Capture data and DNA yields from sea urchin specimens plus surrounding water and sediments collected in 2023 from four locations along the coast of Puerto Rico

Website: https://www.bco-dmo.org/dataset/985892
Data Type: Other Field Results
Version: 1
Version Date: 2025-11-14

Project
» RAPID: The black urchin (Diadema antillarum) massive resurgent die-off: Causes, demographic, and community consequences (Diadema antillarum die-off associated microbiota in Puerto Rico)

Contributors	Affiliation	Role
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Abstract
The dataset provides detailed metadata on sea urchin samples and animal captures made in 2023 as part of a study investigating the reef-dwelling sea urchin Diadema antillarum. Each entry corresponds to an individual sea urchin specimen, with samples identified by a unique sample ID. Samples for microbiota analyses were primarily taken from the gut of the sea urchins, as indicated by the recorded sample type. The collection took place across multiple periods, denoted numerically, with the sampling dates ranging mainly from March to December 2023. The study focused on four main collection sites along the coast of Puerto Rico: Cerro Gordo (abbreviated as CGD), Dorado (DBE), and Luquillo (PTB) and Punta Las Marias (PTM). These locations are well-represented in the dataset, reflecting a geographic spread intended to capture environmental variability. The dataset also includes information on the collectors, dates of laboratory reception, and detailed notes on sample storage, ensuring traceability and consistency throughout the research process.

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Coverage

Location: Puerto Rican coast
Spatial Extent: **N**:18.485 **E**:-65.287 **S**:18.281 **W**:-66.298
Temporal Extent: 2023-03-23 - 2023-12-07

Dataset Description

This dataset is one of multiple datasets from a study investigating sea urchins and associated water and sediment samples from four shallow reef ecosystems in coastal waters surrounding Puerto Rico. All analyses were conducted by the PRINBRE Metabolomics Research Core at the University of Puerto Rico, Medical Sciences Campus, in collaboration with marine biologists from the Institute of Tropical Ecosystem Studies. The study is part of a broader initiative to integrate marine ecology with chemical ecology and metabolomics.

- **Sea urchin capture data, sizes, and DNA yields [this dataset]**
- Metabolites from GC-MS analysis of sea urchin gut samples and associated seawater samples [dataset 986552]
- Raw GC-MS data (metabolomics) data [dataset number TBD]
- Microbiome data 16S rRNA results for sea urchin gut content, sediment, and surrounding seawater [dataset 986537]

This dataset presents field sampling, animal capture data, sizes, and DNA yields.

Methods & Sampling

Study location
The study was conducted at four sites along the northeastern and eastern coasts of Puerto Rico. These sites were selected due to the robust ecological and microbial data on *Diadema antillarum* collected in previous studies (Rodríguez-Barreras et al., 2018, 2021, and 2022). Furthermore, recent observations suggest contrasting levels of disease impact among these sites, ranging from deeply affected to unaffected by the disease. Punta Melones (**PME**, 18°16'51.40"N, 65°17'12.21"W), located in the Luis Peña Natural Reserve in Culebra had a high density of sea urchins (1.5 ind. per m2) before the recent die-off. Nevertheless, Rodríguez-Barreras et al., (2023) observed over 90% mortality of sea urchin at the site. Punta Bandera (**PBA,** 18°23'18.46" N, 65°43'5.52"W), Cerro Gordo reef (**CGO** 18°29'05.54"N, 65°20'21.65"W), and Punta Sardinas (**PSA** 18°28'36.34"N, 66°17'52.48"W), are fringing reefs located on the northern coast of Puerto Rico. These sites exhibited densities around 1.26 ind. per m2.

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Data Manager comment: ***The abstract mentions different collection sites with different site abbreviations:*** Specific collection sites reflect a geographic spread intended to capture environmental variability. Four coastal sites were Cerro Gordo (CGD), Dorado (DBE), Luquillo (PTB), and Punta Las Marias (PTM). ***se[The sites in the abstract should list those in the Methods and vice versa. Please let data manager know which is correct and provide latitude and longitude values (and verify values for coordinates that are in the data table)]***
~ ~ ~ ~

