# Sugar concentrations from the BATS site in the Sargasso Sea, 2001-2004 (Ocean Microbial Observatory project)

Website: https://www.bco-dmo.org/dataset/543771 Data Type: Cruise Results Version: 1 Version Date: 2014-12-16

#### Project

» <u>Transitions in the Surface Layer and the Role of Vertically Stratified Microbial Communities in the Carbon Cycle</u> - <u>An Oceanic Microbial Observatory</u> (Ocean Microbial Observatory)

Contributors	Affiliation	Role
<u>Carlson, Craig A.</u>	University of California-Santa Barbara (UCSB)	Principal Investigator
<u>Giovannoni, Stephen</u>	Oregon State University (OSU)	Co-Principal Investigator
Copley, Nancy	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

#### Abstract

Sugar concentrations and dissolved combined neutral sugar (DCNS) dynamics were measured from samples for DCNS collected monthly to bimonthly between 2001 and 2004 at the BATS study site aboard the R/V Weatherbird II, Western Sargasso Sea.

# Table of Contents

- <u>Coverage</u>
- Dataset Description
  - Methods & Sampling
    - Data Processing Description
- Data Files
- <u>Related Publications</u>
- Parameters
- Instruments
- Deployments
- <u>Project Information</u>
- <u>Funding</u>

# Coverage

**Spatial Extent**: N:31.711 **E**:-64.092 **S**:31.593 **W**:-64.271 **Temporal Extent**: 2001-09-12 - 2004-12-08

# **Dataset Description**

Sugar concentrations and dissolved combined neutral sugar (DCNS) dynamics were measured from samples for DCNS collected monthly to bimonthly between 2001 and 2004 at the BATS study site aboard the R/V *Weatherbird II*. Supporting data provided by the BATS time-series program and are available at (<u>http://bats.bios.edu/)</u>

#### Methods & Sampling

Methodology is from Goldberg et al (2009).

Study site

The BATS site is located at 31°40'N, 64°10'W in the Northwestern Sargasso Sea. There, the surface layer of the water column is thermally stratified during summer and autumn months and concentrations of macronutrients are generally below limits of detection (Steinberg et al., 2001). Sub-tropical mode water

(STMW), formed to the north before subducting with subsequent southerly flow, lies below the surface layer at the BATS site, occupying the 18 °C thermostad between 150 and 400 m (Worthington, 1976; Palter et al., 2005). Deep convective mixing that occurs during winter months can entrain STMW, with elevated nutrient concentrations, into the surface layer, supporting the annual winter/spring phytoplankton bloom.

#### Sample collection

Samples for DOC and DCNS were collected monthly to bimonthly between 2001 and 2004 at the BATS study site aboard the R/V Weatherbird II. Seawater was collected in 12 L Niskin bottles using a conductivity, temperature, and depth (CTD) profiler. Each sample was gravity filtered through an inline 47 mm glass fiber filter (GF/F filters, Whatman) housed in an acid cleaned polycarbonate cartridge (Gelman) and attached directly to the Niskin bottle spigot using silicone tubing. Filtrate was collected in 40 mL combusted glass EPA vials, frozen immediately, and stored at -20 °C until analysis at University of California Santa Barbara. For long-term storage, 4 mL aliquots of sample were transferred into 5 mL glass ampoules, dried in a Savant Speed Vac, sealed with Teflon tape, and stored in sealed polyethylene bags at -20 °C. All plasticware was washed with 10% hydrochloric acid (HCl; Fisher) and flushed thoroughly with UV oxidized Nanopure® water (Barnstead Thermoline). Glass fiber (GF/F) filters and borosilicate vials were combusted at 450 °C for 2-3 h prior to use. All samples were analyzed between October 2004 and July 2006.

To ensure run-to-run comparability, surface (1 m) and deep (200 m) seawater references (same batch) were incorporated in each run. A large batch of reference seawaters were collected during the summer of 2004 from the Santa Barbara Channel, filtered, dried and stored in 5 mL glass ampoules as described above.

