Autotrophy, heterotrophy and niche partitioning in Caribbean sponges sampled June 9, 2019 on reef sites around Bocas del Toro Panama.

Website: https://www.bco-dmo.org/dataset/954735
Data Type: Other Field Results
Version: 1
Version Date: 2025-02-27

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

Contributors	Affiliation	Role
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Abstract
Photosymbionts expand the metabolic capabilities of host sponges, but their potential role in mediating niche partitioning on crowded and oligotrophic coral reefs is unknown. To address this question, we conducted two ex situ isotope tracer experiments with ten of the most ecologically dominant sponge species in the Caribbean. This research was carried out in Bocas del Toro, Panama. To target autotrophic and heterotrophic nutrient acquisition by microbial symbionts, we incubated sponges in seawater laced with the inorganic compounds NaH13CO3 and Na15NO3 under both light and dark conditions. We also measured host sponge heterotrophic feeding rates by incubating the same species with 13C- and 15N-labeled bacterial cells. Sponge cells isolated from sponge species hosting photosymbionts were significantly more enriched in 13C and 15N from inorganic sources, and 72 % of the variation in 13C and 15N enrichment across samples was explained by sponge species identity. Dark enrichment of 13C was minimal, but all species were enriched in 15N in the dark due to heterotrophic microbial nitrogen assimilation. Sponges rapidly consumed bacterial cells, but there was substantial variation in heterotrophic feeding rates among sponge species. When considering all three resource pools (symbiont autotrophy, symbiont heterotrophy, and sponge heterotrophy) and both elements, sponge species identity accounted for over 80 % of variation among specimens; in addition, we observed a clear separation of sponge species along a continuum of heterotrophic feeding on particulate organic matter to autotrophic metabolism via photosymbionts. These data demonstrate that the combined influence of sponge and photosymbiont metabolism enable coexisting sponge species to exploit unique resource pools on Caribbean reefs.

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Coverage

Location: Reef sites around Bocas del Toro Panama. This site is adjacent to the Smithsonian Tropical Research Institute.
Spatial Extent: **Lat**:9.349457 **Lon**:-82.262448
Temporal Extent: 2019-06-09

Methods & Sampling

In June of 2019, individuals of the sponge species *Aiolochroia crassa*, *Amphimedon compressa, Aplysina cauliformis, Aplysina fulva, Iotrochota birotulata, Ircinia bocatorensis* (formerly *Ircinia felix*; Kelly & Thacker 2021)*, Mycale laevis, Niphates erecta, Verongula rigida,* and *Xestospongia muta* were collected by SCUBA from shallow reefs (3-10 m) around the Smithsonian Tropical Research Institute (STRI) in the Bocas del Toro (BDT) archipelago of Panama. Individuals of the species *A. crassa*, *A. compressa, A. cauliformis, A. fulva, I. birotulata, M. laevis, N. erecta,* and *V. rigida* in BDT can be easily partitioned into genetically identical fragments that can be allocated to multiple experiments or treatments. In contrast, the species *I. bocatorensis* and *X. muta* cannot be reliably split into multiple individuals. Thus, to start these experiments, seven large individuals of *A. crassa*, *A. compressa, A. cauliformis, A. fulva, I. birotulata, M. laevis, N. erecta,* and *V. rigida* were collected. Each individual of these species was partitioned into four fragments (an initial sample and one sample each for the heterotrophy experiment and light and dark treatments of the autotrophy experiment). Small individuals (N = 28 each) of *I. bocatorensis* and *X. muta* were also collected and these whole individuals were used in each experiment. All fragments and intact individuals were labeled and initially attached to a small square of plastic window screening that was cable tied to plastic mesh secured to the benthos with cinder blocks. Sponges were acclimated in this shallow (2-3 m) common garden reef near STRI for at least 48 hr before the start of experiments. After 48 hr and prior to the start of experiments, initial samples of each species (N = 7) were removed from this common garden and processed for microbiome (small subsample placed in 95 % EtOH and frozen at -20 °C) and isotope (wrapped in aluminum foil and frozen at -20 °C) analyses.

