



Highlight

Microbial respiration measurements

Microbial respiration is a key process in the carbon cycling of marine pelagic ecosystems and, therefore, its accurate measurement is required for a complete understanding of the ecology of aquatic environments.

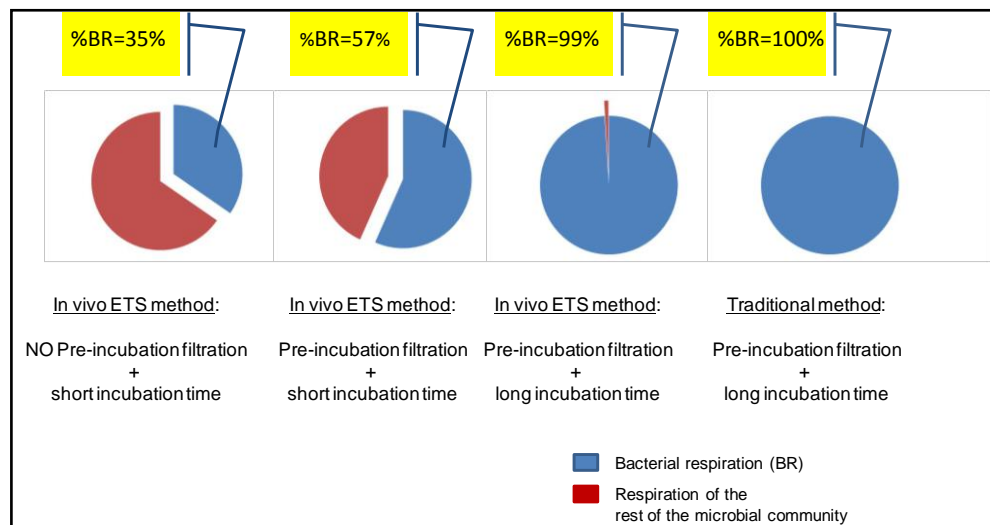
A longstanding scientific enigma exists in oligotrophic subtropical waters: it appears that bacterial respiration contributes up to 90% of total community respiration even though the contribution of bacterial biomass to total biomass is much less. It has been suggested that experimental artifacts including pre-incubation filtration procedures, applied in order to separate bacteria from the rest of the microbial community by means of size-fractionation, and long incubation times used in traditional respiration measurements may lead to erroneous rates, but few field data exist to test this hypothesis.



Size-fractionation filtration system used in the in vivo ETS method.

C-MORE Post-doc Sandra Martinez-Garcia, in a collaboration between the Karl Lab and University of Vigo (Spain), has performed the first experimental assessment of these experimental treatment effects on bacterial respiration (BR) measurements using the 'In vivo Electron Transport System (ETS) activity method'.

She demonstrated that pre-incubation filtration procedures have a significant effect on bacterial respiration and that long-incubation times exacerbate this artifact. Pre-incubation filtration procedures were also shown to affect picoplankton community composition, bacterial production and bacterial growth efficiency. The causes are currently under investigation in C-MORE.





Highlight

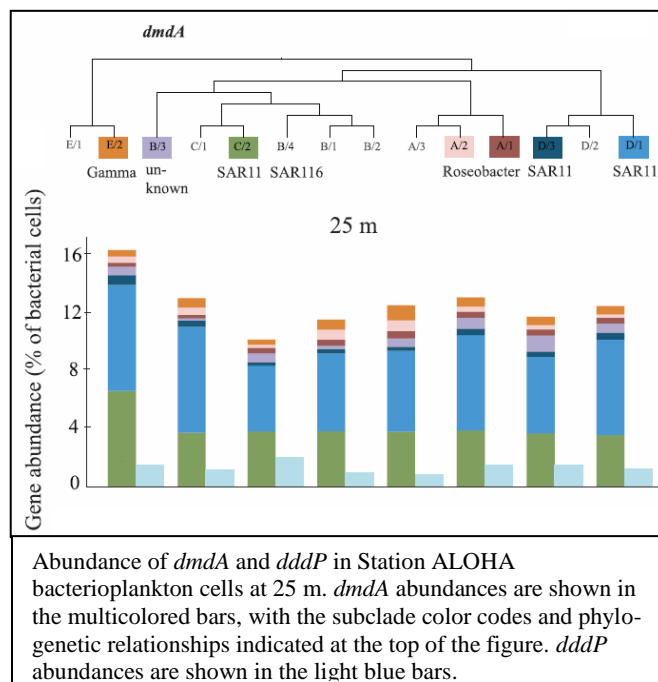
Unveiling the cycle of dimethylsulfoniopropionate in the North Pacific Subtropical Gyre

