Sample log from R/V Atlantis cruise AT18-02 in the Gulf of Mexico Macondo wellhead area 28N 88W, depth 1500 m; 2010 (GoMX - Microbial Response project)

Website: https://www.bco-dmo.org/dataset/3576
Version: 10 November 2011
Version Date: 2011-11-10

Project
» RAPID Deepwater Horizon Oil Spill: The Microbial response to the Deepwater Horizon Oil Spill (GoMX - Microbial Response)

Program
» Gulf of Mexico - Deepwater Horizon Oil Spill (GoMX - DHOS)

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Dataset Description

Sample Log AT18-02 - December Atlantis Samples
Sampling Station, Date, Latitude, Longitude, Distance_To_WH, Temp, Salinity, Oxygen, Depth at each sample station

Methods & Sampling

Generated by BCO-DMO staff from form Dataset5.rtf submitted by Andreas Teske

Data Processing Description

BCO-DMO Processing Notes
Generated by BCO-DMO staff from form Dataset5.rtf submitted by Andreas Teske
Information in columns transposed to rows and o/p as excel file

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Data Files

File
AT18-02_Sample_Log.csv (Comma Separated Values (.csv), 1.17 KB) MD5:4c53d9f020a2b710b7b8599dc7e0467d Primary data file for dataset ID 3576

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Parameters

Parameter	Description	Units
Sampling_Station	Sampling Station Id	text
Date	Date	YYYYMMDD
Latitude	Sample latitude (South is negative)	decimal degrees
Longitude	Sample longitude (West is negative)	decimal degrees
Distance_To_WH	Distance To Well Head (WH)	text
Temp	Temperature	degrees Celsius
Depth	Depth of sample	meters
Salinity	Salinity	PSU
Oxygen	Oxygen	mg/l

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Deployments

AT18-02

Website	https://www.bco-dmo.org/deployment/58735
Platform	R/V Atlantis
Start Date	2010-11-08
End Date	2010-12-03
Description	The AT18-02 cruise sailed from Galveston, Texas and returned to Gulfport, Mississippi. Operations consisted of sediment sampling using the DSV ALVIN, hydrographic characterizations of the water column and sampling of water for geochemical and microbiological characterization using a standard CTD/Rosette, and additional sampling using a multiple corer. See more information from the WHOI cruise planning synopsis. Cruise information and original data are available from the NSF R2R data catalog.

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Project Information

RAPID Deepwater Horizon Oil Spill: The Microbial response to the Deepwater Horizon Oil Spill (GoMX - Microbial Response)

Website: http://sites.google.com/site/teskelab/Home/rapid-response-cruise-1

Coverage: Gulf of Mexico Macondo wellhead area 28N 88W, MC252 and MC118

The Microbial Response to the Deepwater Horizon Oil Spill - a Rapid Response Proposal

Project summary: Intellectual merit. We are applying for funding to conduct a time series of microbiological and geochemical assessments of the consequences of the Deepwater Horizon oil spill offshore the Louisiana coast. We are building our work on a large database of pre-spill baseline microbiology and biogeochemistry that we have been doing at a microbial observatory (Mississippi Canyon 118) near the Deepwater Horizon site since 2005, and have started our first rapid response cruise and sampling program near the spill site as soon as possible, on May 4. We will combine molecular, gene-based analyses of the microbial community structure and function in surface water and underlying sediments; in situ water column dissolved oxygen and light hydrocarbon measurements using advanced sensor technologies (Seaguard system) for deep water plume tracking; and a biogeochemical survey of the sediments and water in the immediate vicinity of and at increasing distance from the oil spill, and on different time scales during follow-up cruises. 16S rRNA and functional gene sequencing of total microbial DNA and RNA from contaminated and clean water and sediments will monitor how the oil-affected microbial community changes in composition and activity. High-throughput pyrosequencing of PCR-amplified rRNA fragments will increase the coverage by approx. three orders of magnitude, and allow for the detection of minority microbial populations that go unnoticed in conventional clone libraries. Special attention will be paid to the enrichment of oil-degrading bacteria in natural samples and in time-series experiments conducted in the lab, to monitor their growth with group-specific PCR, to monitor geochemical changes that are concomitant with the establishment and enrichment of a hydrocarbon-degrading microbial community, and to identify potential carbon incorporation pathways with stable isotope probing of nucleic acids. To summarize, the proposal focuses on molecular and microbiological assessments of hydrocarbon impact, across the spatial and time scales of the Deepwater Horizon oil spill as determined by diagnostic water column oxygen and light hydrocarbon measurements. The water column microbiological and dissolved gas data will be linked to potential impacts on the bacterial activity in bottom sediments through measurements of geochemical indicators of sedimentary anaerobic microbial activity, and porewater analyses of DIC, CH4 and low-molecular weight organic acids, the principal products of hydrocarbon degradation. We coordinate our proposed work and sampling survey with a parallel proposal by our long-term collaborator Dr. Mandy Joye at the University of Georgia, who intends to submit a primarily geochemically oriented RAPID
proposal.

