Molecular Investigations of Marine Microbial Ecology

Microorganisms are major mediators of energy and nutrient cycling in the sea. Although a diverse group of organisms are responsible for these processes, current methods for identifying microbial species and linking them to specific biogeochemical and biological cycles are not well developed. The underlying goal of this project was to apply sensitive and specific molecular biological tools to identify, detect, and quantify microorganisms, and relate them to the biogeochemical processes that they mediate. The organisms studied span the taxonomic spectrum from eukaryotic plankton to prokaryotes to viruses. One of the major goals of the investigators was to integrate their technological developments with those of Chavez et al. (Biogeochemical responses to changes in climate and ocean circulation) to further our understanding of the relationships between microorganisms and larger scale ocean physics, chemistry, and biology.

Principal Investigators

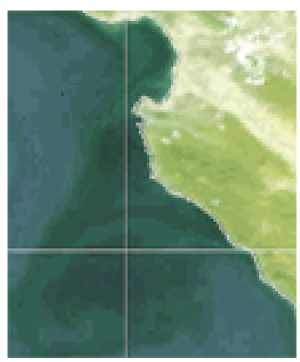
Ed DeLong

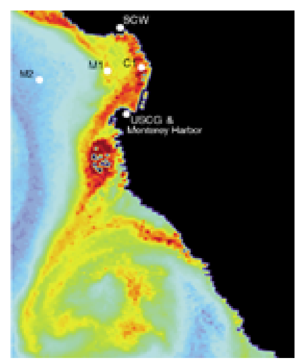
Chris Scholin

Development and application of DNA probes and novel instrumentation for detection and quantification of toxic and nontoxic phytoplankton

A "harmful algal bloom" (HAB) can be thought of as a patch of microalgae (phytoplankton) in the water that through one means or another negatively impacts the health of humans or aquatic wildlife. Disastrous economic impacts often follow in the wake of an HAB event. Challenges associated with the study of HABs underscore a long-standing problem in ocean science: How do we identify and count microorganisms in discrete samples that harbor a great diversity of species, particularly when rapid analysis of a large number of samples is desired routinely? In response to this problem, researchers at MBARI have developed a suite of species-specific DNA probes that allow one to detect, with considerably less effort and higher fidelity than traditional microscopy-based techniques, several different groups of toxic algae that occur along the shores of the U.S. and other countries. The DNA probe tests were designed so as to be useful to the widest range of workers possible. Minimal technical training and laboratory facilities are required to use these tests, and per sample analytical costs have been minimized. The probes are now in use in a number of laboratories around the world. MBARI researchers are developing an instrument that will conduct the probe tests autonomously, *in situ*, thus providing for real-time species detection from remote locations.

Within Monterey Bay, MBARI researchers used the probes to detect a toxic diatom bloom that, for the first time, was linked to the illness and death of California sea lions. The probes provided researchers with the ability to map the distribution of the cells, and through collaboration with workers at the National Oceanographic and Atmospheric Administration's





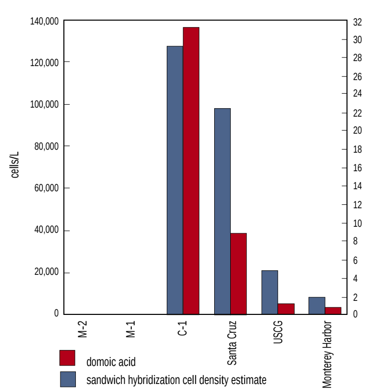


Figure 3. [top left] May 17, 1998 true color satellite image of the California coast during the bloom of toxic diatoms (*Pseudo-nitzschia australis*).

Figure 4. [top right] SeaWIFS image showing Monterey Bay and chlorophyll/sediment signature.

Figure 5. [left] Distribution of *P. australis* cells and associated toxicity during the peak of the bloom.

