

Metabolite and microbiota data from sea urchin specimens and seawater samples collected in 2023 from four locations in coastal waters of Puerto Rico

Website: <https://www.bco-dmo.org/dataset/986552>

Version: 1

Version Date: 2025-10-10

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)

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Abstract

This dataset represents a comprehensive untargeted gas chromatography-mass spectrometry (GC-MS) metabolomic analysis of the reef-dwelling sea urchin *Diadema antillarum*, conducted to investigate how gut microbial and dietary interactions reflect site-specific contamination and environmental stress across Puerto Rican reef ecosystems. Sampling was performed during the dry season (January–March 2024) [DIFFERENT from date range in METHODS??] to minimize seasonal confounders such as terrestrial runoff and salinity fluctuations. Adult urchins were collected from four ecologically distinct coastal sites—Cerro Gordo and Sardinera (Dorado), Punta Bandera (Luquillo), and Culebra Island—representing gradients of biodiversity and anthropogenic exposure. Concurrent seawater samples were collected to evaluate environmental versus organismal accumulation patterns. Metabolites were extracted from gut contents and water samples using a chloroform-methanol-water protocol (2:5:2, v/v/v), derivatized with methoxyamine hydrochloride and MSTFA + 1% TMCS, and analyzed on a Shimadzu GCMS-TQ8050 triple quadrupole system equipped with an Rxi-5MS column. Quality control samples and blanks were distributed throughout runs to ensure analytical reproducibility. Metabolite annotation was performed using the NIST14/EPA/NIH library, and data were normalized, log-transformed, and Pareto scaled prior to multivariate and univariate statistical analysis in MetaboAnalyst 6.0. Principal component analysis (PCA) and two-way ANOVA were used to assess compositional differences across collection sites, with p-values adjusted for multiple testing (FDR < 0.05). The dataset reveals site-specific accumulation of anthropogenic contaminants in *D. antillarum* guts, including nitrile-derived industrial compounds, hydrocarbons, waxes, detergents, and pharmaceutical residues. PCA and hierarchical clustering demonstrated clear segregation of samples by location (PERMANOVA $p = 0.001$), with Culebra urchins exhibiting the highest contaminant load, while seawater profiles showed no significant site variation. These results indicate that urchin gut metabolomes reflect local contamination more sensitively than seawater, highlighting their potential as bioindicators of reef health and anthropogenic stress.

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Coverage

Location: Coastal waters of Puerto Rico

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 [dataset 985892]
- **Metabolites from GC-MS analysis of sea urchin gut samples and associated seawater samples [this dataset]**
- 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 metabolomic profiling data for samples collected from reef ecosystems in coastal waters surrounding Puerto Rico. Specific collection sites included shallow reef habitats known for high biodiversity and ecological sensitivity in Dorado, Luquillo, and Culebra. This study used untargeted gas chromatography-mass spectrometry (GC/MS) metabolomics to analyze gut metabolites of *Diadema antillarum* collected from four sites in Puerto Rico: Cerro Gordo (n=6), Sardinera (n=9), Punta Bandera (n=7), and Culebra (n=11) and water samples.

Methods & Sampling

Sample Collection

The study was conducted at four sites along the northeastern and eastern coasts of Puerto Rico. (CGD=Dorado:Cerro Gordo, DBE=Dorado:Sardinera; PBA=Luquillo:Punta Bandera, and PTM=Culebra). 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 [**what disease?**]

Sea urchin and water samples were collected during March 2023 to December 2023 [**differs from what is mentioned in Abstract??**] a period associated with reduced terrestrial runoff and more stable reef conditions, which minimizes confounding environmental variables in metabolomic analysis. 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-PVSIS-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).

For field sampling, capture, and specimen details, see Related Datasets section below.

Sample Processing

Upon arrival at the Microbiome Laboratory of Dr. Filipa Godoy-Vitorino (University of Puerto Rico Medical School), all sample types were processed (see Supplemental Files section for lab protocols). 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,

examined for signs of albinism, and the size of the tests' equatorial/horizontal diameters measured. All sample types were then stored at -80°C until dissection.

Sea urchins were dissected following the standard protocol to obtain gut pellets. 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. Each fecal pellet was divided into two subsamples for microbial analysis and metabolomic analysis, with a minimum size of 50 milligrams. For sample details, see BCO-DMO dataset 985892.

