Seawater microbial communities within coral reef seawater change over six years in response to disturbance

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Project
» Coral Chorus: The Role of Soundscapes in Coral Reef Larval Recruitment and Biodiversity (Coral Chorus)

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Abstract
Reefs on the southern shore of St. John, United States Virgin Islands (USVI) were targeted for 11 opportunistic sampling events over six years from June 2016 to June 2022. The reefs included (from west to east) Dittlif, Cocoloba, Joels Shoal, Europa, Yawzi, Tektite, Booby Rock, and Ram Head, all of which are within the bounds of Virgin Islands National Park, except for Dittlif. Reef collections included surveys for benthic composition, small volumes of seawater for inorganic and organic nutrients and microbial abundances, larger volumes for microbial biomass and chlorophyll, and CTD casts for temperature and salinity. All collections occurred during daylight hours. Dates of seawater sampling events included June 10-12, 2016, October 28-29, 2016, March 25-28, 2017, July 26-30, 2017, November 27-30, 2017, April 11-13, 2018, November 5-9, 2018, August 6-10, 2020, January 17-24, 2021, October 20-25, 2021, and June 24-29, 2022. These sampling points surround two major stressors to St. John, USVI reefs: two category 5 hurricanes, Irma and Maria, which affected the reefs in September, 2017, and stony coral tissue loss disease, a multi-species disease outbreak that began emerging around St. John, USVI between January-June, 2020. As of August 2020, the disease just began affecting all reefs in the study area, except Europa and Cocoloba. All reefs were impacted by the next sampling in January 2021. To better understand how seawater microorganisms reflect changes in the underlying reef habitat, we sought to 1) identify the changes to the benthic reef habitat over a six year time period with the hypothesis that reefs would become more algal-dominant, 2) examine the changes in the overlying seawater nutrients, cell counts, and temperature, and 3) investigate the concurrent changes in specific microbial groups during that time period.

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Coverage

Spatial Extent: **N**:41.5265 **E**:-64.7048 **S**:18.297 **W**:-70.6731

Temporal Extent: 2016-06-10 - 2022-06-29

Methods & Sampling

Sampling locations and dates:
Reefs on the southern shore of St. John, United States Virgin Islands (USVI) were targeted for 11 opportunistic sampling events over six years from June 2016 to June 2022. The reefs included (from west to east) Dittlif, Cocoloba, Joel's Shoal, Europa, Yawzi, Tektite, Booby Rock, and Ram Head, all of which are within the bounds of Virgin Islands National Park, except for Dittlif. The research operations were coastal, predominantly land-based, lasting 1-3 weeks, with seawater sampling and diver-based reef surveys deployed from a power boat. Reef collections included surveys for benthic composition, small volumes of seawater for inorganic and organic nutrients and microbial abundances, larger volumes for microbial biomass and chlorophyll, and CTD casts for temperature and salinity. All collections occurred during daylight hours. Dates of seawater sampling events included June 10-12, 2016, October 28-29, 2016, March 25-28, 2017, July 26-30, 2017, November 27-30, 2017 (occurred aboard R/V Walton Smith), April 11-13, 2018, November 5-9, 2018 (occurred aboard R/V Walton Smith), August 6-10, 2020, January 17-24, 2021, October 20-25, 2021, and June 24-29, 2022.

These sampling points surround two major stressors to St. John, USVI reefs: two category 5 hurricanes, Irma and Maria, which impacted the reefs in September, 2017, and stony coral tissue loss disease, a multi-species disease outbreak that began emerging around St. John, USVI between January-June, 2020. As of August 2020, the disease just began affecting all reefs in the study area, except Europa and Cocoloba. All reefs were impacted by the next sampling in January 2021.

Field collections:
Surveys were conducted via SCUBA. Point intercept benthic reef surveys were conducted to understand the percent cover of organisms and substrates on each reef. Surveys proceeded using four to six 10-meter (m) long transects, with the underlying biological reef organisms or substrate recorded every 10 centimeters (cm). Transects were collected yearly prior to the 2017 hurricane, then were collected at each field sampling event. Following the emergence of stony coral tissue loss disease in 2020, roving diver surveys to quantify that disease were conducted in January and October 2021. Disease surveys lasted thirty minutes within a 100 m^2 plot and the diver counted all apparently healthy and diseased coral colonies and recorded species level, when possible. At the conclusion of the survey, the area surveyed within the plot was estimated.

