INNER WORKINGS

Research sub buoys prospects for 3D map of marine microbial communities

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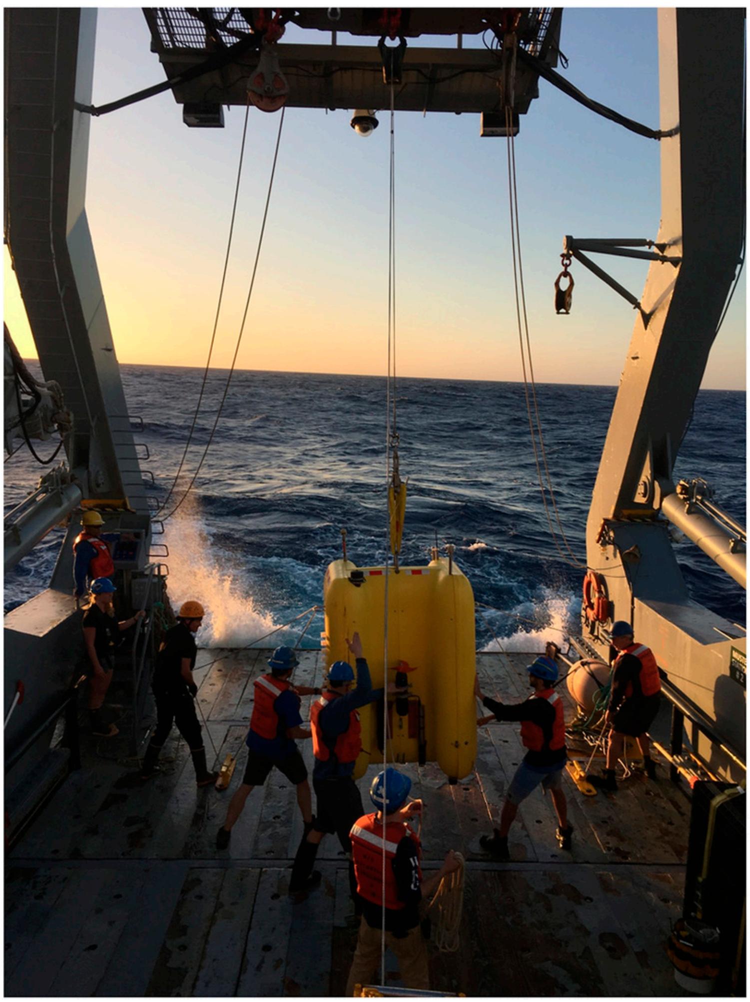
Marine microbes are the foundation of ocean food webs; they are the workhorses that convert carbon, nitrogen, and other essential nutrients into bioavailable forms for all other life in the oceans (1, 2). But only about 10% of these bacteria, archaea, viruses, protists, and fungi can be cultured in the lab—the vast majority must be sampled directly from the ocean (3). Since sampling is expensive and time consuming, research is limited on most ocean microbes. As a result, the water column locales of microbe-driven biogeochemical processes haven't been thoroughly mapped.



The Clio autonomous underwater vehicle, or AUV, is already unlocking secrets of the ocean's microbial and chemical composition. The AUV's unusual shape is streamlined for vertical motion through the water column. Image credit: Mak Saito (Woods Hole Oceanographic Institution, Woods Hole, MA).

Thus far, the tools at researchers' disposal have fallen short. Researchers would like to analyze microbial proteins, DNA, and RNA, as well as sugars, vitamins, and other small organic molecules. And because microbial communities vary at fine spatial scales and can change rapidly from meter to meter throughout the water column, researchers need to be able to sample seawater from precise depths.

An autonomous submarine called Clio could be the answer. Biogeochemist Mak Saito at the Woods Hole Oceanographic Institution in Woods Hole, MA, and his collaborators developed the 6-foot-tall, bright yellow, refrigerator-shaped submarine beginning in 2014. Unmanned and untethered, the autonomous underwater vehicle, or AUV, is among the first specifically designed to sample microbes for studies of marine biogeochemistry. Saito hopes the coming years will reveal a wealth of data that allow researchers to explore the ocean's changing microbial and chemical composition in high resolution.

