

# Nutrient and cell count data from incubations conducted with methylcyclohexane or methylcyclopentane on samples collected from the Gulf of Mexico during June 2015 on R/V Atlantis cruise AT29-02

**Website:** <https://www.bco-dmo.org/dataset/914186>

**Data Type:** Cruise Results

**Version:** 1

**Version Date:** 2023-11-07

## Project

» [Collaborative Research: Chemical and microbiological studies of water-soluble alkanes in the ocean](#) (CASA)

Contributors	Affiliation	Role
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## Abstract

Hydrocarbon incubations were conducted with water collected from 1000 meters depth in the Gulf of Mexico across four stations during cruise AT29-02 on the R/V Atlantis. Sample collection occurred June 16-17, 2015. Each incubation was injected with 10 microliters of methylcyclohexane (MCH) or methylcyclopentane (MCP). Each incubation was monitored for oxygen content using remote optical oxygen sensors. After we observed a respiration signal, we sacrificially harvested each bottle for cell counts, nutrients, and DNA. Incubation time varied from 10-31 days. These data were used to assess who consumes these compounds in the deep Gulf of Mexico, over what time frame, and what metabolic pathways bacteria use to consume them.

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## Coverage

**Spatial Extent:** N:27.64 E:-87.2068 S:27.1933 W:-90.9163

**Temporal Extent:** 2015-06-17 - 2015-06-18

## Methods & Sampling

Seawater samples were collected aboard R/V Atlantis in June 2015 on cruise AT29-02. Methylcyclohexane (MCH) and methylcyclopentane (MCP) incubations were conducted at stations 1 (27° 30.41' N, 87° 12.41' W), 2 (27° 15.00' N, 89° 05.05' W), 3 (27° 11.60' N, 90° 41.75' W), and 4 (27° 38.40' N, 90° 54.98' W) with seawater collected from 1000 meters (m) depth. Seawater collected from the CTD Niskin bottles was transferred to 250-milliliter (mL) glass serum vials using a small length of Tygon tubing. Vials were filled for at least 3 volumes of water to overflow. Care was taken to ensure no bubbles were present before sealing with a polytetrafluoroethylene (PTFE) coated chlorobutyl rubber stopper and crimp cap seal. All bottles, except for unamended blank controls, immediately received 10 mL of MCH or MCP using a gas-tight syringe (Hamilton) and were maintained in the dark at in-situ temperature (4<sup>o</sup> Celsius). Before filling, each serum bottle was fixed with a contactless optical oxygen sensor (Pyroscience, Aachen, Germany) on the inner side with silicone glue,

and afterward were cleaned from organic contaminants with triple rinses of ethanol, 3% hydrogen peroxide, 10% hydrochloric acid, and MilliQ water, and were sterilized via autoclave. Oxygen concentration was monitored approximately every 8 hours with a fiber optic oxygen meter (Pyroscience, Aachen, Germany). Observed changes in oxygen content were normalized to unamended controls to correct for oxygen loss from background respiration processes and variability due to temperature changes. Bloom onset is operationally defined as three consecutive time points with oxygen loss  $>0.21$  micromoles per hour ( $\mu\text{M h}^{-1}$ ). Importantly, incubations were conducted with no added headspace and were limited to in-situ availability of oxygen, as well as for key nutrients, such as nitrogen and phosphorous. At the termination of each respiration experiment, samples were harvested and the microbial community was captured on a 0.22-micrometer ( $\mu\text{m}$ ) polyethersulfone filter. Prior to filtration samples were collected for dissolved inorganic nutrients and bacterioplankton abundance. Samples for prokaryotic cell abundance were fixed with 0.2% paraformaldehyde and quantified using Guava EasyCyte flow cytometer. Dissolved nutrient (nitrate, phosphate, and ammonia) sample collection was conducted following the University of California, Santa Barbara Marine Science Institute Analytical Lab's requirements. Seawater samples from incubations were filtered through a 0.2  $\mu\text{m}$  polyvinylidene into pre-rinsed plastic HDPE 20 mL. Nutrient sample volumes were  $\sim 17$  mL water and stored frozen until analysis. Dissolved nutrient concentrations were analyzed by flow injection analysis (FIA) using the QuikChem 8500 Series 2 (Lachat Instruments, Zellweger Analytics Inc.).

### **Data Processing Description**

The average oxygen loss in unamended controls was averaged. For incubations with hydrocarbons, oxygen loss was normalized to oxygen loss in unamended controls to account for background respiration.

### **BCO-DMO Processing Description**

- Imported original file named "bco\_dmo\_wsa\_incubation\_metadata.csv" into the BCO-DMO system.
- Marked "NA" as a missing data value (missing data are blank/empty in the final CSV file).
- Renamed fields to comply with BCO-DMO naming conventions.
- Added columns for station latitude and longitude, using the location information provided in the metadata.
- Added columns for the sampling date and time in local and UTC time zones.
- Saved the final file as "914186\_v1\_nutrient\_and\_cell\_counts\_incubations.csv".