At each reef, measurements of temperature (ºC) and salinity (psu) were conducted using a CastAway CTD (SonTek, Xylem, San Diego, CA, USA). Next, small volumes of water were collected from both surface and approximately within 1 m of reef depth (referred to as "benthic" depth) for organic nutrients, inorganic nutrients, and microbial cell abundances. Seawater for total organic carbon (TOC) and total nitrogen (TN) was collected into 40-milliliter (ml) combusted borosilicate glass vials. Seawater for inorganic nutrients (phosphate, ammonium, silicate, nitrite plus nitrate), was collected in acid-clean 30 ml HDPE bottles (Nalgene, ThermoFisher Scientific, Waltham, MA, USA). Bottles for all collections were triple-rinsed with seawater prior to collection. Following collection, organic nutrient samples were fixed with 75 microliters (µl) phosphoric acid and stored at room temperature until analysis. A 1.4 ml aliquot from the inorganic nutrient collection was placed into a cryovial for flow cytometry-based analysis for microbial cell abundances, then fixed with paraformaldehyde (1% final concentration, Electron Microscopy Sciences) in the dark for 20 minutes, then frozen in a liquid nitrogen dry shipper and stored at -80º Celsius (C) until analysis. Bottles for inorganic nutrient analysis were kept frozen at -20ºC until analysis. Inorganic and organic nutrients and microbial cell abundances were collected in biological duplicates for the June and October 2016 sampling events, but not for further events. Duplicates from those events were averaged for comparison to all other timepoints, which were sampled in singlicate.

Seawater for microbial biomass and chlorophyll analysis was collected from surface and benthic depths at each reef. For all benthic seawater collections prior to 2019, a groundwater pump (Mini-Monsoon 12V, Proactive Environmental Products, Bradenton, Florida, USA) was used. Beginning in August 2020, an 8L diver-operated Niskin bottle was used to enable more accurate collection of water directly over the reef habitat. Seawater (4L) for chlorophyll (benthic depth only) and for microbial biomass was collected into acid-clean or bleach-clean, then triple seawater-rinsed Platy® water tank bags (Platypus, Cascade Designs, Seattle, WA, USA) or LDPE Nalgene bottles. Samples were kept in a cooler on ice until filtration within 6 hours of collection. Seawater was filtered for both microbial biomass and chlorophyll via peristalsis using a Masterflex L/S peristaltic pump (Cole-Parmer, Vernon Hills, IL, USA) through acid-clean or 10% bleached silicone tubing (L/S, platinum-cured, #96410-24 size, Cole-Parmer) and a 25-millimeter (mm) filter holder (Swinnex-25, Millipore Corporation) with either a 0.2-micrometer (µm) Supor filter (Pall, Port Washington, New York, USA) or GF/F filter. Seawater was filtered 2 liters at a time for technical duplicates. In some cases, the 0.2 µm filter would clog, so less than the 2

liters were filtered. In those cases, the amount filtered was recorded. Prior to November 2018, the 4L chlorophyll sample was filtered through a single GF/F filter. For consistency with other environmental variables (nutrients and microbial abundances), any technical duplicates of chlorophyll (post-2018) were averaged to yield one variable for environmental analyses. All filters were placed in cryovials and frozen in a liquid nitrogen dry shipper and stored at -80ºC upon return to the Woods Hole Oceanographic Institution until analysis.