#### Sample processing

All DCNS samples were analyzed in triplicate following the methodology of Borch and Kirchman (1997) with slight modification of the hydrolysis time (see recovery tests below). Prior to hydrolysis, dried samples were resuspended to the initial volume with Nanopure® water. Samples were then flame sealed and hydrolyzed with H2SO4 (0.85 M; Fisher) for 21 h at 100 °C. Samples were cooled then pipetted into 30 mL polycarbonate tubes that had been pre-cleaned with successive rinses of methanol (Fisher), 0.5 M HCl, 0.5 M NaOH (Fisher), and Nanopure® water. Samples were neutralized with 1.2 Meq CaCO3 that had been precombusted at 450 °C for 2-3 h and vortexed until a pH of ~6 was achieved (Skoog and Benner, 1997). Samples were then placed in a centrifuge and spun at 28,760g for 30 min at room temperature. The supernatant was dispensed by pipette into 7 mL combusted glass scintillation vials equipped with Teflon lined caps and refrigerated (4 °C no longer than 72 h) in the dark prior to desalting. The desalting protocol was conducted according to the methods of Mopper et al. (1992) in 20 mL BioRad (Hercules, CA) HDPE columns that were cleaned with full bed volumes of NaOH (0.5 M), HCl (0.5 M), and Nanopure® water prior to resin loading. Columns were loaded with 7 mL of mixed anion (AG 2-X8, 20-50 mesh, Bio-Rad) and cation (AG 50W-X8, 100-200 mesh, Bio-Rad) exchange resin that were then flushed 3× with two bed volumes of Nanopure® water and dried by purging with ultra high purity He gas. Resin was primed 3 times with 400 µL of sample and purged immediately. Then, 900 µL of sample was added to the resin and let stand for 7 min before collection in 20 mL combusted glass scintillation vials. Sample salinity was randomly checked with a refractometer. Only one lot of mixed anion and cation exchange resin was used throughout this study and was regenerated over the extended period of analysis to ensure consistency in sugar recovery, as demonstrated with reference water runs.

#### HPLC analysis

DCNS were analyzed via pulsed amperometric detection high performance liquid chromatography (PAD-HPLC) using a Dionex (Sunnyvale, CA) Bio-LC 600 equipped with a GS-50 pump, ED-50 detector, and AS-50 autosampler. Chromeleon 6.2 integration software was used for data integration. Sugars were isocratically eluted at 18 mM NaOH (50% w/w, Fisher) and separated with Dionex CarboPac PA-10 analytical and guard columns. The electrochemical detector was equipped with an Au working electrode and an Ag/Cl pH reference electrode. A 200 mM NaOH wash (10 min) was used to minimize CO3 buildup on the columns and was performed after each sample. A known Dionex mono-standard (100 nM) of 6 sugars (fucose, galactosamine, glucosamine, galactose, glucose and mannose) was analyzed every 8th sample to assess variability associated with the electrodes and PA-10 columns. This standard was also used to determine if the PAD-HPLC system was stable for each analytical run. Runs were aborted when the decrease in sensitivity approached 20% of initial standard values. A mono-standard mix of 7 sugars including fucose, rhamnose, arabinose, galactose, glucose, mannose, and fructose (Absolute Standards Inc., Hamden, CT) was used for standardization via a 4point standard curve (10, 75, 125, 250 nM). Desalting and hydrolysis recoveries for aldoses in the quantification standard were within the range of 70-90% and 55-60%, respectively, for all neutral sugars. The values for DCNS in field samples were normalized to hydrolyzed and desalted quantification standards, similar to Kirchman et al. (2001). Concentrations reported have been corrected for blank levels measured with hydrolyzed Nanopure® water. Fructose is degraded or destroyed during acid hydrolysis, and is therefore not reported. Similar to other studies of DCNS in oceanic settings (Borch and Kirchman, 1997; Rich et al., 1997; Kirchman et al., 2001), the peaks for mannose and xylose co-eluted and are referred to as mannose+xylose

#### hereafter.

Vials containing surface and deep reference seawater material processed with every batch of samples were analyzed to track total analytical variability over time. Surface and deep reference waters were analyzed in triplicate at the beginning, middle, and end of each run to assess protocol efficiency, cleanliness and consistency within and between runs.

#### Ancillary data

Supporting data such as DOC concentration, primary production (PP), temperature, and sigma-theta were provided by the BATS time-series program and are available at (<u>http://bats.bbsr.edu/</u>). DOC concentrations were determined according to the method of Farmer and Hansell (2007), and the analytical variability was <2% for field (Hansell and Carlson, 2001; Carlson et al., 2004) and seawater culture samples. There is minimal contribution of particles to TOC at the BATS site (Hansell and Carlson, 2001), and DOC concentrations reported herein reflect values determined from unfiltered samples. The methods used to make the remaining ancillary measurements are described in Knap et al. (1997).