Dimethylsulfoniopropionate (DMSP) is a common sulfur compound produced by phytoplankton, which, when released into seawater, can be rapidly sequestered and metabolized by the bacterioplankton community (turnover time: 3-12 h). DMSP can be metabolized through two different pathways: the *demethylation pathway* which provides bacteria with reduced carbon and sulfur that can be assimilated into biomass or oxidized for energy; and the *cleavage pathway* which yields a 3-carbon compound and dimethylsulfide (DMS), a reduced gas that has a major impact on the global sulfur cycle and climate.

Recent work carried out at Station ALOHA by Daniela del Valle and her colleagues supports the hypothesis that the switch between these two pathways is controlled by the demand of reduced sulfur for protein synthesis. It was found that the assimilation of the DMSP-sulfur into proteins is stimulated by light and that this process is accompanied by a decrease in the production of DMS, indicating that there is indeed a link between these two processes. However, more than 70% of the DMSP-sulfur is neither assimilated into proteins nor found as DMS, but oxidized to sulfate, which points to DMSP as an important source of carbon and energy to the microbial assemblage. We estimated that DMSP can satisfy 20-67% of the bacterial sulfur demand, and up to 9% of the bacterial carbon demand in the surface waters of the oligotrophic North Pacific Subtropical Gyre (NPSG).

It is likely that SAR11 is responsible for the light-stimulated uptake of DMSP-sulfur, based on their need for reduced sulfur and the findings of a recent study by the Karl (UH) and Moran (UGA) laboratories which measured the abundance and diversity of genes encoding bacterial DMS production (*dddP*) and demethylation (*dmdA*) throughout the year at Station ALOHA. SAR11 dominated DMSP cycling and *dmdA* and *dddP* genes were more abundant during periods of high solar radiation.

It was also found that light-stimulated DMSP-sulfur assimilation is tightly coupled to the uptake of leucine; however, *Prochlorococcus* is responsible for the light-stimulated uptake of leucine. This suggests a tight link between photosynthetic production and release of dissolved organic matter by *Prochlorococcus* and the growth and cellular requirements for reduced sulfur of SAR11.





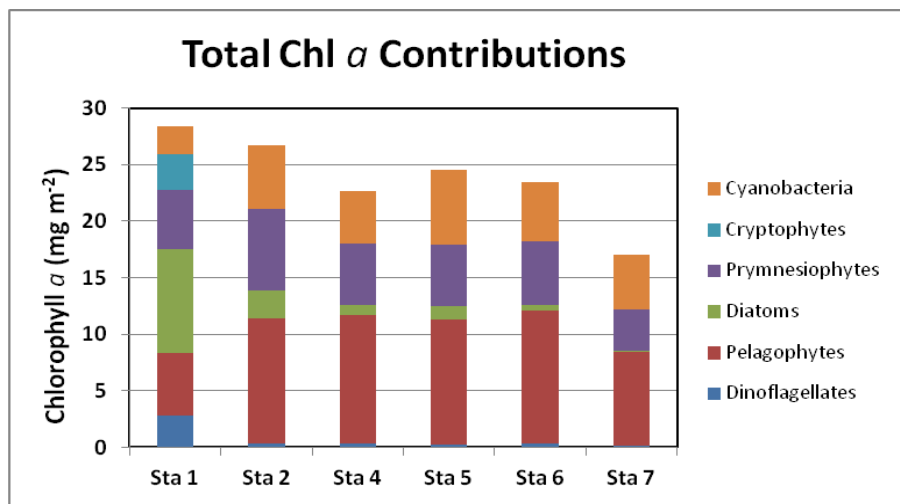
Highlight

Characterization of phytoplankton community structure during the BiG RAPA cruise using HPLC-determined pigment biomarkers