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### **Parameters**

Parameter	Description	Units
Rank	Sample ID	unitless
Type	Incubation type. T0 = time point zero?; MCH = Methylcyclohexane; MCP = Methylcyclopentane ; Blank = blank control.	unitless
Station	Station number	unitless
Station_Latitude	Station latitude; positive values = North	decimal degrees
Station_Longitude	Station longitude; negative values = West	decimal degrees
ISO_DateTime_Local	Date and time of sampling at the station in ISO 8601 format in local time zone = US Eastern (-5:00/-4:00)	unitless
ISO_DateTime_UTC	Date and time of sampling at the station in ISO 8601 format in UTC	unitless
Phosphate	Phosphate concentration	micromolar (uM)
Nitrite	Nitrite concentration	micromolar (uM)
Ammonia	Ammonia concentration	micromolar (uM)
Nitrate	Nitrate concentration	micromolar (uM)
Total_Oxygen_Loss	Total oxygen loss	micromolar (uM)
Normalized_Oxygen_Loss	Normalized oxygen loss	micromolar (uM)
Total_Incubation_Time_Days	Total incubation time in number of days	days
Bloom_Status_verbal	Bloom status. Early = ?, No = ?, Yes = ?	unitless
Cell_Abundance	Cell abundance	cells per milliliter (cells/mL)
Bloom_Onset_Time_Days	Bloom onset time in number of days	days

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## Instruments

<b>Dataset-specific Instrument Name</b>	Millipore Guava EasyCyte 5HT Flow Cytometer
<b>Generic Instrument Name</b>	Flow Cytometer
<b>Generic Instrument Description</b>	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: <a href="http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm">http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm</a> )

<b>Dataset-specific Instrument Name</b>	QuikChem 8500 Series 2 (Lachat Instruments)
<b>Generic Instrument Name</b>	Lachat QuikChem 8500 flow injection analysis system
<b>Generic Instrument Description</b>	The Lachat QuikChem 8500 Series 2 Flow Injection Analysis System features high sample throughput and simple, but rapid, method changeover. The QuikChem 8500 Series 2 system maximises productivity in determining ionic species in a variety of sample types, from sub-ppb to percent concentrations. Analysis takes 20 to 60 seconds, with a sample throughput of 60 to 120 samples per hour.

<b>Dataset-specific Instrument Name</b>	CTD Niskin bottles
<b>Generic Instrument Name</b>	Niskin bottle
<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>	Pyroscience OXSP5 optical oxygen sensors with FireStingO2 oxygen meter
<b>Generic Instrument Name</b>	Oxygen Sensor
<b>Generic Instrument Description</b>	An electronic device that measures the proportion of oxygen (O <sub>2</sub> ) in the gas or liquid being analyzed

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## Deployments

### AT29-02

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/914194">https://www.bco-dmo.org/deployment/914194</a>
<b>Platform</b>	R/V Atlantis
<b>Start Date</b>	2015-06-15
<b>End Date</b>	2015-06-29
<b>Description</b>	More information is available in R2R: <a href="https://www.rvdata.us/search/cruise/AT29-02">https://www.rvdata.us/search/cruise/AT29-02</a>

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## Project Information

**Collaborative Research: Chemical and microbiological studies of water-soluble alkanes in the ocean (CASA)**

**Coverage:** Coal Oil Point, Santa Barbara, CA and Gulf of Mexico

### *NSF Abstract:*

This research project addresses the fate of hydrocarbons that enter the ocean, using geological oil seeps as a natural scientific laboratory. The key issues of intellectual merit that will be addressed focus on the development and application of methodology to determine how the chemical properties of hydrocarbon molecules dictate whether they will be trapped in the ocean's interior or find their way to the atmosphere. The research will further follow the fate of these molecules in the ocean's interior, determining how the ocean's bacterial population responds, and the extent to which responding bacteria will degrade these molecules. The broader impacts of this research will include the training of undergraduate and graduate students in scientific research and at-sea oceanographic training, as well as the dissemination of findings to policy makers striving to understand the fate and effects of hydrocarbons in the ocean.

Hydrocarbons enter the ocean through a combination of natural seepage, anthropogenic discharge and biological production, with profound impacts on ocean biogeochemistry, ecology, and the atmosphere. This research project addresses the chemical and biological processes affecting water-soluble alkanes in the ocean, using natural seeps to study their fluxes, partitioning between ocean and atmosphere, and the bacterial response to their input. The intellectual merit of this research pertains to the behavior of highly volatile hydrocarbons, a class that is abundant in petroleum reservoirs and many crude and refined products, but is poorly understood in the ocean. Volatile hydrocarbons display distinct behaviors compared with traditional oil in that they will partition to seawater or the atmosphere depending on their molecular structure and the context by which they enter the ocean, a combination of characteristics unsuitable for traditional fate and transport models that govern our understanding of liquid oil. This research project addresses this gap in knowledge through a plan to study volatile, water-soluble hydrocarbons in the context of natural seepage, focusing on key questions about their transport and fate, and the ocean's microbial response. Two key questions include: 1) What factors control the partitioning of water-soluble alkanes between water and the atmosphere at natural seeps, and how does this affect their availability to microbes? 2) What genomic and metabolic factors enable the microbial response to the input of water-soluble alkanes and how does the microbial response vary with regional oceanographic and geologic factors such as proximity to and flux from natural seepage? The hypotheses that result from these questions will be tested through a series of oceanographic and laboratory-based experiments designed around natural oil seeps in the Pacific and in the Gulf of Mexico. The results of these studies promise to inform our understanding of the transport, fate, and effects of water-soluble alkanes in the ocean.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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## **Funding**

<b>Funding Source</b>	<b>Award</b>
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-1756947</a>

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