A child-centric microbiology education framework

Cold Seeps (including mud volcanoes!)

Were anyone else's socks damp this morning?
The groundsheet must have a leak and water seeped through it.

Photo by cottonbro from Pexels

Andreas Teske

Department of Earth, Marine and Environmental Sciences, University of North Carolina, Chapel Hill, North Carolina, USA

Cold Seeps

Storyline

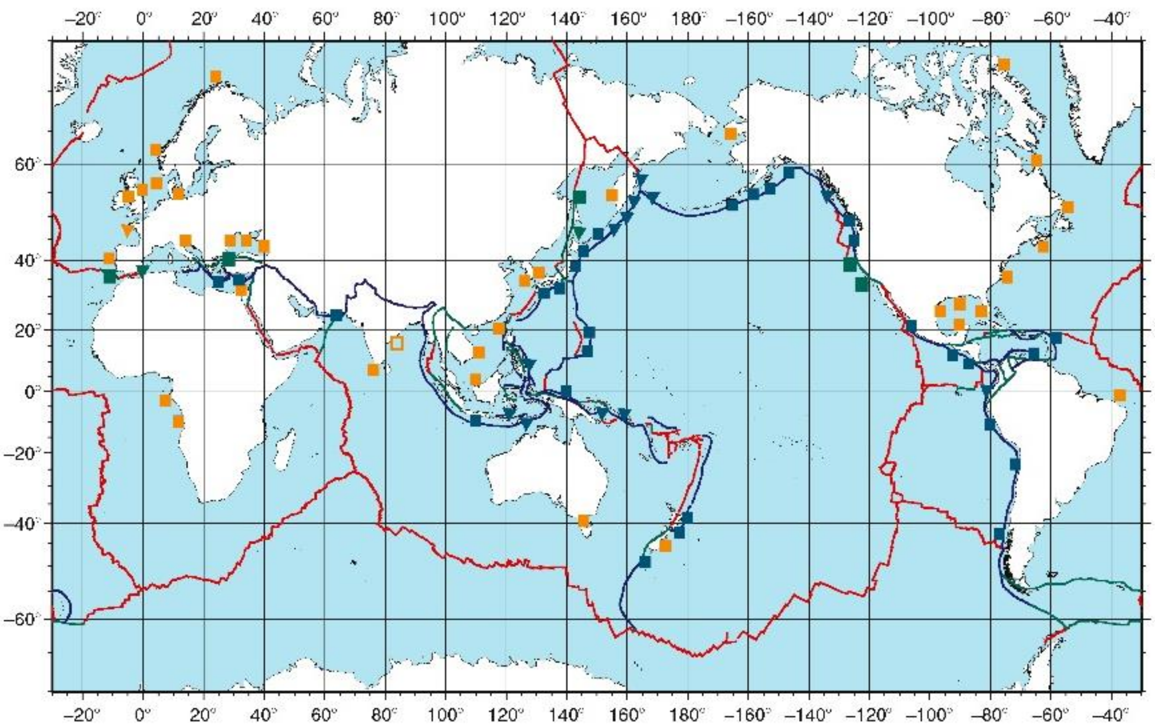
Cold seeps are places where fossil carbon produced in the deep subsurface reaches the surface in the form of gas and petroleum, and sustains unique microbes that use these specific carbon sources, also called hydrocarbons, as carbon and energy sources. Some oil- and gas-dependent microbes live as symbionts inside specialized marine animals that occur only at cold seeps, or they form associations where every microbe has a specific role in hydrocarbon degradation. Without hydrocarbon-degrading microbes, symbionts and microbial associations, oil and gas would accumulate everywhere in the biosphere, and Earth would turn into a toxic dump. Many of the best-studied cold seeps are located in the northern Gulf of Mexico, offshore Mississippi, Louisiana and Texas, and are frequently explored by submersible and deep-sea robots. Hydrocarbon-degrading bacteria in the Gulf of Mexico jumpstarted the microbial oil spill response right after the Deepwater Horizon disaster, and are indispensable in ecosystem restauration.

