676129.
*Methods*

Martin BD, Witten D, Willis AD (2019) Modeling microbial abundances and dysbiosis with beta-binomial regression. arXiv:1902.02776 [stat].
*Methods*

Meyer, J. L., Castellanos-Gell, J., Aeby, G. S., Häse, C. C., Ushijima, B., & Paul, V. J. (2019). Microbial Community Shifts Associated With the Ongoing Stony Coral Tissue Loss Disease Outbreak on the Florida Reef Tract. Frontiers in Microbiology, 10. doi:10.3389/fmicb.2019.02244
*Related Research*

Miller, J., Muller, E., Rogers, C., Waara, R., Atkinson, A., Whelan, K. R. T., Patterson, M., & Witcher, B. (2009). Coral disease following massive bleaching in 2005 causes 60% decline in coral cover on reefs in the US Virgin Islands. Coral Reefs, 28(4), 925–937. https://doi.org/10.1007/s00338-009-0531-7
*Related Research*

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens HH, Szoecs E, Wagner H (2019) Vegan: Community Ecology Package. R package version 25-4. https://cran.r-project.org/package=vegan https://cran.r-project.org/src/contrib/Archive/vegan/vegan_2.5-4.tar.gz
*Software*

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology, 18(5), 1403–1414. doi:10.1111/1462-2920.13023
*Methods*

Rosales, S. M., Clark, A. S., Huebner, L. K., Ruzicka, R. R., & Muller, E. M. (2020). Rhodobacterales and Rhizobiales Are Associated With Stony Coral Tissue Loss Disease and Its Suspected Sources of Transmission. Frontiers in Microbiology, 11. doi:10.3389/fmicb.2020.00681
*Related Research*

Ushijima, B., Smith, A., Aeby, G. S., & Callahan, S. M. (2012). Vibrio owensii Induces the Tissue Loss Disease Montipora White Syndrome in the Hawaiian Reef Coral Montipora capitata. PLoS ONE, 7(10), e46717. doi:10.1371/journal.pone.0046717
*Related Research*

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## Related Datasets

### IsRelatedTo

Beavers, K., Mydlarz, L. (2024) **RNAseq data from apparently healthy and Stony Coral Tissue Loss Disease-affected Montastraea cavernosa coral collected from St. Thomas, US Virgin Islands in 2020.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-12-10 doi:10.26008/1912/bco-dmo.935630.1 [view at BCO-DMO]

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## Parameters

| Parameter | Description | Units |
|---|---|---|
| Sample_ID | sample identifier | unitless |

| | | |
|---|---|---|
| NCBI_BioProject_accession_number | Accession number for the NCBI Sequence read archive | unitless |
| NCBI_BioSample_accession_number | Accession number for the NCBI Sequence read archive | unitless |
| Sample_type | Sample type as reported to NCBI for sequence upload | unitless |
| Sequencing_Strategy | Describes what type of sequencing library preparation we did | unitless |
| Sequencing_Instrument_model | This is the model type of sequencer used for the dataset uploaded to NCBI | unitless |
| Sequencing_strategy_details | This is a more detailed description of how we sequenced. This is included in the methods in more depth. | unitless |
| Reef_Name | This is the local name of the site where samples were collected | unitless |
| Latitude | Latitude | decimal degrees |
| Longitude | Longitude | decimal degrees |
| Coral_species | Coral species (genus species) that was sampled | unitless |
| iSeq_run_number | Identifying name of the sequencing run that the sample was put in | unitless |
| reef_type | Qualitative description of reef type | unitless |
| SCTLD_status | Outbreak status of stony coral tissue loss disease (SCTLD); as epidemic = current outbreak; endemic = post outbreak | unitless |
| SCTLD_duration_months | Number of months that stony coral tissue loss disease has affected the sampled reef | months |
| reef_depth_m | Depth of coral reef | meters |
| Temperature_C | Temperature of seawater taken from Exo2 sonde (SonTek) | degrees Celsius |

| | | |
|---|---|---|
| Salinity_psu | Salinity of seawater as taken from the Exo2 sonde (SonTek) | psu |
| pH | pH of seawater as taken from the Exo2 sonde (SonTek) | pH |
| sample_type | Qualitative description of the way the sample was collected | unitless |
| Disease_Present | Binary identifier where y = disease on coral and n = no disease (healthy) on coral | unitless |
| Disease_Sample_Type | Identifyer where HH = healthy sample from a healthy colony; HD = healthy sample from a diseased colony; and DD = diseased sample from a diseased colony | unitless |
| Project_Colony_Code | Unique identifier to keep track when the same colony was sampled multiple times. First 4 letters refer to coral species. | unitless |
| Lesion_Distribution | Distribution of stony coral tissue loss disease lesion (e.g. focal; multifocal; diffuse) | unitless |
| Lesion_Start | Start of stony coral tissue loss disease lesion (e.g. edge of colony; middle of colony; both) | unitless |
| Lesion_Margin | Appearance of the lesion margin (e.g.recent mortality of lesions next to apparently healthy tissue; bleaching band; discoloration; cyano/other biofilm; etc.) | unitless |
| Disease_Colony_Sample_Distance_cm | distance between apparently unaffected sample from diseased colony and the nearest lesion margin to the sample | centimeters |
| Coral_Clade | Coral species belongs to either complex or robust clade | unitless |
| DNA_extraction_method | Name of DNA extraction kit used on the sample | unitless |
| variable_region_16S | Location in the 16S ribosomal RNA gene targeted for a taxonomic gene marker | unitless |
| Primer_Pair | Pair of primers used to amplify the 16S variable region | unitless |