New research conducted by C-MORE investigator Bob Bidigare has provided novel information about phytoplankton community structure in the South Pacific Ocean. Stepwise multiple linear regression (MLR) analysis was used to model the HPLC pigment data collected for BiG RAPA stations 1-7. Carotenoid pigments were used as biomass proxies for diatoms (fucoxanthin), dinoflagellates (peridinin), cryptophytes (alloxanthin), pelagophytes (19'-butanoyloxyfucoxanthin), cyanobacteria (zeaxanthin), prasinophytes (prasincoxanthin), chlorophytes (lutein), and prymnesiophytes (19'-hexanoyloxyfucoxanthin). Highly significant relationships ($P < 0.001$) were obtained for all carotenoids except prasincoxanthin and lutein, revealing that prasinophytes and chlorophytes were not important phytoplankton community constituents during BiG RAPA:

$$\text{CHLA} = 13.4 + 1.10 \text{ PER} + 3.17 \text{ BUT} + 1.62 \text{ FUCO} + 0.75 \text{ HEX} + 5.83 \text{ ALLOX} + 0.74 \text{ ZEAX}$$

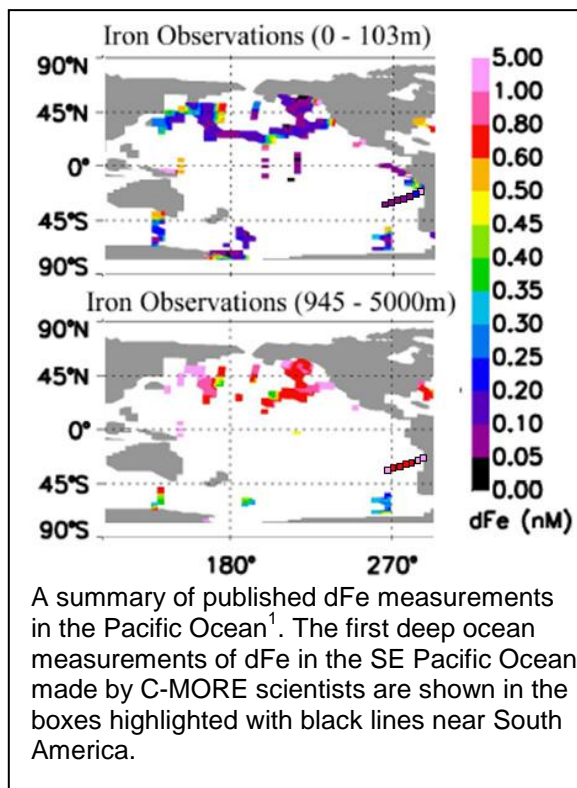
Chlorophyll (Chl) *a* concentrations predicted from regression coefficients and individual carotenoids concentrations were not statistically different from HPLC-measured total Chl *a* concentrations ($\Delta y/\Delta x = 0.98 \pm 0.02$, $R^2 = 0.976$, $P < 0.001$), and the y-intercept was not different for zero ($P = 0.335$). Results of the MLR analysis were used to assess variations in phytoplankton composition and taxon-specific chlorophyll *a* biomass contributions along the BiG RAPA transect. Depth-integrated Chl *a* concentrations decreased with increasing distance from shore. Cryptophytes were only observed at station 1, and were likely associated with the *Mesodinium rubrum* bloom observed in the region. Diatom and dinoflagellate Chl *a* contributions were highest at the inshore stations and decreased monotonically along the transect line. Cyanobacterial pigment biomass was lowest at station 1 ($2.5 \text{ mg Chl } a \text{ m}^{-2}$), and increased and remained fairly constant at stations 2-7 ($5.4 \pm 0.8 \text{ mg Chl } a \text{ m}^{-2}$). Pelagophytes and prymnesiophytes were abundant at all stations and their pigment biomass averaged $9.9 \pm 2.4 \text{ mg Chl } a \text{ m}^{-2}$ and $5.4 \pm 1.2 \text{ mg Chl } a \text{ m}^{-2}$, respectively.