Cold Seeps: the Microbiology

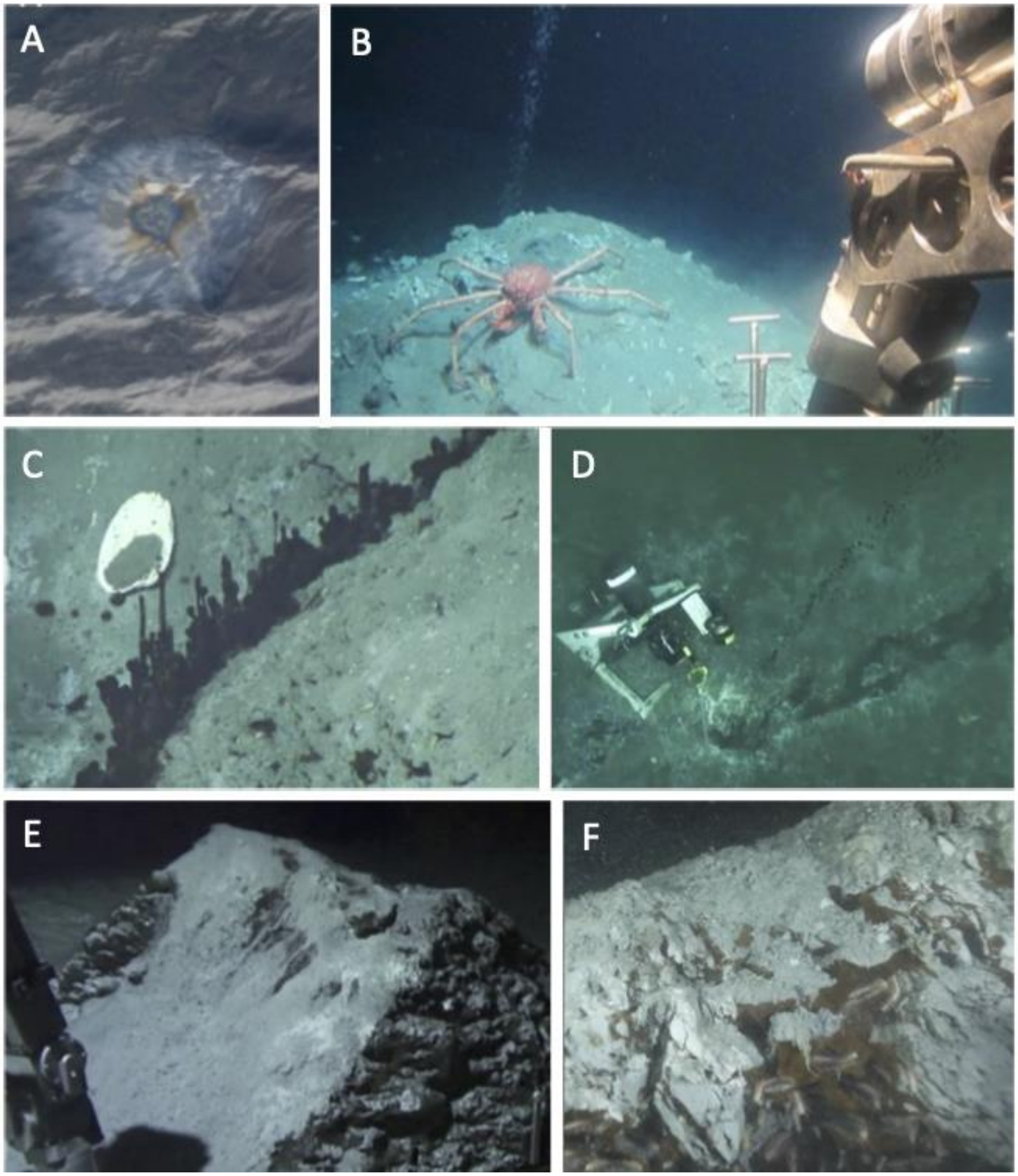
1. ***What are cold seeps?*** In some places, liquids are seeping from the underground that are a lot stranger than water seeping into a tent, and often they smell a lot worse! They contain weird mixtures of water, gases, petroleum and dissolved salts that are decidedly different from spring or rain water. These liquids come from the deep subsurface, hundreds of meters or even several kilometers below the surface, where organic matter from decaying and buried plants is degraded and chemically transformed into fossil carbon. Fossil carbon compounds are also called hydrocarbons because they consist only of carbon and hydrogen and have lost all other biologically important elements, for example nitrogen and phosphorus. This fossil carbon can take the form of gas, predominantly methane, with some ethane and propane (gases that burn easily on a camping stove), but also liquid hydrocarbons of variable composition that are collectively called petroleum. The places where these fossil carbon compounds are seeping from underground are called cold seeps, to reflect the fact that these fossil carbon mixtures are cold when they finally arrive at the surface after a long upward journey through cracks and fissures, although the deep subsurface where they originate can be quite hot.

Cold seeps can occur in many flavors, such as oil seeps and tar pits where petroleum is quietly seeping from the ground (a famous example is the La Brea tar pits in Los Angeles, which have entrapped many ice age animals), bubbly methane seeps that are dominated by gas emissions, and finally mud volcanoes when rising fluids carry a lot of sediment that emerges at the surface just like lava is emerging from a volcano. Many cold seeps contain also hydrogen sulfide, a foul-smelling and toxic gas that is produced by certain types of bacteria (sulfate- and sulfur-reducing bacteria) that degrade organic matter when oxygen is absent. Last but not least, cold seep fluids that make contact with buried salt layers take up salt and arrive at the surface as brines.

