

# Measurements of nutrients, bacterial cell concentration and bacterial production from RV/Endeavor EN556, June 2015 (Patterns of activities project)

**Website:** <https://www.bco-dmo.org/dataset/717577>

**Data Type:** Cruise Results

**Version:** 1

**Version Date:** 2017-10-27

## Project

» [Latitudinal and depth-related contrasts in enzymatic capabilities of pelagic microbial communities: Predictable patterns in the ocean?](#) (Patterns of activities)

Contributors	Affiliation	Role
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## Abstract

Nutrients, bacterial cell counts, and bacterial protein production as measured by  $^3\text{H}$ -leucine incorporation from the 'bulk water experiments'. Water was collected on EN556. See Niskin Bottle and Cast List EN556 to link specific casts and bottles to each experiment: <https://www.bco-dmo.org/dataset/717427>.

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## Coverage

**Spatial Extent:** N:40.7072 E:-68.4037 S:37.6018 W:-71.028

**Temporal Extent:** 2015-06-01

## Dataset Description

Nutrients, bacterial cell counts, and bacterial protein production as measured by  $^3\text{H}$ -leucine incorporation from the 'bulk water experiments'. Water was collected on EN556. See Niskin Bottle and Cast List EN556 to link specific casts and bottles to each experiment: <https://www.bco-dmo.org/dataset/717427>.

## Methods & Sampling

Seawater was transferred to a 20 L carboy that were rinsed three times with water from the sampling depth and then filled with seawater from three Niskin bottles, using silicone tubing that had been acid washed then rinsed with distilled water prior to use. From each carboy, water was dispensed into smaller glass containers that were cleaned and pre-rinsed three times with water from the carboy prior to dispensing. This water was used to measure cell counts, bacterial productivity, and the activities of polysaccharide hydrolases, peptidases, and glucosidases. A separate glass Duran bottle was filled with seawater from the carboy and sterilized in an

autoclave for 20-30 minutes to serve as a killed control for microbial activity measurements.

Bacterial cells were counted by flow cytometry following the procedure described in Gasol and Del Giorgio [2000]. Water samples (2 mL) were fixed with 0.1% glutaraldehyde (final concentration) for 10 min at room temperature in the dark, and stored frozen at -80°C. Prior to analysis, thawed samples were pipetted through a cell strainer (Flowmi, 70 m porosity) and stained with SYBR Green I for 15 min on ice in the dark. Counts were performed on a FACSCalibur flow cytometer (Becton-Dickson) using fluorescent microspheres (Molecular Probes) of 1 µm in diameter as internal size standard. Cells were enumerated according to their green fluorescence and right angle scatter using the FloJo 7.6.1 software.

Samples were analyzed for nutrients and DOC content modified after Grasshoff and Kremling [1999]. Clean and acid washed syringes, tubing, and filter holders were used for each sampling. Duplicate DOC samples were filtered using the same 60 cc syringe through combusted glass fiber filters (Whatman 1825-025) secured within a polycarbonate filter holder into two combusted 20 mL scintillation vials and acidified using 100 µL of 50% phosphoric acid then immediately frozen at -20°C. DOC samples were analyzed by high-temperature catalytic oxidation (HTCO) using a Shimadzu Total Organic Carbon analyzer (TOC-8000A/5050A).

Following DOC sampling, nutrient samples were analyzed for colorimetric determination of ammonia, nitrate, nitrite, and soluble reactive phosphorous [Grasshoff and Kremling, 1999]. Samples were syringe filtered using a 60 cc syringe through 0.2 µm syringe filters (Sartorius Minisart 17823) into 50 mL Falcon tubes and immediately frozen at -20°C aboard ship. A 15 mL subsample was sent to the LSU-College of the Coast & Environment, Wetland, Biogeochemistry, Analytical Services (WBAS) facility for analysis.