Metabolite Sample Analysis

Untargeted gas chromatography-mass spectrometry (GC/MS) metabolomics was used to analyze gut metabolites of *Diadema antillarum* (sea urchin) and water samples. For GC/MS analysis, samples were homogenized in 800 µL of the chloroform methanol-water (v/v 2:5:2) solution, vortexed for 10 min at 4°C using a digital vortex mixer, and centrifuged at 14 K rpm × 10 min at 4°C. The supernatant was collected and evaporated to dryness under a nitrogen gas stream at 50°C (RapidVap, Labconco) and stored at -80°C. After that, dried samples were first derivatized by methoxyamination by adding 30 µL of 20 mg/mL methoxyamine hydrochloride solution in pyridine (Sigma-Aldrich) and incubated at 37°C for 2 hours followed by trimethylsilylation derivatization step performed by adding 30 µL of N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA + 1% TMCS, Sigma-Aldrich) and incubated for 1 hour at 65°C. Samples were centrifuged at 14,000 revolutions per minute (14 K rpm) for 10 min at room temperature. Supernatants were transferred to glass vials. 20 µL of each sample was added to analytical glass vials with inserts and processed by GCMS/MS-TQ8050-2017 (Shimadzu Inc.). Metabolite separation was achieved using a Restek Rxi-5MS column (30 m × 0.25 mm inner diameter, 0.25 µm film thickness) operated in constant pressure mode at 50 kPa with a helium carrier gas flow rate of 0.8 mL/min. The GC oven temperature was programmed for optimal resolution of derivatized metabolites. An AOC-20i+ autosampler (Shimadzu Corporation) provided automated, high-throughput sample injection. Instrument tuning and mass calibration were performed using perfluorotributylamine (PFTBA) standards, achieving a resolution of ~0.60 FWHM at reference ions (m/z 69, 219, 502). Ion source and interface temperatures were maintained at 200 °C and 280 °C, respectively, under stable vacuum conditions (low vacuum: 6.2 Pa; high vacuum: 1.0×10^{-4} Pa). The scan range was m/z 10–700 with a scan speed of 50 Hz. System performance was verified on May 8, 2025, confirming proper sensitivity and resolution. Quality control samples and blanks were distributed throughout runs to ensure analytical reproducibility.

The chromatography conditions were as follows: RXI-5MS (0.25 mm I.D., 0.25 µm D.F., 30 m) (Restek), split injection (ratio = 15), injection volume of 1 µL. The inlet temperature was 280°C, the ion source temperature was 200°C, and the interface temperature was 150°C. The oven temperature was set at 100°C for 1 min and then programmed from 100°C to 290°C at 8°C/min, held at 290°C for 16 min. Helium was the carrier gas at a constant linear velocity of 39 cm/s. Mass Spec conditions: electrospray ionization (ESI) source, full scan mode, electron energy of 70 eV, quadrupole scan range of m/z 35–700. To mitigate systematic bias, the order of sample analysis was randomized, and blanks and quality control samples were evenly distributed among the injections to monitor instrument stability.

Data Processing Description

Microbial characterization

The downstream microbial analyses were performed locally in QIIME2 (version 2024.05) and include demultiplexing, trimming at 250 bp, and denoising. The 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 is this?]** ecological and metabolic function inference (FAPROTAX, Louca et al. 2016) and putative biomarker data (LefSe) were computed.

Metabolomics

The GC/MS raw data that were obtained were processed using the Labsolution Postrun analysis software

(Shimadzu Inc.) equipped with the NIST14/2014/EPA/NIH database to identify the metabolites, following peak integration through the Labsolution Postrun analysis software and multiple searches in the mass spectral library database. To evaluate significant differences in metabolite levels detected by GC/MS across all samples, peak intensities were acquired and processed using MetaboAnalyst 6.0 (Pang et al., 2024). Briefly, data obtained from sea urchin gut samples were normalized by sample weight and 1 ml of water samples, each data set was saved as an Excel table and also saved as .csv file. These tables were uploaded to the MetaboAnalyst 6.0 web page, log₁₀-transformed, and Pareto scaled. Statistical analyses included principal component analysis (PCA) with group differences assessed using the PERMANOVA test as well as hierarchical clustering via heatmap using Euclidean distance and the Ward algorithm. Additionally, two-way ANOVA was conducted and resulting p-values were adjusted for multiple comparisons using the false discovery rate (FDR) method. A significance threshold (α) of 0.05 was applied.