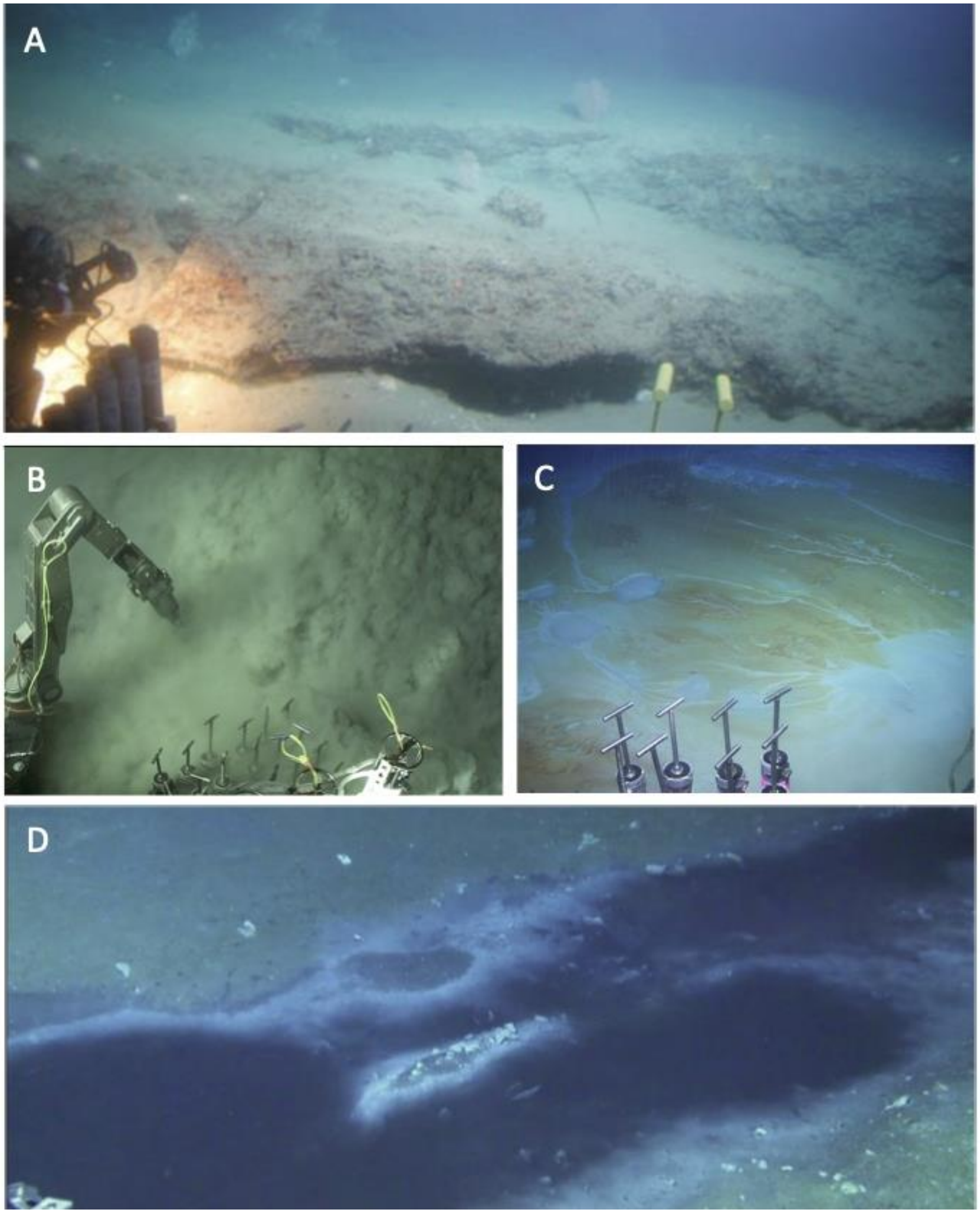
2. ***Where do cold seeps occur?*** Many cold seeps are located in the ocean, in an offshore ring around the continents where massive sediments and buried organic material have accumulated and set the stage for producing fossil carbon. Many cold seep sites harbor gas hydrates, an ice-like compound where gas molecules (mostly methane) are surrounded by water molecules in crystal-like structures that are only stable at cold temperatures and high pressure. Methane hydrates are the dominant form of fossil carbon on the planet. Perhaps the best-studied examples are in the northern Gulf of Mexico where cold seeps of all types extend all the way from Florida to Texas and Mexico.



Methane seep locations with well characterized chemistry, seep fauna and hydrocarbon-degrading microbes. Seeps on active continental margins, mostly around the Pacific Ocean, are indicated in blue and green, and seeps on passive margins, mostly in the Atlantic Ocean and the Gulf of Mexico, appear in orange. Red lines mark mid-ocean spreading centers, blue lines mark subduction zones. From: E. Suess, Marine Cold Seeps. Chapter 24 in Handbook of Hydrocarbon Microbiology, 2010. Edited by Kenneth Timmis. Springer.