Prior to the start of the experiment, irradiance was measured at multiple depths from collection sites around STRI using a LI-COR LI-193 underwater spherical quantum sensor. Vertical light extinction coefficients averaged 0.355, indicating that our average irradiance (between 10 am and 4 pm) of over 3000 µmol photons m-2 s-1 at the surface (as measured by the STRI weather platform; Paton 2019) corresponded to over 500 µmol photons m-2 s-1 at 5 m depth.

The experiment was carried out for seven days in a Latin square design with one replicate of each of the ten sponge species used per day. During the evening prior to the experiment, sponges were collected from the common garden reef and dark-adapted overnight in a common flow-through seawater tank covered in thick plywood. At the start of the experiment, sponges were transferred to either 4 L or 6 L (for larger *I. bocatorensis* and *X. muta* individuals) experimental containers filled with 0.7 µm (GF/F) filtered seawater that was dosed with NaH13CO3 (0.1 g L-1) and Na15NO3 (0.1 g L-1) as in Freeman et al. (2013). These containers were resting within flowing seawater tables that acted as a temperature bath (Fig. S1). Two fragments of a replicate of *A. crassa*, *A. compressa, A. cauliformis, A. fulva, I. birotulata, M. laevis, N. erecta,* and *V. rigida* or individuals of *I. bocatorensis* and *X. muta* were each relegated to an experimental container in one of two flowing seawater tables: a light treatment that was exposed to direct, ambient light and a dark treatment that was under the roof of the wet lab at STRI and covered with a sheet of thick plywood. Light loggers placed within this dark tank during all seven days of the experiment consistently measured zero irradiance. Water circulation in each experimental container was maintained using a submersible pump. Water temperature in experimental containers was measured every 30 min and, if needed, ice packs were added to the temperature bath to maintain a target temperature of 29-30 °C. Ambient irradiance was monitored every 15 min using the STRI weather station (Paton 2019; in irradiance units of W m-2), and layers of window screening were added to the light treatment as needed in order to maintain an irradiance level in the experimental containers that approached irradiance at 3 m depth (1000 µmol photons m-2 s-1) where almost all of our species were found. A conversion factor of 1 W m-2 to 457 µmol photons m-2 s-1 was used for this calculation. At the end of the experiment (6 hr), sponge tissue was rinsed in 0.7 µm filtered, unlabeled seawater, a small subsample was removed for microbiome analyses and preserved in 95% EtOH as outlined above, and the remaining tissue was wrapped in aluminum foil, and frozen at -20 °C for transport back to Charleston, SC. Sponges were later weighed to the nearest 0.001 g after thawing.

The bacterium *Oceanicola batsensis* (NCMA B15 from https://ncma.bigelow.org/) was purchased to serve as

the primary bacterium for the heterotrophy experiment, but local heterotrophic bacteria were also cultured from the water near STRI. To isolate ambient bacteria, 250 mL of seawater from the site's flow through system (that acquires water from a seagrass and hardbottom habitat approximately 100 m offshore) was filtered through a 0.22 µm polycarbonate filter. Sterile techniques were employed in a countertop, portable hood sterilized with 70% EtOH. The filter was placed into sterile 15 ml conical tubes with 8 ml of 0.2 µm filtered seawater and shaken gently. The filter was plated face down, and the water was streaked out onto the following agar preparations: 1.5% Difco marine broth 2216 with 0.2 µm unamended filtered sea water (FSW) and 1.5 % Difco marine broth 2216 with 0.7 µm FSW 1.5 % + 10 mM NH4Cl. These culturing techniques led to the formation of two colonies: JHB59-1.1 was a yellow, smooth colony isolated on 1.5% Difco marine broth 2216, and JHB59-3.2 were clear colonies isolated on 0.2 µm filtered seawater 1.5 % + 10 mM NH4Cl. Each of these, along with the purchased *O. batsensis*, were enriched in 13C and 15N for the heterotrophy experiments to measure sponge cell isotopic enrichment as a proxy for cell removal and assimilation.

