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

Website: https://www.bco-dmo.org/dataset/954439
Data Type: experimental
Version: 1
Version Date: 2025-04-23

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

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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. With Nanoscale secondary ion mass spectrometry (NanoSIMS), we evaluated the respective roles of host and microbial symbiont cells in the uptake and recycling of three resource pools (dissolved organic matter: DOM, particulate organic matter: POM, and inorganic nutrients: NaHCO3 and NaNO3) over 72 hr. NanoSIMS analysis revealed enrichment of individual host and symbiont cells over time and highlighted the 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, dissolved organic matter 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 and lab 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: 2021-07-27 - 2021-08-19

Methods & Sampling

Sample collection**:** Samples were collected on August 1, 2021. Lab work and experiments were 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 13C-labeled glucose (35 µM) and galactose (35 µM), and 13C and 15N-labeled cell-free amino acids (range from 39-790 nM) and urea (2.2 µM). The second resource pool, POM, consisted of 15N- and 13C-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 x105 cells ml-1 for photosynthetic cells and 1.01 x 106 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 MgSO4.7H2O), 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

Scanning electron microscopy and nanoSIMS***:*** Due to the high cost of SEM and especially NanoSIMS analysis, only one HMA species (*A. cauliformis*) and one LMA species (*I. birotulata*) from each experiment was selected for high-resolution analyses, and only four time-points (including t=0h) were included. One replicate each of *A. cauliformis* and *I. birotulata* per time point per experiment was analyzed via nanoSIMS; these species were chosen based on better histological preservation than *X. muta* and *N. digitalis* as evaluated during electron microscopy, and replicates were selected based on median enrichment of bulk tissue to approximate the mean as closely as possible. Samples from t = 0 h, 0.5 h, 12 h, and 75 h were analyzed from each experiment, with at least three different areas containing diverse cell types analyzed per sample. Individual cells identified for analysis are referred to as regions of interest (ROI), and a total of 9,179 ROI were analyzed across 26 holobiont samples, including natural abundance samples.

To prepare samples for SEM and NanoSIMS, similar methods to Hudspith et al.10 were followed. Cross-sections stored in 1.5X PHEM buffer were post-fixed for 1.5 h with 1 % (w/v) osmium tetroxide before being dehydrated with a graded ethanol series and embedded in Embed 812. Semithin (500 nm) sections were cut using an RMC Products Powertome XL ultramicrotome at the Electron Microscopy Center at Clemson University, Anderson, SC, USA. Semithin sections were then mounted onto silicon wafers and stained with Uranyless (a non-radioactive substitute for uranyl acetate) and lead citrate for SEM analysis. Scanning electron microscopy (SEM) was carried out to find areas within sponge tissue containing diverse cell types, provide sample maps, and serve as a correlative method to confidently identify individual ROI analyzed during NanoSIMS. SEM was performed at Clemson University's Warren Lasch Conservation Center in North Charleston, SC, USA, using a Hitachi S-3700N SEM set to backscattered electron (BSE) imaging at an accelerating voltage of 5 kV. SEM images were taken at a maximum magnification of 3,000X, with several images of the same area taken at lower magnification so that finding areas of interest during NanoSIMS was possible. All SEM images included at least one choanocyte chamber with multiple mesohyl cells and symbiont cells within 30 µm of the choanocyte chamber, allowing for efficient analysis of diverse cell types during

NanoSIMS.

After SEM was complete, samples were coated with 5 nm gold-palladium for NanoSIMS analysis at the Nano Shared Facilities Laboratory at Stanford University, Stanford, CA, USA. NanoSIMS analysis was performed using a CAMECA 50L nanoscale secondary ion mass spectrometer to visualize cellular enrichment of individual host and symbiont cells. During NanoSIMS analysis, samples were bombarded with a 16kV Cs+ primary ion beam to detect the secondary ions of 12C12C-, 12C13C-, 12C14N-, 12C15N-, using a mass resolving power of at least 7,000. Raster scans of 30 µm and 256 x 256 pixels were taken using 1 ms beam dwell time per pixel and repeated 50 times.