Hydrocarbon seepage from seafloor to sea surface at Oil Mountain, the largest natural oil seep in the Gulf of Mexico. A) Oil droplet spreading in a rainbow-colored oil sheen on the sea surface after rising from the seafloor at 1200 m depth. B) Sediment-covered hydrate mound with shell hash, methane bubble stream and resident crab. C) Dark-brown viscous hydrocarbon fingers emerge from the sediment at the base of a petroleum-stained hydrate mound. Rising oil droplets detach periodically from the tips. D) Automated seafloor camera recording a stream of oil droplets emerging from sediment. E) A massive hydrate outcrop, ca. 2 m high. F) Close-up view with ice worms in the crevices of the brown-stained hydrate, contrasting with the light-grey sediment cover. Photos: A by A. Teske, UNC Chapel Hill; D and F by I. MacDonald, Florida State University; B, C and E by *Alvin* Group, Woods Hole Oceanographic Institution.

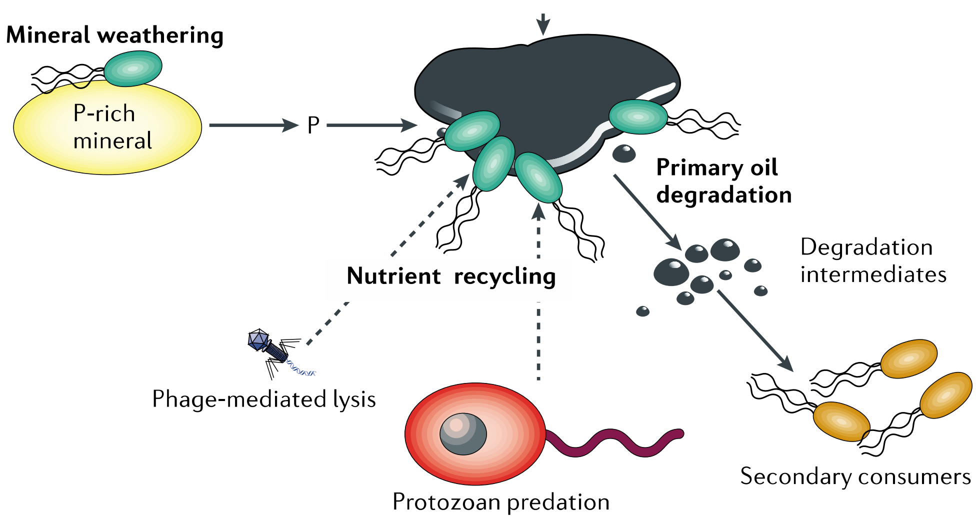


Methane hydrates, brine lakes and mud volcanoes in the Gulf of Mexico. A) The largest seafloor hydrate outcrop in the Gulf of Mexico, "Sleeping Dragon", at Mississippi Canyon, 900 m depth. B) Sampling a turbulent mud flow. C) Small mud volcanoes and mud flows, stained by small amounts of dissolved iron. D) Seafloor brines, formed by salt exclusion from hydrates, with sulfidic sediments forming a grey-whitish halo of sulfur precipitates. Photos: A by S.B. Joye, UGA Athens; B, C, and D, by *Alvin* Group, Woods Hole Oceanographic Institution.

3. ***Cold seep microbes: the specialists and generalists.*** Cold seeps harbor distinctive microbes that use hydrocarbons as carbon source and gain energy by respiration, just as other life forms (including humans) thrive by eating food and respiring with oxygen. Since hydrocarbons are tough substrates and difficult to degrade, microbes have special enzyme systems and biochemical pathways that deal specifically with hydrocarbons and degrade them ultimately to CO_2.