A day prior to each experiment, colonies of *O. batsensis*, JHB59-1.1, and JHB59-3.2 were used to create starter cultures with 4 ml of marine broth. The evening prior to the experiment, the starter culture was added to 400 ml of marine broth, and the media was amended with 13C6H12O6 (Glucose) (0.4. g L-1), NaH13CO3 (0.1 g L-1), 15NH4Cl (0.2 g L-1), and Na15NO3 (0.1 g L-1). Dual carbon and nitrogen labels were used because one strain of *O. batsensis* does not utilize glucose and to ensure adequate 15N enrichment. All isotopic tracer compounds had an atom % of the heavy atom of C and N (13C and 15N) of at least 98%. Cultures were grown in an incubator at 29°C with rocking motion. On the morning of the experiment, the OD600 concentration was recorded for the overnight cultures using a spectrophotometer and then cells were pelleted by centrifugation in 50 ml Falcon tubes at 4500 x g for 10 min. Cells were resuspended in 12 ml of 0.7 µm filtered seawater for use in the experiment. Each day, the remainder of culture was stored in RNAlater and stored at -20 °C for future microbial 16S rDNA analysis.

The heterotrophy experiment was conducted over four consecutive days in a Latin square design with at least one replicate (one replicate on day #1 and two replicates in days two, three, and four) of each of the ten sponge species used per day. On the evening prior to the experiment, sponges were collected from the common garden reef and dark-adapted overnight in a common flow-through seawater tank. As above, at the start of the experiment, sponges were transferred to either 4 L or 6 L experimental containers filled with 0.7 µm (GFF) filtered seawater resting within a flow table with running seawater, and water circulation in experimental containers was maintained using a submersible pump for 120 min. Sponges were dosed with 13C- and 15N-enriched bacteria using a pipette at an initial average concentration of 3x106 microbial cells per ml. An initial 1 ml water sample was collected immediately at T = 0 for future flow cytometry analysis and this was repeated at 30 min intervals for T = 30, 60, 90, and 120 min. Data from the 90 min sampling were ultimately excluded from the study due to preservation issues. Samples were also collected from a control (without a sponge) tank dosed with the same concentration of bacteria as the sponge tanks each day; one control tank was included on day 1 and two controls tanks were included in days 2, 3, and 4 of the experiment. All water samples were added to a 2 ml screw top cryovial tube with 0.5% paraformaldehyde (final concentration), held at 4 °C for one hr, and then frozen at -20 °C. Water temperature was recorded every 30 min and ice packs were added to the surrounding water bath as needed. After 120 min of incubation with the enriched bacteria, flowing seawater was added to each tank via individual plastic tubing and tanks were flushed with ambient water for one hr to allow for the purging of enriched bacteria that might be transiting the sponge aquiferous system but had not yet been consumed. Following this, sponge tissue was rinsed in 0.7 µm filtered, unlabeled seawater, a small subsample was removed for microbiome analyses and preserved in 95% EtOH as outlined above, and the remaining tissue was wrapped in aluminum foil, and frozen at -20 °C for transport back to Charleston, SC. Sponges were later weighed to the nearest 0.001 g after thawing.

To determine the clearance rate for all ten sponge species, heterotrophic bacteria abundance was enumerated at each time point using flow cytometry. Cell counting was performed using a BD Biosciences Accuri C6 Plus Model flow cytometer at the Smithsonian Marine Station in Fort Pierce, Florida. The methods for staining heterotrophic bacteria with SYBR Green 1. Each day, fluoresce bright Plain YG 1.0 µm beads were added at 1.0 x 106 to 0.2 µm filtered seawater and run as a size standard, and a blank consisting of 1 ml TE (10 mM Tris-HCL, 1 mM EDTA) buffer and 10 µl 50x SYBR Green was also analyzed to differentiate signal from the TE buffer (distinct crescent shape output on the display) from the signal of experimental samples.

