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# Deepwater Horizon and the Rise of the Omics

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# DEEPWATER HORIZON
# AND THE RISE OF THE
# MICS

Microbial genomics techniques came of age following the Deepwater Horizon spill, offering researchers unparalleled insights into how ecosystems respond to such environmental disasters.

By Joel E. Kostka, Samantha Joye, and Rita Colwell

*Photograph of oil beneath the surface of the Gulf of Mexico following the Deepwater Horizon spill (background). In the inset, microscopic specimens of* Candidatus Maconimonas diazotrophica *are visible both inside and around the edges of oil droplets (large round shapes) in this microscope image. Credits: Rich Matthews/AP images (photo); Shutterstock/CoreDESIGN (DNA illustration); and Shmruti Karthikeyan (inset)*

Almost everywhere scientists have looked on or near Earth's surface—from ice-buried Antarctic lakes to arid, ultraviolet-baked deserts and ecosystems ranging from pristine to heavily polluted—they have found abundant and often highly diverse populations of microorganisms. Microorganisms, or microbes, are everywhere; they are adaptable, and they play key roles in element cycling and ecosystem functioning in nearly every environment on Earth.

Microbes are the great decomposers in ecosystems, breaking down dead and dying organic matter and recycling major nutrients for use by plants. And by reacting rapidly and adapting to changing conditions, they act as first responders in helping restore balance and stability to ecosystems after such disturbances as pollution or catastrophic storms. Microbes are, for example, intimately involved in ecosystem responses to oil spills.

Like organic matter derived from modern-day primary production, oil formed over geologic time can act as a carbon source that fuels microbial growth and metabolism. Hydrocarbon-degrading microbes have been studied for decades and are thought to be ubiquitous and diverse and to have adapted to consuming oil over millions of years [*Head et al.*, 2006]. And biodegradation mediated by indigenous microbial communities is considered the primary fate of most petroleum (oil and gas) that enters the marine environment through natural mechanisms like seeps [*Leahy and Colwell*, 1990].

As researchers began unveiling the complexity of microbial communities and illuminated fundamentals of how they operate in recent decades, though, much remained unclear about their structure and functioning in nature. The reason for this was in part because of a shortage of techniques for studying them. Because of their small size, microbes evade easy observation, and most cannot be cultured in the laboratory. At the time of the Exxon Valdez oil spill in 1989, for example, environmental microbiology was a relatively nascent field. But in the past decade, a variety of so-called omics techniques, focused on parsing the genetic makeup of cells, have emerged and offered researchers powerful new ways to study microbial communities and the roles played by specific groups of microbes.

## Omics Emerge

The 2010 Deepwater Horizon (DWH) oil spill in the Gulf of Mexico is the largest accidental oil discharge into a marine environment for which a proportional emergency response effort was mounted. In contrast to the Valdez spill, the last major spill affecting the United States before 2010, the DWH discharge occurred in deep water, with extraordinarily large volumes of chemical dispersant applied during emergency response efforts.

The DWH spill was also the first major environmental disaster for which genomics technologies had matured to such an extent that they could be deployed to quantify microbial responses over large spatial and temporal scales. As a result, the field of environmental genomics matured during the past decade in parallel with the DWH response. Technical advances in genomics enabled direct, comprehensive analyses of the microbes in their natural habitat, be it oil-contaminated or uncontaminated seawater or sediments. Researchers studying the effects of the DWH spill presided over an explosion of microbial genomics data that enabled major advances in oil spill science and allowed scientists to answer the question, What microbes are there?, in complex communities in unprecedented detail.

Metagenomics, the sequencing of all genes for all organisms in a sample, enabled determinations of the full range of microbial species present. It also provided assessments of these organisms' metabolic potential to carry out important ecosystem processes like photosynthesis and the degradation of certain carbon compounds. Application of metatranscriptomics, the sequencing of active or expressed genes, provided opportunities to decipher the functions or activities of those same microbes in nature, essentially answering the question, What are they doing?

