Microbiome dynamics of coral reef and cleanerfish from ecological surveys, in situ manipulations, and laboratory experiments conducted from 2020-2021

Website: https://www.bco-dmo.org/dataset/906740
Data Type: Other Field Results
Version: 1
Version Date: 2023-08-30

Project
» Collaborative Research: Cleaning stations as hubs for the maintenance and recovery of microbial diversity on coral reefs. (Cleanerfish microbiomes)

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Abstract
Coral reefs host some of the most iconic symbiotic interactions in nature and are host to the highest diversity of life on the planet. Cleaning symbiosis, wherein small fish or shrimp remove external parasites and associated microorganisms from specific clients, is common on coral reefs. Sites on the reef occupied by cleaners, or "cleaning stations", attract a wide variety of fish species that engage in direct physical contact with the cleaner. In this study, we used a combination of ecological surveys, in situ manipulations, and laboratory experiments to examine the unique features of cleaning stations to understand transfer of bacterial and archaeal symbionts amongst fish and within coral reef environment. We used microbial 16S rRNA gene amplicons, environmental parameters, and other molecular tools to evaluate the dynamics between coral microbiomes, cleanerfish skin microbiomes, and the environment. This dataset contains metadata describing sequenced samples, including sample name, data deposition accession records, and measurements at the time of sample collection.

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Coverage

Spatial Extent: **N**:24.8261 **E**:-64.8109 **S**:17.7734 **W**:-80.8137
Temporal Extent: 2020-11-17 - 2021-11-16

Methods & Sampling

Coral reef cleaning stations were identified for survey at locations in Marathon, Florida (USA), St. Croix (US

Virgin Islands), and Puerto Rico. SCUBA divers and snorkelers performed site surveys and fish observations at cleaning sites. All molecular samples were stored at -80 degrees Celsius or liquid nitrogen dry shipped until DNA extraction.

After observation, cleanerfish and damselfish were captured using individual hand nets and transferred to individual sealed plastic bags. Immediately upon capture, fish were taken to the lab and swabbed on both sides of the body with tubed sterile cotton swabs. Sampling was performed using gloves, and nets were submerged in a 30% bleach solution and rinsed with fresh water prior to each use.

At select coral cleaning stations, sterile clay hexagon tiles were deployed 1 meter above coral heads to develop biofilms. After 6-7 days, the tiles were recovered with minimal handling into Whirlpak bags and transported to the laboratory. The coral heads were also sampled for their mucus, and water was collected in 60-milliliter (mL) syringes from within 30 centimeters of the coral heads. Sterile swabs were used to collect samples from the tiles and coral mucus, and stored in 1.5 mL microcentrifuge tubes.

From select locations, water samples to examine macronutrients were collected from each station into acid-washed polypropylene bottles, frozen, and analyzed with a continuous segmented flow system at Oregon State University to resolve nitrate, nitrite, ammonium, phosphate, and silicate. Water for non-purgeable organic carbon (TOC) and organic nitrogen was collected from the stations into combusted, borosilicate EPA vials and acidified using 75 mL phosphoric acid. Samples were analyzed using a Shimadzu TOC-VCSH total organic carbon analyzer equipped with a TNM-1 module at Woods Hole Oceanographic Institution. To quantify abundances of Prochlorococcus, Synechococcus, picoeukaryotes, and unpigmented (heterotrophic) microorganisms, water (1.4 mL) was collected at the stations, preserved using paraformaldehyde, and frozen in liquid nitrogen vapors.

DNA extractions were performed on swabs of coral, damselfish, and cleanerfish mucus; swabs of tiles; and water filters. DNeasy PowerBiofilm DNA extraction kits (Qiagen, Germantown, MD, USA) were used with a modified protocol for the swab samples. Lysing reagents were added directly to the sample vial with the swab, vortexed, then the swab was re-oriented in the tube so it was cotton-side-up and was spun for 1 minute at 13,000 g to release the liquid absorbed by the swab. The liquid from the swab and the lysing reagents were added to the bead tube, and the manufacturer instructions were followed for the rest of the protocol. For water filters, the filter and lysing reagents were added directly to the bead tube, and then the manufacturer protocols were followed. DNA extraction controls (sterile hydroflock swabs, unused 0.22-micrometer (µm) filters, and unused bead tubes) were completed following the steps above.

Data Processing Description

FlowJo software (Tree Star, Inc.) was used for flow cytometry.

BCO-DMO Processing Description

- Imported original file "bco-dmo_submission.xlsx" into the BCO-DMO system.
- Separated latitude and longitude values into separate columns where they were combined.
- Saved the final file as "906740_v1_sequence_sample_information.csv".

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Data Files

File
906740_v1_sequence_sample_information.csv (Comma Separated Values (.csv), 122.66 KB) MD5:1619826a4b4085a3d7ae06ab176446f1 Primary data file for dataset ID 906740, version 1.