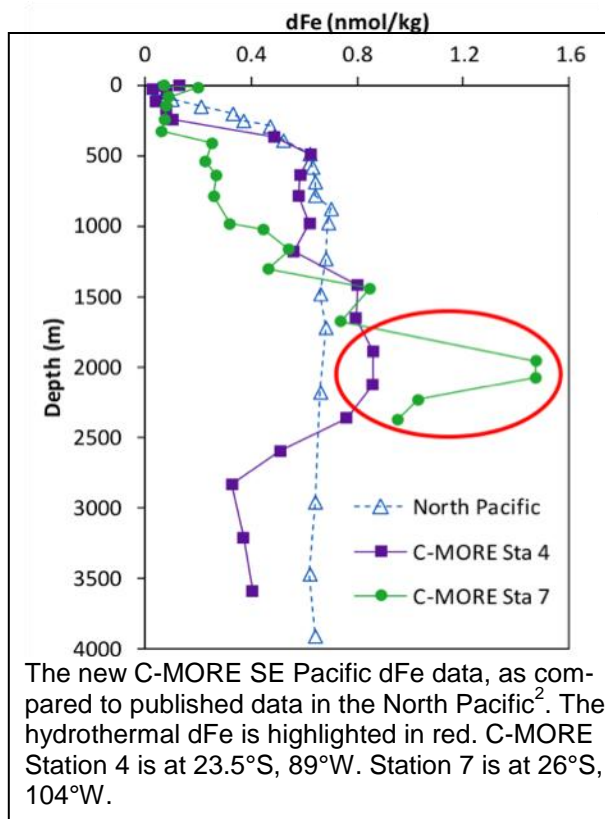
First deep ocean dissolved iron data in the Southeast Pacific Ocean reveals significant hydrothermally-derived iron

Dissolved iron (dFe) is a required micronutrient for marine phytoplankton, essential for photosynthesis, nitrogen acquisition, and nitrogen fixation. Models estimate that iron is the limiting nutrient for primary production in up to 40% of the surface ocean. Linking microbial processes to dFe cycling, however, is difficult because of the paucity of data on the global marine dFe distribution.



solved Fe in the deep Pacific than was previously thought to be present. As can be seen in Figure 2, there is a large enrichment of dFe over typical North Pacific dFe concentrations at 2000m. This enrichment can be attributed to hydrothermal vent activity in the nearby East Pacific Rise. Many more dFe measurements in the deep South Pacific are needed to assess the extent of this hydrothermal Fe, but this preliminary data indicates that hydrothermal vents may be a much greater source of dFe to the global ocean than was previously believed.

One region where almost no dFe measurements have been is the Southeast Pacific Ocean; in fact, as the figure at left shows, there is no published dFe data in the deep SE Pacific Ocean at all. The first measurements of dFe deeper than 1000m in this large ocean region were made by C-MORE scientist Ed Boyle and his graduate student Jessica Fitzsimmons on the 2010 C-MORE BiGRAPA cruise (led by Chief Scientist Dan Repeta), which sailed from Arica, Chile, to Easter Island. These measurements reveal much more dis-



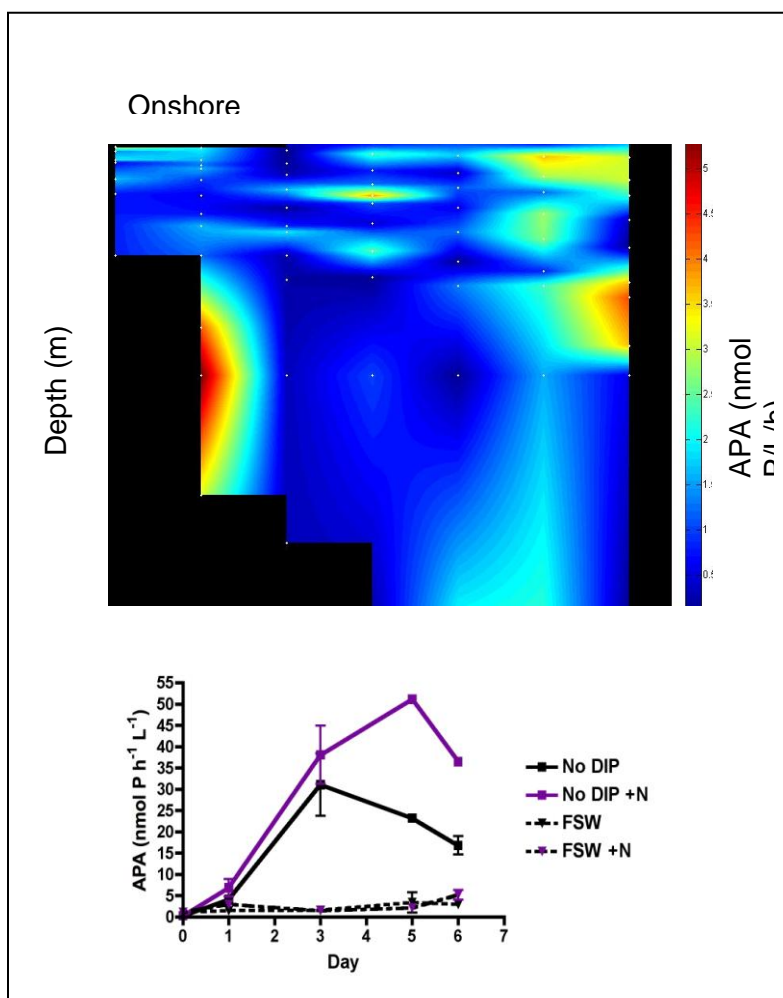
¹ Moore, JK & Braucher, O. 2008. Biogeosciences, 5:631-656. ² Martin *et al.*, 1989. Deep-Sea Research, 36:649-680.