Each 1 ml experimental water sample was thawed and vortexed to ensure bacterial cells were fully resuspended. 100 µl of each sample was added to 900 µl of TE buffer and 10 µl of 50x SYBR Green in a new 1.5 ml centrifuge tube. This tube was inverted, incubated in a -20 °C freezer for 15 min in the dark, and run on the BD C6 flow cytometer at a flow rate of 35 µl min-1 for 2 min. Samples were excited by a 488 nm laser and cell populations were detected using a 533/30 filter (FL-1), forward and side scatter with a threshold of 700 on FL-1. To reduce potential contamination across samples, the flow cytometer was backflushed, rinsed for one min with milliQ water at high flow rate (66 µl min-1), and the sample nozzle was wiped in between each sample.

If a water sample (experimental or control) was analyzed more than once, then an average concentration of cells from all runs was ultimately used for a concentration value in the equations below.

The isolation of microbial and sponge cells from bulk sponge tissue followed methods adapted from Freeman et al. (2013). Sponge tissue was minced into small pieces using a sterile razor blade, added to 50 ml falcon tubes, and the sponge pieces were allowed to thaw for an hr at room temperature before being covered with ice-cold calcium-magnesium-free artificial seawater (CMFASW) amended with Ethylenediaminetetraacetic acid (EDTA; as in Freeman & Thacker 2011). Samples were gently homogenized with a mortar and pestle 55x to dissociate the sponge and bacterial cells from the sponge matrix. This solution was filtered through 50 µm Nitex nylon mesh and lightly squeezed to retain undissociated cells and large spicules.

The resulting filtrate was poured into 50 ml centrifuge tubes and topped with CMFASW-EDTA for a final concentration of 50 ml. The filtrate for each sponge species was centrifuged at low speed to first isolate sponge cells and then high speeds to isolate microbial cells. To optimize sample purity, unique methods and speeds were used for each species (as in Freeman & Thacker 2011, Freeman et al. 2013; Table S3). To isolate initial sponge cell fractions (containing some microbial cells), the above filtrate was centrifuged, and the supernatant after two to four low-speed rounds of centrifugation was decanted into a new 50 ml Falcon tube that was then centrifuged at 8000 RCF to obtain a microbial pellet (Table S3). Following this high-speed spin, the supernatant was poured off and the microbial pellet was stored at -20 °C. The initial pellets from low-speed centrifugation contained primarily sponge cells but needed to be purified again at lower speeds to remove microbial cells. Sponge pellets from consecutive low-speed centrifugation were resuspended in 15 ml of CMFASW-EDTA and combined. After each sponge pellet cleaning spin, the supernatant was discarded, and the cells were resuspended in 15 ml CMFASW-EDTA for the following spins. Purified sponge and microbial cell pellets were transferred to 2 ml micro-centrifuge tubes and centrifuged at 7200 RCF for 5 min at 4 °C. The supernatant was discarded, and the pellets were stored at -20 °C. Bulk sponge tissue was used for *Amphimedon compressa* because a pure sponge pellet could not be obtained due to cells rupturing during the dissociation process and because *A. compressa* has a very low abundance of microbial cells such that the microbial cell pellets lacked the biomass required for isotope analyses. Sponge and microbial fraction purity were verified using fluorescence and phase contrast microscopy at 400X (as in Freeman & Thacker 2011).