NanoSIMS images were processed using openMIMS software for Fiji. Individual ROI were manually drawn by outlining individual cells, and cells were identified as choanocytes, mesohyl cells, symbiont cells, or cyanobacteria. Mesohyl cells here include multiple cell types, such as amoebocytes and sclerocytes, though spherulous cells were excluded as minimal enrichment was observed within any of these cells, which is consistent with previous studies. SEM images were used to confirm cell identifications. Daily scans of an unenriched control sample of sponge tissue were taken. Enrichment values of each daily control scan were compared to the bulk enrichment measured from the same sample to calculate a correction factor:

to compensate for any machine drift during NanoSIMS analysis, where RsampleIRMS represents the 12C15N/12C14N or 12C13C/12C12C ratio measured during bulk tissue analysis, and RsamplenanoSIMS represents the 12C15N/12C14N or 12C13C/12C12C ratio measured during each daily scan of the same unenriched control sample. There were four days (out of twenty-one days during which NanoSIMS analyses were conducted) that control scans were not taken, so the mean correction factor (mean ± SD CF for 12C15N-/12C14N- was 1.00 ± 0.01, and for 12C13C-/12C12C- was 1.05 ± 0.02) was applied to these samples.

To determine whether a cell was considered enriched, the isotope value of each cell analyzed within experimental samples was compared to the mean isotope value of all cells of the same type within the same sponge species from unenriched control (t=0h) samples, using a conservative threshold of three standard deviations above mean natural abundance values as the differentiator between enriched vs unenriched cells. Due to the high cost and low throughput of NanoSIMS analysis, replication at the holobiont level is often quite limited. Thus, while multiple areas were imaged within each sample, only one replicate per species per timepoint per experiment was analyzed, so no statistical analyses are presented here. Instead, descriptive statistics, including mean enrichment of the different ROI cell groups associated with each species at each time point, as well as the percentage of cells within each group that were above the enrichment threshold are used to describe trends in the uptake and retention of 13C and 15N derived from different resources in each symbiosis.

BCO-DMO Curation Notes

* 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	
954439_v1_nanosims.csv	(Comma Separated Values (.csv), 1.88 MB) MD5:ad68ca284b25c519b6c7d3805a82633a
Primary data file for dataset ID 954439, version 1	

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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
Roi_name	number of roi (region of interest) within a given sample - each roi corresponds to one cell	unitless
Roi_group	gives cell type (various host cell types (choanocytes, pinacocytes, c chamber [choanocyte chamber] vs symbiont cells) of each roi	unitless
area	gives number of pixels within each roi from a 256x256 pixel image	pixels^2
mean13C	mean 13C12C/12C2 ratio (x10000) in each roi	ratio (x10000)
stddev13C	standard deviation of 13C12C/12C2 ratio (x10000) in each roi	ratio (x10000)
R_13C12C_12C2	mean 13C12C/12C2 ratio in each roi	ratio
corr_R_13C_13C	same as "R 13C12C/12C2" but corrected for machine drift and divided by 2 to give 13C:12C ratio in each roi	ratio
d_13C_12C	delta 13C value of each roi calculated using an unlabeled bulk tissue sample as a control	delta 13C in permille
species	sponge species from which tissue sampe was taken; Aplysina cauliformis or Iotrochota birotulata	unitless
Collection_date	Collection date of sponge samples	unitless
time	timepoint that sample was collected during experiment	unitless

SEM_region	numbered region within tissue crossection of the correlated Scanning Electron Microscopy image for each NanoSIMS image.	unitless
mean15N	mean 15N12C/14N12C ratio (x10000) in each roi	ratio (x10000)
stddev15N	standard deviation of 15N12C/14N12C ratio (x10000) in each roi	ratio (x10000)
R_15N_14N	mean 15N12C/14N12C ratio in each roi	ratio
corr_R_15N_14N	same as "R 15N/14N" but corrected for machine drift	ratio
d_15N_14N	delta 15N value of each roi calculated using an unlabeled bulk tissue sample as a control	delta 15N in permille
experiment	AD, AL, DOM, and POM signify if samples were collected during experiments focused on uptake of: inorganic nutrients in darkness, inorganic nutrients in the light, dissolved organic matter, and particulate organic matter, respectively.	unitless
lat	latitude of collection site for sponge individuals, south is negative	decimal degrees
lon	latitude of collection site for sponge individuals, south is negative	decimal degrees

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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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