Bulk isotope data from sponges collected in Summerland Key in Florida between July 27 - August 19, 2021

Website: https://www.bco-dmo.org/dataset/954470
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Project
» Collaborative Research: Investigations into microbially mediated ecological diversification in sponges (Ecological Diversification in Sponges)

Contributors	Affiliation	Role
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Abstract
Sponges represent one of the oldest extant animal phyla, and their associations with microbial symbionts have likely played a critical role in their success on oligotrophic coral reefs. For example, variation in the abundance, diversity, and community composition of microbiomes across host species may drive niche partitioning due to differential assimilation and recycling of nutrients across sponge species. By coupling stable isotope analysis of bulk sponge tissue with cellular-level resolution using Nanoscale secondary ion mass spectrometry (NanoSIMS), we tested for evidence of partitioning of three major resource pools (dissolved organic matter: DOM, particulate organic matter: POM, and inorganic nutrients: NaHCO3 and NaNO3) among four emergent Caribbean sponge species. We also evaluated the respective roles of host and microbial symbiont cells in the uptake and recycling of these resources over 72 hr. Results from bulk (holobiont tissue including both sponge and microbial cells) stable isotope analysis show niche partitioning, with strong interspecific variation in the relative exploitation of each resource pool. NanoSIMS analysis revealed differing roles of host and symbiont cells in the uptake and recycling of diverse sources of carbon and nitrogen within two of these species. For instance, DOM uptake was mediated by microbial symbionts in one species and by host cells in another species. Together , these results demonstrate that associations with microbial symbionts have facilitated niche partitioning by allowing host sponges to acquire and recycle diverse nutrient pools via unique mechanisms. Field work for this research was carried out in the Florida Keys during June/August of 2021.

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Coverage

Location: Florida Keys patch reefs off Summerland Key and Mote Marine Laboratory

Spatial Extent: **Lat**:24.56381897 **Lon**:-81.40839451
Temporal Extent: 2021-08-01

Methods & Sampling

Sample collection**:** Fieldwork was conducted at Mote Marine Laboratory in Summerland Key, FL, USA, between July 27 and August 19, 2021. Replicate individuals of the four sponge species *Aplysina cauliformis*, *Iotrochota birotulata*, *Niphates digitalis*, and *Xestospongia muta* were collected from a shallow (7 m depth) patch reef (24°33' N, 81°24' W) offshore of Summerland Key (24°39' N, 81°26' W) via dive knives (with the spongocoel intact for the barrel/tube sponges by cutting the sponge from the substrate) using SCUBA, and were placed into plastic bags filled with seawater before being brought to the surface. After collection, all sponges were kept submerged in ambient seawater in large, sealed plastic bags in an insulated cooler during transit back to the lab. Upon return to Mote Marine Laboratory, sponges were attached with cable ties to plastic window screens, and held in large, flow-through raceways for 24 h for acclimatization. At no point during this process were sponges exposed to air.

Only healthy sponges (sponges confirmed to be actively pumping via use of fluorescein dye, and without any signs of necrosis) were processed for and used in experiments. For each experiment (and each treatment in the autotrophy experiment), four large individuals of each of the rope sponges *I. birotulata* and *A. cauliformis* collected from the reef were partitioned into seven subsamples for sampling at discrete time points. Both *X. muta* and *N. digitalis* were only sampled at four timepoints during each experiment because they have barrel/tube morphologies that cannot be successfully partitioned and subsampled, requiring the sacrifice of an entire individual for each timepoint. Therefore, sixteen small individuals of each of these two species were collected from the reef for each experiment. Experimental replicates were defined as follows: a replicate of *X. muta* consisted of one individual attached to a plastic window screen, which was placed in an 8 L, clear food-grade container; a replicate of *N. digitalis* consisted of four small individuals attached to a plastic window screen, which was placed into a 17.9 L, clear food-grade container; and replicates of *A. cauliformis* and *I. birotulata* consisted of all seven subsamples from the same individual attached to a plastic window screen, which was placed into a 17.9 L, clear food-grade container. In total, there were four replicates of each species collected at each time point in each experiment, with the exception the DOM experiment, during which multiple individuals, including all individuals of *A. cauliformis*, developed necroses in experimental tanks after 24h. The effected individuals were eliminated, limiting replication to only two timepoints (24h and 72h) during the chase for all species. Due to necroses of all individuals of *A. cauliformis* after 24h, the DOM experiment was repeated for this species only, using three healthy replicates.

