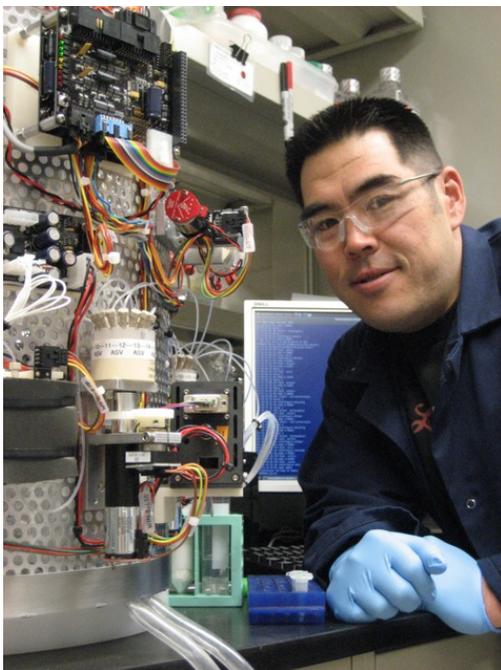




Ecogenomic sensors built for ocean research offer a new paradigm for assessing water quality

Poor water quality that arises at the land-sea interface is a primary challenge to human health. People who swim in sewage-polluted waters are subject to a slew of pathogens that can cause everything from gastrointestinal and respiratory illnesses to ear, nose and throat infections and skin rashes. The health costs associated with gastrointestinal illnesses caused by contaminated beach waters is estimated to total \$21- \$51 million annually in southern California alone. Development of rapid methods for detecting pathogens and other organisms indicative of sewage effluents is thus a high priority. Use of molecular analytical techniques, such as quantitative PCR, is one means by which public health officials and resource managers aim to improve water quality monitoring. However, current protocols require that samples be returned to a laboratory for analysis where they are processed in batch mode hours later to reveal targets of interest. The same limitation of requiring that samples be returned to a laboratory prior to analysis also limits many ocean research and monitoring programs.



The Center for Ocean Solution's Early Career Fellow, Kevan Yahamara, with the inner workings of the Environmental Sample Processor. (photo: A. Boehm)

Researchers at the Monterey Bay Aquarium Research Institute and partners comprising the Center for Microbial Oceanography Research and Education (C-MORE) have worked to overcome this limitation through the development of the environmental sample processor (ESP). The ESP allows for application of molecular analytical tests *in situ*. The instrument is completely autonomous and supports two-way communications for transmitting results of analyses as well as for downloading instructions so that its mode of operation can be altered remotely. Development and testing of the ESP was made possible by funding from NSF, NASA, and the Keck, David and Lucille Packard, and Gordon and Betty Moore Foundations.

While C-MORE investigators aim to utilize the ESP in an open ocean setting, researchers from Stanford University's Center for Ocean Solutions (<http://www.centerforoceansolutions.org/>) are looking to utilize ESP technology for water quality testing. The COS team, in conjunction with collaborators at the Southern California Coastal Water Research Project (SCCWRP) and NOAA's National Marine Fisheries Service, is evaluating the utility of the ESP to determine if a remote water quality monitoring network based on DNA probe technology is feasible. C-MORE, through MBARI, is loaning ESP instrumentation necessary to conduct these tests as one element of the Center's technology transfer program.



Going with the flow: tracking coherent microbial populations to better understand how environmental perturbations modulate gene expression in the open sea



The drifting Environmental Sample Processor (in the foreground) with the research vessel Western Flyer off central California. Image: Phil Sammet © 2010 MBARI

Modern molecular biological techniques have revolutionized our understanding of the diversity, function and community structure of marine microorganisms. For field research, molecular analytical analyses are often applied to discrete samples wherein the microbial community structure and function is referenced against physical, chemical and bulk biological conditions that are assessed at the instant samples are collected. However, the biological community collected at any instant is derived from past as well as present conditions. Thus, there is an inherent difficulty in establishing detailed cause and effect relationships since the history of the population in question is generally not known. A team from the Monterey Bay Aquarium Research Institute and its counterparts from the Center for Microbial Oceanography Research and Education (C-MORE) are working to overcome this limitation by developing submersible autonomous platforms that are capable of actively identifying, tracking and sampling coherent microbial populations so that changes in their community structure and gene expression can be assessed over time in response to changing environmental conditions. The long term goal is to develop a system that is capable of both sample archival as well as in situ molecular analytical analyses so that targeted sampling and analyses can be accomplished in remote locations without necessarily requiring a human presence at sea. NSF, the David and Lucille Packard, and Gordon and Betty Moore Foundations are supporting this effort.