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## Instruments

| | |
|---|---|
| **Dataset-specific Instrument Name** | Illumina iSeq 100 System (Illumina Inc., San Diego, CA, USA) |
| **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** | centrifuge |
| **Generic Instrument Name** | Centrifuge |
| **Dataset-specific Description** | Used to separate tissue, mucus, and microbes from seawater |
| **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** | Qubit 2.0 fluorometer |
| **Generic Instrument Name** | Fluorometer |
| **Dataset-specific Description** | Used to quantify PCR purified products |
| **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. |

| | |
|---|---|
| **Dataset-specific Instrument Name** | mini8 (miniPCR, Cambridge, MA, USA)and BentoLab (Bento Bioworks Ltd, London, UK) |
| **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) |

| | |
|---|---|
| **Dataset-specific Instrument Name** | Exo2 multiparameter sonde (YSI, Yellow Springs, OH, USA) |
| **Generic Instrument Name** | YSI EXO multiparameter water quality sondes |
| **Dataset-specific Description** | Used for seawater sampling |
| **Generic Instrument Description** | Comprehensive multi-parameter, water-quality monitoring sondes designed for long-term monitoring, profiling and spot sampling. The EXO sondes are split into several categories: EXO1 Sonde, EXO2 Sonde, EXO3 Sonde. Each category has a slightly different design purpose with the EXO2 and EXO3 containing more sensor ports than the EXO1. Data are collected using up to four user-replaceable sensors and an integral pressure transducer. Users communicate with the sonde via a field cable to an EXO Handheld, via Bluetooth wireless connection to a PC, or a USB connection to a PC. Typical parameter specifications for relevant sensors include dissolved oxygen with ranges of 0-50 mg/l, with a resolution of +/- 0.1 mg/l, an accuracy of 1 percent of reading for values between 0-20 mg/l and an accuracy of +/- 5 percent of reading for values 20-50 mg/l. Temp ranges are from-5 to +50 degC, with an accuracy of +/- 0.001 degC. Conductivity has a range of 0-200 mS/cm, with an accuracy of +/-0.5 percent of reading + 0.001 mS/cm and a resolution of 0.0001 - 0.01 mS/cm. |

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## Project Information

**RAPID: Collaborative Research: Predicting the Spread of Multi-Species Coral Disease Using Species Immune Traits (Multi-Species Coral Disease)**

**Coverage**: St. Thomas, U.S. Virgin Islands

*NSF Award Abstract:*
Coral reef ecosystems provide substantial economic resources to the societies of the United States Virgin Islands (USVI) and other US locations in the forms of tourism, fishing and coastal protection. However, reefs are among the most threatened marine environments, and coral disease is having a devastating impact on these valued systems. In early 2019, a multi-species rapid tissue loss disease matching the description of stony coral tissue loss disease (SCTLD) was found severely affecting a reef off the southwest coast of St. Thomas in the US Virgin Islands (USVI). SCTLD has been devastating coral reef communities in southeast Florida for the last four years, and was very recently reported from disparate areas around the Caribbean, including Mexico, Jamaica, and St. Martin. Rapid surveys by the investigators at the University of the Virgin Islands believe that a 50 km2 area southwest of St. Thomas is the initial incidence area of the disease, but will likely spread across the USVI, British Virgin Islands, and Puerto Rico. This study performs experiments to understand how this disease affects coral species immune traits and compares the microbiology and physiology of disease samples in the USVI to samples from Florida. It also examines how changing the species composition of a coral community affects the spread and impact of the disease. The overall aim is to produce a model to predict the impact of multi-species disease spread on reefs based on coral species assemblages. The project contributes to the research training of at least 2 undergraduates, 2 M.S. students, and 3 Ph.D. students, who benefit from cross-investigator mentoring. The research team includes representatives to the Coral Disease Advisory Committees for the USVI and Florida, which ensures rapid communication of findings to management bodies in both regions.

Coral disease is a significant and increasing threat to Caribbean coral reef systems. Recent results demonstrate that coral species immune traits can predict disease resistance, and thus, forecast impacts to coral community structure, under multi-species coral disease. The onset of this epizootic in the USVI offers an unprecedented opportunity to test hypotheses about the impact of coral resistance, tolerance and immune traits on disease spread during the early stages of an outbreak that could profoundly change the diversity of

Caribbean reefs. It is hypothesized that the abundance of highly susceptible species dictates 1) the onset of disease at reef sites downstream of the initial incidence area, and 2) the spread of disease within reef sites. Furthermore, 3) downstream reef sites where highly susceptible species are removed or treated show lower immune responses in all susceptible corals, later onset of disease, and slower within-site disease spread. To test these hypotheses, two experiments directly compare species responses to disease exposure and test the effect of species assemblage on coral immune function and disease spread. Results from these experiments aim to inform a generalizable model to predict the impact of multi-species disease spread on reefs based on coral species assemblages. Results of this project include direct comparison of the USVI disease to Florida SCTLD and a better understanding of how the abundance of highly susceptible host species impacts the spread of disease during the early onset of a multi-species panzootic.

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-1928761 |


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