(NOAA) Marine Biotoxins Laboratory in Charleston, South Carolina, their associated toxicity was revealed as well. When this information was combined with satellite imagery, it was apparent that the bloom of toxic algae occurred in a narrow band along the shore (Figure 4). Chemical and physical properties of this plume suggest a water mass enriched with nutrients washed into the nearshore zone from excessive El Niño-driven rain. A consortium of scientists from MBARI, NOAA, the University of California, the Marine Mammal Center, California Department of Health Services, Colorado State Diagnostic Laboratory, and National Wildlife Health Center, along with others, demonstrated that the toxic algae were consumed by anchovies. When sea lions fed on those fish they acquired the toxin, and in turn suffered from its ill effects.

Development of methods for detection, quantification, and biogeochemical characterization of dominant planktonic prokaryotes

Despite their high abundance and ecological significance, a large fraction of marine microorganisms remains virtually unknown and unstudied. This is because many, perhaps most, naturally occurring microorganisms cannot be readily identified using standard methods. Several new technologies were developed for identifying and quantifying marine microbial species and applied in the water column and in marine sediments. One new technique developed in 1998 used new types of RNA probes, to microscopically visualize picoplankton, the small microorganisms in the 0.2 to 2.0 micrometer size range. The sensitivity of the new technique allowed for routine quantitation of several planktonic microbial groups never before quantified or visualized microscopically. Newly discovered picoplankton taxa (known as archaea) were shown to be abundant, regular components of the Monterey Bay microbiota. One archaeal group exhibited considerable variability, blooming in surface waters in response to changing conditions. The MBARI-developed microscopic technique was robust enough to share with other researchers at the University of Hawaii, who are now using it to track similar microorganisms in the Central North Pacific gyre, at the Hawaii Ocean Time Series site.

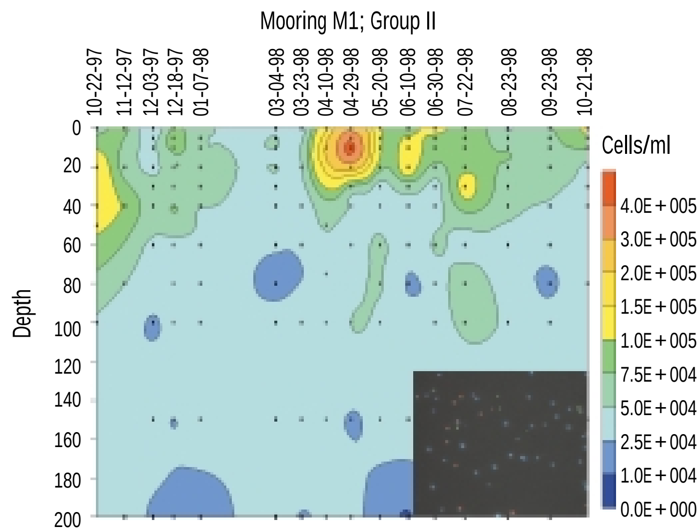


Figure 6. Contour plot of planktonic archaea (Group 2) at station M1 in Monterey Bay. [Inset] Identification of specific picoplankton (100 m depth, station M1) using molecular probes. Red and green cells represent different planktonic archaea types. The blue stain depicts the bacterial cells in the field.

Benthic microbes are also poorly characterized, and their activities not thoroughly understood. Using a new, multidisciplinary approach with collaborators at Woods Hole Oceanographic Institution (WHOI), analysis of sediment microbes has provided new insights into a globally important but poorly understood process, anaerobic methane oxidation. Until now, the specific microorganisms responsible for this pervasive biogeochemical process were unknown. Molecular biological analyses conducted at MBARI identified a major new microbial group responsible for anaerobic methane oxidation in marine sediments. Parallel analyses of stable isotope composition, conducted collaboratively at WHOI, provided compelling evidence that these new microbes are indeed responsible for anaerobic methane oxidation. This breakthrough is now providing data that will facilitate studies of the globally important, but enigmatic process of anaerobic methane oxidation in the sea.