Laboratory processing:
Samples for organic nutrients were analyzed on a Shimadzu TOC-VCSH TOC analyzer (Hansell and Carlson, 2001), using a TNM-1 module to generate non-purgeable total organic carbon (TOC) and total nitrogen (TN). The inorganic nutrient samples were analyzed at Oregon State University as described previously (Becker et al., 2020), and used a Technicon AutoAnalyzer II (SEAL Analytical) and Alpkem RFA 300 Rapid Flow Analyzer to generate concentrations of ammonium, phosphate, silicate, nitrite, and nitrate plus nitrite. The microbial abundance samples were analyzed as described in Becker et al. (2020) at the University of Hawaii SOEST Flow Cytometry Facility. The facility used a Beckman-Coulter Altra flow cytometer (Beckman Coulter Life Sciences). The seawater samples were stained with Hoechst 33342 DNA stain and excited with 488 nm and UV wavelengths (Campbell and Vaulot, 1993; Monger and Landry, 1993). Signals were collected and then processed in FlowJo software (Tree Star) to generate populations and abundances (cells per milliliter) of Prochlorococcus, Synechococcus, eukaryotic phytoplankton ("picoeukaryotes"), and non-pigmented bacteria and archaea. Non-pigmented bacteria and archaea are mostly heterotrophic and referred to as "heterotrophic microbes" in this study (Monger and Landry, 1993; Marie et al., 1997).

Chlorophyll was extracted with 90% acetone using standard methods (JGOFS, 1996). Briefly, filters were thawed and placed individually in 5 ml of 90% acetone and capped. If the filter was dark, 10 ml of acetone was used. After a 24-hour extraction in the dark at 4°C, the tubes were vortexed and centrifuged and ~3 ml of solvent was measured on an AquaFluor fluorometer at 664 nanometer (nm) (Turner Designs handheld 800446) fitted with a red-sensitive photomultiplier. Before and after analysis, blanks including air, 90% acetone, and a black standard were run. After each measurement, samples were acidified with two drops of 10% hydrochloric acid, and re-measured to assess phaeopigment concentration. Readings volume-corrected and concentrations generated with a standard curve. Chlorophyll from June and October 2016 was assessed using high-performance liquid chromatography.

DNA extraction of duplicate 0.22 µm filters proceeded as described previously (Becker et al., 2020) using a method that combines physical and chemical lysis with column purification (Santoro et al., 2010). In addition to the seawater filters, DNA from 14 blank filters was extracted as a DNA extraction control. Briefly, the filters were subjected to chemical lysis with a sucrose-EDTA and 10% SDS lysis buffer and physical lysis with a 15 bead-beating step. The DNeasy Blood and Tissue Kit (Qiagen) was then used to purify the lysate. Resulting DNA was diluted 1:100 in UV-sterile PCR-grade water in preparation for PCR. Single, barcoded PCR reactions per sample were run to amplify the small subunit (SSU) ribosomal RNA (rRNA) gene of bacteria and archaea using primers 515F and 806R (Apprill et al., 2015; Parada et al., 2016). In addition to samples, genomic DNA from Microbial Mock Community B (even, low concentration), v5.1L, for 16S rRNA Gene Sequencing, HM-782D, was used as a sequencing control and PCR-grade water was used as a negative PCR control. PCR reactions (50 µl) contained the following: 2 µl DNA template, 0.5 µl of GoTaq DNA Polymerase (Promega), 1 µl each of forward and reverse primers at 10 µM, 1 µl of 10 mM deoxynucleoside triphosphate (dNTP) mix (Promega), 5 µl $MgCl_2$, 10 µl GoTaq 5X colorless flexi buffer (Promega), and 29.5 µl nuclease-free water. Reactions proceeded with: 95°C for 2 minutes; 28 cycles of 95°C for 20 seconds, 55°C for 15 seconds, and 72°C for 5 minutes; and finally 72°C for 10 minutes before holding at 4ºC. PCR products were all purified with the QIAquick 96 PCR purification kit (Qiagen) or MinElute PCR Purification Kit (Qiagen). Concentrations of purified barcoded PCR products were measured with the Qubit 2.0 fluorometer. Each barcoded sample was diluted to 1 nanogram per microliter (ng/µl) and pooled. Samples were sequenced across four runs to maximize read output per sample on an Illumina MiSeq 2 x 250 bp sequencing at the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana-Champaign or the BioMicro center at Massachusetts Institute of Technology. All resulting fastq files were downloaded and used for further analysis. Fastq files were also uploaded to the NCBI sequence read archive under BioProject accession number PRJNA936592.

