Hydrocarbon concentrations, DIC isotopes, nutrients, and cyanobacteria counts from samples collected on R/V Neil Armstrong cruise AR16 in the western north Atlantic during May 2017

Website: https://www.bco-dmo.org/dataset/826878
Data Type: Cruise Results
Version: 1
Version Date: 2020-10-16

Project
» Collaborative Research: Do Cyanobacteria Drive Marine Hydrocarbon Biogeochemistry? (Cyanobacteria Hydrocarbons)

Contributors	Affiliation	Role
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Abstract
Hydrocarbon concentrations, DIC isotopes, nutrients, and cyanobacteria counts from samples collected on R/V Neil Armstrong cruise AR16 in the western north Atlantic during May 2017.

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Coverage

Spatial Extent: **N**:40.4212167 **E**:-64.1637167 **S**:29.0470333 **W**:-71.399
Temporal Extent: 2017-05-04 - 2017-05-20

Methods & Sampling

***in situ* Sampling and Quantification of Hydrocarbon Production**
Water was collected with a rosette equipped with 12 L Niskin bottles just after sunrise (~ 8 AM) for all sampling except for the diel experiment. Salinity, density, temperature, fluorescence and percent photosynthetically active radiation (% PAR) were measured semi-continuously for each hydrocast. For diel sampling, a Lagrangian framework was used by following deployed particle traps set just below the DCM (150 m) and sampled at six-hour intervals through a full 24-hour cycle. Sampling targeted six light-penetration levels with depths held constant following initial collection, plus the DCM, which is a depth-variable feature. Water was collected from the Niskin into 2 L polycarbonate bottles via a polyvinyl chloride tube equipped with a 200 m mesh to filter out large zooplankton.

For *in situ* hydrocarbon concentration measurements, water in the 2 L polycarbonate bottles was immediately

filtered through a 0.22 m Teflon filter under gentle vacuum with an oil-less vacuum pump. For the hydrocarbon production experiment ^{13}C-bicarbonate tracer solution (with 45 g/L NaCl to sink the tracer to the bottom of the bottle) made from ^{13}C-sodium bicarbonate (Cambridge Isotope Laboratories Inc., ^{13}C 99%) was added to the 2L polycarbonate bottles to achieve a 480 ‰ enrichment in seawater DIC. Dark control bottles were covered completely beforehand with aluminum foil before tracer addition and kill control bottles were treated with Zinc Chloride to 2% $ZnCl_2$ (m/v) before tracer addition. 2 L bottles were then immediately placed into black mesh bags to attenuate light to the value from which it was collected (either 30%, 10% or 1% PAR) and placed into on-board seawater incubators with a continuous flow of surface water; this was marked as the start of incubation. Bottles were harvested at 0 hour (initial), 5, 10, 20 and 30 hour (final) time points for the 30% PAR light bags and at t = 0 hour and t = 30 hour final for the 10% and 1% light levels, care was taken to reduce light exposure in the ship-board laboratory when preparing for incubation by placing bottles into covered tubs. A 2 mL aliquot was taken for ^{13}C-DIC prior to filtration. Filters were placed into pre-combusted aluminum foil packets and immediately frozen at -20 C for later analysis.

Hydrocarbon Extraction and Analysis
A modified Bligh-Dyer method was used to extract hydrocarbons from membranes of frozen cells collected on Teflon filters. Dodecahydrotriphenylene (internal standard) and C23 ethyl ester (chromatographically remote secondary internal standard) were added to the dry filter before extraction. Once extracted into dichloromethane, sodium sulfate was added for drying, ~40 L of toluene was added to prevent complete dryness of the extracts and then the solution was rotary evaporated to ~30 L and placed into a 2 mL GC-vial with a combusted glass insert. Before analysis, a small volume of C23 methyl ester (external standard) was added. All glassware and solid chemicals were pre-combusted before use. Concentration analysis was done on a gas chromatograph flame ionization detector (GC-FID). GC-FID was performed with a 30 m x 0.25 mm ID, 0.25 m pore size, fused silica Restek 13323 Rxi-1 MS Capillary Column with a splitless 2 L injection. Initial oven temperature was at 70 °C held for 2 minutes, a 3 °C min^{-1} ramp to 120 °C, then a 6 °C min^{-1} ramp to the final temperature of 320 °C. A standard mix of pentadecane, heptadecane, internal standard, external standard and transesterification standard was run to calibrate response factors for every batch of samples (~20 per batch). Blanks were run every ~ six samples and peaks were manually integrated, there were no co-eluting peaks for pentadecane or heptadecane. Comprehensive two-dimensional chromatography was used on select samples to check for other hydrocarbons, contaminants, and quality of blank filters run through the extractive process.