Gene sequences are collected from the environment in fragments. Recent improvements in bioinformatics tools, which use high-performance computing to stitch these fragments back together into the genomes of individual microbial species, have allowed scientists to reconstruct microbial genomes over large scales, revealing the incredible diversity and complexity of microbial communities.
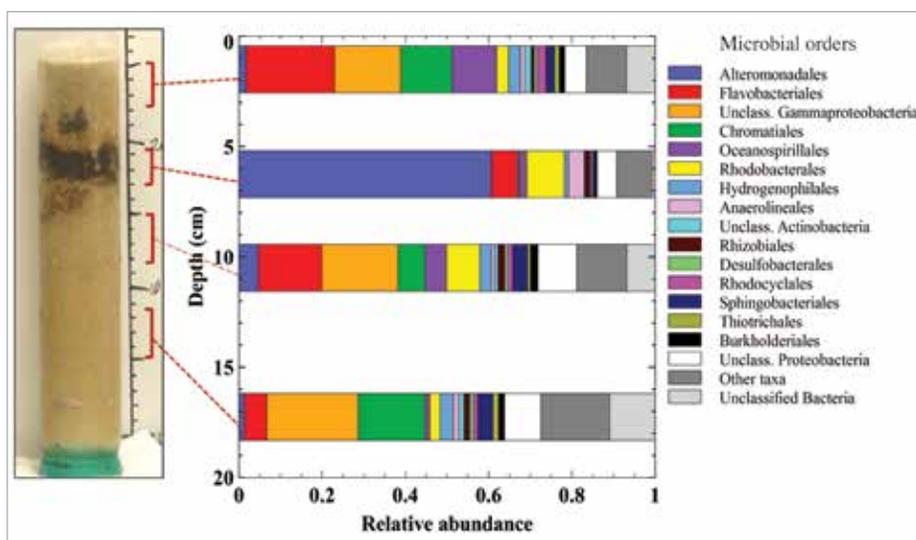


This sand core (left) collected on 30 June 2010 at Pensacola Beach, Fla., contains a pronounced oiled layer (dark brown). More than 50% of the microbes in that layer belonged to genus Marinobacter (in the Alteromonadales order), a known hydrocarbon-degrading microbial group, far more than in sands below and above the oiled layer. Credit: Markus Huettel

Jonathan Delgardio and Will Overholt of the Georgia Institute of Technology sample sand layers on 20 October 2010 at Pensacola Beach, Fla., which was heavily polluted by weathered oil after Deepwater Horizon discharge. Researchers used genomics to track how microbial communities changed in response to the oil by comparing oiled sand layers to pristine sands. Credit: Markus Huettel

Through a systems approach that incorporates genomics along with knowledge and tools from a range of other disciplines (e.g., biogeochemistry and oceanography), researchers can now monitor and assess ecosystem health—and identify disturbances that might otherwise go unnoticed—by analyzing microbial populations that both act as stewards for and represent bioindicators of ecosystems. With these efforts, global ecosystems can be better protected and, when necessary, restored in the face of diverse environmental stressors.

**Transformative Discoveries**
Prior to 2010, most studies of microbes associated with oil spills were conducted by growing them in the laboratory using pure cultures or enrichments. Consequently, we had a very limited understanding of the types and distribution of oil-degrading microorganisms—and of what they actually do—in the environment, because the vast majority of microorganisms in the natural environment have yet to be cultured. But in the wake of the DWH spill, multidisciplinary

scientific partnerships enabled transformative discoveries detailing how microbes respond to petroleum discharges and facilitate ecosystem recovery.

Many of these partnerships were supported by the Gulf of Mexico Research Initiative (GoMRI), created with a $500 million, 10-year commitment from BP to fund an independent scientific research program dedicated to studying oil spill impacts and mitigation, particularly in the Gulf of Mexico. GoMRI has funded 17 international consortia and thousands of investigators (bit .ly/GoM-Research).

Armed with genomics tools, GoMRI researchers showed that oil-degrading microbes are, indeed, nearly ubiquitous, found almost everywhere around the world in low abundance even when crude oil is absent. These microbes, part of the pool of low-abundance species known as the rare biosphere, harbor a specialized metabolic capacity to use oil as a food source—a capability that can be rapidly activated upon exposure to oil [*Kleindienst et al.,* 2015].

From deep ocean waters to shallow coastal sediments, hydrocarbon-degrading bacteria responded profoundly to oil contamination after the DWH spill, increasing in abundance and expressing genes involved in hydrocarbon metabolism over days to months. It was shown in some cases that microbial communities were composed of up to 90% oil-degrading species after exposure to hydrocarbons [*Kleindienst et al.,* 2015; *Huettel et al.,* 2018].

