Flux of organic carbon for sponges at Conch Reef, Key Largo, FL, and Carrie Bow Cay, Belize as sampled in 2016.

Website: https://www.bco-dmo.org/dataset/840792
Data Type: Other Field Results
Version: 1
Version Date: 2021-02-15

Project
» Testing the sponge-loop hypothesis for Caribbean coral reefs (Sponge_Loop)

Contributors	Affiliation	Role
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Abstract
The sponge loop hypothesis proposes that sponges on coral reefs absorb large quantities of dissolved organic carbon (molecules such as carbohydrates) that are released by seaweeds and corals and return it to the reef as particles in the form of living and dead cells, or other cellular debris. In this dataset, carbon flux was quantified for sponges to test the sponge-loop hypothesis in the field. Sponges were sampled from Conch Reef off of Key Largo, Florida (24° 56.9' N, 80° 27.2' W), and reefs off Carrie Bow Cay in Belize (16° 56.9' N, 80° 27.2' W), in June and July 2016. At each location, a total of 2-7 individuals of sponge species common throughout the Caribbean were haphazardly selected for study between 15 and 20 meter depths. Sponge species were chosen that exhibit morphologies that distinctly separate incurrent from excurrent flow; these include barrel, vase and tube-forming species. Of the species investigated, Agelas tubulata (cf. conifera), Verongula gigantea, V. reiswigi, and Xestospongia muta are considered HMA species (High Microbial Abundance) and Callyspongia plicifera, C. vaginalis, Mycale laxissima, and Niphates digitalis are considered LMA species (Low Microbial Abundance). An additional species, the HMA sponge Ircinia strobilina, was selected for study on Conch Reef only. With the exception of A. tubulata, only individuals with a single osculum were studied for each species. Additionally, only sponges with no obvious signs of disease or tissue damage and not fouled with algae or colonized by epibionts (e.g. zoanthids) were included.

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Coverage

Spatial Extent: **N**:24.94833 **E**:-80.4533 **S**:16.8 **W**:-88.0767
Temporal Extent: 2016-06-01 - 2016-07-19

Methods & Sampling

At each location, a total of 2-7 individuals of sponge species common throughout the Caribbean were

haphazardly selected for study between 15 and 20 meter depths. Sponge species were chosen that exhibit morphologies that distinctly separate incurrent from excurrent flow; these include barrel, vase and tube-forming species. Of the species investigated, Agelas tubulata (cf. conifera), Verongula gigantea, V. reiswigi, and Xestospongia muta are considered HMA species (high icrobial abundance) and Callyspongia plicifera, C. vaginalis, Mycale laxissima, and Niphates digitalis are considered LMA species (low microbial abundance). An additional species, the HMA sponge Ircinia strobilina, was selected for study on Conch Reef only. With the exception of A. tubulata, only individuals with a single osculum were studied for each species. Additionally, only sponges with no obvious signs of disease or tissue damage and not fouled with algae or colonized by epibionts (e.g. zoanthids) were included.

Paired 1.5 liter incurrent (ambient) and excurrent seawater samples were collected from each sponge with 100 milliliter syringes, 5 millimeter diameter tip opening, as previously described (McMurray et al. 2016) for measurements of live particulate organic carbon (LPOC), total particulate organic carbon (POC), and dissolved organic carbon (DOC). Incurrent seawater samples were collected adjacent to the ostia that lines the external sponge surface and excurrent samples were slowly collected from approximately 5 centimeters below the osculum within the atrium (inner empty space) of each sponge and at a rate lower than the excurrent water velocity to avoid contamination from ambient seawater. Samples thus represent an integration of approximately 10 to 20 minutes of sponge feeding. Following seawater collection, the velocity of excurrent seawater at the centerline of the osculum of each sponge was measured using a Sontek Micro acoustic Doppler velocimeter mounted on a tripod for 3 minutes at 2 hertz (Hz). The dimensions of each sponge were subsequently measured using a flexible measuring tape. Sponge volume, excluding the spongocoel, was calculated by approximating the morphology of each individual as a geometric solid.

