Flow cytometry data from Incurrent and Excurrent flow samples collected from shallow artificial reef sponges and seawater in the Florida Keys, USA from Apr 2021 to Aug 2021

Website: https://www.bco-dmo.org/dataset/969846
Data Type: Other Field Results
Version: 1
Version Date: 2025-08-29

Project
» Collaborative Research: Investigations into microbially mediated ecological diversification in sponges (Ecological Diversification in Sponges)

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Abstract
Sponges are a dominant component of coral reefs worldwide and in the Caribbean, where their biomass exceeds reef-building corals. For almost a quarter century, the success of sponges in the Caribbean has been linked to their filter-feeding ability. However, recent work demonstrated that coexisting sponges on Caribbean reefs host unique communities of bacteria that might allow sponges to access multiple pools of nutrients unavailable to other organisms. In this project, the investigators will test the hypothesis that ecologically dominant sponge species in the Caribbean have unique metabolic strategies mediated by their associations with microbes living within the sponge body. In this dataset, we present flow cytometry data for an artificial reef experiment where sponges of 10 species were placed on this temporary reef from April to August 2021 and sampled using a VacuSIP. VacuSIP methods capture incurrent (In) and excurrent (Ex) water from each sponge specimen. Incurrent represents the microbes available for the sponge to consume via filter feeding, and excurrent represents the microbes that remain once sponges have consumed their preferred taxa. Flow cytometry data was generated with the help of Bigelow Laboratory for Ocean Sciences. Samples were analyzed for the presence of photosynthetic microbes (Synechococcus, Prochlorococcus, photosynthetic eukaryotes) and heterotrophic bacteria (high nucleic acid and low nucleic acid). The present data represents raw data provided by the Bigelow Laboratory for Ocean Sciences, as well as the collection metadata, water sample type, and sponge species information.

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Coverage

Location: These data were generated on shallow reefs (<10m) in the Florida Keys, USA.
Spatial Extent: **Lat**:24.562003 **Lon**:-81.408711
Temporal Extent: 2021-04-01 - 2021-08-31

Methods & Sampling

Location
Samples were collected on shallow reefs (<10m) in the Florida Keys, USA. The temporary artificial reef was constructed adjacent to patch reefs within the Looe Key special preservation area. At the conclusion of the experiment, the reef was completely dismantled. The environment was well mixed by wave action within minimal tidal influence.

Collection and Analysis

One-milliliter of seawater samples were collected via VacuSIP and preserved in paraformaldehyde (0.5% final concentration), flash frozen in liquid nitrogen, and then shipped to Bigelow Laboratory for Ocean Sciences. At this laboratory, the following methodology was used:

A ZE5 Cell Analyzer flow cytometer (Bio-Rad, Hercules, CA, USA) was used to measure optical properties of single cells from each sample and quantify requested populations. To ensure accurate calibration of the flow cytometer, ZE5 QC beads (Bio-Rad, Hercules, CA, USA) were run daily.

Picophytoplankton (less than 3 µm) and nanophytoplankton (3-20 µm) were analyzed using a slight modification of the method described in Lomas et al., 2010. Immediately after thawing at room temperature, 300-400 µl of sample was prescreened through 70 µm mesh and run at a flow rate of 1 µl sec-1. Particles were excited with a 488 nm blue laser and data acquisition was triggered on red fluorescence. Signals were recorded from detectors with bandpass filters for forward scatter (FSC), right angle light scatter (SSC) and fluorescence emission in red (692/80 nm) indicative of chlorophyll a, and orange (593/52 nm) for phycoerythrin. Data files were analyzed from logarithmic dot plots based on fluorescence and characteristic light scattering properties (DuRand & Olson, 1996) using FlowJo 10.6 software (FlowJo, 2023) (Becton Dickinson & Company, San Jose, CA, USA). Total pico and nano phytoplankton populations were identified based upon cell size and red fluorescence. Phycoerythrin containing cell populations were determined by orange fluorescence. Based upon these gating criteria, the number of cells in each identified population was enumerated and converted to cell abundances using the processed sample volume and adjusted for dilution by preservative.

For total bacteria analysis, samples were thawed, diluted 1:10 with Tris EDTA (TE) Buffer pH 8.0 and stained using a 10x working stock of SYBR Green I Nucleic Acid Stain (Thermofisher Scientific, USA) at room temperature in the dark for 15 min using the protocol of Marie et al. (2005). At a flow rate of 0.5 µl sec-1, 180 µl of the diluted sample was run. Particles were excited with a 488 nm blue laser and data acquisition was triggered on green fluorescence. Signals were recorded from detectors with bandpass filters for forward scatter (FSC), right angle light scatter (SSC) and fluorescence emission in green (525/35nm). Data files were analyzed from two logarithmic scatter plots based on fluorescence and characteristic light scattering properties. Total bacteria counts were identified based on size and presence of green fluorescence and counts were converted to cell abundances using the volume of sample processed including adjustments for preservation, dilution and staining.