To model this system, MBARI teamed with microbial ecologists from MIT and deployed a free-drifting Environmental Sample Processor (ESP) roughly 100 miles from the central California coast for five days. The ESP is a robotic underwater molecular biology lab that is about the size of a kitchen garbage can. It was suspended below a surface float and allowed roam freely while it performing a range of tasks from collecting water samples and carrying out a number of DNA probe tests in real-time, to preserving samples for examination after the instrument was recovered. At the same time, an autonomous underwater vehicle circled the drifting ESP, automatically altering its trajectory by tracking the position, heading and speed of the drifter, thereby providing a volume view around the instrument as it meandered at the whim of ocean currents.



Highlight

Unveiling microbial diversity in the oceans through single cell genomics

One of the great challenges in microbial oceanography is understanding the diversity of the microbes in the sea. Traditionally, researchers have done this by comparing strains isolated into culture or by looking at the complex 'meta-genome' of the entire microbial community harvested from the wild. Neither of these approaches can unveil the genetic makeup of co-existing wild cells, free of the biases introduced by culturing. Researchers in the NSF Center for Microbial Oceanography: Research and Education (C-MORE) have been working on an approach which, combined with fast and inexpensive DNA sequencing technologies, could revolutionize the way oceanographers study microbial diversity in the sea and its consequences.

C-MORE Post-docs Sebastien Rodrigue (now at U. Sherbrooke) and Rex Malmstrom (now at the DOE's JGI), working in C-MORE PI Sallie Chisholm's laboratory, developed a single cell genomics pipeline to sort individual microbial cells— in their case the tiny photosynthetic cell, *Prochlorococcus* – away from the millions of other cells in a teaspoon of ocean water. They then amplify the cell's DNA so it could be sequenced, allowing them to see the genetic make up of the cells, how they differ from one another, and how they are adapted to particular environments.

Using water samples obtained during the C-MORE sponsored BULA cruise to the S. Pacific (Figure 1), Rodrigue and Malmstrom were able to analyze the genetic composition of 5 *Prochlorococcus* cells. The genome sequences of just these 5 cells added over 600 new genes to the global *Prochlorococcus* 'pan-genome' – i.e. the total number of unique genes in the global population of this species. Considering that the average cell has 2000 genes, this suggests that there is an extraordinary amount of novelty in each *Prochlorococcus* cell. Among the exciting genetic features discovered in these cells is the potential for specialized iron acquisition mechanisms, and evidence that viruses have inserted themselves into the genomes of these microbes. The latter could provide a mechanism for generating some of the extraordinary genetic diversity seen within this group.

This approach is now being applied to the analysis of hundreds of single cells from the global oceans to further explore the astonishing diversity within this globally distributed photosynthetic cell.

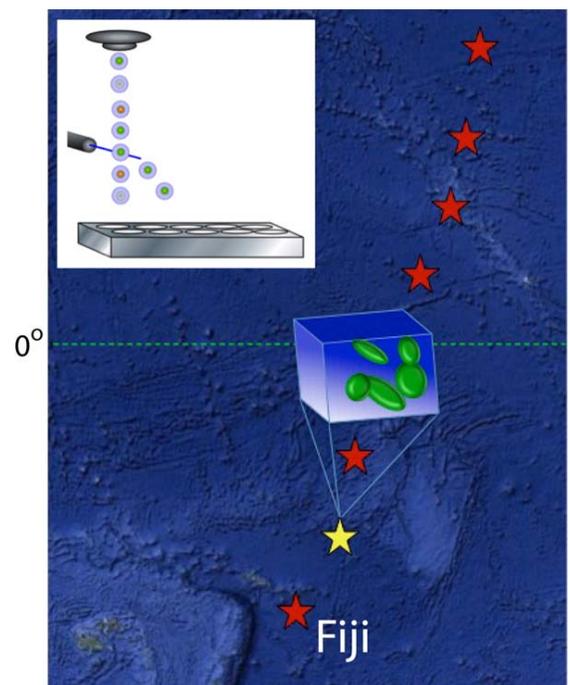


Figure 1.