To quantify the flux of LPOC in the form of picoplankton, 5 milliliters of both incurrent and excurrent seawater samples were preserved in electron microscopy grade glutaraldehyde at a final concentration of 0.1% in cryovials for 10 minutes in the dark and subsequently frozen in liquid nitrogen and stored at -80°C until flow cytometry analysis. Phytoplankton (Prochlorococcus (Pro), Synechococcus (Syn), and photosynthetic pico- and nanoeukaryotes (Euk)) and bacterioplankton (high nucleic acid bacteria (HNA) and low nucleic acid bacteria (LNA)) in seawater samples were enumerated using a BD FACSCelesta Flow Cytometer and populations characterized as previously described (McMurray et al. 2016). Briefly, cells were excited with a 488 nanometer laser and forward scatter, side scatter, green fluorescence (530 ± 30 nm), orange fluorescence (575 ± 26 nm), and red fluorescence (695 ± 40 nm) emissions were measured. Phytoplankton were analyzed for 10 minutes at high flow rate and heterotrophic bacteria were stained with SYBR Green-I as previously described (Marie et al. 1997) and analyzed at low flow rate for 5 minutes. Picoplankton were classified based on their characteristic flow cytometric signatures relative to standard fluorescent microspheres following standard population gating schemes. Carbon (C) content of each type of picoplankton was estimated using standard cell conversions used in previous studies of sponge feeding: 53 femtogram (fg) of carbon per cell for Pro, 470 femtogram of carbon per cell for Syn, 1496 femtogram of carbon per cell for Euk, and 20 femtogram of carbon per cell for HNA and LNA bacteria.

To quantify sponge-mediated flux of particulate organic carbon (POC) and dissolved organic carbon (DOC), the remaining seawater from each sample was filtered through a 100 micron (µm) mesh that excluded particles greater than the size of incurrent ostia and subsequently through a precombusted GF/F glass fiber filter under low pressure. Filters were individually wrapped in aluminum foil and frozen until analysis of POC. Twenty milliliters (20 mL) of the filtrate from each sample was transferred to an EPA precleaned glass vial, acidified with 100 microliter (µL) of 50% phosphoric acid, and stored at 4°C until analysis of DOC. Particulate organic carbon was measured using a CE Elantech NC2100 combustion elemental analyzer after filters were dried at 50°C and subsequently exposed to hydrochloric acid fumes for 24 hours. DOC was measured using high temperature catalytic oxidation with a Shimadzu TOC 5050 analyzer. Calibration was achieved with standards diluted from a stock solution of potassium hydrogen phthalate and both standards and deep seawater consensus reference material (Batch 9, lot #09-09, Hansell Laboratory, University of Miami, RSMAS) were interspersed with samples for quality assurance and control. Each seawater sample was run in duplicate and each analysis tube was injected three to five times for a coefficient of variance < 1.5%. The approximate analytical precision of the instrument was 2 micromoles of carbon per liter of seawater (2 umols C/ L seawater). All plastic used for sample collection was soaked in a 0.5 molar HCl bath for at least 24 hours and then thoroughly rinsed in ultrapure water before use and all glassware and aluminum foil used to process samples were combusted at 450° for > 4 hours prior to use. We note that some samples were discarded due to potential contamination while processing in the field; therefore, a small number of individuals are lacking flux estimates for one or two of the three carbon pools investigated.

Detrital carbon in incurrent and excurrent seawater samples was estimated as the portion of total POC not accounted for by LPOC (i.e. Detritus = POC – LPOC). Sponge specific filtration rates, or carbon flux (carbon per second per liter of sponge), of DOC, LPOC, and detritus were calculated as: where Cin and Cex are the

incurrent and excurrent concentrations of each carbon pool (carbon per milliliter), Vsponge is sponge tissue volume (L), and Q is the volume flow or pumping rate for each sponge (milliliter per second). Positive and negative flux estimates therefore represent consumption and production of a particular carbon pool, respectively. For Q, we assumed that the mean excurrent velocity for each sponge was equivalent to the velocity of seawater measured at the osculum centerline with an Acoustic Doppler Velocimeter (i.e. plug flow), and volume flow was calculated as the product of the centerline excurrent velocity and the osculum area; for X. muta, the mean excurrent seawater velocity for each sponge was corrected for the uneven velocity distribution across the osculum due to the morphology of the spongocoel (Eq. 3, McMurray et al. 2014).