Over time, successions of microbial populations bloomed as they consumed the different hydrocarbon compounds of oil and responded to environmental factors [*Kostka*

*et al.,* 2011; *Yang et al.,* 2016; *Kleindienst et al.,* 2015]. Genomics research revealed that different microbial species are adapted to degrade specific types of hydrocarbon compounds (e.g., natural gases, straight-chain aliphatics, or aromatics) depending on environmental conditions like temperature and nutrient availability. These discoveries underscore the natural capacity of microbes in the Gulf of Mexico and elsewhere to bioremediate petroleum hydrocarbons.

**How Oil Affects Ecosystems**
Scientists have long hypothesized that the fate and impacts of oil in ecosystems are determined by interplays between the physical and chemical characteristics of the environment and by hydrocarbon chemistry and biogeochemical processes largely mediated by microbes. However, the complexity of these interactions has impaired our ability to decipher exactly how ecosystem functioning is affected by oil.

Oil can be a food source for some microbes, but it can be toxic to others, resulting in adverse effects on microbially mediated ecosystem services like the breakdown of organic matter and the regeneration of nutrients. Following the DWH discharge, GoMRI researchers observed through multiple lines of evidence that liq-

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uid and gaseous hydrocarbons from the spill rapidly entered the microbial food web and persisted for years [*Fernández-Carrera et al.,* 2016; *Rogers et al.,* 2019; *Chanton et al.,* 2020], with major implications for carbon and nutrient cycling through the environment. Genomics-enabled research revealed, for example, that ecosystem functions related

*A sheen of oil coats the surface of the Gulf of Mexico in June 2010, as ships work to help control the Deepwater Horizon spill. Credit: kris krüg, CC BY-NC-SA 2.0 (bit.ly/ccbyncsa2-0)*

to the microbial nitrogen cycle were drastically affected by oil.

Petroleum is made mostly of carbon and contains relatively small amounts of major nutrients like nitrogen and phosphorus. Thus, scientists expected that oiled environments would likely become limited in major nutrients, with negative effects potentially rippling through entire food webs. In numerous investigations of DWH-contaminated seawater and sediments, genes for nitrogen fixation—a process carried out by some microbes that involves converting inorganic nitrogen gas into fixed forms like ammonium, which can be used by all organisms—were shown to increase many times relative to pristine conditions, even when adjusted for the overall abundance of microbes present.

For example, a metagenomic time series revealed an increase in the abundance of genes that encode for nitrogen fixation (via the enzyme nitrogenase) that coincided with an increase in genes related to hydrocarbon degradation pathways [*Rodriguez-R. et al.,* 2015]. This increase then dissipated when the oil and associated hydrocarbon compounds disappeared. In addition, the abundances of genes related to degradation of specific hydrocarbon classes, such as alkanes and polycyclic aromatics, could be directly correlated with concentrations of the corresponding classes.

Genomic data were corroborated by research using isotopic tracers, which showed the incorporation of inorganic nitrogen into the microbial food web [*Fernández-Carrera et al.,* 2016]. Nitrogen-fixing microbes, also called diazotrophs, are well known to support crop growth in agricultural ecosystems and photosynthetic production in the open ocean [*Zehr et al.,* 2016], but nitrogen fixation by oil degraders in response to hydrocarbon exposure is a new discovery. The recognition that oil-degrading bacteria can supply themselves with nitrogen indicates that the microbial food web can compensate, at least to an extent, for influxes of nutrient-poor oil. Studies by GoMRI researchers further revealed that as overall microbial diversity declined in oil-contaminated environments, the oil selected for a few very abundant microbial species with the dual capability to fix nitrogen and degrade oil.

### "Superbug" Discovered

Fertilizing water with nitrogen and phosphorus to stimulate microbial growth is a common bioremediation strategy for oil spill cleanup; it was used, for example, during the Valdez spill in 1989 [*Bragg et al.,* 1994]. But fertilizers are costly and difficult to apply over large scales and may result in unintended ecosystem consequences. Thus, practitioners charged with cleaning up after oil spills dream of a "superbug"—one that's native to the contaminated environment and capable of removing all components of oil while also generating its own nutrients.