Bacterial protein production was measured from 3H-leucine incorporation by heterotrophic bacteria using the cold trichloroacetic acid (TCA) and microcentrifuge extraction method [as in Kirchman, 2001]. All work was performed aboard ship. In brief, triplicate live samples of 1.5 mL seawater as well as one 100% (w/v) TCA-killed control were incubated with 23 µL of L-[3,4,5-3H(N)]-Leucine (PerkinElmer, NET460250UC) for between 4 and 24 hours in the dark at as close to in situ temperature as possible. Live samples were then killed with 89 µL of 100% (w/v) TCA and centrifuged (10,000 rpm at 4°C for 10 min) to pelletize cell material. The supernatant liquid was removed and 1 mL of 5% (w/v) TCA solution was added, followed by vortex mixing and centrifugation. Supernatant removal, mixing, and centrifugation were repeated using 1 mL of 80% ethanol solution. Finally, the supernatant liquid was removed and each sample was dried overnight. After drying, 1 mL of scintillation cocktail (ScintiSafe 30% Cocktail, Fisher SX23-5) was added and incorporated radioactivity was measured using an LSA scintillation counter (PerkinElmer Tri-Carb 2910TR). Leucine incorporation rate was calculated from the incorporated radioactivity, compared to 1 mL of scintillation cocktail spiked with 23 µL of L-[3,4,5-3H(N)]-Leucine radioactivity, divided by incubation time.

## Data Processing Description

### BCO-DMO Processing Notes:

- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- added columns with lat and lon in decimal degrees
- added cruise\_id column
- replaced 'NA' and blank cells with 'nd' (no data)
- removed the text 'cast00-' and 'stn0-' from data records for cast and station, leaving the digits in place
- changed longitude to negative degrees, denoting West
- reduced the decimal precision of temp to 2
- removed from display the following column with no data: Leu\_3H\_rep\_3

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## Data Files

File
<b>EN556_bulk_nuts_counts_production.csv</b> (Comma Separated Values (.csv), 3.10 KB) MD5:02002bdfef780380889caeabcbdd2c90
Primary data file for dataset ID 717577

## Related Publications

Gasol, J. M., & Del Giorgio, P. A. (2000). Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. *Scientia Marina*, 64(2), 197-224.

doi:[10.3989/scimar.2000.64n2197](https://doi.org/10.3989/scimar.2000.64n2197)

*Methods*

Grasshoff, K., Kremling, K., & Ehrhardt, M. (Eds.). (1999). *Methods of Seawater Analysis*.

doi:[10.1002/9783527613984](https://doi.org/10.1002/9783527613984)

*Methods*

Kirchman, D. (2001). Measuring bacterial biomass production and growth rates from leucine incorporation in natural aquatic environments. *Marine Microbiology*, 227-237. doi:10.1016/S0580-9517(01)30047-8

[https://doi.org/10.1016/S0580-9517\(01\)30047-8](https://doi.org/10.1016/S0580-9517(01)30047-8)

*Methods*

## Parameters

Parameter	Description	Units
cruise_id	cruise identifier	unitless
cast	cast number	unitless
station	station number	unitless
depth_id	depth description: sequence of depths sampled with 1 is surface and higher numbers at greater depths	unitless
depth_m	actual depth at which water collected	meters
lat_degdecmin	latitude formatted as degrees.decimal_minutes	degrees and decimal minutes
lon_degdecmin_W	longitude formatted as degrees.decimal_minutes	degrees and decimal minutes
lat_dec	latitude; north is positive	decimal degrees
lon_dec	longitude; north is positive	decimal degrees
temp	water temperature as determined by CTD	degrees Celsius
salinity	salinity as determined by CTD	per mil

timepoint	sampling time point (0; 1; 2; etc.) post-incubation	unitless
time_elapsed_hr	hours elapsed to reach a specific timepoint	hours
NO2_NO3_uM	nitrite plus nitrate concentration	micromolar
NH4_uM	ammonium concentration	micromolar
PO4_uM	phosphate concentration	micromolar
DOC_uM	dissolved organic carbon concentration	micromolar
cells_per_mL	prokaryotic cell counts per mL seawater	cells/milliliter
Leu_3H_rep_1	replicate 1 of leucine incorporation rate (bacterial protein production)	pico mol leucine/hour
Leu_3H_rep_2	replicate 2 of leucine incorporation rate (bacterial protein production)	pico mol leucine/hour

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## Instruments

<b>Dataset-specific Instrument Name</b>	
<b>Generic Instrument Name</b>	Centrifuge
<b>Dataset-specific Description</b>	Used to concentrate cell material.
<b>Generic Instrument Description</b>	A machine with a rapidly rotating container that applies centrifugal force to its contents, typically to separate fluids of different densities (e.g., cream from milk) or liquids from solids.