#### Data analyses

Multivariate statistical analysis (EOF) was performed to assess vertical and temporal variability of organic carbon constituents including concentrations of bulk DOC, bulk DCNS, and individual neutral sugars (i.e. fucose, rhamnose, arabinose, galactose, glucose, and mannose+xylose) measured from 2001 to 2004 (n=228 time points) over the upper 250 m at the BATS study site. All data were mean-centered and normalized to their standard deviation at each sampling depth (i.e. 0, 40, 80, 100, 140, 250 m). Correlation coefficients and p-values between EOF modal amplitudes, mol% DCNS values, DCNS yield, temperature, and sigma-theta were calculated with Statview 5.0 (SAS). Figures were made using Deltagraph and Matlab and all contour plots were generated using Ocean Data View (Schlitzer, 2007).

#### Seawater cultures

Seawater culture experiments using natural assemblages of heterotrophic bacterioplankton followed the methods of Carlson et al. (2004). They were designed to assess the turnover of DCNS and DOC that accumulated in the stratified surface seawater at or in the vicinity of the BATS study site. Seawater was collected at BATS in September of 2005 aboard the R/V Weatherbird II and along the A20 (30 °54'N, 52°20'W) US CLIVAR Repeat Hydrography transect in October of 2003 aboard the R/V Knorr. Upon recovery of the CTD, a filtrate of surface seawater was collected in a clean polycarbonate carboy by gravity filtration through a 0.2 μm pore size 142 mm Costar Membra-Fil filter housed in a 142 mm plastic filter holder. Costar Membra-Fil filters leach DOC upon initial use (Carlson et al., 2004), and so were flushed with >2 L of Nanopure® water and >0.5 L of seawater prior to collecting the filtrate to prevent organic contamination. Whole surface seawater was diluted by 70% with the 0.2 µm filtrate for all experimental treatments, and final volumes were 10 and 8 L respectively for the BATS and A20 experiments. All cultures were incubated at in situ temperatures in the dark in Precision laboratory incubators for 8-31 days. Bacterioplankton samples for cellular abundance were collected and fixed with 0.2 µm filtered 10% formalin (final concentration 3.5%; Fisher). These samples were stored at 4 °C until slides were prepared (within 48 h of collection). Cells were filtered onto 0.2 µm polycarbonate filters pre-stained with Irgalan black that were subsequently stained with 4'-6'-diamidino-2phenylidole (DAPI) according to the methods of Porter and Feig (1980). An Olympus AX70 or BX-51 epifluorescence microscope was used to enumerate DAPI stained cells.

#### **Data Processing Description**

#### **BCO-DMO Processing:**

- added conventional header with dataset name, PI name, version date, reference information
- renamed parameters to BCO-DMO standard
- added UNOLS cruise id's

[ table of contents | back to top ]

Data Files

File
Sugars.csv(Comma Separated Values (.csv), 29.29 KB) MD5:51d2490e4e1fca960db883dc1917084c
Primary data file for dataset ID 543771

[ table of contents | back to top ]

# **Related Publications**

Goldberg, S. J., Carlson, C. A., Hansell, D. A., Nelson, N. B., & Siegel, D. A. (2009). Temporal dynamics of dissolved combined neutral sugars and the quality of dissolved organic matter in the Northwestern Sargasso Sea. Deep Sea Research Part I: Oceanographic Research Papers, 56(5), 672–685. doi:10.1016/j.dsr.2008.12.013 Methods

[ table of contents | back to top ]

#### **Parameters**

Parameter	Description	Units
cruise_id	UNOLS cruise identification	unitless
cruise_id2	BATS cruise during which sample was collected	unitless
cruise_code	BATS cruise code	unitless
station	BATS station label	unitless
cast_type	B for bottle type of cast	unitless
ISO_DateTime_UTC	date and time at start of cast [UTC] formatted as yyyy-mm- ddThh:mm:ss.sss	yyyy-MM- dd'T'HH:mm:ss.SSS
lon	longitude at start of cast; east is positive	decimal degrees
lat	latitude at start of cast; north is positive	decimal degrees
depth	CTD depth	meters
year_decimal	decimal year formatted as yyyy.fraction_of_year	
depth_n	bottle target depth	meters
fucose	concentration of Fucose	nanomoles/liter
rhamnose	concentration of Rhamnose	nanomoles/liter
arabinose	concentration of Arabinose	nanomoles/liter
galactose	concentration of Galactose	nanomoles/liter
glucose	concentration of Glucose	nanomoles/liter
mannose	concentration of Mannose	nanomoles/liter
DCNS	dissolved combined neutral sugar concentration in micromolar carbon units	nanomoles/liter

[ table of contents | back to top ]