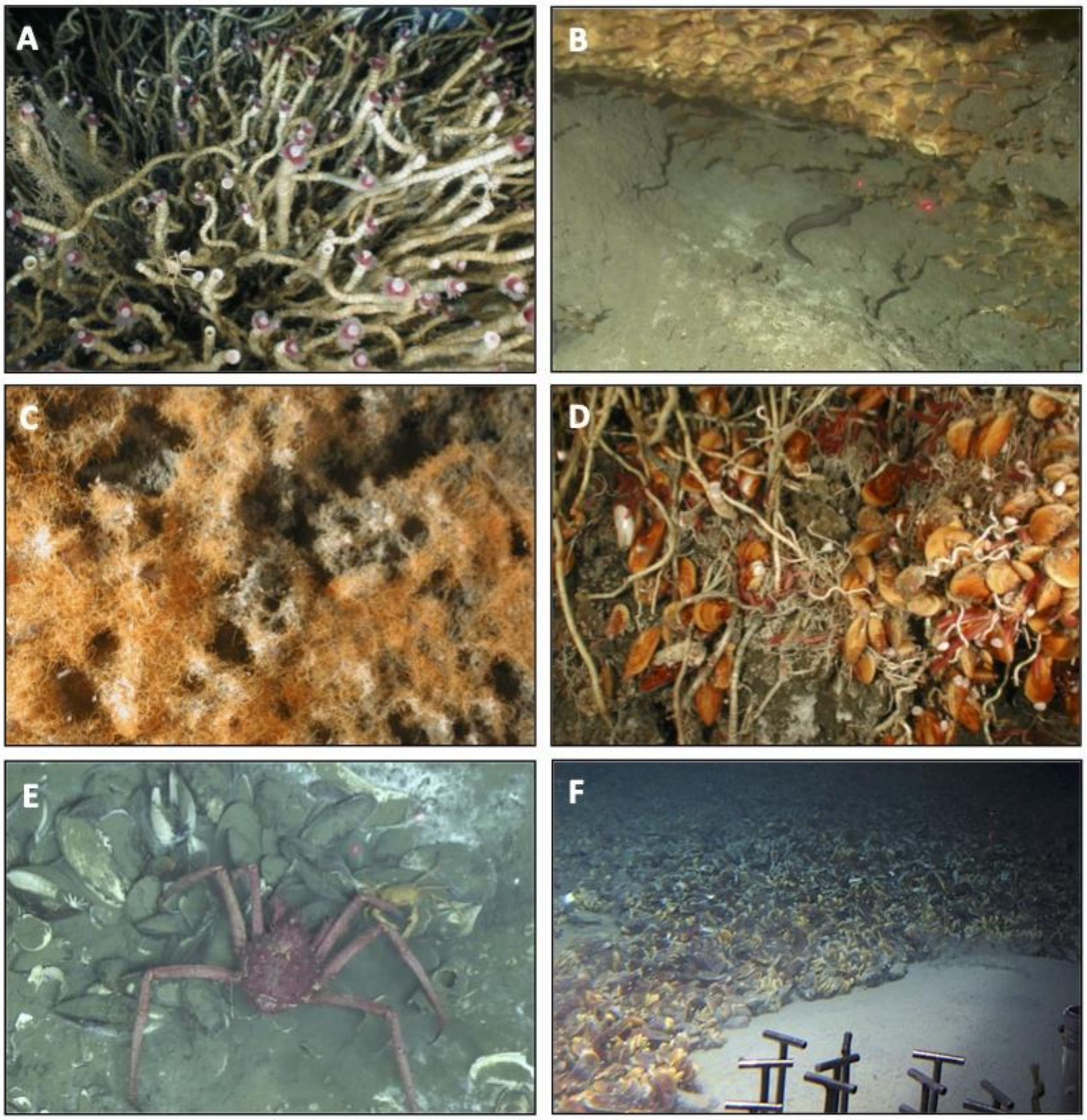
Since oxygen runs out very quickly under the sediment surface, many hydrocarbon-degrading microbes have specialized in anaerobic pathways that work without oxygen, for example by respiring not with oxygen but with sulfate, a common salt ion in seawater. Depending on the types of hydrocarbon molecules that are available, different microorganisms are selected that are specialized to crack specific types of hydrocarbon molecules. Although these capabilities are specific and work only for one type of substrate but not for others, the hydrocarbon-degrading microbial community consists of so many different types of microbes taking turns for each other that ultimately no hydrocarbon compound escapes microbial degradation entirely.

As soon as hydrocarbon-degrading specialists have performed the biochemically most challenging reactions, many reaction products are easier to degrade, and microbial generalists with a more widely applicable biochemical repertoire then participate in this second layer of hydrocarbon degradation and create a microbial food web that distributes the hydrocarbon-derived carbon resources throughout the entire microbial ecosystem. Interestingly, hydrocarbon-degrading microbial partnerships can become quite specialized, in the sense that one particular microbe performs the initial steps while a particular partner complements its function, for example by removing a degradation product that would otherwise build up, or by performing an energy-gaining respiration step that the primary hydrocarbon degrader by itself cannot do. These microbial partnerships are called syntrophic associations and play a major role in hydrocarbon degradation.



A microbial degradation network. The network indicates that oil biodegradation involves more biological components than just the microorganisms that directly attack oil (the primary oil degraders) and shows that the primary oil degraders interact with these components. Oil-degrading bacteria are shown in green. Solid arrows indicate material fluxes, and broken arrows indicate direct interactions (for example, virus infection followed by cell lysis, and predation by protozoa). From Head et al., 2006.

4. ***Unique animals specialized for life around cold seeps.*** Marine hydrocarbon seeps harbor unusual animal life, for example worms and mussels that harbor hydrocarbon-degrading symbiotic bacteria which, by degrading hydrocarbons, provide microbial biomass and nutrition for their host animals. The animals are so dependent on their hydrocarbon-degrading symbionts that they would not even survive outside their seep habitat. Some inhabitants of hydrocarbon seeps are even specialized to particular kinds of hydrocarbon seeps; while some seeps release gases, such as methane or light alkanes, others emit liquid petroleum or even solid hydrocarbons, such as asphalt.