Data Processing Description

For all analyses, assumptions of normality and homogeneity of variances were checked with box and residual plots and data were transformed as needed or nonparametric tests were used. Log10-transformed incurrent carbon concentrations were compared between locations (Conch Reef and Carrie Bow Cay) and carbon pools (DOC, LPOC, detritus) with a 2-way ANOVA and significant interactions were evaluated by tests of simple main effects. Specific filtration rates were compared between locations, sponge species, and carbon pools using the Scheirer-Ray-Hare extension of the Kruskal-Wallis test; V. gigantea and V. reiswigi were excluded from this analysis due to insufficient replication for these species at Carrie Bow Cay and Conch Reef, respectively. To test the hypothesis that sponges are net producers (or consumers) of detritus, paired t-tests were used to compare the concentrations of detritus in incurrent and excurrent seawater for each species. Statistical analyses were conducted with the following statistical software:
- SAS Software (version 9.1.3 for Windows; SAS Institute) and
- SPSS Statistics Software (version 22 for Windows; IBM).

BCO-DMO Processing description:

- Converted Date to ISO date format.
- Missing data identifiers ‘NA’ and '.' replaced with 'nd' (BCO-DMO's default missing data identifier).
- Adjusted field/parameter names to comply with database requirements
- Added a conventional header with dataset name, PI names, version date

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Related Publications

IBM Corp. (2013). IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.
Software

Marie, D., Partensky, F., Jacquet, S., and Vaulot, D. (1997) Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. Applied and Environmental Microbiology 63: 186-193. https://aem.asm.org/content/63/1/186.short
Methods

McMurray, S. E., Johnson, Z. I., Hunt, D. E., Pawlik, J. R., & Finelli, C. M. (2016). Selective feeding by the giant barrel sponge enhances foraging efficiency. Limnology and Oceanography, 61(4), 1271–1286. doi:10.1002/lno.10287
Methods

McMurray, S., Pawlik, J., & Finelli, C. (2014). Trait-mediated ecosystem impacts: how morphology and size affect pumping rates of the Caribbean giant barrel sponge. Aquatic Biology, 23(1), 1–13. doi:10.3354/ab00612
Methods

McMurray, S., Stubler, A., Erwin, P., Finelli, C., & Pawlik, J. (2018). A test of the sponge-loop hypothesis for emergent Caribbean reef sponges. Marine Ecology Progress Series, 588, 1–14. https://doi.org/10.3354/meps12466
Results

SAS Institute Inc. (2004) SAS 9.1.3 version for Windows (release August 2004). Cary, NC: SAS Institute Inc.

Software

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Parameters

Parameter	Description	Units
ISO_Date	Date of water sample collection	%Y-%m-%d
Latitude	Latitude	decimal degress
Longitude	Longitude (West is negative)	decimal degress
spongeid	Unique identifier for each sponge sampled	unitless
species	Sponge species	unitless
site	Location of sponge	unitless
depth	Depth of sponge	feet
volflow	Volume flow (i.e. pumping rate) for each sponge	mililiters per second (ml/s)
temp	Water temperature at time of sampling	degrees Celsius
hgt	Height of sponge	centimeters (cm)
od	Mean diameter of sponge osculum	centimeters (cm)
bd	Diameter of sponge base	centimeters (cm)
ioh	Mean depth of sponge osculum	centimeters (cm)
iod	Mean depth of inner base of sponge osculum	centimeters (cm)
uMCproin	Carbon in incurrent water samples in the form of Prochlorococcus cells	micromoles of carbon per liter of seawater (umol C/L)
uMCproex	Carbon in excurrent water samples in the form of Prochlorococcus cells	micromoles of carbon per liter of seawater (umol C/L)