Data Processing Description

Raw data is presented here. Data analysis includes univariate and multivariate statistics.

BCO-DMO Curation Notes

- Imported "Flow_cytometry_BCO-DMO.csv" into BCO-DMO system

- Converted "Lat" and "Lon" to decimal degrees to conform with BCO-DMO guidance
- Converted "Datetime" (only included date) to YYYY-MM-DD ISO 8601 date format to conform with BCO-DMO guidance
- Removed "Day " part of string of values representing day of collection to convert value to an integer
- Renamed fields to comply with BCO-DMO naming conventions, removing units, special characters, and spaces
- Exported file as "969846_v1_flow_cytometry_incurrent_excurrent_flow.csv"

Taxonomic names were checked using the World Register of Marine Species Taxa Match tool (WoRMS). All names matched a known name exactly, except Amphimedon compressa (https://www.marinespecies.org/aphia.php?p=taxdetails&id=166666), which was recently superseded by Amphimedon nodosa (https://www.marinespecies.org/aphia.php?p=taxdetails&id=1814728) earlier in 2025 after work for this dataset was completed. Submitter requested Amphimedon compressa be used in the dataset. The URI locator provides access to the latest taxonomic information.

Problem Description

No problems were encountered with these methods.

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Data Files

File
969846_v1_flow_cytometry_incurrent_excurrent_flow.csv (Comma Separated Values (.csv), 17.48 KB) MD5:6fc77eb3349552379bbf54f046b96918 Primary data file for dataset ID 969846, version 1

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Related Publications

Durand, M. D., & Olson, R. J. (1996). Contributions of phytoplankton light scattering and cell concentration changes to diel variations in beam attenuation in the equatorial Pacific from flow cytometric measurements of pico-, ultra- and nanoplankton. Deep Sea Research Part II: Topical Studies in Oceanography, 43(4–6), 891–906. https://doi.org/10.1016/0967-0645(96)00020-3
Methods

FlowJo, LLC. (2023) FlowJo™ Software Version 10.6 [software application] Becton, Dickinson and Company. https://docs.flowjo.com/flowjo/getting-acquainted/10-6-release-notes/10-6-exhaustive-release-notes/
Software

Lomas, M. W., Steinberg, D. K., Dickey, T., Carlson, C. A., Nelson, N. B., Condon, R. H., & Bates, N. R. (2010). Increased ocean carbon export in the Sargasso Sea linked to climate variability is countered by its enhanced mesopelagic attenuation. Biogeosciences, 7(1), 57–70. https://doi.org/10.5194/bg-7-57-2010
Methods

Marie, D., Simon, N., and Vaulot, D. (2005). "Phytoplankton cell counting by flow cytometry," in Algal culturing techniques. Ed. R. A. Andersen (San Diego: Academic Press), 253–267. https://books.google.com/books?hl=en&lr=&id=-qWHAwAAQBAJ&oi=fnd&pg=PA253&dq=Phytoplankton+cell+counting+by+flow+cytometry&ots=XrosAbvlO2&sig=kegVhlxLGyvRTQ5r67D38jJQkbI#v=onepage&q=Phytoplankton%
Methods

Vaulot, D., Courties, C., & Partensky, F. (1989). A simple method to preserve oceanic phytoplankton for flow cytometric analyses. Cytometry, 10(5), 629–635. https://doi.org/10.1002/cyto.990100519
Methods

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Related Datasets

IsRelatedTo

Easson, C. G., Freeman, C. J., Fiore, C. L., Thacker, R. W. (2025) **16S microbiome metadata collected from shallow artificial reef sponges and seawater in the Florida Keys, USA from Apr 2021 to Aug 2021.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-02-21
doi:10.26008/1912/bco-dmo.953999.1 [view at BCO-DMO]
Relationship Description: Water samples for each of these datasets were sampled simultaneously and later divided among analyses.

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Parameters

Parameter	Description	Units
Collection_Date	Date of sample collection	unitless
Latitude	Latitude of sample collection; North is positive	decimal degrees
Longitude	Longitude of sample collection; West is negative	decimal degrees
Sample_ID	Sample identifier	unitless
Species	Sponge species sampled	unitless
Species_Abbreviation	Abbreviation of sponge species	unitless
In_or_Ex	Incurrent or Excurrent water sampled	unitless
Replicate	Replicate number	unitless
Collection_day	Day of collection	unitless
TotPhyto_Conc	Total Phytoplankton (Syn + Pro+ Photosynthetic Eukaryotes)	mL-1
Syn_Conc	Synechococcus concentration	mL-1
Pro_Conc	Prochlorococcus concentration	mL-1
Photosynthetic_Eukaryotes	Photosynthetic Eukaryote concentration	mL-1
TotBact_Conc	Total Heterotrophic bacteria (HNA + LNA)	mL-1
HNA_Conc	High nucleic acid bacteria	mL-1
LNA_Conc	Low Nucleic acid bacteria	mL-1
LSID	Life Science Identifier (LSID) for the sponge species	unitless
AphiaID	Unique identifier for the sponge species in the Aphia database	unitless