Known Issues/Problems:
In some cases, data are missing. This is noted as either "not collected", which indicates that the sample does not exist, or "not applicable" meaning the data were not relevant to the sampling design.

Data Processing Description

Environmental data processing:

Data from point-intercept benthic survey transects were grouped into different benthic categories, including bleached coral, crustose coralline algae (CCA), cyanobacterial mats (CYAN), diseased coral, hard coral, macroalgae, ramicrusta, soft coral, sponge, substrate (a combination of rubble, pavement, sand, and rock), turf algae, and other (a combination of millepora and hydroids, zoanthids, and other invertebrates, dead coral, and eelgrass). Counts of each category were transformed to a percent and averaged over the 4-6 transects per reef at each timepoint. Those values were used for data analyses. Data from the disease surveys were used to generate disease prevalence on reefs in January 2021 and October 2021 by dividing the number of coral colonies afflicted by stony coral tissue loss disease by the total number of colonies identified.

BCO-DMO Processing:
- converted data from Excel format to csv.

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Data Files

File
usvi_time-series.csv (Comma Separated Values (.csv), 157.75 KB) MD5:fbe91e7c4a079ded90c75f9e7e31c486 Primary data file for dataset ID 892971

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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

Becker, C., Weber, L., Suca, J., Llopiz, J., Mooney, T., & Apprill, A. (2020). Microbial and nutrient dynamics in mangrove, reef, and seagrass waters over tidal and diurnal time scales. Aquatic Microbial Ecology, 85, 101–119. https://doi.org/10.3354/ame01944
Methods

Campbell, L., & Vaulot, D. (1993). Photosynthetic picoplankton community structure in the subtropical North Pacific Ocean near Hawaii (station ALOHA). Deep Sea Research Part I: Oceanographic Research Papers, 40(10), 2043–2060. doi:10.1016/0967-0637(93)90044-4
Methods

Hansell, D. A., & Carlson, C. A. (2001). Biogeochemistry of total organic carbon and nitrogen in the Sargasso Sea: control by convective overturn. Deep Sea Research Part II: Topical Studies in Oceanography, 48(8-9), 1649–1667. doi:10.1016/s0967-0645(00)00153-3 https://doi.org/10.1016/S0967-0645(00)00153-3
Methods

JGOFS (1996) Protocols for the joint global ocean flux study (JGOFS) core measurements (Report 19), Bergen, Norway: IOC SCOR.
Methods

Marie, D., Partensky, F., Jacquet, S., & Vaulot, D. (1997). Enumeration and Cell Cycle Analysis of Natural Populations of Marine Picoplankton by Flow Cytometry Using the Nucleic Acid Stain SYBR Green I. Applied and Environmental Microbiology, 63(1), 186–193. doi:10.1128/aem.63.1.186-193.1997
Methods

Monger, B. C., & Landry, M. R. (1993). Flow Cytometric Analysis of Marine Bacteria with Hoechst 33342 †. Applied and Environmental Microbiology, 59(3), 905–911. doi:10.1128/aem.59.3.905-911.1993
Methods

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2015). 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

Santoro, A. E., Casciotti, K. L., & Francis, C. A. (2010). Activity, abundance and diversity of nitrifying archaea and bacteria in the central California Current. Environmental Microbiology, 12(7), 1989–2006. doi:10.1111/j.1462-2920.2010.02205.x
Methods