Nature may have provided just such an organism. Guided by metagenomic field data, GoMRI researchers patched together the genomes of microbes thought to be diazotrophs that also degraded oil in marine sediments. After looking at the potential metabolisms of these microbes, they isolated a particular microorganism from the field samples. They used hexadecane, a
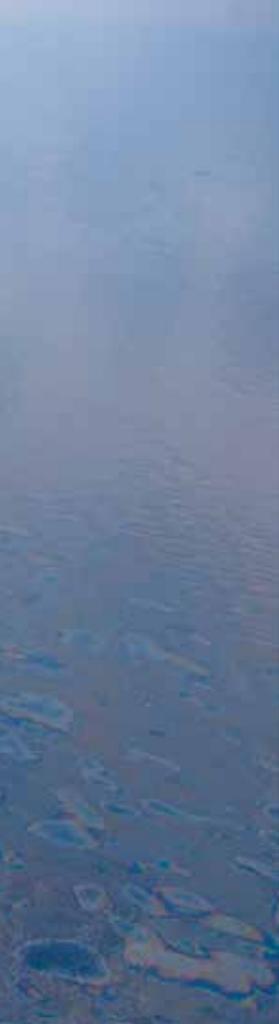
hydrocarbon, as the sole carbon and energy source and did not provide any nitrogen [*Karthikeyan et al.,* 2019]. Sequencing confirmed that the genome of the newly isolated microbe, KTK-01, contains genes that encode for nitrogen fixation and hydrocarbon degradation pathways as well as for biosurfactant production, all of which together facilitate growth in a nitrogen-limited, oiled environment.

Comparisons with genomes from publicly available data sets collected in other studies revealed that the newly isolated microbe—provisionally named *Candidatus Macondimonas diazotrophica* for the Macondo oil that was discharged during the DWH disaster—represents a novel genus of Gammaproteobacteria, a class that includes *Escherichia coli* and *Salmonella*, among many others. The screening also revealed a remarkable distribution of sequences identical or almost identical to those in KTK-01 in hydrocarbon-contaminated sediments from coastal ecosystems across the globe: Microbes with genomes matching this sequence often made up about 30% of their total communities but were almost absent in pristine sediments or seawater. *Macondimonas* thus appears to play a key ecological role in the natural responses to oil spills in coastal environments around the world and could prove to be a useful model organism for further studying such responses.

### Oil Contamination Biomarkers

The ultimate goal of GoMRI-supported genomic research is to translate genomic findings into actionable information to help scientists monitor and restore ecosystem health in the face of natural or human-made disasters. Through examination of the organisms, genes, and metabolic pathways present in microbial communities, researchers can take the pulse of an ecosystem and identify functional deficits or gains in the communities that affect the overall health of the ecosystem. Such genomic indicators serve as biomarkers to guide mitigation strategies, much like blood tests can point physicians to disease diagnosis and treatment options.

During the DWH response, microbial genomics techniques have demonstrated the potential to develop effective genetic proxies or biomarkers for recording oil inputs, exposure regimes, and hydrocarbon degradation. Oil-induced ecosystem disruptions were identified by a reduction in community diversity; an overgrowth of certain species; or the emergence of novel genes, metabolic pathways, and ecosystem functions. For example, *Macondimonas* was shown to dominate microbial communities in oiled beach sands, and a large increase in the abundance of nitrogen fixation genes signified nutrient limitation and disruptions to the nitrogen cycle initiated by oiling [*Karthikeyan et al.,* 2019]. Further, a decline in the abundance of chemolithoautotrophic nitrifying microorganisms in oiled sediments followed by the rebound of these microbes in recovered sands provided evidence of ecosystem recovery [*Huettel et al.,* 2018].

### Preparedness for Response and Restoration

Efforts supported by GoMRI to characterize responses of microbial communities in Gulf of Mexico ecosystems following the DWH oil spill generated knowledge with far-reaching impacts and spurred a wealth of discoveries. And newly developed tools and approaches have shown the proof of principle for deployment as part of the emergency response tool kit.

The need for ongoing research on these fronts is great because the risk of future oil spills like DWH remains as the petroleum industry continues tapping ultradeep marine wells for oil and gas production and because chemical dispersants—which may be toxic to organisms—remain the major response strategy. But lessons learned from DWH research so far can be applied to developing new mitigation strategies and improvements in predictive capabilities for responding to future environmental disturbances, such as those caused by extreme weather events or climate change.

For the first time, a data-driven approach for oil spill response and mitigation is possible. With advanced genomic tools and scientific expertise, microbiologists can quickly and inexpensively analyze field samples to provide essential information about microbial ecosystems before, during, and after spills.

We envision a future in which omics measurements enable assessment of environmental risks, identification of ecosystem deficits, selection of appropriate mitigation plans, and monitoring of ecosystem recovery and in which scientists play key roles in informing practitioners to improve response and restoration preparedness for future environmental disasters.

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