Highlight

A new method tests variability in alkaline phosphatase activity along a gradient and with novel experimental treatments in the South East Pacific

The South East Pacific (SEP) is characterized by a gradient in nutrients from the coast out to the gyre. Despite this gradient, dissolved inorganic phosphate concentrations (DIP), and dissolved organic phosphorus (DOP) concentrations along this SEP transect, even out in the gyre, are much higher than some systems like the western North Atlantic. Alkaline phosphatase is an enzyme that hydrolyzes DOP to bioavailable DIP, in a manner that is typically regulated by DIP availability. Here a study was undertaken on the C-MORE SEP cruise (BiG RAPA) by WHOI researcher Sonya Dyhrman to examine alkaline phosphatase activity (APA) in depth profiles along the transect. APA in near surface waters was increased in the gyre relative to the more coastal stations, but even in the gyre APA was relatively low (< 4 nmol P/L/h) relative to the western North Atlantic which can be ~ 45 nmol P/L/h. This is consistent with the elevated DIP in the SEP. An enduring question is if the activity of this enzyme would increase if DIP were reduced. For the first time, a group of researchers from WHOI, UH, and OSU were able to address this question. Using a novel experimental approach, DIP was removed from the seawater using a brucite stripping method, and then a concentrated fraction of the local microbial community was added back to examine if APA would increase in no DIP treatments relative to filtered seawater controls (FSW). Nitrate was also added in separate treatments to alleviate potential limitation. Strikingly, maximal rates of APA hydrolysis reached levels seen in low DIP systems (~ 40 nmol P/L/h), when DIP was removed. This underscores DIP control over near surface APA in this system, and this finding would not have been possible without this multi-institution collaboration.



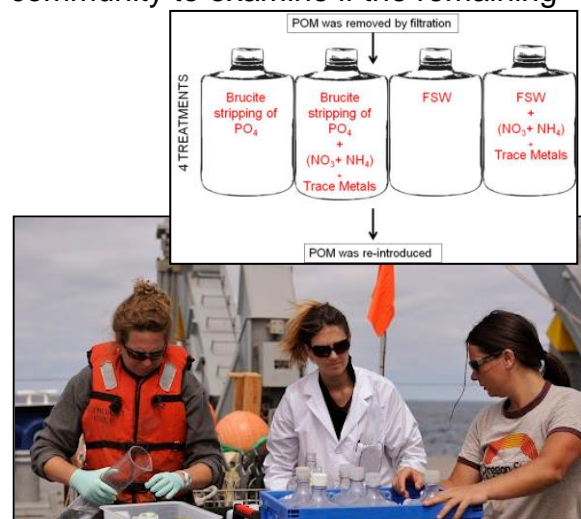


Highlight

A new method tests the bioavailability of dissolved organic phosphorus during a C-MORE cruise in the South East Pacific

The South East Pacific (SEP) is characterized by high nutrient concentrations in the waters adjacent to the Chilean coast, but low nutrient concentrations (oligotrophic) in the mid- South Pacific Subtropical Gyre (SPSG), near Easter Island. The steep gradient in nutrient concentrations affects the level of marine production, the composition of the microbial community, and the operation of major biogeochemical cycles in ways that are not fully understood. The SEP is still the most sparsely sampled oceanic region of the global ocean from hydrodynamic, biological, and biogeochemical points of view.