Compound-specific and Dissolved Inorganic Carbon Isotope Measurements
Compound-specific isotope analysis was performed after concentration analysis on a gas chromatograph combustion isotope ratio mass spectrometer (GC/C-IRMS) with a Trace GC (Thermo Finnigan) set up to a GC-C/TC III (FinniganTM) interface and a Deltaplus XP isotope ratio mass spectrometer (Thermo Finnigan). A J & W Scientific DB-5 Capillary column (30 m, 0.25 mm, 0.25 m) was used with 2 L manual injections. Temperature ramp was conducted starting at 70 °C and held for 2 minutes, then a 3 °C min-1 ramp to 120 °C, hold for 0 minutes, then a 6 °C min-1 ramp to 185 °C, hold for 0 minutes then a 120 °C min^{-1} ramp to 290 °C, hold for 3 minutes. Inlet temperature was 260 °C, flow rate was held at 2.2 mL He min-1 with a splitless injection held for 0.5 minutes after injection. Isotope ratio accuracy was calibrated with a C14 fatty acid methyl ester Schimmelmann reference material to Vienna PeeDee Belemnite. Precision was accounted for with a standard mix of nC15, nC16 and nC17 at ~1.2 ng L-1 and was run between every batch of ~20 samples. Peaks were manually integrated after establishing the baseline, analytical precision was ~0.9 ‰ δ13C for pentadecane. Dissolved inorganic carbon 13C isotope ratio measurements were made on a Gas Bench II (Thermo Finnigan) interfaced to the same Deltaplus XP isotope ratio mass spectrometer (Thermo Finnigan) used for the compound-specific analysis. Sample preparation and analysis were followed closely to the protocol outlines by the University of California, Davis, Stable Isotope Facility (https://stableisotopefacility.ucdavis.edu/dictracegas.html).

Cell Counts and Dissolved Nutrient Analysis
Sampling for nutrients and cell counts was conducted on the CTD cast immediately before the casts for hydrocarbon sampling (~ 1-hour difference), these casts were all at ~sunrise. Parallel sampling was conducted with the same cast water for the diel sampling. Flow cytometry analysis was performed by the Bigelow Laboratory for Ocean Sciences using a slightly modified protocol from Lomas et al., 2010. Samples were fixed with paraformaldehyde (0.5% final concentration) and stored at ~4 °C for 1-2 hours before long term storage in liquid nitrogen. An Influx cytometer was used with a 488 nm blue excitation laser, appropriate Chl-a (692 ± 20 nm) and phycoerythrin (585 ± 15 nm) bandpass filters, and was calibrated daily with 3.46 µm Rainbow Beads (Spherotech Inc. Lake Forest, Illinois, USA). Each sample was run for 4–6 min (~0.2–0.3 ml total volume analyzed), with log-amplified Chl-a and phycoerythrin fluorescence, and forward and right-angle scatter signals recorded. Data files were analyzed from two-dimensional scatter plots based on red or orange fluorescence and characteristic light scattering properties using FlowJo 9.8 Software (Becton Dickinson, San Jose, CA). Pico-autotrophs were identified as either *Synechococcus* or *Prochlorococcus*, pico-eukaryotes based upon cell size

and the presence or absence of phycoerythrin, respectively. Nutrients were analyzed by the University of Washington Marine Chemistry Laboratory.

Data Processing Description

BCO-DMO Processing:
- replaced "NA" with "nd" as missing data identifier;
- renamed fields to conform with BCO-DMO naming conventions (e.g. no special characters; names must begin with letters)
- converted date format to YYYY-MM-DD;
- replaced commas with semi-colons in the sample column;
- made longitude values to negative to indicate degrees West.

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Data Files

File
hydrocarbon_data.csv (Comma Separated Values (.csv), 130.65 KB) MD5:2f33ba6bdf9b86965426e52630146fcd Primary data file for dataset ID 826878