Insert: Laser-based micro-fluidic system that allows researchers to isolate thousands of individual microbes into individual wells, select those of particular interest, and study their genetic composition.

Map: C-MORE cruise track in S. Pacific. Analysis of the genetic content of five *Prochlorococcus* cells (each 1/100 the width of a human hair) has revealed tremendous diversity and novel adaptations.

Diagnosing ecosystems through the genes of ocean microbes

The mission guiding the NSF Center for Microbial Oceanography: Research and Education (C-MORE) is “*Linking Genomes to Biomes*”. A powerful approach to doing this is by comparing the gene content of populations of specific microbes from similar, but distinct, habitats. The genes microbes carry reflect the environmental variables that have been most significant in shaping their indigenous populations. Thus, by comparing populations from different oceans, noting differences in their collective gene content, and identifying what functions those genes carry out in the cells, one can begin to identify the most important selective pressures shaping these populations in different oceans. These pressures are what drive evolutionary change in all living things – leading to the “survival of the fittest” through Darwinian natural selection.

C-MORE scientist Sallie Chisholm (MIT) and her graduate student Maureen Coleman – now a post-doc at Cal Tech – used the smallest and most abundant microbes in open ocean systems as ‘reporters’ to help them understand the most important differences between the N. Atlantic and N. Pacific subtropical ocean gyres (Figure 2) from the point of view of their microbial inhabitants. The two groups they studied, *Prochlorococcus* and *Pelagibacter*, have fundamentally different lifestyles: The former gains carbon and energy through photosynthesis, while the latter survives on the carbon compounds produced by the photosynthesis of others.

The major difference that emerged from the study was the overwhelmingly higher frequency of P-related genes in the Atlantic populations of both *Prochlorococcus* and *Pelagibacter* relative to their Pacific cousins.

These genes function in phosphorus acquisition and metabolism, and their over-representation in the Atlantic populations is consistent with the relatively low concentrations of phosphorus in the Atlantic vs. Pacific oceans. Interestingly, the photosynthetic *Prochlorococcus* has a different repertoire of P-related genes than *Pelagibacter*, suggesting that the two types of microbes may have evolved over time to exploit different phosphorus sources, thus reducing competition for this limited resource.

The details of this study were in the October 10th 2010 issue of the Proceedings of the National Academy of Sciences of the United States of America.