Previous expeditions to this region have documented large reservoirs of dissolved organic phosphorus (DOP) in the surface ocean of the SEP and SPSG. It has been suggested that these DOP pools are the byproduct of a prolonged build up of recalcitrant P compounds, potentially unavailable to fuel microbial growth. A collaborative effort by WHOI, OSU and UH scientists sought to counter this emerging paradigm using a novel experimental approach conducted along a gradient of microbial habitats stretching from the productive SEP to the oligotrophic blue waters of the SPSG. We have hypothesized that DOP utilization is (1) limited by the delivery of other essential elements, specifically nitrogen and iron and that (2) if nutrient limitation were released, residual and highly bioavailable inorganic phosphate would preclude DOP uptake. We quantitatively removed phosphate (DIP) from seawater using a brucite stripping approach, and then added back a concentrated fraction of the microbial community to examine if the remaining DOP was bioavailable. Nitrate and iron were added in separate treatments to alleviate potential limitation, and further drive the microbial community to use P, either DIP or DOP. This resulted in four treatments, which were assayed at a coastal, mesotrophic and an oligotrophic station. In these experiments changes in heterotrophic and phytoplankton population abundance, alkaline phosphatase activity, DIP and ATP turnover, inorganic and organic nutrients were monitored over a roughly week-long incubation period.



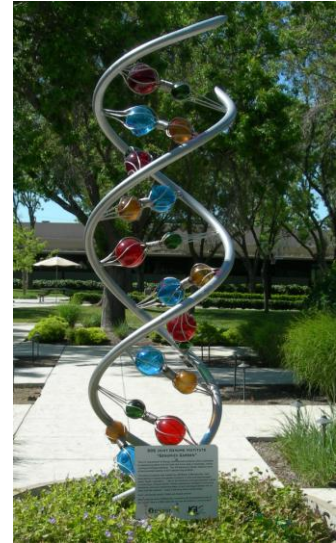
The most salient finding from these experiments were that DOP pools appear to be labile and available for microbial consumption and that both removal of preferred inorganic pools and addition of nitrate stimulate DOP utilization. Notably, DOP drawdown was enhanced in the oligotrophic zone relative to coastal stations. Additionally, we have shown that brucite stripping is a powerful and effective approach that will allow us to expand upon our understanding of P cycling in other regions.



Highlight

C-MORE EDventures – Participation in the Microbial Genomics and Metagenomics workshop at the Department of Energy’s Joint Genome Institute

The rapidly increasing number of microbial genome sequences available for study requires straightforward tools to store, administrate, and characterize the data in order to respond to scientific questions. Postdoctoral scholar Jana Grote had the tremendous opportunity to participate in the five-day workshop “Microbial Genomics and Metagenomics” offered by the U.S. Department of Energy Joint Genome Institute (DOE JGI) in Walnut Creek, CA. This workshop is highly recommended for users of JGI software tools, and its overall goal is to provide excellent training in microbial genomic and metagenomic data analysis.



The workshop consisted of two days of intensive seminars and three days of hands-on tutorials. The group of 50 participants was diverse in nationality, level of scientific career (students, postdocs, and faculty) and field of interest in microbial genomics, ranging from human health related questions to strictly environmental issues. However, the workshop was highly successful in delivering excellent training to the participants using the DOE JGI Integrated Microbial Genomes (IMG) platform and associated tools for analysis of microbial genomic and metagenomic data sets. Generally, IMG allows navigating in the microbial genome data along three key dimensions: genomes (organism), functions (terms and pathways), and genes. The workshop consisted of introductory seminars, presentations of up to date scientific case studies, and hands-on tutorials given by members of the JGI Genome Biology Program, the Biological Data Management & Technology Center, and invited scientists. Live demos covering e.g. functional annotation, quality control, and functions and pathways were followed by hands-on exercises and discussion of common problems and new tools e.g. handling and exploration of expression data (transcriptomics and proteomics). The workshop was completed by a poster session where Jana Grote had the occasion to discuss her own research followed by a JGI facilities tour giving the opportunity to see the newest JGI sequencing machines.