Data and Sample Collection
Each site was surveyed on months 0, 3, 6, and 9 to collect samples from March 2023 to December 2023, between the hours of 10:00 and 13:00. We set up eight belt transects of 20 m2 (10 meters x 2 meters) parallel to the coast to estimate density. Transects were positioned at least 5 meters apart from each other, at depths ranging from 1 to 3 meters; as sea urchin abundance tends to be higher at these depths. All individuals within each transect were counted. These data were used to estimate sea urchin density (i.e., the number of urchins per transect per site). At each site, between 1 and 3 healthy sea urchin specimens were collected, for a total of 45 individuals. Additionally, we measured the horizontal test diameter (*td*) of individuals collected from the transects to assess the size distribution at each reef. A total of 50 individuals per reef were measured using a caliper. If necessary, sea urchins found outside of the transects were also measured until reaching 50 individuals per reef, following Mercado-Molina et al., (2015) and Rodríguez-Barreras et al. (2018, 2023). We also measured the tests of dead and diseased sea urchins when possible. Based on test diameter, sea urchins were categorized into three size classes: small or juvenile ($td \leq 4.0$ cm), medium or young adult ($4.01 < td \leq 6.0$ cm), and large or adult ($td \geq 6.01$ cm). These data were used to construct a size-frequency distribution (Miller et al., 2003; Lugo-Ascorbe, 2004; Rodríguez-Barreras et al., 2014; Rodríguez-Barreras et al., 2023).

Additionally, 1 liter of seawater (in a sterile glass bottle) and 50 mL of sediment (in a sterile plastic tube) were

collected during each survey. Sampling was approved by the Department of Natural and Environmental Resources of Puerto Rico permit #DRNA-2022-IC-026 (O-VS-PVSlS-SJ-01291-06052022). The IACUC permit previously approved for Rodríguez-Barreras used in a former sea urchin-microbiota project was renewed until September 29, 2026 (UPRRCM # A530118).

Sample processing
Upon arrival at the Microbiome Laboratory of Dr. Filipa Godoy-Vitorino (University of Puerto Rico Medical School), all sample types were processed according to standard lab operating procedure (see Supplemental Files). Sediment was divided into 50 mL tubes. Seawater was filtered and then divided into two aliquots--one for downstream microbial analyses and the other for metabolomic analyses--with a minimum of 50 milliliters of seawater per sample. Sea urchins were anesthetized, and the size across equatorial diameter was measured with calipers. At that time, the presence of albinism on the sea urchin test was recorded. All sample types were then stored at -80°C until analysis (and dissection).

The sea urchins were dissected following the standard protocol (Tosado et al., 2023) to obtain gut pellets. At the height of the maximum diameter, sea urchins were opened with an equatorial cut and the digestive tract removed. This includes gut tissue, esophagus, stomach, and intestine which are then rinsed with autoclaved sea water. The gut food pellets are voided by gentle shaking. Each pellet was divided into two subsamples for microbial and metabolomic analysis with a minimum size of 50 milligrams. At the end of the sampling period (December 2023), a total of 45 sea urchin gut pellets, 14 sediment samples, and 16 seawater samples were collected.

DNA extraction
The DNA of the 3 sample types (gut pellets, sediments, sea water) was then extracted using DNeasy PowerSoil Pro kit (QIAGEN LLC, Germantown Road, Maryland, United States) and quantified using Qubit® dsDNA HS assay kit. We normalized the DNA to 4nM during the 16S rRNA gene library preparation process. Using region-specific primers that include sequencer adapter sequences used in the Illumina flowcell, we used the Earth Microbiome Project standard protocols (Caporaso et al., 2023) <-- [***added this. Please confirm***] to amplify the hypervariable region V4 of the 16S ribosomal RNA gene (~291 bp) using the universal bacterial primers 515F (5′GTGCCAGCMGCCGCGGTAA3′) and 806R (5′GGACTACHVGGGTWTCTAAT3′). Amplicons were measured with a plate reader (Infinite® 200 PRO, Tecan) and PicoGreen (Invitrogen). Volumes of all the products were combined into one tube after they were quantified, resulting in an equimolar representation of each amplicon. This pool was then cleaned up using AMPure XP Beads (Beckman Coulter), and finally quantified using a fluorometer (Qubit, Invitrogen). Customized sequencing was outsourced to Argonne National Laboratory in Illinois, USA, utilizing a 2 × 150 bp paired-end sequencing kit with an Illumina MiSeq. The sequencer's reads and the metadata that went along with them were uploaded to QIITA study ID 15616 (version 2024.02).

Data Processing Description

[Data manager comment: Was this dataset only capture data and DNA yields? If so, isn't the content in this section more appropriate for the microbiome dataset? https://www.bco-dmo.org/dataset/986537
If the downstream microbial analyses are not part of this dataset, then this section content should be removed.