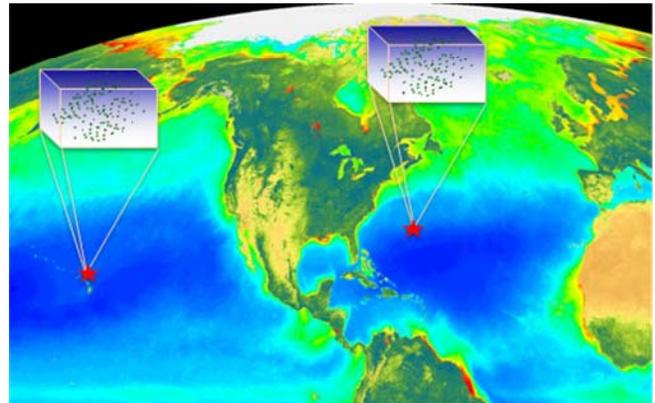
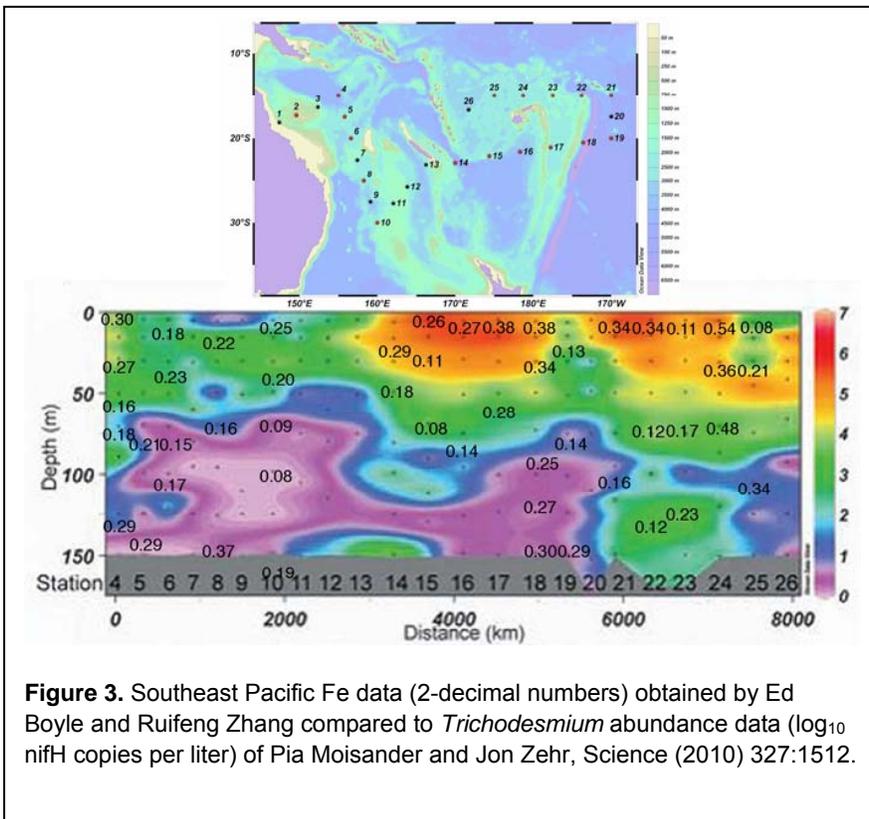


Figure 2. Sample sites for this study (red stars): NSF-sponsored time-series stations HOT (Pacific) and BATS (Atlantic). *Prochlorococcus* populations were collected from each site and their genetic content was compared to help us understand what environmental variables are most important in driving the evolution and metabolism of these populations.



An association between nitrogen fixers and iron in the southwest Pacific

Because nitrogen fixation enzymes require multiple atoms of iron, nitrogen fixers are assumed to require more iron than ordinary phytoplankton (which require iron for photosynthetic activity). However, there is only very indirect evidence for the notion that the iron supply is linked to the abundance and activity of nitrogen fixing organisms. This lack of evidence is mainly the result of the extreme paucity of valid Fe data in the ocean, particularly data collected in tandem with appropriate biological information. In 2007, at the invitation of C-MORE collaborator John Zehr, we obtained Fe data on the same cruise as multiple biological N-fixing parameters. We find that the areas where Fe is higher than average are also areas of higher *Trichodesmium* abundance.





Highlight

Bimodal Effects of Ocean Acidification on Marine Phytoplankton

It is generally believed that phytoplankton will benefit from the Ocean Acidification due to lower cost of carbon acquisition. Although most species of marine phytoplankton operate carbon concentration mechanisms, these mechanisms consume energy and resources. Under conditions of increasing $p\text{CO}_2$ /decreasing pH, significant fraction of this energy and resources can be redirected to support other metabolic process, resulting in higher rates of primary production, higher level of nitrogen fixation, and faster growth. Experiments performed with natural phytoplankton populations of Monterey Bay support this notion, but only within relatively narrow range of pH changes (Figure 4). At ΔpH exceeding 0.25 units the rates of photosynthetic electron transport begin to decrease, indicating that there are two opposite effects of pH shift on the primary production in phytoplankton: the positive effects of increased carbon availability at the site of carbon

fixation, and the negative effects of decreasing pH on the processes of light utilization, charge separation, and photosynthetic electron transport. The threshold pH level and the net effect of pH shift on the photosynthetic performance are likely to be controlled by the local conditions of nutrients and energy supply.