## Instruments

Dataset- specific Instrument Name	CTD
Generic Instrument Name	CTD - profiler
Generic Instrument Description	The Conductivity, Temperature, Depth (CTD) unit is an integrated instrument package designed to measure the conductivity, temperature, and pressure (depth) of the water column. The instrument is lowered via cable through the water column. It permits scientists to observe the physical properties in real-time via a conducting cable, which is typically connected to a CTD to a deck unit and computer on a ship. The CTD is often configured with additional optional sensors including fluorometers, transmissometers and/or radiometers. It is often combined with a Rosette of water sampling bottles (e.g. Niskin, GO-FLO) for collecting discrete water samples during the cast. This term applies to profiling CTDs. For fixed CTDs, see <a href="https://www.bco-dmo.org/instrument/869934">https://www.bco-dmo.org/instrument/869934</a> .

Dataset- specific Instrument Name	PAD-HPLC
Generic Instrument Name	High-Performance Liquid Chromatograph
Dataset- specific Description	DCNS were analyzed via pulsed amperometric detection highperformance liquid chromatography (PAD-HPLC) using a Dionex (Sunnyvale, CA) Bio-LC600 equipped with a GS-50 pump, ED-50 detector, and AS-50 autosampler. Chromeleon 6.2 integration software was used for data integration.
	A High-performance liquid chromatograph (HPLC) is a type of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of the mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by high pressure pumping of the sample mixture onto a column packed with microspheres coated with the stationary phase. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.
Datasat	

Dataset- specific Instrument Name	Niskin bottle
Generic Instrument Name	Niskin bottle
Dataset- specific Description	12 liter Niskin bottles
	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.

[ table of contents | back to top ]

# Deployments

#### **BATS\_cruises**

Website	https://www.bco-dmo.org/deployment/58883
Platform	Unknown Platform
Report	http://bats.bios.edu/bats-data/
Start Date	1988-10-20
Description	Bermuda Institute of Ocean Science established the Bermuda Atlantic Time-series Study with the objective of acquiring diverse and detailed time-series data. BATS makes monthly measurements of important hydrographic, biological and chemical parameters throughout the water column at the BATS Study Site, located at 31 40N, 64 10W.

#### [ table of contents | back to top ]

## **Project Information**

Transitions in the Surface Layer and the Role of Vertically Stratified Microbial Communities in the Carbon Cycle - An Oceanic Microbial Observatory (Ocean Microbial Observatory)

Website: http://www.bios.edu/research/projects/oceanic-microbial-observatory/

Coverage: Bermuda Atlantic Time-Series study site

#### (Adapted from the NSF award abstract)

The premise of this project is that stratified bacterioplankton clades engage in specialized biogeochemical activities that can be identified by integrated oceanographic and microbiological approaches. Specifically, the objective of this project is to assess if the mesopelagic microbial community rely on diagenetically altered organic matter and subcellular fragments that are produced by microbial processes in the euphotic zone and delivered into the upper mesopelagic by sinking or mixing. In past efforts this microbial observatory had greater success cultivating members of the euphotic zone microbial community, and revealed an unanticipated growth requirement for reduced sulfur compounds in alphaproteobacteria of the SAR11 clade. Genomic information showed that intense competition for substrates imposes trade-offs on bacterioplankton - there are regions of N dimensional nutrient space where specialists win. We postulate that specific growth requirements may explain some the regular spatial and temporal patterns that have been observed in upper mesopelagic bacterioplankton communities, and the difficulties of culturing some of these organisms.

The specific objectives of this project are: 1) to produce 13C and 15N labeled subcellular (e.g., soluble, cell wall, and membrane) and DOM fractions from photosynthetic plankton cultures and use stable isotope probing to identify specific clades in the surface and upper mesopelagic microbial community that assimilate fractions of varying composition and lability. 2) to use fluorescence in situ hybridization approaches to monitor temporal and spatial variability of specific microbial populations identified from the SIP and HTC experiments. To increase resolution we will use CARD-FISH protocols. 3) to measure the proteomes of bacterioplankton communities to identify highly translated genes in the surface layer and upper mesopelagic, and community responses to seasonal nutrient limitation. 4) and, to cultivate these organisms via high throughput culturing (HTC) by pursuing the hypothesis that they require specific nutrient factors and/or diagenetically altered organic substrates. Complete genome sequences from key organisms will be sought and used as queries to study patterns of natural variation in genes and populations that have been associated with biogeochemically important functions.

[ table of contents | back to top ]

# Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	<u>OCE-0802004</u>

[ table of contents | back to top ]