<b>Dataset-specific Instrument Name</b>	LSA scintillation counter, PerkinElmer Tri-Carb 2910TR
<b>Generic Instrument Name</b>	Liquid Scintillation Counter
<b>Dataset-specific Description</b>	Used to measure incorporated radioactive 3H-leucine
<b>Generic Instrument Description</b>	Liquid scintillation counting is an analytical technique which is defined by the incorporation of the radiolabeled analyte into uniform distribution with a liquid chemical medium capable of converting the kinetic energy of nuclear emissions into light energy. Although the liquid scintillation counter is a sophisticated laboratory counting system used to quantify the activity of particulate emitting ( $\beta$ and $\alpha$ ) radioactive samples, it can also detect the Auger electrons emitted from 51Cr and 125I samples. Liquid scintillation counters are instruments assaying alpha and beta radiation by quantitative detection of visible light produced by the passage of rays or particles through a suitable scintillant incorporated into the sample.

<b>Dataset-specific Instrument Name</b>	20 liter Niskin bottles
<b>Generic Instrument Name</b>	Niskin bottle
<b>Dataset-specific Description</b>	Used to collect water for large volume mesocosm experiments
<b>Generic Instrument Description</b>	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.

<b>Dataset-specific Instrument Name</b>	
<b>Generic Instrument Name</b>	Shipboard Incubator
<b>Generic Instrument Description</b>	A device mounted on a ship that holds water samples under conditions of controlled temperature or controlled temperature and illumination.

<b>Dataset-specific Instrument Name</b>	Shimadzu Total Organic Carbon analyzer (TOC-8000A/5050A)
<b>Generic Instrument Name</b>	Total Organic Carbon Analyzer
<b>Dataset-specific Description</b>	Used to measure dissolved organic carbon concentration in the samples.
<b>Generic Instrument Description</b>	A unit that accurately determines the carbon concentrations of organic compounds typically by detecting and measuring its combustion product (CO <sub>2</sub> ). See description document at: <a href="http://bcodata.whoi.edu/LaurentianGreatLakes_Chemistry/bs116.pdf">http://bcodata.whoi.edu/LaurentianGreatLakes_Chemistry/bs116.pdf</a>

## Deployments

### EN556

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/717216">https://www.bco-dmo.org/deployment/717216</a>
<b>Platform</b>	R/V Endeavor
<b>Start Date</b>	2015-04-27
<b>End Date</b>	2015-05-02
<b>Description</b>	Project: Latitudinal and Depth-Related Contrasts in Enzymatic Capabilities of Pelagic Microbial Communities. Cruise track obtained from rvddata.us control-point navigation ( <a href="http://www.rvddata.us/catalog/EN556">http://www.rvddata.us/catalog/EN556</a> )

## Project Information

### **Latitudinal and depth-related contrasts in enzymatic capabilities of pelagic microbial communities: Predictable patterns in the ocean? (Patterns of activities)**

**Coverage:** Atlantic Ocean, Arctic Ocean, Pacific Ocean, Greenland

#### *NSF Award Abstract:*

Heterotrophic microbial communities are key players in the marine carbon cycle, transforming and respiring organic carbon, regenerating nutrients, and acting as the final filter in sediments through which organic matter passes before long-term burial. Microbially-driven carbon cycling in the ocean profoundly affects the global carbon cycle, but key factors determining rates and locations of organic matter remineralization are unclear. In this study, researchers from the University of North Carolina at Chapel Hill will investigate the ability of pelagic microbial communities to initiate the remineralization of polysaccharides and proteins, which together constitute a major pool of organic matter in the ocean. Results from this study will be predictive on a large scale regarding the nature of the microbial response to organic matter input, and will provide a mechanistic framework for interpreting organic matter reactivity in the ocean.

Broader Impacts: This study will provide scientific training for undergraduate and graduate students from underrepresented groups. The project will also involve German colleagues, thus strengthening international scientific collaboration.

## Funding

<b>Funding Source</b>	<b>Award</b>
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1332881</a>