Broader Impact. The results of this project will identify which bacterial and archaeal populations, and in which sequence, respond to oil spill events in marine sediments and the water column, and the attendant – often beneficial - biogeochemical consequences of this massive restructuring of the microbial community and their

activities. Most importantly, this project provides comprehensive microbial and geochemical coverage of different marine habitats (deep and shallow marine sediments, water column, surface) on the geographical and time scale of the oil spill as it is unfolding. This independent analysis will contribute to an observation-based and results-oriented reference database that, at present, the various interested players in the Deepwater Horizon saga cannot provide (http://www.nytimes. com/2010/05/21/science/earth/21conflict.html?hp).

Scientific rationale for RAPID Response proposal. The natural microbial community in the seabed and in the water column plays a critical role in alleviating the consequences of oil spills, by oxidizing and remineralizing the hydrocarbons completely to C02. Aerobic and anaerobic hydrocarbon-degrading bacteria perform this function in the oxic water column and surficial sediments, and in anoxic marine sediments, respectively (Teske 2010). The petroleum hydrocarbons massively impact the microbial community, its composition and activity. Ultimately, microorganisms that respond to the oil spill and oxidize most of the petroleum hydrocarbons will grow from small natural seed populations (e.g., Bordenave et al. 2007, Röling et al. 2002, 2004), while highly recalcitrant petroleum residues undergo very slow degradation and remain detectable in the sediment for many decades (Pearson et al. 2008). The Deepwater Horizon oil spill represents a unique opportunity to employ state-of-the-art microbiological, molecular biological, and geochemical approaches to quantify these changes in microbial community composition and activity in response to the oil spill, and to monitor their temporal and spatial dynamics in the aerobic water column and the anoxic sediment, starting with the immediate aftermath and continuing several months after the oil spill. These microbial processes are at the foundation of natural and anthropogenic oil spill remediation, and their thorough, integrated microbiological and geochemical investigation in parallel field and laboratory experiments is overdue. The project is coordinated with our long-term collaborator Dr. Samantha Joye (Univ. of Georgia), who is submitting a complementary RAPID proposal focused on biogeochemistry.