Field incubation experiments confirm this notion (Figure 5). During the OPEREX cruise in NPSG Kolber and Tozzi have observed null, or negative effects of increased $p\text{CO}_2$ level on the phytoplankton growth rates under ambient nutrient levels (Fig. 5A), but positive effects under conditions of enriched nutrients (Fig. 5B). During the BiGRAPA cruise, similar null or negative effects of increased $p\text{CO}_2$ were observed under ambient nutrient conditions (Fig. 5C), but no positive effects were observed under enriched nutrients levels (Fig. 5D). These data indicate that it may be premature to declare the phytoplankton as the ultimate winner under high $p\text{CO}_2$ /low pH conditions of the Future Ocean.

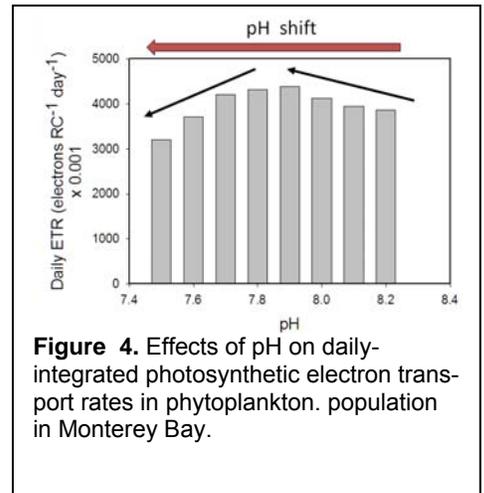


Figure 4. Effects of pH on daily-integrated photosynthetic electron transport rates in phytoplankton population in Monterey Bay.

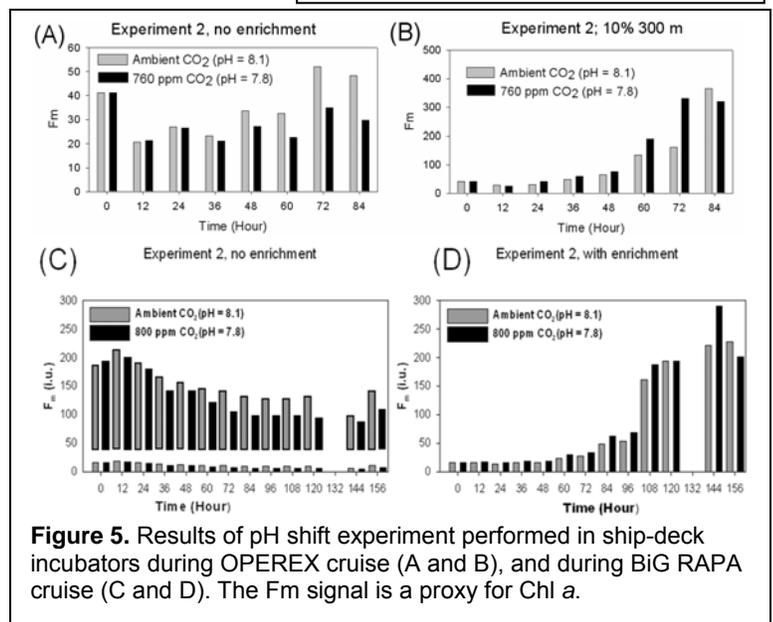


Figure 5. Results of pH shift experiment performed in ship-deck incubators during OPEREX cruise (A and B), and during BiGRAPA cruise (C and D). The F_m signal is a proxy for Chl *a*.

Differentiating among strains of *Crocospaera watsonii*

Crocospaera watsonii is a species of nitrogen-fixing cyanobacteria that plays an important role in carbon and nitrogen cycling in the world's oceans. In tropical and subtropical areas, nitrogen provided by *C. watsonii* populations enhances photosynthesis by phytoplankton and thereby provide more nutrients for all higher trophic levels. A number of strains of *C. watsonii* have been isolated from various ocean basins over the last few decades, and interesting differences have been observed in these strains. For example, some strains have a larger average cell size, accompanied by higher per-cell rates of nitrogen fixation. The strains with larger cell sizes also produce an extracellular matrix, made of a type of sugar called exopolysaccharide (EPS), in which the cells are embedded. The physiological differences among the various *C. watsonii* strains may significantly alter their relative contributions to the marine ecosystem, especially in terms of nitrogen and carbon cycling.