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Parameters

Parameter	Description	Units
sample	sample identification	unitless
station	station number	unitless
depth	depth	meters
PAR	% photosynthetically active radiation	unitless
id	description: iso = isotope added; env, = no isotope added; dark = dark control; kill = killed with ZnCl; trap = sediment trap particle sample	unitless
replicate	replicate; A, B, or C	unitless
time	duration of incubation	hours
time_inc	time incubation started; format: HH:MM	unitless
time_harvest	time water was filtered; format: HH:MM	unitless
act_inc_time	duration of incubation	hours
cast_number	cast number of cruise	unitless
date_collected	date of collection; format: YYYY-MM-DD	unitless
time_collected	time of collection; format: HH:MM	unitless
lat	Latitude	degrees North
long	Longitude	degrees East
temp	water temperature	degrees Celsius

density	potential seawater density	kilograms per cubic meter +1000 (kg/m^3 + 1000)
fluor	fluorescence	milligrams chl per cubic meter (mg/m^3 Chl)
fluor_avgd_1m	fluorescence averaged at that depth with fluorescence found one meter above and below	milligrams chl per cubic meter (mg/m^3 Chl)
fluor_avgd_2m	fluorescence averaged at that depth with fluorescence found two meters above and below	milligrams chl per cubic meter (mg/m^3 Chl)
nC15	pentadecane concentration	nanograms per liter (ng/L)
C17	heptadecane concentration	nanograms per liter (ng/L)
C13_12C_nC15	13C isotope value pentadecane	permill to VPDB
C13_12C_DIC	13C isotope value dissolved inorganic carbon	permill to VPDB
sd_13C_DIC	standard deviation of 13C/12C_DIC measurements done in triplicate	permill to VPDB
Total_phyto	number of all counted phytoplankton	number per milliliter (#/mL)
Syn_count	number of Synechococcus cells per mL	number per milliliter (#/mL)
Pro_count	number of Prochlorococcus cells per mL	number per milliliter (#/mL)
pEu_count	number of picoeukaryote phytoplankton per mL	number per milliliter (#/mL)
nEu_count	number of nanoeukaryote phytoplankton per mL	number per milliliter (#/mL)
Hetero_count	number of heterotrophic bacteria per mL	number per milliliter (#/mL)
depth_flowcyt	depth of flow cytometry sample	meters
pro_notes	notes of flow cytometry measurement	unitless
phosphate	concentration of phosphate	micrograms per liter (ug/L)
silicone	concentration of silicone	micrograms per liter (ug/L)
nitrate	concentration of nitrate	micrograms per liter (ug/L)
nitrite	concentration of nitrite	micrograms per liter (ug/L)
ammonium	concentration of ammonium	micrograms per liter (ug/L)
TPP	total particulate phosphorus	nanomolar (nM)
TPP_stdev	standard deviation of TPP measurements	nanomolar (nM)
N_P	ratio of N to P	unitless
recovery_IS	percent (%) recovery of internal standard for nC15 extractions	unitless (percent)
notes	notes	unitless

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Instruments

Dataset-specific Instrument Name	Sea-Bird SBE-911+
Generic Instrument Name	CTD Sea-Bird SBE 911plus
Generic Instrument Description	The Sea-Bird SBE 911 plus is a type of CTD instrument package for continuous measurement of conductivity, temperature and pressure. The SBE 911 plus includes the SBE 9plus Underwater Unit and the SBE 11plus Deck Unit (for real-time readout using conductive wire) for deployment from a vessel. The combination of the SBE 9 plus and SBE 11 plus is called a SBE 911 plus. The SBE 9 plus uses Sea-Bird's standard modular temperature and conductivity sensors (SBE 3 plus and SBE 4). The SBE 9 plus CTD can be configured with up to eight auxiliary sensors to measure other parameters including dissolved oxygen, pH, turbidity, fluorescence, light (PAR), light transmission, etc.). more information from Sea-Bird Electronics

Dataset-specific Instrument Name	gas chromatograph flame ionization detector (GC-FID)
Generic Instrument Name	Flame Ionization Detector
Generic Instrument Description	A flame ionization detector (FID) is a scientific instrument that measures the concentration of organic species in a gas stream. It is frequently used as a detector in gas chromatography. Standalone FIDs can also be used in applications such as landfill gas monitoring, fugitive emissions monitoring and internal combustion engine emissions measurement in stationary or portable instruments.