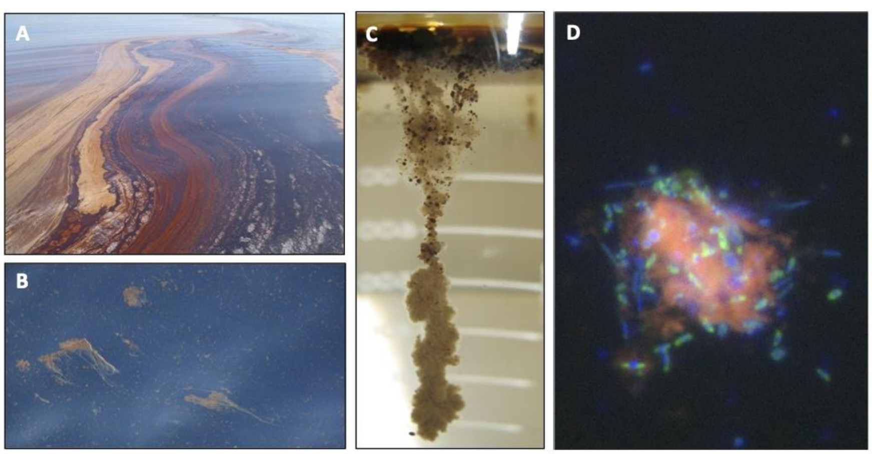


Cold seep fauna in the Gulf of Mexico. A) Tube worm colony harbor sulfide- and methane-oxidizing symbiotic bacteria, red gills are emerging from worm tubes. B) Methane hydrate with specialized worms that graze on methane-oxidizing bacteria (*Alvin* dive 4567). C) Sulfide-oxidizing bacterial mats in close-up. D) symbiont-bearing mussels and tube worm roots .E) crabs feeding on symbiont-dependent clams (*Alvin* dive 4696). E) Massive colony of symbiotic mussels (*Alvin* dive 4641). Photos A, C, D by I. MacDonald, Florida State University. Photos B, E, F by Alvin group, Woods Hole Oceanographic Institution.

New types of hydrocarbon-degrading symbioses are still being discovered, and we do at present not even know how many hydrocarbon-dependent symbiont-bearing animals exist and how they contribute to marine ecology. Thus, hydrocarbon seeps should be protected to preserve this unique biodiversity that occurs nowhere else on Earth.

5. Hydrocarbon-degrading microbes protect the biosphere from oil suffocation. Bacteria are the only organisms that can consume methane, hydrocarbons and petroleum; therefore they are essential for the removal of methane, and for the cleanup of hydrocarbon contamination and oil spills. Without bacteria that degrade hydrocarbons, oil spills would last forever and spread uncontrollably since human cleanup efforts simply cannot reach all places to which oil spreads. The environmental effects of major accidents, such as the Deepwater Horizon oil spill, would last many times longer than they did – perhaps indefinitely longer - in a world without microbial oil degradation. Only microorganisms can spread everywhere and follow expanding hydrocarbon contamination in water and soil. For example, to clean up a petroleum-contaminated beach would require washing practically every single oil-coated sand grain. And, even if this were possible, the contaminants would be in the wash, and the problem of what to do with the spilled oil would still remain. Only oil-degrading bacteria can change the oil to CO_2 and microbial biomass, and by reaching every single oil-coated sand grain they can – given enough time – truly remove the hydrocarbons.

6. Cold seeps are natural reservoirs of primed hydrocarbon-degrading microbes, ready and eager to deal with human oil spill accidents. Hydrocarbon seeps are natural oil spills where hydrocarbons migrate from the deep underground to the surface reservoirs where hydrocarbon-degrading bacteria thrive and stand ready to spread through the ocean once an oil spill occurs. Marine areas with natural hydrocarbon seeps are considered "primed" for such rapid microbial community responses, for example the seep-rich Gulf of Mexico where microbial degradation got underway within two weeks after the start of the Deepwater Horizon oil spill.



Oil-degrading bacteria in the Gulf of Mexico. A) Oil from the Deepwater Horizon oil spill floating at the sea surface near the wellhead. B) Microbial flocs growing on floating oil droplets forming within a few days of the onset of the oil spill. C) Microbial flocs grown in the lab in seawater with a few drops of Deepwater Horizon oil. D) Microscopic image of oil-degrading bacteria, stained blue and green, growing around an oil-soaked particle in pink. Photos A, B, D by L. McKay; Photo C by K. Ziervogel, UNC Chapel Hill.

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 14. Conserve and sustainably use the oceans, seas and marine resources for sustainable development**. Petroleum oil pollution of marine systems occurs naturally, via cold seeps, and through human activities, principally, the extraction of oil from offshore reservoirs and its transportation via shipping. Hydrocarbon-degrading microbial communities around cold seeps have evolved to handle the amounts released, so largely prevent oil-mediated ecological damage. In contrast, petroleum oil released through accidents, especially in locations remote from cold seep sites, causes massive ecological damage of marine wildlife. Eventually, however, hydrocarbon-degrading microbes from cold seeps, and from oil-producing micro-algae and cyanobacteria, reach the oil, multiply, degrade it and thus begin to mitigate further ecological damage. Without the hydrocarbon degraders, some regions of marine systems would become oil:water emulsions with none of the animal life that we know and treasure.