Despite observed physiological differences, previous genetic studies of natural populations of *C. watsonii* were unable to differentiate between strains because all known strains shared identical DNA sequences for the genetic markers being used. In order to identify the genetic basis for the physiological differences among strains, researchers at University of California, Santa Cruz and the NSF Science and Technology Center for Microbial Oceanography: Research and Education (CMORE) compared the entire genomes of two *C. watsonii* strains, one of the large-cell, EPS producing type and one of the small-cell type that does not produce EPS. This comparison revealed that while the DNA sequence in most of the genomes was nearly identical, there was a large difference between the two strains in the number of mobile genetic elements per genome, especially transposase genes. This suggested that genetic movement, including insertions and deletions of pieces of the genomes, is an important mechanism by which the strains have evolutionarily diverged from each other. This was further supported by finding transposase genes adjacent to genomic regions that showed insertions or deletions when the two genomes were aligned, as shown in Figure 6. The region in this figure also contains genes known to be important in EPS synthesis, which are present only in the genome of the EPS-producing strain.

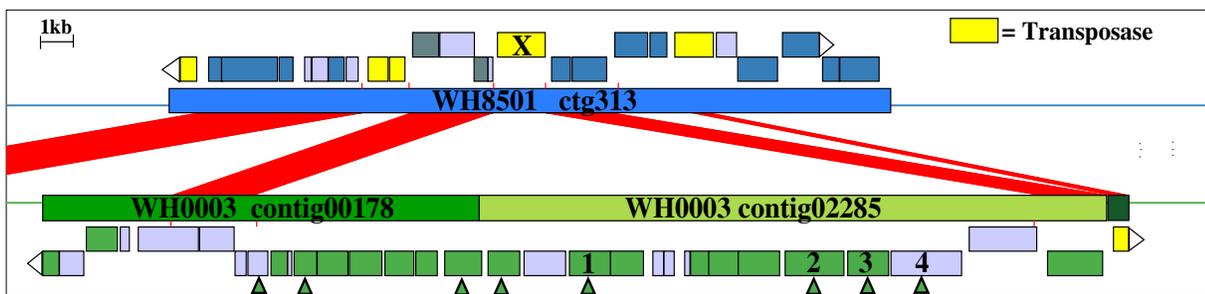


Figure 6. Alignment of DNA sequence for genomic regions from two *C. watsonii* strains. Strain WH8501 (top, in blue) is a small-cell, non-EPS producing type, and strain WH0003 (bottom in green) is a large-cell, EPS-producing type. The red lines between the genomes indicate regions that are identical (DNA sequence identity of >99%) between the two genomes, and regions without red connections have no homology, and are specific to the genome of that strain. Genes coded by the DNA sequence are indicated as boxes above (WH8501) or below (WH0003) the genomic regions, separated into rows based on direction with the direction indicated by the first or last gene in each row. Transposase genes that encode mobile genetic element functions are indicated in yellow, and green arrowheads indicate genes that encode EPS synthesis (or related functions) and are only found in the WH0003 genome.

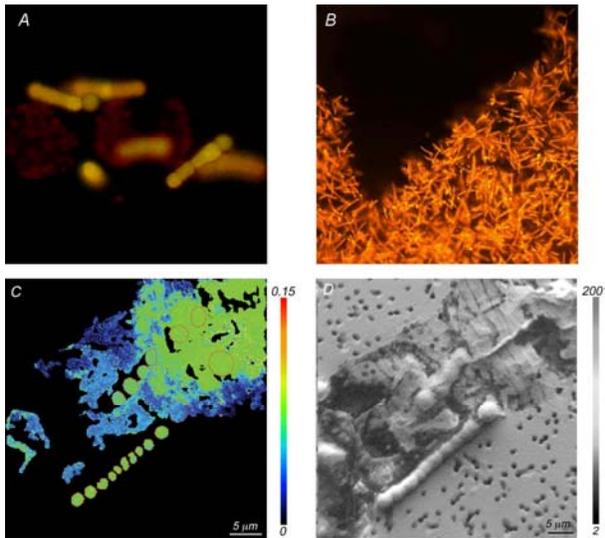
Identifying such genes which help explain the physiological differences among the strains was one of the main goals of the genome comparison. The DNA sequences for these strain-specific genes can also now be used as genetic markers which, unlike previous markers, can differentiate between the different types of *C. watsonii* in the environment.