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Instruments

Dataset-specific Instrument Name	VacuSIP
Generic Instrument Name	Discrete water sampler
Dataset-specific Description	One milliliter of seawater samples were collected via VacuSIP and preserved in paraformaldehyde (0.5% final concentration), flash frozen in liquid nitrogen, and then shipped to Bigelow Laboratory for Ocean Sciences.
Generic Instrument Description	A device that collects an in-situ discrete water sample from any depth and returns it to the surface without contamination by the waters through which it passes, such as a water bottle.

Dataset-specific Instrument Name	ZE5 Cell Analyzer flow cytometer (Bio-Rad, Hercules, CA, USA)
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	A ZE5 Cell Analyzer flow cytometer (Bio-Rad, Hercules, CA, USA) was used to measure optical properties of single cells from each sample and quantify requested populations. To ensure accurate calibration of the flow cytometer, ZE5 QC beads (Bio-Rad, Hercules, CA, USA) were run daily.
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. Description from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm

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Project Information

Collaborative Research: Investigations into microbially mediated ecological diversification in sponges (Ecological Diversification in Sponges)

Coverage: Caribbean coast of Panama

NSF Award Abstract:
Coral reefs represent a paradox because, despite their immense productivity and biodiversity, they are found in nutrient-poor habitats that are equivalent to "marine deserts." High biodiversity is often associated with a division of resources that allows many types of organisms to coexist with minimal competition. Indeed, unlike many other organisms on coral reefs, sponges are adapted to efficiently remove bacteria, phytoplankton, and dissolved organic matter from seawater by filter-feeding. Sponges are a dominant component of coral reefs worldwide and in the Caribbean, where their biomass exceeds that of reef-building corals. For almost a quarter century, the success of sponges in the Caribbean has been linked to their filter-feeding ability. However, recent work demonstrated that coexisting sponges on Caribbean reefs host unique communities of bacteria that might allow sponges to access multiple pools of nutrients that are not available to other organisms. In this project, the investigators will test the hypothesis that ecologically dominant sponge species in the Caribbean have unique metabolic strategies that are mediated by their associations with microbes that live within the sponge body. This research will combine manipulative field experiments with a novel combination of modern analytical tools to investigate both filter-feeding by sponge hosts and the metabolic pathways of their microbes. This work will advance our understanding of the ecological and evolutionary forces that have helped shape the species present on Caribbean coral reefs. Additionally, this project will support three early-career investigators and provide training opportunities for graduate and undergraduate students at Nova Southeastern University, Appalachian State University, Stony Brook University, and Smithsonian Marine Station. The investigators will also develop innovative outreach programs that expand existing platforms at their institutions to increase public engagement and scientific literacy.

Marine sponges have been widely successful in their expansion across ecological niches in the Caribbean, with biomass often exceeding that of reef-building corals and high species diversity. However, whether this success is linked to efficient heterotrophic filter-feeding on organic carbon in the water column or to their evolutionary investment in microbial symbionts is yet to be fully elucidated. Microbial symbionts expand the metabolic capabilities of host sponges, supplementing heterotrophic feeding with inorganic carbon and nitrogen, mediating the assimilation of dissolved organic matter, and facilitating recycling of host-derived nitrogen. Despite these benefits, microbial symbiont communities are widely divergent across coexisting sponge species and there is substantial variation in host reliance on symbiont-derived carbon and nitrogen among host sponges; therefore, these associations likely mediate the ecological diversification of coexisting sponge species. The goal of this project is to test this transformative hypothesis by adopting an integrative approach to assess the individual components of holobiont metabolism (i.e., microbial symbionts and sponge host) in ten of the most common sponge species in the Caribbean. The investigators will isolate autotrophic and heterotrophic metabolic pathways and explore potential links between microbial symbiont community composition and the assimilation of particulate and dissolved organic matter (POM and DOM) from seawater. This project will elucidate whether Caribbean sponge species are on similar or divergent evolutionary trajectories, and will provide information that is critical for our understanding of how conditions in the Caribbean basin have shaped the evolution of benthic organisms.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1915949
NSF Division of Ocean Sciences (NSF OCE)	OCE-1756799
NSF Division of Ocean Sciences (NSF OCE)	OCE-1929293
NSF Division of Ocean Sciences (NSF OCE)	OCE-1756114
NSF Division of Ocean Sciences (NSF OCE)	OCE-1756249
NSF Division of Ocean Sciences (NSF OCE)	OCE-1756171

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