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Parameters

Parameter	Description	Units
sample_name	Generic name of a sample.	unitless
BioProject_accession	Project accession number for the NCBI Sequence Read Archive.	unitless
BioSample_accession	Accession number for a specific sample in the NCBI Sequence Read Archive	unitless
collection_date	Date of collection	unitless
depth_m	Depth of reef	meters (m)
geo_loc_name	Broad location of the collection as Country: State	unitless
latitude	Station latitude, south is negative	decimal degrees
longitude	Station longitude, west is negative	decimal degrees
site	Name of the reef or location sampled. For laboratory controls sampled in an artificial environment, we specified either control or mock.	unitless
depthtype	qualitative description of whether the water sample was collected at the surface waters or within 1 meter of the reef benthos, or benthic depth.	unitless
Replicate	Replicate for technical duplicates of seawater samples	unitless
Library	ID of sequencing library in which the sample was run.	unitless
Sequences	number of DNA sequences in each sample.	number of reads
yearmonth	year and month in which a sample was collected	unitless
prehurricane	yes or no referring to whether a sample was collected prior to the hurricane or not	unitless
disease	yes or no referring to whether there was or was not active stony coral tissue loss disease on the reef when it was sampled.	unitless
disturbance	most recent major reef disturbance, either hurricane or disease. Historic refers to general collective disturbances to the reefs prior to 2017 hurricanes.	unitless
temp_C	Temperature of seawater taken from CastAway CTD (SonTek)	degrees Celsius
salinity	Salinity taken from the CastAway CTD	practical salinity units
npoc_um	Non-purgeable organic carbon, referred to as total organic carbon, in reef seawater	micromolar (uM)
tn_um	Total nitrogen, which includes organic and inorganic nitrogen	micromolar (uM)
po4_um	Phosphate in reef seawater	micromolar (uM)
no2no3_um	Nitrite plus nitrate concentrations in reef seawater	micromolar (uM)

silicate_um	Silicate concentrations in reef seawater	micromolar (uM)
no2_um	Nitrite concentrations in reef seawater	micromolar (uM)
nh4_um	Ammonium concentrations in reef seawater	micromolar (uM)
Chl_ug_per_l	Amount of chlorophyll-a in the reef seawater	micrograms per liter (ug/L)
Phaeo_ug_per_l	Amount of phaeophytin in the reef seawater	micrograms per liter (ug/L)
pro_per_ml	Number of prochlorococcus cyanobacteria cells per milliliter in reef seawater	cells per milliliter
syn_per_ml	Number of Synechococcus cyanobacteria cells per milliliter in reef seawater	cells per milliliter
peuk_per_ml	Number of photosynthetic picoeukaryote cells per milliliter in reef seawater	cells per milliliter
hbact_per_ml	Number of unpigmented bacteria and archaea, also referred to as heterotrophic microbes, cells per milliliter in reef seawater	cells per milliliter
BleachCoral	bleached coral cover in the reef habitat	relative abundance
CCA	crustose coralline algae cover in the reef habitat	relative abundance
CYAN	filamentous cyanobacterial mat cover in the reef habitat	relative abundance
DiseasedCoral	diseased coral cover in the reef habitat	relative abundance
HardCoral	hard, or stony, coral cover in the reef habitat	relative abundance
Macroalgae	macroalgal cover in the reef habitat	relative abundance
Other	other cover including millepora and hydroids, zoanthids, and other invertebrates, dead coral, and eelgrass in the reef habitat	relative abundance
Ramicrusta	Invasive Ramicrusta textilis encrusting alga cover in the reef habitat	relative abundance
SoftCoral	Soft coral cover in the reef habitat	relative abundance
Sponge	sponge cover in the reef habitat	relative abundance
Substrate	Substrate includes sand, pavement, and rubble in the reef habitat	relative abundance
TurfAlgae	turf algal cover in the reef habitat	relative abundance

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Instruments

Dataset-specific Instrument Name	Alpkem RFA 300 Rapid Flow Analyzer
Generic Instrument Name	Alpkem RFA300
Generic Instrument Description	A rapid flow analyser (RFA) that may be used to measure nutrient concentrations in seawater. It is an air-segmented, continuous flow instrument comprising a sampler, a peristaltic pump which simultaneously pumps samples, reagents and air bubbles through the system, analytical cartridge, heating bath, colorimeter, data station, and printer. The RFA-300 was a precursor to the smaller Alpkem RFA/2 (also RFA II or RFA-2).