Baseline work. A rapid response cruise on RV Pelican out of Cocodrie, LA, in the immediate aftermath of the Deepwater Horizon oil spill (May 5 - May 16, 2010) performed a coordinated sediment (box coring) and water sampling program of the oil spill area by the Teske and Joye labs; to our best knowledge, no other academic research vessel was on-site sampling so early. For detailed day-by-day cruise accounts and tables of the sampling plan, see the Teske lab website http:// sites.google.com/site/teskelab/Home/rapid-response-cruise-1). Reference station samples southeast of the spill site (station 1) were followed by a gradient from the immediate neighborhood of the spill site (st. 2, 500m distance) northwest towards a long-term microbial observatory 8.6 miles distant from the spill site (sts 3-8), then a second southeast-northwest transect further east (sts 16-19), a large hexagon of approx. 20 miles diameter, with the spill site in the South-west corner (sts 20-26), and a near-shore station at the Mississippi mouth, South Pass (st. 27). The transects traversed heavily contaminated areas just northwest of the spill site (stations 2-8) and ca. 10-20 miles northeast and east-north-east of the spill site (sts 22, 23) **(Fig. 1)**. Thus, we have **1)** contaminated samples that are as close as possible, spatially and in time, to the onset of the spill, **2)** uncontaminated sediment and water samples nearby (sts 1, 27), and **3)** samples in the general path of the oil but without visible surface oil at the time of sampling (sts 16-19). Follow-up cruises are in preparation, to survey the oil spill region and to monitor the development of the microbial and biogeochemical impact of the oil spill in the field over time. At the time of writing this proposal, two PhD students (Lisa Nigro and Tingting Yang) of the Teske lab have joined the RV Walton Smith (May 25-June10, see UNOLS ship schedule) sailing from Gulfport, MS (Chief Scientists on the two cruise legs, Mandy Joye, UGA and Ian MacDonald, FSU) and focusing on deep water plume sampling. A RV Pelican cruise from Sept 26-Oct 2 has a UNC contingent led by the Martens group scheduled for installation of in-situ monitoring equipment, and presents an additional sampling opportunity. We are looking into bunk space or at least sampling opportunities on RV Pelican in June (14-26) and in September 2010 (Sept 9-15); these cruises are being scheduled by the NOAA/NIUST gas hydrate consortium.

Baseline samples 2005-present. Several microbial field and laboratory studies have demonstrated the importance of a time series for oil spill studies, as the microbial community evolves and changes continuously over many weeks and months (e.g., Bordenave et al. 2007) and shows the microbial imprint of the contamination even several decades after the spill (Pearson et al. 2008). The Teske and Martens labs have five years of microbiological and biogeochemical baseline data from benthic sediments at our long-term microbial observatory at Mississippi Canyon (MC) 118, 8.6 miles northwest of the spill site at ca. 900 meters depth. Here, naturally occurring gas hydrates and microbial mats are sustained by methane-rich seep fluids. Base line data include an extensive bacterial and archaeal sequence database of ribosomal RNA and functional gene sequences, and their geochemical framework, e.g. methane and sulfate porewater concentrations, sulfate reduction rates, methane oxidation rates (Lloyd et al. 2010), and sediment chemistry investigations by the Martens lab including pore water chemistry (Lapham et al. 2008). Sediment accumulation rates measurements spanning the study area provide detailed information about Holocene sedimentation processes (Ingram et al. submitted) that form the baseline for long-term hydrocarbon burial in benthic sediments. Sediments were sampled repeatedly since fall 2005, mostly deep gravity cores of 1-4 meters length (sampling campaigns in October 2005 and April 2008 with RV Pelican) and targeted push cores taken by submersible or ROV from

microbial mats, with bare sediments as reference (Johnson Sea Link (JSL-II), Sept 2006; RV Brooks McCall, July 2009). This multi-year database documents the natural state of the benthic microbial community, and its organic matterdegrading activity via sulfate reduction and methanogenesis, for > four years before the oil spill impact. This database reinforces our focus on the sediment community after the oil spill, and to extend these data towards detailed surface water and water column surveys.

Methods and Workplan

16S rRNA and functional gene clone libraries. Isolation of total RNA, reverse transcription of rRNA, and subsequent PCR amplification of cDNA with general bacterial and archaeal primers, followed by clone library construction and sequencing, provides a qualitative picture of the diversity of metabolically active microbial communities in the sediments (Biddle et al. 2006, Sørensen and Teske 2006) and in the water column (Teske et al. 1996). We have optimized protocols for efficient and quantitative DNA and RNA recovery from Gulf of Mexico sediments, incl. hydrocarbon seep sediments (Lloyd et al. 2006, 2010a, 2010b).