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Data acquisition, peak integration, and spectral library matching were performed using LabSolutions GCMS ver. 4.45 (Shimadzu Corporation), which facilitated real-time MRM optimization, quantitative peak integration, and spectral deconvolution.

MetaboAnalyst 6.0 Data Analysis Platform (University of Alberta, Canada; <https://www.metaboanalyst.ca>) was used for downstream data processing, normalization, and statistical analysis of GC-MS results. CSV tables were uploaded, log₁₀-transformed, and Pareto-scaled before conducting multivariate analyses including PCA, PERMANOVA, hierarchical clustering, and two-way ANOVA. This platform enabled visualization of group differences and metabolite patterns across reef sites.

BCO-DMO Processing Description

- Pivoted the sea urchin gut metabolite data so each row is a complete observation with a single record of analysis with associated metadata
- Pivoted the water metabolite table so each row is a complete observation
- Combined the sea urchin and water metabolite data and added column for sample type
- Added latitude and longitude columns based on site locations
- Sorted data by metabolite, then collection site and sample type

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Related Publications

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](https://doi.org/10.1038/nmeth.3869)

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

Pang, Z., Lu, Y., Zhou, G., Hui, F., Xu, L., Viau, C., Spigelman, A. F., MacDonald, P. E., Wishart, D. S., Li, S., & Xia, J. (2024). MetaboAnalyst 6.0: towards a unified platform for metabolomics data processing, analysis and interpretation. *Nucleic Acids Research*, 52(W1), W398–W406. <https://doi.org/10.1093/nar/gkae253>

Methods

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., Ruiz-Díaz, 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

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

Godoy-Vitorino, F., Kardas, E., Ruiz-Díaz, 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](#)]

Godoy-Vitorino, F., Toledo-Hernandez, C., Kardas, E., Chorna, N., Rodriguez-Barreras, R., Ruiz-Díaz, C. P. (2025) **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.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-11-14 <http://lod.bco-dmo.org/id/dataset/985892> [[view at BCO-DMO](#)]

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Parameters

Parameter	Description	Units
Collection_Site	Sample collection reef location in Puerto Rico. Full site names recorded as Dorado:Cerro Gordo (CGD), Dorado:Sardinera (DBE), Luquillo:Punta Bandera (PBA), Culebra (PTM)	unitless
Latitude	Latitude of sampling site	decimal degrees
Longitude	Longitude of sampling site	decimal degrees
Sample_ID	Unique identifier assigned to each individual sample. Composite alphanumeric code indicates site and replicate. Format: SITE ABBREVIATION + batch - replicate number (e.g., CGD 1-1 for Cerro Gordo, DBE 1-2 for Sardinera, PBA 2-1 for Punta Bandera, PTM 3-1 for Culebra).	unitless
Sample_type	Biological/environmental sample category (either Seawater or Sea urchin guts for Diadema antillarum gut content)	unitless
Metabolite	Compound name identified by GC-MS using the NIST14/EPA/NIH spectral library. Each metabolite name corresponds to the best spectral match confirmed by retention time and ion fragmentation pattern.	unitless
Retention_time	Chromatographic elution time measured on Restek SH-Rxi-5MS; precision typically ± 0.01 -0.05 min.	unknown
Exact_mass	[Need input] Exact mass; the calculated mass of an ion or molecule with the most intense molecule/ion peak in mass spec data	unknown
Main_class	[Need input] Main class of molecule? Or main chemical class?	unitless
Counts_or_Intensity_maybe	[Need input]	unknown
Sample_weight	[Need input] (in what units?)	unknown
Sample_Site	Abbreviation for collection site location	unitless