The Evidence Base, Further Reading and Teaching Aids

"The Adventures of Zach and Molly", by Jim Toomey, award winning cartoonist of 'Sherman's Lagoon', an educational cartoon series that promotes ocean education and ocean literacy. Best for grade school. https://ecogig.org/zackandmolly

"Beyond Blue" by E-Line Media, an ocean-themed video game that provides an immersive experience exploring and learning about the ocean, ocean biology, and ocean conservation. https://www.youtube.com/watch?v=MEi2vA3kyFI

BBC Blue Planet II - The Deep. Drop-dead gorgeous.

The frame grabber system of deep-sea submersible *Alvin*, accessible for everyone, provides a clickable photo tour of *Alvin* dives since 1988, with one photo every 30 seconds. Something for geeks: Not edited or filtered and therefore authentic *Alvin* geek fare, but you have to search for the gems. Image quality improves in recent *Alvin* dives, especially since 2014. http://4dgeo.whoi.edu/alvin

Schmidt Ocean Institute provides high-resolution curated deep-sea images from ROV expeditions around the world, has themed expedition records archived, and provides real-time links to ongoing expeditions on R/V *Falkor*. https://schmidtocean.org

The Ocean Exploration Trust provides high-resolution curated deep-sea footage from ROV expeditions around the world, has an archive of themed video collections (in my opinion, by far the best in existence), and provides real-time links to ongoing expeditions on R/V *Nautilus*. (https://nautiluslive.org/expedition). Especially the 2015 Gulf of Mexico Expedition and 2021 Cascadia margin expedition provide excellent cold seep footage. Pick expeditions of interest on the index site: https://nautiluslive.org/expedition-index

Davidova I.A., C.R. Marks, J.M. Suflita. 2018. Anaerobic Hydrocarbon Degrading *Deltaproteobacteria*. In: McGenity T. (eds) Taxonomy, Genomics and Ecophysiology of Hydrocarbon-Degrading Microbes. Handbook of Hydrocarbon and Lipid Microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-60053-6_12-1

Dubilier, N., C. Bergin, and C. Lott. 2008. Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. Nature Reviews Microbiology 6:725-740. (*Everything about microbial symbiosis in cold seeps, hot vents, and many other ecosystems*)

Fisher, C., H. Roberts, E. Cordes, and B. Bernard. 2007. Cold seeps and associated communities of the Gulf of Mexico. *Oceanography* 20(4):118–129. https://doi.org/10.5670/oceanog.2007.12. (*informative and concise*)

Head, I.M., D.M. Jones and WF.M. Roling. 2006. Marine Microorganisms make a meal of oil. Nature Reviews Microbiology 4:173-182. (*Still the most readable and perceptive introduction to oil-degrading microbes*)

Kniemeyer, O., F., Musat, S.M. Sievert, K. Knittel, H. Wilkes, M. Blumenberg, W. Michaelis, A. Classen,C. Bolm, S.B. Joye, F. Widdel. 2007 Anaerobic oxidation of short-chain hydrocarbons by marine sulphate-reducing bacteria. Nature 449:898–901. (*Pioneering microbiology and biochemical study on novel hydrocarbon-degrading bacteria and their pathways*)

Joye, S.B. 2019. The Geology and Geobiology of Hydrocarbon Seeps. Annual Review of Earth and Planetary Science, https://doi.org/10.1146/annurev-earth-063016-020052. (*emphasizes the interaction of geology, chemistry and biology*)

MacDonald, I.R., F.J. Reilly, J.N.L. Guinasso, J.M. Brooks, R.S. Carney, W.A. Bryant, T.J. Bright. 1990. Chemosynthetic mussels at a brine-filled pockmark in the northern Gulf of Mexico. Science 248:1096–1099. (*The discovery of hydrocarbon-fueled symbiosis*)

Suess, E. 2010. Marine Cold Seeps. Chapter 24 in Handbook of Hydrocarbon Microbiology, 2010. Edited by K. Timmis. Springer. (*Good Introduction from a geological perspective*)

Teske A. 2019. Hydrocarbon-degrading anaerobic microbial communities in natural oil seeps. In: McGenity TJ (ed), Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology, Handbook of Hydrocarbon and Lipid Microbiology. Springer, Cham. doi: 10.1007/978-3-319-60063-5_3-2

Teske, A., and S.B. Joye. 2020. The Gulf of Mexico: An introductory survey of a seep-dominated seafloor landscape. Pp. 69-100. In: Marine Hydrocarbon Seeps - Microbiology and Biogeochemistry of a Global Marine Habitat (eds. A. Teske and V. Carvalho). Springer. (*a guided tour of the northern Gulf of Mexico cold seeps, in an amply illustrated book on marine cold seeps and their microbial inhabitants. Get the online version for color)*