Pyrosequencing survey. To generate more comprehensive microbial community profiles, we will employ a tag sequencing strategy that combines the use of amplicons of the V6 hypervariable region of SSU rRNA as proxies for the presence of individual phylotypes (Operational Taxonomic Units, OTUs) with massively parallel sequencing on a Roche Genome Systems FLX sequencer (GS-FLEX) at UNC Chapel Hill (Dept of Genetics). This strategy provides assessments of microbial diversity, evenness, and community structure at a scale 100- to 1000-fold finer than that possible from cloning and capillary sequencing of longer SSU rRNA amplicons (Sogin et al. 2006); it also has the capacity to detect very low abundance taxa. Mixtures of multiple primers will insure recovery of all known bacterial V6 rRNA regions (Huber et al. 2007). We have applied the method successfully to microbial community analysis of Golf of Mexico hydrocarbon seep sediments and benthic sediments nearby (Lloyd et al. 2010).

Molecular detection of hydrocarbon-oxidizing bacteria and archaea. Based on our sequence databases, we will monitor aromatics- and alkane-degrading bacteria and archaea that respond to oil spills. Among the aromatics- and alkane-degrading sulfate-reducing bacteria (Teske 2010), the Desulfobacterium anilini group is a primary target, a monophyletic cluster of exclusively hydrocarbonoxidizing deltaproteobacteria (Figure 2, left. Phylogenetic tree of petro-leum-degrading sulfatereducing deltaproteo-bacteria. Blue, alkane oxidizers; purple, aromatics oxidizers. From Teske 2010). This cluster is abundant in natural hydrocarbon seeps (Lloyd et al. 2006, 2010) and is accessible for 16S rRNA and dsrAB PCR primer design, incl. group-specific q-PCR (Kniemeyer et al. 2003). Methanogenic archaea also participate in hydrocarbon degradation, via syntrophic associations of bacterial n-alkane degraders (Zengler et al. 1999) with mixed hydrogenotrophic and acetoclastic methanogen communities that are consistently detected in natural and anthropogenic oil seeps, spills and leaks (see Teske 2010 for review). For an in-depth survey of methanogens, we will use the mcrA functional gene (Dhillon et al. 2005).

Water column dissolved oxygen and light hydrocarbon concentration measurements will guide and inform water column microbial sampling. We will utilize a proven suite of chemical sensors for these measurements including fast response optodes, a Non-Dispersive Infrared (NDIR) light hydrocarbon sensor and several METS methane sesors along with conductivity and pressure (depth) sensors. The sensors are interchangeable and will all connect (via 3 m long cables) and report to an Aanderaa Data Instruments (AADI) Seaguard logger, sufficiently compact (50 cm long, ca, 20 cm diameter) to be mounted directly on a rosette water sampler used for microbial sampling (Fig. 3, left: Seaguard datalogger with four sensors). It can transmit real-time data if cabled back to the ship but independently collects data continuously with a duty cycle of approximately 30 seconds using onboard Li batteries. The Martens lab will deploy a similar multi-sensor package designed for sediment-water gas flux measurements on the seafloor at MC-118 during the late September 2010 RV Pelican cruise (Sept 26-Oct 2). We expect to find direct linkages between water column microbial communities and light hydrocarbon degradation during initial stages of oil dispersal (covered by samples on the May 4-9 and May 25-June 8 cruises), followed by a slow return to background conditions that will be defined through comparisons between the seafloor multi-sensor system already funded as a dedicated component of the MC-118 seafloor observatory near the oil leakage site. The multi-sensor Seaguard system that we propose can be mounted on ROVs for survey work as opportunities arise with or without power from the ROV. It will be fully compatible with the existing SSD ROV now in use at the MC-118 Seafloor observatory.