The purified and frozen sponge and microbial fractions and subsamples of *A. compressa* tissue were lyophilized for 24 hr. Dried *A. compressa* was crushed with a mortar and pestle into a fine powder and transferred into 1.5 ml centrifuge tubes, and all dried pellets were placed in a 65 °C drying oven for 12 hr to remove any residual moisture. Samples were then acidified to remove any carbonate by exposure to 12 mol L-1 HCl vapors for 12 hr in a sealed desiccator. Residual acid was removed following acidification by placing samples back into a 65 °C drying oven for 12 hr. Dried samples were weighed into tared tin capsules (Costech) to the nearest 0.001 mg (1.8 – 2.0 mg) using a microbalance. Crushed capsules were arranged into a 96-well plate and submitted for stable isotope analysis at the Smithsonian Institution OUSS/MCI Stable Isotope Mass Spectrometry Facility in Suitland, MD. Isotope ratios (δ13C and δ15N) of each fraction were measured on a Thermo Delta V Advantage mass spectrometer in continuous flow mode coupled to an Elementar vario ISOTOPE Cube Elemental Analyzer (EA) via a Thermo Conflo IV. All calculations of raw isotope values are performed with Isodat 3.0 software. Analytical precision at the Smithsonian Institution OUSS/MCI Stable Isotope Mass Spectrometry Facility is ± 0.2 ‰.

Sponge and microbial cell isotopic (15N and 13C) enrichment was initially expressed in delta (δ) notation in permil units (0‰; in δ13C and δ15N values) but because initial natural abundance values were available for all species, enrichment for both experiments was ultimately expressed as change (Δ) in δ13C and δ15N (Δδ13C and Δδ15N value when compared to initial samples). These initial and experimental samples were matched for sponge genotype (same individual split among initial and experimental samples for *A. cauliformis*, *A. crassa*, *A. fulva*, *I. birotulata*, *M. laevis, N. erecta*, *V. rigida*, but not for *I. bocatorensis* and *X. muta* because these sponges cannot be easily and successfully partitioned into multiple individuals. Thus, for *I. bocatorensis* and *X. muta*, all seven initial (non-experimental) replicates were pooled to obtain an average natural abundance value for sponge and microbial cell fractions of each species. In addition, due to sample loss, there were only two initial *A. compressa* tissue samples; these two samples were used to obtain an initial average δ13C and δ15N value of holobiont tissue. These average initial δ13C and δ15N values were then subtracted from the δ13C and δ15N values of microbial and sponge cell fractions (or holobiont for *A. compressa*) from individual experimental replicates to obtain Δδ13C and Δδ15N values for *A. compressa*, *I. bocatorensis*, and *X. muta*.

Because 13C and 15N enrichment in the light treatment was derived from both autotrophic and heterotrophic microbial metabolism, but 13C and 15N enrichment in the dark treatment was only influenced by heterotrophic microbes, we isolated the role of autotrophic microbes in holobiont C and N metabolism in the light by subtracting 13C and 15N enrichment (calculated as Δδ13C and Δδ15N) in the dark treatment from total 13C and 15N enrichment (Δδ13C and Δδ15N) in the light treatment for each individual sponge sample. These values

were ultimately expressed as % phototrophic and % heterotrophic metabolism according to the contribution of each pathway to total 13C and 15N enrichment in the light. Because *I. bocatorensis* and *X. muta* samples were not matched (the same genotype was not used in both light and dark experiments), 13C and 15N enrichment by phototrophic symbionts was measured by subtracting the average Δδ13C andΔδ15N values in the dark from average Δδ13C and Δδ15N values in the light.

Heterotrophic efficiency of particulate organic matter cell removal was calculated using clearance rate at an intermediate (60 min) and final (120 min) time point. Clearance rate (CR) represents the volume of water cleared of suspended particles per unit of time and is corrected by mass of the sponge according to the equation:

CR= (V/txm) ln(C*o*/C*t*)

where V is the volume of the experimental tanks in ml (4000 ml or 6000 ml), t is time in hr (one or two hr), m is the wet mass of the sponge, C0 is the starting concentration of bacteria in the tank, and Ct is the concentration of bacteria at each time point (T = 60 min and T = 120 min). Because the concentration of labeled heterotrophic bacteria in control tanks varied minimally over the 120 min incubations with no significant decrease in cell number, clearance rates in experimental tanks were not corrected based on controls