Pulse-chase experiments**:** Three separate "pulse-chase" experiments were carried out to track the uptake and recycling of labeled compounds from three different resource pools. The first resource pool, DOM, consisted of ^{13}C-labeled glucose (35 µM) and galactose (35 µM), and ^{13}C and ^{15}N-labeled cell-free amino acids (range from 39-790 nM) and urea (2.2 µM). The second resource pool, POM, consisted of ^{15}N- and ^{13}C-labeled *Synechococcus* spp. bacteria incubated for 36 h in seawater containing 1.18 µM $NaH^{13}CO_3$, 3.67 µM $^{15}NH_4Cl$, and 0.117 M $Na^{15}NO_3$, and *Oceanicola batsensis* incubated in 0.55 µM galactose, 1.18 µM $NaH^{13}CO_3$, 3.67 µM $^{15}NH_4Cl$, and 0.117 µM $Na^{15}NO_3$. Finally, the third resource pool of inorganic compounds consisted of $Na^{15}NO_3$ (0.117 µM) and $NaH^{13}CO_3$ (1.18 µM). Labeled bacterial cells were added to filtered seawater for the POM experiment at a final combined concentration of 4.65 x10^5 cells ml^{-1} for photosynthetic cells and 1.01 x 10^6 for heterotrophic cells (measured at the Center for Aquatic Cytometry at Bigelow Laboratory for Ocean Sciences). Concentrations of labeled resources were chosen to approximate the concentrations of corresponding inorganic and organic sources of carbon and nitrogen found in Caribbean seawater as closely as possible, as well as to match concentrations used in previous studies. In all incubations, experimental water was 0.7 µm (GF/F) filtered prior to the addition of tracers or labeled bacteria. Sponges were "pulsed" for 3 h in water containing these isotopically enriched resources, and then sponges were held in unenriched, flowing seawater for a 72-h "chase" period. Experimental samples were collected during the pulse at t=0.5 h, and during the chase at final times of t=12 h, 48 h, and 75 h (minus the 48h sampling in the DOM experiment). Prior to the start of the pulse, one of the seven subsamples of *A. cauliformis* and *I. birotulata*, and four individuals each of *X. muta* and *N. digitalis* were sampled and processed to serve as t = 0 h (natural abundance) samples.

To start the pulse, each experimental replicate was added to a food grade container containing filtered seawater and labeled compounds or food bacteria, as well as a recirculating pump used to maintain flow. Replicates of *X. muta*were held in 8 L, clear food grade containers holding 6 L of filtered seawater, while replicates of *I. birotulata*, *A. cauliformis*, and *N. digitalis* were held in 17.9 L containers holding 15 L of filtered

seawater. These containers were all held in a large 4.6 m-diameter outdoor tank with a shallow seawater bath to control temperature in experimental tanks holding sponges. At the end of the pulse, the water level in the outdoor tank was raised to flood experimental tanks with unlabeled seawater (supplied from the canal adjacent to Mote Marine Lab, drawn from 10 m, and filtered through sand to 40 µm) throughout the chase. Temperature measurements were taken at regular intervals (30 min), and an additional 25°C supply of well water, with salinity equal to that of seawater, was supplemented to maintain a tank temperature between 29-32°C. During the day for all experiments, irradiance measurements were taken every 30 min to monitor for deviation outside of the irradiance that sponges experience at depth, which was 800 to 1000 µmol photons m^{-2} s^{-1} measured at depth during cloudless daytime conditions at the collection site. One layer of standard shade cloth was sufficient to keep irradiance within the normal limits of what the sponges experience at depth, and adjustments during the experiment were only made when irradiance went below the 800-1000 µmol photons m^{-2} s^{-1} range under cloudy conditions. When irradiance decreased, we removed the layer of shade cloth to increase exposure of the sponges to ambient sunlight.

To assess the role of both autotrophic and heterotrophic symbionts in the assimilation of inorganic compounds, the autotrophy experiment included both light and dark treatments. For this reason, each large individual of *I. birotulata* and *A. cauliformis* was partitioned into 14 subsamples, and 32 small individuals each of *N. digitalis* and *X. muta* were collected for this experiment. Half of the subsamples or individuals of each species were randomly assigned to a dark treatment (covered in opaque plastic tarp with irradiance values from HOBO loggers that detected 0 Lux throughout the pulse), and the other half to a light treatment (exposure to ambient irradiance as in other experiments). Samples in the dark treatment were covered during the pulse but were exposed to ambient irradiance during the chase so that we could monitor nutrient retention and recycling under ambient (light-dark cycle) conditions.