uMCsynin	Carbon in incurrent water samples in the form of Synechococcus cells	micromoles of carbon per liter of seawater (umol C/L)
uMCsynex	Carbon in excurrent water samples in the form of Synechococcus cells	micromoles of carbon per liter of seawater (umol C/L)
uMCpkin	Carbon in incurrent water samples in the form of pico- and nanoaukaryote cells	micromoles of carbon per liter of seawater (umol C/L)
uMCpkex	Carbon in excurrent water samples in the form of pico- and nanoaukaryote cells	micromoles of carbon per liter of seawater (umol C/L)
uMChnain	Carbon in incurrent water samples in the form of high nucleic acid (HNA) bacteria cells	micromoles of carbon per liter of seawater (umol C/L)
uMChnaex	Carbon in excurrent water samples in the form of high nucleic acid (HNA) bacteria cells	micromoles of carbon per liter of seawater (umol C/L)
uMClnain	Carbon in incurrent water samples in the form of low nucleic acid (LNA) bacteria cells	micromoles of carbon per liter of seawater (umol C/L)
uMClnaex	Carbon in excurrent water samples in the form of low nucleic acid (LNA) bacteria cells	micromoles of carbon per liter of seawater (umol C/L)
uMCvirin	Carbon in incurrent water samples in the form of virus cells	micromoles of carbon per liter of seawater (umol C/L)
uMCvirex	Carbon in excurrent water samples in the form of virus cells	micromoles of carbon per liter of seawater (umol C/L)
uMdocin	Dissolved organic carbon (DOC) in incurrent water samples	micromoles of carbon per liter of seawater (umol C/L)
uMpocin	Particulate organic carbon (POC) in incurrent water samples	micromoles of carbon per liter of seawater (umol C/L)
uMlpocin	Live particulate organic carbon (LPOC) in incurrent water samples	micromoles of carbon per liter of seawater (umol C/L)
uMdetritusin	Detrital organic carbon in incurrent water samples	micromoles of carbon per liter of seawater (umol C/L)
tocin	Total organic carbon (TOC) in incurrent water samples	micromoles of carbon per liter of seawater (umol C/L)

uMdocex	Dissolved organic carbon (DOC) in excurrent water samples	micromoles of carbon per liter of seawater (umol C/L)
uMpocex	Particulate organic carbon (POC) in excurrent water samples	micromoles of carbon per liter of seawater (umol C/L)
uMlpocex	Live particulate organic carbon (LPOC) n excurrent water samples	micromoles of carbon per liter of seawater (umol C/L)
uMCdetritusex	Detrital organic carbon in excurrent water samples	micromoles of carbon per liter of seawater (umol C/L)
tocex	Total organic carbon (TOC) in excurrent water samples	micromoles of carbon per liter of seawater (umol C/L)
docre	Sponge retention efficiency for dissolved organic carbon	percent (%)
pocre	Sponge retention efficiency for particulate organic carbon	percent (%)
lpocre	Sponge retention efficiency for live particulate organic carbon	percent (%)
detcre	Sponge retention efficiency for detrital organic carbon	percent (%)
tocre	Sponge retention efficiency for total organic carbon	percent (%)
virre	Sponge retention efficiency for virus cells	percent (%)
hnare	Sponge retention efficiency for high nucleic acid (HNA) bacteria cells	percent (%)
lnare	Sponge retention efficiency for low nucleic acid (LNA) bacteria cells	percent (%)
synre	Sponge retention efficiency for Synechococcus cells	percent (%)
prore	Sponge retention efficiency for Prochlorococcus cells	percent (%)
peukre	Sponge retention efficiency for pico- and nanoeukaryote cells	percent (%)

docsfr	Specific sponge filtration rate for dissolved organic carbon	umol/s/L sponge
pocsfr	Specific sponge filtration rate for particulate organic carbon	umol/s/L sponge
lpocsfr	Specific sponge filtration rate for live particulate organic carbon (LPOC)	umol/s/L sponge
detCsfr	Specific sponge filtration rate for detrital organic carbon	umol/s/L sponge
tocsfr	Specific sponge filtration rate of total organic carbon (TOC)	umol/s/L sponge
vcellsSfr	Specific sponge filtration rate of virus cells	umol/s/L sponge
hcellsSfr	Specific sponge filtration rate of high nucleic acid (HNA) bacteria cells	umol/s/L sponge
lcellsSfr	Specific sponge filtration rate of low nucleic acid (LNA) bacteria cells	umol/s/L sponge
scellsSfr	Specific sponge filtration rate of Synechococcus cells	umol/s/L sponge
pcellsSfr	Specific sponge filtration rate of Prochlorococcus cells	umol/s/L sponge
pkcellsSfr	Specific sponge filtration rate of pico- and nanoeukaryote cells	umol/s/L sponge

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Instruments

Dataset-specific Instrument Name	Sontek Micro acoustic Doppler velocimeter
Generic Instrument Name	Acoustic Doppler Velocimeter
Generic Instrument Description	ADV is the acronym for acoustic doppler velocimeter. The ADV is a remote-sensing, three-dimensional velocity sensor. Its operation is based on the Doppler shift effect. The sensor can be deployed either as a moored instrument or attached to a still structure near the seabed. Reference: G. Voulgaris and J. H. Trowbridge, 1998. Evaluation of the Acoustic Doppler Velocimeter (ADV) for Turbulence Measurements. J. Atmos. Oceanic Technol., 15, 272–289. doi: http://dx.doi.org/10.1175/1520-0426(1998)0152.0.CO;2