BCO-DMO Processing Description

* split latitude & longitude into own column
* converted date to iso format (yyyy-mm-dd)
* Adjusted parameter names to comply with database requirements

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Data Files

File	
954735_v1_autotrophy.csv	(Comma Separated Values (.csv), 54.54 KB) MD5:bb02e47d70aceea5e8085aa0368b6d1e
Primary data file for dataset ID 954735, version 1	

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Related Publications

Bocas del Toro - Physical Monitoring | Smithsonian Tropical Research Institute. (n.d.). https://striresearch.si.edu/physical-monitoring/bocas-del-toro/ https://biogeodb.stri.si.edu/physical_monitoring/research/bocas
Related Research

Freeman, C. J., & Thacker, R. W. (2011). Complex interactions between marine sponges and their symbiotic microbial communities. Limnology and Oceanography, 56(5), 1577–1586. Portico. https://doi.org/10.4319/lo.2011.56.5.1577
Methods

Freeman, C. J., Thacker, R. W., Baker, D. M., & Fogel, M. L. (2013). Quality or quantity: is nutrient transfer driven more by symbiont identity and productivity than by symbiont abundance? The ISME Journal, 7(6), 1116–1125. https://doi.org/10.1038/ismej.2013.7
Methods

KELLY, J. B., & THACKER, R. W. (2021). New shallow water species of Caribbean Ircinia Nardo, 1833 (Porifera: Irciniidae). Zootaxa, 5072(4), 301–323. https://doi.org/10.11646/zootaxa.5072.4.1
Methods

Protocols and standards : Earthmicrobiome. earthmicrobiome RSS. (n.d.). http://earthmicrobiome.org/protocols-and-standards/

Parameters

Parameter	Description	Units
Species	Sponge species (Acau: Aplysina cauliformis; Acra: Aiolochroia crassa; Aful: Aplysina fulva; Acom: Amphimedon compressa; Ibiro: Iotrochota birotulata; Ifel: Ircinia felix; Mlae: Mycale laevis; Nere: Niphates erecta; Vrig: Verongula rigida; Xmut: Xestospongia muta).	unitless
Full_scientific_name	Complete scientific name of each sponge species	unitless
Date	Date of sample collection	unitless
Lat	Latitude of collection site, south is negative	decimal degrees
Lon	Longitude of collection site, west is negative	decimal degrees
Replicate	Replicate number (1-10)	unitless
Fraction	Microbial cell fraction (Bac) or sponge cell fraction (Sponge) isolated from bulk sponge tissue; holobiont (combined bulk tissue including both microbial and sponge cells for Amphimedon compressa)	unitless
Initial_d15N	Nitrogen isotope ratio in Initial, wild-caught samples	permille (‰)
Initial_wt_percN	Percent nitrogen content in initial, wild-caught samples	percentage (%)
Initial_Atm_perc15N	Percent 15N in initial, wild-cauight samples	percentage (%)
Initial_d13C	Carbon isotope ratio in Initial, wild-caught samples	permille (‰)
Initial_wt_percC	Percent carbon content in initial, wild-caught samples	percentage (%)
Atm_perc13C	Percent 13C in initial, wild-caught samples	percentage (%)
Dark_d15N	Nitrogen isotope ratio in samples from dark experimental treatment	permille (‰)