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Parameters

Parameter	Description	Units
BioProject_accession	SRA BioProject Accession number	unitless
BioSample_accession	SRA BioSample Accession number	unitless
sample_name	Sample name	unitless
SRA_accession	SRA Data Accession number	unitless
collection_date	Collection date	unitless
geo_loc_name	Geographical location, in State: Location	unitless
host	Host organism scientific name	unitless
lat	Station latitude, south is negative	decimal degrees
lon	Station longitude, west is negative	decimal degrees
isolation_source	Sample isolation	unitless
host_common_name	Host organism common name	unitless
host_disease	Host disease state	unitless
host_condition	Host condition	unitless
host_coral_cleaner_goby_pretreatment	Whether host coral was pre-treated	unitless
host_coral_reef_id	Host coral site ID	unitless
location_survey_date	Survey date	unitless
location_mean_total_species	Survey mean number of species present	unitless
FCM_Pro_mL	Abundance of prochlorococcus	cells per milliliter (cells/mL)

FCM_Syn_mL	Abundance of synechococcus	cells per milliliter (cells/mL)
FCM_Peuk_mL	Abundance of picoeukaryotes	cells per milliliter (cells/mL)
FCM_Hbac_mL	Abundance of heterotrophic bacteria	cells per milliliter (cells/mL)
NPOC_uM	Particulate organic carbon	micromolar (uM)
TN_uM	Total organic nitrogen	micromolar (uM)
phosphate	Phosphate	micromolar (uM)
nitrate_nitrite	Nitrate+nitrite	micromolar (uM)
silicate	silicate	micromolar (uM)
NO2	NO2	micromolar (uM)
ammonium	ammonium	micromolar (uM)
nitrate	Nitrate	micromolar (uM)

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Instruments

Dataset-specific Instrument Name	Illumina MiSeq
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	Altra flow cytometer (Beckman Coulter)
Generic Instrument Name	Flow Cytometer
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

Dataset-specific Instrument Name	SCUBA
Generic Instrument Name	Self-Contained Underwater Breathing Apparatus
Generic Instrument Description	The self-contained underwater breathing apparatus or scuba diving system is the result of technological developments and innovations that began almost 300 years ago. Scuba diving is the most extensively used system for breathing underwater by recreational divers throughout the world and in various forms is also widely used to perform underwater work for military, scientific, and commercial purposes. Reference: https://oceanexplorer.noaa.gov/technology/technical/technical.html

Dataset-specific Instrument Name	Shimadzu TOC-VCSH total organic carbon analyzer equipped with a TNM-1 module
Generic Instrument Name	Shimadzu Total Organic Carbon Analyzer TOC-VCPH
Generic Instrument Description	The Shimadzu Total Organic Carbon Analyzer TOC-VCPH is a PC-controlled, total organic carbon analyzer (high-sensitivity model), designed to measure total carbon (TC), inorganic carbon (IC), total organic carbon (TOC), and non-purgeable organic carbon (NPOC); an optional accessory enables the measurement of particulate organic carbon (POC) and total nitrogen (TN) as well. The instrument uses the 680 degrees Celsius combustion catalytic oxidation method to analyze aqueous samples, and optionally solid and gas samples.

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Project Information

Collaborative Research: Cleaning stations as hubs for the maintenance and recovery of microbial diversity on coral reefs. (Cleanerfish microbiomes)

Coverage: Eastern Caribbean

NSF Award Abstract:

Biodiversity in the ocean is influenced by interactions between disparate organisms which ultimately shape population, community, and ecosystem dynamics. Symbiotic interactions involving subsets of species can have

disproportionate impacts on communities, reaching well beyond each interacting species. Coral reefs host some of the most iconic symbiotic interactions in nature and are host to the highest diversity of life on the planet. Cleaning symbiosis, wherein small fish or shrimp remove external parasites and associated microorganisms from specific clients, is common on coral reefs. Sites on the reef occupied by cleaners, or “cleaning stations”, attract a wide variety of fish species that engage in direct physical contact with the cleaner. These highly used territories are viewed both as “clinics of the sea”, where parasitized and sickly fish seek the service of cleaners, but also as potential “garbage dumps”, where unnecessary parasites and other microorganisms are removed. This project seeks to understand the role of cleaning symbiosis transferring microbes in coral reef environments. This research supports training for U.S. graduate students and for undergraduates from Arkansas State University, a primarily undergraduate institution that includes a large population of first-generation college students. These students participate in field site research and have opportunities to visit the Woods Hole Oceanographic Institution for broader exposure to ocean science and more specific laboratory training. The project strengthens international collaboration and further builds on the existing relationships between the team of scientists and resource managers, local divers, fishers, and boat operators, as well as K-12 schools and environmental education programs, and will therefore contribute to local economies. Outreach efforts include a film highlighting this research and publicly accessible narratives shared through press releases and an on-line magazine.

While the benefits of cleaning to reef ecosystem health have been extensively studied, the cleaning costs for cleaner species and the role of cleaning stations as potential sinks for microbial diversity and possibly even pathogens have never been assessed. Here, the researchers utilize the unique features of cleaning stations to understand transfer of bacterial and archaeal symbionts amongst fish and within coral reef environment. The study capitalizes on cleanerfish access to multiple variety of hosts or clients within stations to address new questions about how cleanerfish act as vectors to transfer microorganisms between hosts on a reef and if and how these microorganisms may play a broader functional role in reef resilience. Specifically, the project addresses the following hypotheses: 1) Cleanerfishes serve as keystone regulators of microbial communities, enhancing microbial community diversity and transferring key microbial species between clients, and 2) Cleanerfishes are a particularly important contributor to reef resilience, facilitating recovery of the microbiome following disturbance. The research team uses an integrative interdisciplinary approach involving field and laboratory observations and experiments, and molecular-based tools. The core research team includes experts in cleaning mutualisms, fish behavior, coral reef ecology, and microbial ecology. The proposed project aims to link behavior of individual organisms with ecosystem-level process.

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-2022955
NSF Division of Ocean Sciences (NSF OCE)	OCE-2023420
NSF Division of Ocean Sciences (NSF OCE)	OCE-2204963

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