Sample fixation***:*** After sampling during pulse-chase experiments, sponges were rinsed with 0.7 µm-filtered, unenriched seawater and lightly blotted with a paper towel before weighing to the nearest 0.001 g to obtain a wet weight. Afterwards, ~2 mm cross sections were collected in duplicate using a razor blade and placed into 2 mL cryovials to be fixed for SEM and NanoSIMS analysis; the remaining bulk tissue was frozen at -20°C for isotope analysis. The 2 mm cross-sections of sponge tissue were fixed in 2.5% (w/v) glutaraldehyde + 1% (w/v) paraformaldehyde in 1.5X PHEM buffer (1.5X PHEM (90 mM PIPES, 37.5 mM HEPES, 15 mM EGTA, 3 mM MgSO4.7H_2O), and 9% (w/v) sucrose at pH 7.4) and stored at 4°C for 12h before being rinsed 3x with PHEM buffer containing no fixative. Finally, 0.1 mL of fixative solution (10% of the concentration in the original solution) was added back to each sample to prevent any fouling during long-term storage at 4°C. After being taken out of storage, cross-sections of sponge tissue were rinsed once more in fixative-free 1.5X PHEM buffer, digested in 5% hydrofluoric acid to break down siliceous spicules within the sponge tissue (note this was carried out for all samples to be consistent, even though *A. cauliformis* lacks spicules), and rinsed twice and stored in 1.5X PHEM buffer.

Data Processing Description

Bulk stable isotope analysis: To prepare for bulk isotope analysis, a small subsample of frozen tissue from each replicate of each species was placed into a scintillation vial and lyophilized overnight to remove seawater. Dried samples were homogenized with a mortar and pestle, and homogenized tissue was acidified with 12N HCl fumes in an acid chamber to remove carbonate before being weighed into tin capsules to the nearest 0.0001 mg using a Mettler Toledo XPR microbalance at Hollings Marine Lab in Charleston, SC, USA. Samples were analyzed at the Smithsonian MCI Stable Isotope Mass Spectrometry Laboratory using a Thermo Delta V Advantage mass spectrometer in continuous flow mode coupled to an Elementar Vario ISOTOPE Cube Elemental Analyzer via a Thermo Conflo IV. Enrichment is expressed in delta notation ($\delta^{13}C$ and $\delta^{15}N$) in units of permille.

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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Related Publications

Hudspith, M., Rix, L., Achlatis, M., Bougoure, J., Guagliardo, P., Clode, P. L., Webster, N. S., Muyzer, G., Pernice, M., & de Goeij, J. M. (2021). Subcellular view of host–microbiome nutrient exchange in sponges: insights into the ecological success of an early metazoan–microbe symbiosis. Microbiome, 9(1). https://doi.org/10.1186/s40168-020-00984-w
Methods

Hudspith, M., de Goeij, J. M., Streekstra, M., Kornder, N. A., Bougoure, J., Guagliardo, P., Campana, S., van der Wel, N. N., Muyzer, G., & Rix, L. (2022). Harnessing solar power: photoautotrophy supplements the diet of a low-light dwelling sponge. The ISME Journal, 16(9), 2076–2086. https://doi.org/10.1038/s41396-022-01254-3
Methods

Rix, L., Ribes, M., Coma, R., Jahn, M. T., de Goeij, J. M., van Oevelen, D., Escrig, S., Meibom, A., & Hentschel, U. (2020). Heterotrophy in the earliest gut: a single-cell view of heterotrophic carbon and nitrogen assimilation in sponge-microbe symbioses. The ISME Journal, 14(10), 2554–2567. https://doi.org/10.1038/s41396-020-0706-3
Methods

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Parameters

Parameter	Description	Units
Experiment	Autotrophy dark, Autotrophy light, dissolved organic matter, or particulate organic matter refer to experiments measuring uptake of inorganic nutrients in dark, inorganic nutrients in light, dissolved organic matter, and particulate organic matter, respectively.	unitless
time	Timepoint at which samples collected (in hours) during experiment. T=0 samples were collected prior to experimentation.	unitless
replicate	Replicate number; typically n=4 for each species at each timepoint in each experiment.	unitless
species_abbreviation	Abreviated codes for species names of each sample. Acau = Aplysina cauliformis, Ibir = Iotrochota birotulata, Ndig = Niphates digitalis, Xmut = Xestospongia muta.	unitless
Species_name	Scientific name	unitless
Collection_date	Collection date of sponge samples	unitless
lat	Latitude of collection site for sponge individuals, west is negative	decimal degrees
lon	Longitude of collection site for sponge individuals, south is negative	decimal degrees
Linear_corr_d15N	Delta 15N value for each sample	permille (‰)
wt_perc_N	Weight percent of N in sample	percentag (%)
Atm_perc_15N	Atom percent 15N (of total N)	percentag (%)
Linear_corr_d13C	Delta 13C value for each sample	permille (‰)
wt_perc_C	Weight percent of C in sample	percentag (%)
Atm_perc_13C	Atom percent 13C (of total C)	percentag (%)

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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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