Porewater concentration analyses will track the geochemical indicators of anaerobic petroleum degradation via microbial “alkane cracking” (Zengler et al. 1999), for example LMW organic acids that might build up in excess of normal concentrations in marine sediments, and stimulation of sulfate reduction or methane production by these substrates. Dissolved organic acids will be quantified via HPLC using the method of Albert and Martens (1997). Sulfate will be analyzed post-cruise via standard ion chromatographic techniques. DIC stable isotopes and concentrations will be determined by standard GC/IR/MS. The chemical composition of petroleum at different stages of microbial decomposition will be analyzed in collaboration with

Prof. Damian Shea at North Carolina State University (email in suppl. Documents).

Enrichments and Stable isotope probing. Our collaborator Dr. Tony Gutierrez (Marie Curie postdoc. fellow in the Aitken lab in the Dept. of Environ. Engineering at UNC), will perform enrichments and stable isotope probing of petroleum-assimilating and aerobic water and sediment bacteria, mostly representatives of fast-growing gamma-proteobacterial genera such as Alkanivorax, Pseudomonas, Idiomarina, Cycloclasticus (Röling et al. 2004) from selected highly contaminated and uncontaminated water and sediment samples, using 13C-labeled alkane and aromatics substrates.

Time course experiments in the laboratory. In collaboration with faculty colleague Dr. Carol Arnosti and her postdoc Dr. Kai Ziervogel, we will set up time course laboratory experiments with clean and oil-contaminated or amended sediment and water samples, to monitor the enrichment of oil-degrading bacteria, the overall changes in microbial community structure, the changing patterns of hydrolytic enzyme expression (see Arnosti et al. 2009, and Ziervogel and Arnosti 2008 for Gulf of Mexico examples), geochemical change towards reduced conditions (electron acceptor depletion, organic acids and DIC accumulation from fermentation and terminal oxidation pathways), and the changing 13C signature in bacterial rRNA. Although laboratory setup provides only a selective analog of field experiments, the microbial community structure changes clearly in both cases, and shows strong selection for hydrocarbon-degrading bacteria (Alkanivorax) (Röling et al. 2002, 2004). External postdoc collaborators (UNC Marine Sciences Alumni) will perform specific time course experiments with experimental variations on their own (see letters of support in Suppl. Documents).

13C and 14C analysis of bacterial RNA. To differentiate microbial utilization of photosynthetic and hydrocarbon derived biomass, we will use 13C and 14C characterization of total and group-specific ribosomal RNA, a method that we are currently using to differentiate microbial carbon sources in the naturally hydrocarbon-rich Guaymas Basin hydrothermal sediments (MacGregor et al., 2006, Pearson et al. 2008). We will also target potential petroleum degraders identified by the isolation and enrichment experiments described above.

Broader impact. The results of this project will identify which bacterial and archaeal populations, and in which sequence, respond to oil spill events in marine sediments and the water column. Further, this project provides comprehensive microbial and geochemical coverage of affected marine habitats (deep and shallow marine sediments, water column, surface) on the geographical and time scale of the unfolding oil spill; this independent analysis will contribute to an observation-based record of the oil spill impact that many players in the oil spill saga (BP, conflicted government agencies) may not always provide.

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Program Information

Gulf of Mexico - Deepwater Horizon Oil Spill (GoMX - DHOS)

Coverage: Northern Gulf of Mexico

Grants for Rapid Response Research (RAPID)
The RAPID funding mechanism is used for proposals having a severe urgency with regard to availability of, or access to data, facilities or specialized equipment, including quick-response research on natural or anthropogenic disasters and similar unanticipated events.

GOM - Broader Impacts
The need to understand the impact of this largest oil spill to date on ecosystems and biochemical cycling is self evident. The consequences of the disaster and accompanying clean up measures (e.g. the distribution of dispersants) need to be evaluated to guide further mediating measures and to develop and improve responses to similar disasters in the future. Would it be advantageous if such oil aggregates sink, or should it rather remain suspended? Possibly measures can be developed to enhance sinking or suspension (e.g. addition of ballast minerals) once we understand their current formation and fate. Understanding the particle dynamics following the input of large amounts of oil and dispersants into the water is a prerequisite to develop response strategies for now and in the future.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1045115

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