Dark_wt_percN	Percent nitrogen content in samples from dark experimental treatment	percentage (%)
Dark_Atm_perc15N	Percent 15N in samples from dark experimental treatment	percentage (%)
Dark_d13C	Carbon isotope ratio in sample from dark experimental treatment	permille (‰)
Dark_wt_percC	Percent carbon content in samples from dark experimental treatment	percentage (%)
Dark_Atm_perc13C	Percent 13C in samples from dark experimental treatment	percentage (%)
Dark_Change_in_d15N	Change in nitgrogen isotope value in dark treatment relative to initial samples	permille (‰)
Dark_Change_in_d13C	Change in carbon isotope value in dark treatment relative to initial samples	permille (‰)
Light_d15N	Nitrogen isotope ratio in samples from light experimental treatment	permille (‰)
Light_wt_percN	Percent nitrogen content in samples from light experimental treatment	percentage (%)
Light_Atm_perc15N	Percent 15N in samples from light experimental treatment	percentage (%)
Light_d13C	Carbon isotope ratio in sample from light experimental treatment	permille (‰)
Light_wt_percC	Percent carbon content in samples from light experimental treatment	percentage (%)
Light_Atm_perc13C	Percent 13C in samples from light experimental treatment	percentage (%)
Light_Change_in_d15N	Change in nitgrogen isotope value in light treatment relative to initial samples	permille (‰)
Light_Change_in_d13C	Change in carbon isotope value in light treatment relative to initial samples	permille (‰)

Light_photo_d15N	Enrichment in 15N in the light treatment attributed to photosymbionts (total enrichment in the light-enrichment in the dark due to heterotrophic bacteria)	permille (‰)
light_photo_d13C	Enrichment in 13C in the light treatment attributed to photosymbionts (total enrichment in the light-enrichment in the dark due to heterotrophic bacteria)	permille (‰)
Hetero_d15N	Enrichment in 15N in the dark attributed to heterotrophic bacterial metabolism	permille (‰)
hetero_d13C	Enrichment in 13C in the dark attributed to heterotrophic bacterial metabolism	permille (‰)
POM_heterotrophy_enriched_d13C	Enrichment in 13C from consuming bacteria labeled in 13C	permille (‰)
POM_heterotrophy_enriched_d15N	Enrichment in 15N from consuming bacteria labeled in 15N	permille (‰)
Heterotrophy_POM_change_in_d13c	Change in carbon isotope value following consumption of 13C labeled bacterial cells relative to initial samples	permille (‰)
Heterotrophy_POM_change_in_d15N	Change in nitgrogen isotope value following consumption of 15N labeled bacterial cells relative to initial samples	permille (‰)
POM_T60_CR	Clearance rate at 60 minutes in heterotrophy experiment based on cell counts via flow cytometry	mililiters/hour
POM_T120_CR	Clearance rate at 120 minutes in heterotrophy experiment based on cell counts via flow cytometry	mililiters/hour

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Instruments

Dataset-specific Instrument Name	BD Biosciences Accuri C6 Plus Model
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	Cell counting was performed using a BD Biosciences Accuri C6 Plus Model flow cytometer at the Smithsonian Marine Station in Fort Pierce, Florida.
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	Thermo Delta V Advantage mass spectrometer in continuous flow mode coupled to an Elementar vario ISOTOPE Cube Elemental Analyzer (EA) via a Thermo Conflo IV
Generic Instrument Name	Mass Spectrometer
Dataset-specific Description	Isotope ratios (δ13C and δ15N) of each fraction were measured on a Thermo Delta V Advantage mass spectrometer in continuous flow mode coupled to an Elementar vario ISOTOPE Cube Elemental Analyzer (EA) via a Thermo Conflo IV.
Generic Instrument Description	General term for instruments used to measure the mass-to-charge ratio of ions; generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components.

Dataset-specific Instrument Name	LI-COR LI-193 underwater spherical quantum sensor
Generic Instrument Name	Photosynthetically Available Radiation Sensor
Dataset-specific Description	Irradiance was measured at multiple depths from collection sites around STRI using a LI-COR LI-193 underwater spherical quantum sensor.
Generic Instrument Description	A PAR sensor measures photosynthetically available (or active) radiation. The sensor measures photon flux density (photons per second per square meter) within the visible wavelength range (typically 400 to 700 nanometers). PAR gives an indication of the total energy available to plants for photosynthesis. This instrument name is used when specific type, make and model are not known.

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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-1929293

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