Being able to identify and quantify the different types of *C. watsonii* in the environment will lead to a better understanding of the populations of this important nitrogen fixer, including the identification of physical or chemical ocean niches where each type is more likely to be found. This will result in a better understanding and better predictions of how those populations contribute to the carbon and nitrogen cycles in the oceans.



Highlight

Nitrogen fixation by heterocystous symbiotic cyanobacteria



In 2004, during the HOT 165 cruise to station ALOHA (22 45'N, 158 0'W) in the subtropical North Atlantic Ocean, Dr. Rachel A. Foster, isolated one of the heterocystous cyanobacterial symbionts, *Calothrix* SC01, which lives in close association with a large chain forming diatom, *Chaetoceros* (photos at left). The *Chaetoceros* diatoms with associated *Calothrix* SC01 were hand-picked with pulled micro-pipets and placed in media without nitrogen (a). Subsequently within 2 months a large colony formed (b) and has been maintained. Recently Foster and her colleagues at UCSC, Nicole Goebel and Jonathan P Zehr, reported more information on the genetic identification and photo-physiology for this first symbiotic isolate from

the open ocean.¹ The N₂ fixation rates and transfer of fixed N to the diatom partners was also recently reported by Foster, Zehr and colleagues at the Max Planck Institute for Marine Microbiology using a high resolution nanometer scale secondary ion mass spectrometry (nanoSIMS) (C and D). N₂ fixation and transfer had not been measured or shown in this symbiosis before, and with nanoSIMS, ¹⁵N/¹⁴N was easily visualized in both the *Calothrix* symbionts and the host *Chaetoceros* diatoms.² In addition, the genome for the *Calothrix* symbiont was recently sequenced and is estimated at 6.6 Mb and 41% GC content. A closer look at the nif operon, which encodes the nitrogenase enzyme for N₂ fixation, identified nickel (Ni) and cobalt (Co) transporters upstream, which is in contrast to other heterocystous cyanobacteria (*Anabaena* and *Nostoc* spp.)(Figure 7, below).

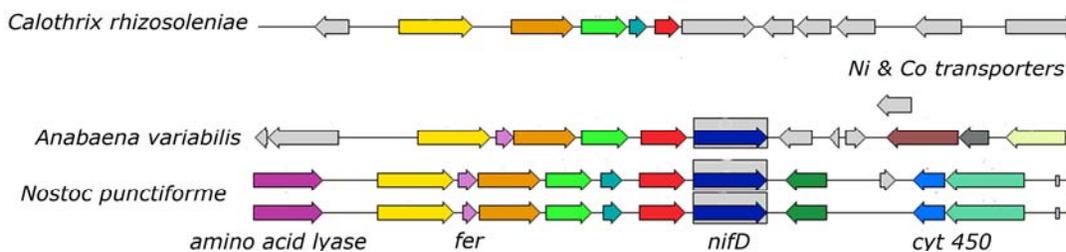


Figure 7. Diagram of nif and adjacent operons in *Calothrix*.

Using a variety of molecular genetic methodologies and analytical tools, we have revealed new and unexpected information for these relatively under-studied populations known to be important to the N and C cycling in the global oceans.

¹ Foster RA, Goebel NL, and JP Zehr. 2010 Isolation of *Calothrix rhizosoleniae* (cyanobacteria) strain SC01 from *Chaetoceros* (Bacillariophyta) spp. diatoms of the subtropical North Pacific Ocean. J. Phycol. 46: 1028-1037.

² Foster RA, Kuypers MMM, Vagner T, Paerl RW, Musta N, and JP Zehr. 2011. Nitrogen fixation and transfer in open ocean diatom-cyanobacterial symbioses. ISME *in press*.