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	Beckman-Coulter Altra flow cytometer
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	AquaFluor fluorometer at 664 nm (Turner Designs handheld 800446) fitted with a red sensitive photomultiplier
Generic Instrument Name	Fluorometer
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	Masterflex L/S peristaltic pump (Cole-Parmer, Vernon Hills, IL, USA)
Generic Instrument Name	Pump
Generic Instrument Description	A pump is a device that moves fluids (liquids or gases), or sometimes slurries, by mechanical action. Pumps can be classified into three major groups according to the method they use to move the fluid: direct lift, displacement, and gravity pumps

Dataset-specific Instrument Name	groundwater pump (Mini-Monsoon 12V, Proactive Environmental Products, Bradenton, Florida, USA)
Generic Instrument Name	Pump
Generic Instrument Description	A pump is a device that moves fluids (liquids or gases), or sometimes slurries, by mechanical action. Pumps can be classified into three major groups according to the method they use to move the fluid: direct lift, displacement, and gravity pumps

Dataset-specific Instrument Name	Shimadzu TOC-VCSH total organic carbon analyzer using a TNM-1 module
Generic Instrument Name	Shimadzu TOC-V Analyzer
Generic Instrument Description	A Shimadzu TOC-V Analyzer measures DOC by high temperature combustion method.

Dataset-specific Instrument Name	CastAway CTD (SonTek, Xylem, San Diego, CA, USA)
Generic Instrument Name	SonTek CastAway-CTD
Generic Instrument Description	The Sontek CastAway-CTD (manufactured by Xylem) is a handheld castable instrument that provides instantaneous profiles of temperature, salinity, and sound speed. Each cast is referenced with both time and location using its built-in GPS receiver. The CastAway software displays profiles of the casts in addition to mapping the locations of the data collection points. The CastAway-CTD has a 5 Hz response and sampling rate, accurate to 0.1 (PSS-78), 0.05° Celsius. Conductivity range is 0 to 100,000 µS/cm. Temperature range is -5° to 45° Celsius. Pressure range is 0 to 100 decibars. Further specs and information can be found on the manufacturer's website: https://www.xylem.com/en-us/brands/wtw/wtw-products/castaway-ctd/

Dataset-specific Instrument Name	Technicon AutoAnalyzer II (SEAL Analytical)
Generic Instrument Name	Technicon AutoAnalyzer II
Generic Instrument Description	A rapid flow analyzer that may be used to measure nutrient concentrations in seawater. It is a continuous segmented flow instrument consisting of a sampler, peristaltic pump, analytical cartridge, heating bath, and colorimeter. See more information about this instrument from the manufacturer.

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Project Information

Coral Chorus: The Role of Soundscapes in Coral Reef Larval Recruitment and Biodiversity (Coral Chorus)

NSF Award Abstract:
Coral reef ecosystems host some of the highest biodiversity of life per unit area on Earth and harbor about one-quarter to one-third of all marine animals. Reef-associated animals are a major source of protein for millions of people, and reefs offer shoreline protection and provide a significant source of tourism revenue, especially in developing countries. Factors that influence supply and settlement of young (larval) fish, coral, and associated animals can have large impacts on reef ecosystem and population structure, and learning more about these can help improve understanding of how to maintain the benefits provided by coral reefs. This study will lead to a detailed, mechanistic understanding of how young larvae use natural sounds to orient toward, locate, and select preferred settlement habitat. The approach will combine detailed field measurements and experiments to isolate key soundscape variables that impact coral reef larvae.

For marine communities, such as those on coral reefs, factors influencing larval supply and settlement can have major impacts on community structure and population replenishment. There are now some indications that sound plays an important role in attracting larvae to suitable settlement habitat. There is little understanding of what soundscape habitat information is available to larvae and how differences and variability in sound can influence settlement. This project will include comprehensive experiments, environmental measurements, and modeling with the goal of understanding the role of sound in influencing larval recruitment and local biodiversity. The investigators will measure in situ settlement of larval fish and coral in relation to different soundscapes and habitat conditions in a marine protected area using traditional larval sampling methods, moored acoustic recorders, and a suite of environmental observations. Controlled and calibrated environmental playback experiments will isolate soundscape components and determine specific and fundamental acoustic cues larvae use to orient and settle. The spatial and temporal variability of soundscape cues and components across reef habitats will be established. Finally, the project will determine the relevant ranges of sound plumes that larvae may encounter through direct measurements of the sound fields of multiple reefs.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1536782
National Oceanic and Atmospheric Administration (NOAA)	NA19OAR4320074

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