Dataset-specific Instrument Name	Influx 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. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

Dataset-specific Instrument Name	gas chromatograph flame ionization detector (GC-FID)
Generic Instrument Name	Gas Chromatograph
Generic Instrument Description	Instrument separating gases, volatile substances, or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC)

Dataset-specific Instrument Name	gas chromatograph combustion isotope ratio mass spectrometer (GC/C-IRMS)
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Generic Instrument Description	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

Dataset-specific Instrument Name	Deltaplus XP isotope ratio mass spectrometer (Thermo Finnigan)
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Generic Instrument Description	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

Dataset-specific Instrument Name	12 L Niskin bottles
Generic Instrument Name	Niskin bottle
Dataset-specific Description	Water was collected with a rosette equipped with 12 L Niskin bottles.
Generic Instrument Description	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

Dataset-specific Instrument Name	Gas Bench II
Generic Instrument Name	Thermo-Fisher Scientific Gas Bench II
Generic Instrument Description	An on-line gas preparation and introduction system for isotope ratio mass spectrometry that is designed for high precision isotope and molecular ratio determination of headspace samples, including water equilibration, carbonates and atmospheric gases. The instrument allows for the use of a dual viscous flow inlet system of repetitive measurements of sample and standard gas on a continuous flow isotope ratio mass spectrometer (CF-IRMS) system. The sample volume is the sample vial (instead of a metal bellows), and the reference gas volume is a pressurized gas tank. The instrument consists of a user programmable autosampler, a gas sampling system, a maintenance-free water removal system, a loop injection system, an isothermal gas chromatograph (GC), an active open split interface, a reference gas injection system with three reference ports, and one or two optional LN2 traps for cryofocusing. The gas sampling system includes a two port needle which adds a gentle flow of He into the sample vial, diluting and displacing sample gas. Water is removed from the sample gas through diffusion traps. The loop injector aliquots the sample gas onto the GC column, which separates the molecular species. The reference gas injection system allows accurate referencing of each sample aliquot to isotopic standards. The system can be used with several options including a carbonate reaction kit that allows injection of anhydrous phospohric acid into sample vials.

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Deployments

AR16

Website	https://www.bco-dmo.org/deployment/747056
Platform	R/V Neil Armstrong
Start Date	2017-05-03
End Date	2017-05-22

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Project Information

Collaborative Research: Do Cyanobacteria Drive Marine Hydrocarbon Biogeochemistry? (Cyanobacteria Hydrocarbons)

Coverage: North Atlantic Sub-tropical Gyre

NSF Award Abstract:
While the release of petroleum hydrocarbons into the ocean is recognized as an environmental and human hazard, a recent study has estimated that on an annual basis, the release of natural hydrocarbons by a single phytoplankton group (cyanobacteria) contributes at least ten times more total hydrocarbon to the surface ocean. This project will be the first in-depth study of the latent biogeochemical cycling of this huge pool of biogenic hydrocarbons. Using field studies, laboratory incubations of cyanobacteria, and state-of-the art chemical analysis, the researchers will examine the molecular structures, rates and mechanisms of production and removal, and the environmental conditions that control the cycling of this major pool of oceanic hydrocarbons. The results of this study will reveal significant new knowledge for improved understanding of a

major carbon cycle in the ocean. Additionally, data could indicate a role for cyanobacterial hydrocarbons in preparing natural marine bacteria to respond to, and degrade petroleum spills, as well as a possible atmospheric impact (e.g. cloud formation) resulting from air-sea exchange of certain components of the hydrocarbon pool.

This project will support undergraduate and graduate students, a postdoctoral investigator, and a new faculty member, and will engage participants from minority-serving institutions in California and North Carolina. Plans are also included to establish links with oil spill and biofuel researchers in order to evaluate additional practical applications for the data resulting from this study.

The annual production of 308,000,000 - 771,000,000 tons of hydrocarbons by cyanobacteria has recently been reported and is a factor of 10 larger than marine petroleum hydrocarbon input from spills and natural seeps. Consequently, these biogenic hydrocarbons almost certainly have significant implications for the carbon cycle and the bacterial community composition in the ocean but have never been the subject of rigorous study. This project will investigate the distribution, partitioning, and cycling of biogenic hydrocarbons in the ocean, focusing on the abundance and molecular diversity of biogenic hydrocarbons in relation to cyanobacterial populations; the extent to which volatilization to the atmosphere acts as a sink for biogenic hydrocarbons; and the rate at which hydrocarbons are produced by cyanobacteria and consumed by hydrocarbon-degrading bacteria. Field studies across natural gradients in phytoplankton community structure and abundance will employ state of the art chemical analysis to evaluate the distribution of biogenic hydrocarbons, and together with incubation experiments will determine quantitative rates for biogenic hydrocarbons cycling in the surface ocean. Laboratory studies will augment field studies by assessing hydrocarbon production and loss mechanisms under carefully controlled laboratory conditions. Together, the project will obtain a quantitative understanding of this important component of the oceanic carbon cycle.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1756667
NSF Division of Ocean Sciences (NSF OCE)	OCE-1635562
NSF Division of Ocean Sciences (NSF OCE)	OCE-1634478

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