Glossary

Brine. Saline water, of higher salinity than seawater, with a salt composition that reflects its geological origin from dissolving subsurface salt deposits. The massive Jurassic-age salt layers that are embedded in the sediments underlying the northern Gulf of Mexico produce numerous brines that rise to the seafloor where they form brine pools or brine lakes which do not mix with the overlying seawater. Since the Gulf of Mexico brines contain subsurface hydrocarbons, many brine lakes are surrounded by cold seep fauna with hydrocarbon-utilizing bacterial symbionts. Curious marine animals face instant pickling when they dive or step into a brine pool. Pickled fish, crabs etc. are a common sight; their degradation by salt-tolerant bacteria imparts a rich menu of organic substrates and biogenic odors to a brine lake, which turns it into an attractive source of nutrients and at the same time a trap for marine life.

Continental margin: transition zone at the edge of continents where the shallow (0-200m) continental shelf drops towards the deep ocean basins (3000-4000 m) or abyssal trenches (>6000m). Due to high photosynthetic productivity and terrestrial runoff, continental margin sediments accumulate buried organic matter that is transformed at depth to fossil carbon, gas and petroleum, and then migrates to the surface along fault lines that fracture the sediment layers.

Hydrocarbon. A chemical compound that consists of the elements carbon and hydrogen, excluding other biologically important elements. Fossil carbon is dominated by hydrocarbons.

Methane. The simplest and most abundant hydrocarbon in nature, consisting of one carbon atom linked to four hydrogen atoms (CH_4). Methane can be produced by high-temperature degradation of more complex hydrocarbons (thermal cracking), or it can be produced microbially from hydrogen and CO_2, or from simple organic compounds (acetate, methanol, formate) by methane-producing (methanogenic) microorganisms, members of the archaea.

Hydrate. Ice-like crystal where a gas molecule (in nature, mostly methane) is surrounded by a cage-like arrangement of water molecules. Hydrates are stable only under a combination of high pressure and cold temperatures, as found in deep marine sediments, or closer to the surface in permanently cold arctic soils (permafrost). Methane hydrates are the most abundant form of fossil carbon on Earth, but are increasingly destabilized by globally warming temperatures; melting hydrates send methane gas into the ocean and finally into the atmosphere where it acts as a powerful greenhouse gas.

Fossil carbon. Biomass of photosynthetic origin (produced by plants) that has accumulated in deep sediments where oxygen is excluded, and under high pressure and temperature has been transformed into chemically distinct compounds that are dominated by carbon and hydrogen, whereas other biologically essential elements (Nitrogen, phosphorus, to a lesser extent sulfur) have been lost. Fossil carbon can occur as gas (methane, ethane, propane etc), as liquid petroleum, or as solid residues (kerogen). Fossil carbon serves as fuel and chemical raw material for the current (unsustainable) stage of human civilization; biologically it is hard to degrade and requires specialized microbes.

Petroleum. General term for complex mixtures of liquid hydrocarbons in nature, and the source for refined liquid hydrocarbon fuels of standardized composition. Petroleum seeps are perhaps the oldest source for hydrocarbons used by humans, seeps of viscous petroleum (bitumen) were tapped as waterproofing material in ancient Mesopotamia.

Sulfide, or more accurately hydrogen sulfide: Dominant sulfur compound (dissolved as S^{2-}, HS^-, or as the gas H_2S) in cold seep fluids, very toxic when inhaled but a favored energy source for many microorganisms, produced from elemental sulfur or seawater sulfate either geothermally or by microbial activity of sulfur- and sulfate-respiring microbes. The gas hydrogen sulfide (H_2S) has an unmistakable odor of rotten eggs or very ripe saltmarsh sediments; while it is certainly not suitable for classroom experiments, marine microbiologists regard it positively as an indicator for a thriving microbial ecosystem.

Symbiosis. The association of two different species for mutual benefit. In cold seeps, symbiosis appears in some of its most extreme and interesting forms, when a marine invertebrate (clams, mussels, diverse worms) harbors hydrocarbon-degrading bacteria in its own body tissues; for example, mussels and clams harbor these bacteria in the epithelial cells of their gills. The bacteria produce biomass that provide nutrition of the host animal, and the host provides a stable environment for the microbes, since it can adjust the supply of their carbon substrate (in most cases, methane) and oxygen.

Syntrophy, or syntrophic association: An association of commonly two types of microbes with distinct but complementary physiological capabilities. One microbe degrades a substrate and produces a compound that is taken up and utilized by the second microbe; by acting jointly, the microbes perform a process that would be impossible to achieve by either of them on their own. Chemically challenging processes, for example hydrocarbon degradation, are often the domain of syntrophic partner microbes, or syntrophs.