Microbial characterization

The downstream microbial analyses were performed locally in QIIME2 (version 2024.05) and include demultiplexing, trimming at 250 bp, denoising. Denoising method was done using DADA2 (Callahan et al., 2016) and included quality filtering, removing chimeric sequences, combining paired-ends, and eliminating singleton reads in order to join, denoise, and duplicate sequences. The bacterial sequences were classified using the Greengenes reference database and taxonomy files (McDonald et al., 2024), trained with classify-sklearn function in QIIME2. Microbiota analyses (diversity and composition) were divided into four sets: (1) sample types (gut digesta pellet, seawater, or sediment), (2) gut digesta pellet between the different collection locations, (3) gut digesta pellet between the different collection periods, and (4) gut digesta pellet between the different collection locations considering the location periods. For each set, beta- and alpha-diversity were calculated, and taxonomic compositions (taxa barplots) and putative biomarker taxa, e.g, MaAsLin (Mallick et al., 2021) and/or LEfSe (Segata et al., 2011) are represented. To correct for difference in extraction dates, those were added from the metadata as a reference for the MaAsLin analysis. In addition, for collection

locations (set of analyses#2) ***[What does this mean? where is this 'set of analyses #2"?]***, ecological and metabolic function inference (FAPROTAX, Louca et al., 2016) and putative biomarker data (LefSe) were computed.

BCO-DMO Processing Description

- Imported the file "Metadata_Sea_urchin_NSF2023_oct2025.csv" into the BCO-DMO system.
- Converted Excel dates (45010, 45031, etc) to yyyy-mm-dd format
- Converted other dates from mm/dd/yy format to yyyy-mm-dd format
- Added latitude and longitude columns based on the sampling site locations

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Related Publications

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., … Asnicar, F. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology, 37(8), 852–857. doi:10.1038/s41587-019-0209-9
Software

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

Caporaso, J.G, Ackermann, G., Apprill, A., Bauer, M., Berg-Lyons, D., Betley, J., Fierer, N., Fraser, L., A. Fuhrman, J., A. Gilbert, J., Gormley, N., Humphrey, G., Huntley, J., K. Jansson, J., Knight, R., L. Lauber, C., A. Lozupone, C., McNally, S., M. Needham, D., … Weber, L. (2023). Earth Microbiome Project (EMP) 16S Illumina Amplicon Protocol v2. https://doi.org/10.17504/protocols.io.kqdg3dzzl25z/v2
Methods

Gonzalez, A., Navas-Molina, J. A., Kosciolek, T., McDonald, D., Vázquez-Baeza, Y., Ackermann, G., DeReus, J., Janssen, S., Swafford, A. D., Orchanian, S. B., Sanders, J. G., Shorenstein, J., Holste, H., Petrus, S., Robbins-Pianka, A., Brislawn, C. J., Wang, M., Rideout, J. R., Bolyen, E., … Knight, R. (2018). Qiita: rapid, web-enabled microbiome meta-analysis. Nature Methods, 15(10), 796–798. https://doi.org/10.1038/s41592-018-0141-9
Software

Louca, S., Parfrey, L. W., & Doebeli, M. (2016). Decoupling function and taxonomy in the global ocean microbiome. Science, 353(6305), 1272–1277. https://doi.org/10.1126/science.aaf4507
Software

Mallick, H., Rahnavard, A., McIver, L. J., Ma, S., Zhang, Y., Nguyen, L. H., Tickle, T. L., Weingart, G., Ren, B., Schwager, E. H., Chatterjee, S., Thompson, K. N., Wilkinson, J. E., Subramanian, A., Lu, Y., Waldron, L., Paulson, J. N., Franzosa, E. A., Bravo, H. C., & Huttenhower, C. (2021). Multivariable association discovery in population-scale meta-omics studies. PLOS Computational Biology, 17(11), e1009442. https://doi.org/10.1371/journal.pcbi.1009442
Software

McDonald, D., Jiang, Y., Balaban, M., Cantrell, K., Zhu, Q., Gonzalez, A., Morton, J. T., Nicolaou, G., Parks, D. H., Karst, S. M., Albertsen, M., Hugenholtz, P., DeSantis, T., Song, S. J., Bartko, A., Havulinna, A. S., Jousilahti, P., Cheng, S., Inouye, M., … Knight, R. (2023). Greengenes2 unifies microbial data in a single reference tree. Nature Biotechnology, 42(5), 715–718. https://doi.org/10.1038/s41587-023-01845-1
Software

Mercado-Molina, A. E., Ruiz-Diaz, C. P., Pérez, M. E., Rodríguez-Barreras, R., & Sabat, A. M. (2015). Demography of the threatened coral Acropora cervicornis: implications for its management and conservation. Coral Reefs, 34(4), 1113–1124. https://doi.org/10.1007/s00338-015-1341-8
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