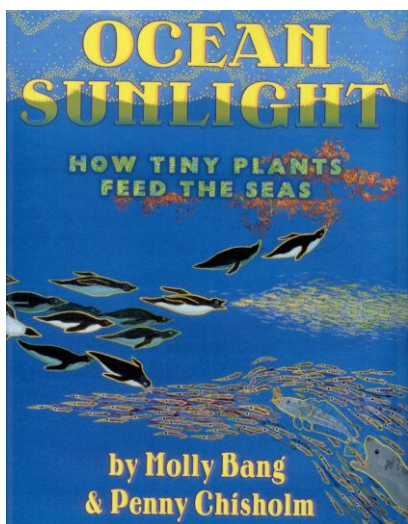
Jana Grote highly valued this workshop as an excellent training opportunity which significantly enhanced her skills in microbial genomics and metagenomics, one of the fastest growing fields in microbial oceanography at the moment.



Highlight

Ocean Sunlight: A book about the oceans for children of all ages

The allure of the oceans is timeless. This alien world is fascinating to children and adults alike. Yet most people do not understand the basic processes that sustain life in the oceans. For example, it is not broadly appreciated that essentially all life in oceans is dependent on microorganisms – the phytoplankton. Using solar energy, these tiny photosynthesizers fix 50 billion tons of carbon dioxide into sugars each year. This fixed carbon sustains the food chains of the sea.



Published by Scholastic, Blue Sky Press, 2012

ton in the thin film of sunlit surface waters supply food for all the deep sea animals.

The book may be of interest not only to children, but also to their parents and teachers, who can find extensive notes at the end of the book elaborating on some of the more important concepts.

To help spread the word about this, and other important ocean processes, Sallie (“Penny”) Chisholm, MIT Professor and a member of the NSF Center for Microbial Oceanography and Research (C-MORE), has written a book – with co-author and illustrator Molly Bang – for children. The book is a sequel to Bang and Chisholm’s 2009 book *Living Sunlight*, in which they describe photosynthesis and the role it plays in ecosystems on land. This book, *Ocean Sunlight*, deals with ocean photosynthesis, and delves into concepts that are not usually described to young readers, or even adults, but are fundamental to understanding how the Earth works. For example, the book introduces the concept of exponential growth, and nutrient limitation, as well as system dynamics – i.e. how things can be simultaneously ever changing and appearing to stay the same. It also explores how phytoplank-

KIRKUS REVIEWS – April 1, 2012

OCEAN SUNLIGHT - *Starred* Review
Molly Bang and Penny Chisholm

“An awe-inspiring lesson in photosynthesis goes under the sea. As in [Bang and Chisholm’s] previous *Living Sunlight* (2009), the sun addresses readers to explain the role of solar energy to support the chain of life—this time in the ocean.... Then the focus ...[is] first near the surface, where phytoplankton grow and multiply, and then to the depths, where nutrient-rich ‘marine snow’ sifts down to feed creatures who live away from sunlight. The transformation of sunlight, water and carbon dioxide into phytoplankton (*‘the great invisible pasture of the sea’*), on which feed zooplankton and progressively larger animals, is set against background paintings of rich marine blues and greens. The churning and recycling of these nutrients is shown again to be a gift of the sun: *‘My sunlight powers winds that build great storms and mix the water layers of the seas.’* ... Readers will want to visit more than once to capture both the science and the abundant sense of celebration here.”

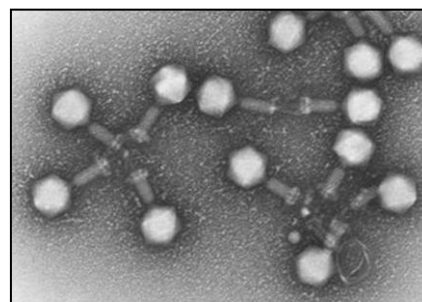


Highlight

Marine virus exploits bacterial host's regulation to activate its own genes

Phosphorus is often a limiting nutrient in marine ecosystems and research has shown that its availability exerts strong selective pressure on the photosynthetic bacterium *Prochlorococcus*, the most abundant photosynthetic cell in the oceans. Like most bacteria, *Prochlorococcus* has a two-component sensing system that triggers the expression of phosphorus-acquisition genes in low-phosphorus ocean environments. The viruses that infect *Prochlorococcus*, redirect its metabolism so they can reproduce, also have phosphorus-acquisition genes – acquired from their hosts over the course of evolution. These genes in the virus presumably facilitate host acquisition of phosphorus to fuel their own need for phosphorus to replicate their DNA. But how this intimate co-evolutionary dance is actually regulated at the cellular level has been a mystery.