Clio could become an integral tool for BioGeoSCAPES, a nascent program planning to study microbial, biological, and chemical oceanography, including making a three-dimensional map of marine microbial communities and ocean chemical cycling over the next decade. Acquiring such a map could have important implications—from elucidating the locations of metabolic pathways that help facilitate the release of potent greenhouse gases, to reducing the oxygenation of seawater, to revealing novel processes and compounds for medicine or pollution remediation.

Sample Size

Already, Clio is at work collecting samples. In October 2018, Saito led the sub on a research cruise 60 miles off the coast of Bermuda. Unpublished preliminary

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A variety of diving robots survey aquatic ecosystems. Here the long-range AUV Tethys patrols Lake Erie to detect harmful cyanobacteria that can poison drinking water. Image credit: Ben Yair Raanan © 2019 Monterey Bay Aquarium Research Institute.

analysis of Clio's samples from the expedition reveals that cyanobacterial protein expression varies with depth, hinting at different chemical processes throughout the water column, Saito says. His recent findings are not the first to examine protein expression in marine microbes. But past methods to collect these microbes were much more time and labor intensive, relying on pumps lowered over the side of a ship on a cable. The new submarine, with many more high-volume samples per cruise, should help researchers more quickly see widespread patterns.

Before Clio, a few other diving robots carried similar scientific instruments, and other ocean-going robots did also sample marine microbes. For instance, one 2019 study used two long-range AUVs and a surface robot to map and sample open ocean phytoplankton communities near Hawaii (4). These and other AUVs built at California's Monterey Bay Aquarium Research Institute contain shoebox-sized laboratories, which can sample, filter, preserve, and in some cases analyze genetic material from seawater, all while the submarine is diving. The Monterey AUVs aren't solely dedicated to one thing. Sonar or video sensors can also outfit the subs for wildlife research, and the vehicles can swap instruments to serve different research purposes.

Clio was designed for the singular purpose of sampling large volumes of seawater vertically through the water column for biogeochemical analyses, explains project leader John "Chip" Breier, a geochemist and engineer at The University of Texas Rio Grande Valley campus, in Port Isabel, and adjunct at Woods Hole Oceanographic. Although each Clio vehicle costs several hundred thousand dollars to build, the sub should pay for itself quickly in saved time, Saito adds. And hence, it should facilitate more thorough mapping of microbial communities, and their behaviors, throughout the water column.

Textbook diagrams of biogeochemical cycling often use arrows to illustrate the transition of one compound into another from land to sea to atmosphere. "But what you don't realize is that every one of those arrows is actually the metabolism of microbes," says microbial oceanographer Alyson Santoro at the University of California, Santa Barbara. Microbes are behind the scenes, driving these major planetary cycles. The marine carbon cycle, for example, involves photosynthetic organisms called phytoplankton that drift near the ocean's surface, where they combine water and light to build their tissues with organic carbon. Predators such as heterotrophic protists eat the phytoplankton, passing the carbon up the food chain, and release waste into the water, including nitrogen, phosphorous, iron, copper, and zinc. In this way, the estimated 3.1×10^{28} individual bacterial cells and 1.3×10^{28} individual archaea drifting through the seas each change the chemistry of their immediate surroundings and collectively change the chemistry of whole oceans (5).

Researchers want to know which chemical cycles each kind of microbe influences, especially in the lesser-studied deep ocean, and at what spatial scales. Over the next decade, potential BioGeoSCAPES data will set a baseline to monitor how the oceans may change in the future.*

Careful Collection

One way to understand which chemical cycles microbes influence is to find the proteins that different microbial populations are expressing; they're often enzymes converting nutrients between organic and inorganic forms. However, collecting marine microbes for protein analysis can be difficult. Unlike DNA and RNA, which researchers can amplify from a few liters of seawater, proteins instead must be isolated from cells directly. Individual marine microbial cells are so tiny and dilute that collecting enough of them for protein analysis requires filtering tens to hundreds of liters of seawater.