Rodríguez-Barreras, R., Montañez-Acuña, A., Otaño-Cruz, A., & Ling, S. D. (2018). Apparent stability of a low-density Diadema antillarum regime for Puerto Rican coral reefs. ICES Journal of Marine Science, 75(6), 2193–2201. https://doi.org/10.1093/icesjms/fsy093
Related Research

Rodríguez-Barreras, R., Pérez, M. E., Mercado-Molina, A. E., Williams, S. M., & Sabat, A. M. (2014). Higher population densities of the sea urchin Diadema antillarum linked to wave sheltered areas in north Puerto Rico Archipelago. Journal of the Marine Biological Association of the United Kingdom, 94(8), 1661–1669. https://doi.org/10.1017/s0025315414000666 https://doi.org/10.1017/S0025315414000666
Related Research

Rodríguez-Barreras, R., Ruiz-Diaz, C. P., Quiñones-Otero, M. A., & Toledo-Hernández, C. (2023). Uneven demographic consequences of the 2022 disease outbreak for the sea urchin Diadema antillarum in Puerto Rico. PeerJ, 11, e16675. Portico. https://doi.org/10.7717/peerj.16675
Related Research

Ruiz-Barrionuevo, J. M., Kardas, E., Rodríguez-Barreras, R., Quiñones-Otero, M. A., Ruiz-Diaz, C. P., Toledo-Hernández, C., & Godoy-Vitorino, F. (2024). Shifts in the gut microbiota of sea urchin Diadema antillarum associated with the 2022 disease outbreak. Frontiers in Microbiology, 15. https://doi.org/10.3389/fmicb.2024.1409729
Results

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. Genome Biology, 12(6). https://doi.org/10.1186/gb-2011-12-6-r60
Software

Tosado, E., Rodriguez, R. and Godoy, F., and Kardas, E. (2023) Microbiome Lab SOP: Collection and Laboratory Processing of Sea Urchins. PR-INBRE Metabolomics Research Core, University of Puerto Rico.
Methods

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Related Datasets

IsRelatedTo

Chorna, N., Toledo-Hernandez, C., Ruiz-Diaz, C. P., Kardas, E., Godoy-Vitorino, F. (2025) **Metabolite and microbiota data from sea urchin specimens and seawater samples collected in 2023 from four locations in coastal waters of Puerto Rico.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-10-10 http://lod.bco-dmo.org/id/dataset/986552 [view at BCO-DMO]

Godoy-Vitorino, F., Kardas, E., Ruiz-Diaz, C. P., Toledo-Hernandez, C., Rodriguez-Barreras, R., Chorna, N. (2025) **Microbiome data 16S rRNA results for sea urchin gut content, sediment, and surrounding seawater from sampled collected in 2023 from four locations along the coast of Puerto Rico.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-10-10 http://lod.bco-dmo.org/id/dataset/986537 [view at BCO-DMO]

Results

University of California San Diego Microbiome Initiative. uropean Nucleotide Archive (ENA) (2023-11-20). Microbiota associated with the 2022 Diadema antillarum die-off in Puerto Rico [Project: PRJEB70304]. Retrieved from https://www.ebi.ac.uk/ena/browser/view/PRJEB70304.

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Parameters

Parameter	Description	Units

sample_ID	Sample identification indicating location, period, and specimen number of the sample (urchin gut, water, or sediment)	unitless
loc	Site location	unitless
latitude	Latitude of sampling site	decimal degrees
longitude	Longitude of sampling site	decimal degrees
loc_abbrev	Site location abbreviation	unitless
period	Period of collection of the samples (period 1 = Mar/Apr; period 2 = May; period 3 = Aug/Sep; period 4 = Dec)	unitless
specimen_num	Specimen identification number indicating the number of the individual gut specimen, or water (w) or sediment (s) samples	unitless
sample_type	Sample type (sea urchin gut pellet, water, or sediment)	unitless
health_status	Health status of the collected specimen (if sea urchin)	unitless
SU_equatorial_diameter	Size of the sea urchin specimen at the equatorial plate level	centimeters (cm)
DNA_yield_1st_extr	Yield of DNA after the first DNA extraction	nanograms per microliter (ng/uL)
DNA_yield_2nd_extr	Yield of DNA after the second DNA extraction	nanograms per microliter (ng/uL)
final_DNA_yield	Final DNA yield; if 2 extractions, both extracted DNA portions are combined and SpeedVac'ed to concentrate DNA	nanograms per microliter (ng/uL)
method_DNA_extraction	Method used for DNA extraction; simple dna extraction, or double extraction followed by speedvac	unitless
date_collection	Date of sample collection	unitless
collectors	Name of person (people) who collected the sample	unitless
date_lab_reception	Date that the sample was received at Dr. Godoy-Vitorino's Microbiome Laboratory	unitless

receiver	Name of person who received the sample at the lab	unitless
date_processed	Date the sample was processed	unitless
dissector_filtrator	Name of person who processed the sample either by dissecting (urchins) or filtering (water)	unitless