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Instruments

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	Eppendorf 5810 R Refrigerated Centrifuge
Generic Instrument Name	Centrifuge
Dataset-specific Description	The Eppendorf 5810 R (Eppendorf AG, Hamburg, Germany; purchased 2012) is a high-capacity refrigerated benchtop centrifuge used for sample clarification before GC-MS analysis. It supports swing-bucket and fixed-angle rotors up to 400 mL and reaches 14,000 rpm (20,913 × g). The temperature range (−9 °C to +40 °C) and microprocessor-controlled cooling system ensure reproducible processing of temperature-sensitive metabolomic samples.
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	dry block incubator
Generic Instrument Name	Incubator
Dataset-specific Description	A temperature-controlled dry block incubator was set to 37 °C for methoxyamination and 65 °C for silylation.
Generic Instrument Description	A device in which environmental conditions (light, photoperiod, temperature, humidity, etc.) can be controlled. Note: we have more specific terms for shipboard incubators (https://www.bco-dmo.org/instrument/629001) and in-situ incubators (https://www.bco-dmo.org/instrument/494).

Dataset-specific Instrument Name	Labconco RapidVap model 7900000/01
Generic Instrument Name	Labconco RapidVap Vacuum Evaporation System
Dataset-specific Description	The Labconco RapidVap Vacuum Evaporation System (model 7900000/01), purchased in 2017, was used to evaporate solvent extracts to dryness prior to derivatization.
Generic Instrument Description	The Labconco RapidVap Vacuum Evaporation System (Labconco Corporation, Kansas City, MO) is a benchtop, multi-sample evaporator that combines vacuum, 1000 W dry-block heating (up to 100 °C), and vortex motion (up to 1000 rpm) to concentrate samples efficiently. It accommodates up to 110 samples in PTFE-coated aluminum blocks and glassware, with programmable temperature, vacuum, and time profiles. The instrument includes automatic vacuum shutoff, audible/visual alarms, and a chemical-resistant, PTFE-coated chamber with phenol-free gaskets, ensuring safe unattended operation.

Dataset-specific Instrument Name	AOC-20i+ autosampler (Shimadzu Corporation)
Generic Instrument Name	Laboratory Autosampler
Dataset-specific Description	An AOC-20i+ autosampler (Shimadzu Corporation) provided automated, high-throughput sample injection.
Generic Instrument Description	Laboratory apparatus that automatically introduces one or more samples with a predetermined volume or mass into an analytical instrument.

Dataset-specific Instrument Name	calibrated micropipette
Generic Instrument Name	pipette
Dataset-specific Description	Calibrated micropipettes were used to transfer reagent, ensuring consistency in steps of the metabolomics workflow
Generic Instrument Description	A pipette (or pipettor) is a laboratory tool (measuring device) designed for the accurate measurement and transfer of precise volumes of liquid. It traditionally included a graduated tube, but can now be manual or digital.

Dataset-specific Instrument Name	Shimadzu GC-MS-TQ8050 Ultra
Generic Instrument Name	Shimadzu GC-MS Triple Quadrupole Mass Spec 8050 Ultra
Dataset-specific Description	All metabolomic analyses were conducted using a Shimadzu GCMS-TQ8050 Ultra (Shimadzu Corporation, Kyoto, Japan), purchased in 2017. This ultra-high-sensitivity triple quadrupole GC-MS/MS system integrates a high-performance gas chromatograph with a triple quadrupole mass spectrometer. Metabolite separation was achieved using a Restek Rxi-5MS column (30 m × 0.25 mm I.D., 0.25 µm film thickness) operated in constant pressure mode at 50 kPa with a helium carrier gas flow rate of 0.8 mL/min. The GC oven temperature was programmed for optimal resolution of derivatized metabolites.
Generic Instrument Description	The Shimadzu GCMS-TQ8050 Ultra (Shimadzu Corporation, Kyoto, Japan), is an ultra-high-sensitivity triple quadrupole GC-MS/MS system that integrates a high-performance gas chromatograph with a triple quadrupole mass spectrometer, operated under standard electron ionization (EI) at 70 eV with a high-efficiency ion source. The system supports multiple acquisition modes, including full-scan and multiple reaction monitoring (MRM).

Dataset-specific Instrument Name	digital vortex mixer
Generic Instrument Name	vortex mixer
Dataset-specific Description	Sample homogenization and derivatization were performed using standard laboratory equipment, including a digital vortex mixer.
Generic Instrument Description	A vortex mixer is an electrical rotator that blends or mixes substances or ingredients, in whirling or rotary motion, for homogenizing samples.

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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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