Dataset-specific Instrument Name	CE Elantech NC2100 combustion elemental analyzer
Generic Instrument Name	Elemental Analyzer
Generic Instrument Description	Instruments that quantify carbon, nitrogen and sometimes other elements by combusting the sample at very high temperature and assaying the resulting gaseous oxides. Usually used for samples including organic material.

Dataset-specific Instrument Name	BD FACSCelesta Flow Cytometer
Generic Instrument Name	Flow Cytometer
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

Dataset-specific Instrument Name	Shimadzu TOC 5050 analyzer
Generic Instrument Name	Total Organic Carbon Analyzer
Generic Instrument Description	A unit that accurately determines the carbon concentrations of organic compounds typically by detecting and measuring its combustion product (CO2). See description document at: http://bcodata.whoi.edu/LaurentianGreatLakes_Chemistry/bs116.pdf

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Project Information

Testing the sponge-loop hypothesis for Caribbean coral reefs (Sponge_Loop)

Coverage: Conch Reef, Key Largo, Florida, USA; Carrie Bow Cay, Belize

NSF Abstract:
Sponges are bottom-dwelling animals that dominate Caribbean reefs now that reef-building corals have been declining for decades. Sponges feed by filtering huge volumes of seawater, providing a mechanism for recycling organic material back to the reef. A new theory has been proposed called the "sponge-loop hypothesis" that is potentially the most important new concept in marine ecology in many years, because it seeks to explain Darwin's Paradox: how do highly productive and diverse coral reefs grow in desert-like tropical seas? The sponge loop hypothesis proposes that sponges on coral reefs absorb the large quantities of dissolved organic carbon (molecules such as carbohydrates) that are released by seaweeds and corals and return it to the reef as particles in the form of living and dead cells, or other cellular debris. This project will use a rigorous set of techniques to test the sponge-loop hypothesis in the field on ten of the largest and most common sponges on Caribbean reefs. For each species, the contributions of particles and dissolved organic carbon to sponge nutrition will be measured, as well as the production of cellular particles in the seawater flowing out of the sponge. For selected sponge species, the concentration of dissolved organic carbon entering the sponge will be experimentally enhanced to determine the capacity of the sponge to absorb this potential food source, and to gauge its effect on the production of cellular particles. This project will provide STEM education and training for postdoctoral, graduate and undergraduate students and public outreach in the form of easily accessible educational videos. Further, this project is important for understanding the carbon cycle on coral reefs where the effects of climate change and ocean acidification may be tipping the competitive balance toward non-reef-building organisms, such as sponges.

The cycling of carbon from the water-column to the benthos is central to marine ecosystem function; for coral reefs, this process begins with photosynthesis by seaweeds and coral symbionts, which then exude a substantial portion of fixed carbon as dissolved organic carbon (DOC) that may be lost to currents and tides. But if sponges, with their enormous water filtering capacity, can return DOC from the water column to the reef, it would represent a major unrecognized source of carbon cycling. The "sponge-loop hypothesis" has the potential to transform our understanding of carbon cycling on coral reefs. Building on preliminary data from studies of the giant barrel sponge, this project will investigate each of the three components of the sponge-loop hypothesis for ten common barrel, vase and tube-forming species that span a range of associations with microbial symbionts, from high microbial abundance (HMA) to low microbial abundance (LMA) in the sponge tissue. Specifically, the experimental approach will include InEx techniques (comparative sampling of seawater immediately before and after passage through the sponge), velocimetry, and flow cytometry to determine whether each species consumes DOC and produces particulate organic carbon (POC) in the form of cellular detritus. Then, for species that consume DOC, the same techniques will be used in manipulative experiments that augment the amount of DOC from three categories (labile, semi-labile and refractory) to determine the types of DOC consumed by sponges. In addition to testing the sponge-loop hypothesis, this project will use molecular techniques to investigate the differences among HMA and LMA sponge species, targeting the microbial symbionts that may be responsible for DOC uptake.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1558580

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