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Instruments

Dataset-specific Instrument Name	Illumina MiSeq at Argonne National Lab
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Customized sequencing was outsourced to Argonne National Laboratory in Illinois, USA, utilizing a 2 × 150 bp paired-end sequencing kit with an Illumina MiSeq.
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset-specific Instrument Name	caliper
Generic Instrument Name	calipers
Dataset-specific Description	Sea urchin tests were measured using a caliper
Generic Instrument Description	A caliper (or "pair of calipers") is a device used to measure the distance between two opposite sides of an object. Many types of calipers permit reading out a measurement on a ruled scale, a dial, or a digital display.

Dataset-specific Instrument Name	Infinite® 200 PRO, Tecan Plate Reader
Generic Instrument Name	plate reader
Dataset-specific Description	Amplicons were measured with a plate reader (Infinite® 200 PRO, Tecan) and PicoGreen (Invitrogen) dye.
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.

Dataset-specific Instrument Name	Invitrogen Qubit fluorometer
Generic Instrument Name	Qubit fluorometer
Dataset-specific Description	Volumes of all the products were combined into one tube after they were quantified, resulting in an equimolar representation of each amplicon which was then quantified using a fluorometer (Qubit, Invitrogen).
Generic Instrument Description	Benchtop fluorometer. The Invitrogen Qubit Fluorometer accurately and quickly measures the concentration of DNA, RNA, or protein in a single sample. It can also be used to assess RNA integrity and quality. Manufactured by Invitrogen, Carlsbad, CA, USA (Invitrogen is one of several brands under the Thermo Fisher Scientific corporation.)

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Project Information

RAPID: The black urchin (Diadema antillarum) massive resurgent die-off: Causes, demographic, and community consequences (Diadema antillarum die-off associated microbiota in Puerto Rico)

Website: https://storymaps.arcgis.com/stories/40c072999aab4662b5fff6555a8c77b9

Coverage: Caribbean coral reefs. Fringing reefs located in the northern coast of Puerto Rico.

In recent decades, many marine species in Caribbean coral reef ecosystems have been impacted by disease. A well-documented mass mortality event affecting the long-spined black sea urchin Diadema antillarum in the early 1980s stands out because it had wide-ranging impacts on reef ecosystems. The urchins function as gatekeeper grazers, feeding mainly on macroalgae and preventing algae from overgrowing reefs. In the 1980s, an unknown disease killed over 90% of these urchins across the Caribbean, changing the reefscape from coral to algal dominated. Nearly 40 years later, black sea urchin populations have yet to recover. In early 2022, a

new mortality event of D. antillarum was reported along the Caribbean, including Puerto Rico. This RAPID project is identifying the microbes involved in the current mortality event. The investigators are also assessing urchin populations under contrasting environmental conditions and disease incidences. Results are providing a better understanding of the causes and consequences of disease in Diadema, and insights learned may help prevent or mitigate future mortality events. The project is providing training for underrepresented undergraduate and graduate students in microbiology, bioinformatics, biochemistry, and ecology.

With use of multi-omics technology, this RAPID project is advancing our understanding of the current die-off of Diadema in the Caribbean, including host-pathogen interactions and how these are influenced by environmental factors. The investigators are pursuing the following questions: (1) Are the microbial and biochemical profiles of diseased urchins similar to healthy ones? (2) Do environmental factors, i.e., temperature, salinity, pH, and dissolved oxygen, influence disease incidence? (3) Are different size classes of urchins differentially being affected by the disease? This project is focusing on four study sites along the eastern and northern coast of Puerto Rico, where demographic and biochemical data have been previously collected. At three-month intervals, healthy and diseased D. antillarum urchins are being evaluated for changes in the microbiome using metagenomic and untargeted metabolomic strategies. In addition, urchins from eight transects per site are being counted and measured to determine how disease modulates life-history traits, i.e., population size-structure and density, and to assess disease incidence. The relationship between disease incidence and environmental measurements is being assessed. These multidisciplinary approaches and cutting-edge techniques, combined with the existing demographic and microbial data, make this a one-of-a-kind project to study the progression and effects of the disease at the metabolic, microbial, population, and ecosystem levels.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2243580

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