Traditionally, biogeochemists have collected high-volume seawater samples by lashing together a series of large water pumps and lowering them over the ship's side on a mile-long, heavy cable, Saito explains. The pumps drop to depth and filter seawater over a polymer mesh for a few hours, snaring microbes in the mesh's fibers. Saito and Santoro coauthored a recent study in *Nature Geoscience* using this traditional approach to filter microbes from thousands of liters of seawater on two expeditions to the Central Pacific in 2011 and in 2016 (6).

The cruises sampled microbes from stretches of open ocean with low concentrations of oxygen in the mesopelagic zone, between 200 and 1,000 meters deep. These oxygen-minimum zones are known to contain bacteria and archaea that breathe chemicals besides oxygen and produce nitrogenous byproducts. Many reactions involved in the marine nitrogen cycle happen in oxygen minimum zones. Hence, Saito and collaborators set out to assess the relative abundance of the microbial proteins known to catalyze nitrogen reactions at these sites.

The researchers found that the enzyme nitrite oxidoreductase, which converts nitrogen from animal waste into less-toxic inorganic forms, is surprisingly abundant in oxygen-minimum zones in the Central Pacific. Nitrite oxidoreductase also uses a lot of iron, Santoro says. Iron is typically studied in surface waters, where sunlight drives photosynthesis, a process that can be iron-limited. These findings suggest that deeper areas may warrant closer study in the marine iron cycle, perhaps revealing ways that the nitrogen and iron cycles are intertwined.

Diving In

The *Nature Geoscience* study combined data from two cruises, one in 2011 and one in 2016. But if Clio had been available, Saito could have collected more samples, more efficiently, and dove deeper with less ship time, he says.

The new AUV works by pumping seawater over filters, explains lead engineer Michael Jakuba of Woods Hole Oceanographic. Inside Clio's sunny yellow plastic kayak skin, an aluminum skeleton holds high-volume water pumps, called SUPR (Suspended Particulate Rosette) samplers. The samplers filter biological and chemical samples from a range of depths throughout the water column.

Once Clio finishes a dive, surfaces, and is hoisted back onto the ship, researchers carry the SUPR samplers into an onboard clean room that Saito affectionately calls "the bubble." In lab coats and gloves, the oceanographers slice each filter into small pieces then freeze the pieces in hundreds of cryovial tubes for future proteomics, genomics, metallomics, and other analyses, he says. Back in his lab in Woods Hole, Saito isolates proteins from the filtered microbial cells. He uses chemical solutions to digest the mix of proteins into smaller peptides and finally injects the peptides into a mass spectrometer. Computational analysis helps identify the original protein.

Before the pandemic, Clio was headed to the tropical Pacific next year, to sample microbes from low oxygen zones 300 to 1,000 meters deep. That expedition is now possibly delayed to 2022. "We know these oxygen-minimum zones exist throughout the ocean in certain regions, but we know little about them," says biological oceanographer Maria T. "Maite" Maldonado at the University of British Columbia in Vancouver, Canada. She was one of the conveners of the 2018 planning workshop for the potential future BioGeoSCAPES program. She hopes that Clio will offer up an unprecedented window on those zones' microbial communities and their influence on nutrient cycling through the water column. Previous research has shown that oxygen-minimum zones release the potent greenhouse gas nitrous oxide (7). And studies show that over the last several decades, oxygen-minimum zones have expanded in size as a result of ocean warming (8), taking up a wider layer of the water column and hypothesized to lead to less-productive fisheries in the United States, Chile, and India, among other countries (9).

Clio does have limitations. It can dive as deep as 6,000 meters, beyond which its housings would implode under the pressure, Jakuba says. In the future, he hopes to update Clio's design to allow it to dive farther, into deep ocean trenches where it could discover new ocean biogeoscapes. There is no comparable instrument to collect biological samples from trenches, Maldonado says. "Who knows," she adds, "maybe with Clio we will discover new environments we don't know about right now."

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