Post-doctoral researcher Qinglu Zeng at MIT, working in Sallie Chisholm's laboratory – part of the NSF Center for Microbial Oceanography: Research and Education (C-MORE) – has recently shed light on this puzzle. By studying the gene expression of the bacteria and virus in phosphorus-rich and phosphorus-limited environments he determined that both host and virus respond to changes in environmental nutrient levels: the host to nutrient levels in the seawater, and the virus to nutrient levels within the host. He has shown that when these viruses infect cells they 'tune in' to the host's sensing system to see if it is phosphorus stressed. Thus the virus has ways of using the signals from the host to turn on its own phosphorus scavenging genes and augment the host's system for drawing phosphorus from seawater into the cell. This helps satisfy the host's need for phosphorus, which in turn is used by the virus for its own DNA synthesis and replication. The virus progeny then explodes the host and are released back into the ocean where they can invade other bacteria and repeat the process. This is the first demonstration of a virus of any kind – including all those studied in biomedical research – exploiting the host's own nutrient-uptake regulatory machinery.



Electron micrograph of *Prochlorococcus* viruses. Each is about 1/1000 the width of a human hair. Image by M. Sullivan and B. Li.

C-MORE Post-doc Libusha Kelly looked for evidence of this pirated viral regulatory mechanism in virus cultures and in DNA from viruses in the wild, and found that it is wide spread. More importantly, she found that it is more frequent in low-phosphorus environments – consistent with the view that viruses are more likely to retain phosphorus acquisition genes in environments where their hosts are phosphorus stressed. This is true of the host cells as well, which was the subject of another C-MORE study carried out by Maureen Coleman, also of the Chisholm Lab. These advances in our understanding of the intertwined processes of virus and host cells, and how they are shaped by the ocean's nutrient regimes, are contributing to C-MORE's goal of linking genomes to biomes.

Some of this work appeared in a paper by Chisholm and Qinglu Zeng in the Jan. 24, 2012 issue of *Current Biology*.



Highlight

ProPortal: Facilitating cross-scale studies of the most abundant photosynthetic cell in the ocean

Researchers in Sallie Chisholm's lab at MIT have developed a unique public database "ProPortal" for the marine microorganism, *Prochlorococcus*. This microbe is the smallest and most abundant photosynthetic cell on the planet, with an estimated 10^{27} cells in the global oceans. Each cell contains very few genes – around 2000 – which contain the minimum information required to create life from sunlight, water, and carbon dioxide. Their global population, however – the '*Prochlorococcus* collective' – has an extraordinary amount of diversity. Theoretical projections suggest that there are 50,000 unique genes in this global 'federation' of tiny powerhouses – about twice the number of genes in the human genome. Collectively these genes contain the information necessary for *Prochlorococcus* to dominate diverse ecological niches in the oceans.

Chisholm and her colleagues are facilitating studies of *Prochlorococcus*' complexity, and its role in the oceans, by collecting much of the data on this single organism in "ProPortal". The goal is to provide cross-referenced data encompassing the full diversity of this group, its close relatives, and the viruses that infect them. The portal houses genomic data from cultures as well as metagenomic databases from the global oceans. It also houses data on how *Prochlorococcus*' gene transcription, which is the first step in acclimating to new conditions, responds to environmental perturbations such as the light and nutrient changes. Finally, it archives the genomes of viruses that infect *Prochlorococcus*, identifies the genes that they have in common, and tracks how they are co-expressed as the virus infects the cell – giving us a view of the intimate co-evolution between the virus and its host.

One of the challenges of microbial oceanography is connecting processes at the gene level to those at the ecosystem, and biosphere level – the mission of the NSF-Sponsored Center for Microbial Oceanography and Research (C-MORE), which provides some support for ProPortal. By studying a single microorganism – *Prochlorococcus* – from its genetic composition to the ecology of its global population, and collecting all the data in this cross-referenced portal, they hope to draw researchers from diverse fields to the study of this globally important microorganism, and foster collaborations across fields.

Clipping from home page of ProPortal (<http://proportal.mit.edu/>), developed by Katherine Huang, Libusha Kelly, and Huiming Ding, members of the Chisholm Lab. Though "unadvertised" to date, there has been a steadily growing number of visits to ProPortal since its inception in 2009. It hit 7000 visits this year, coming from many countries around the globe. Published in 2011 in *Nucleic Acids Research*.

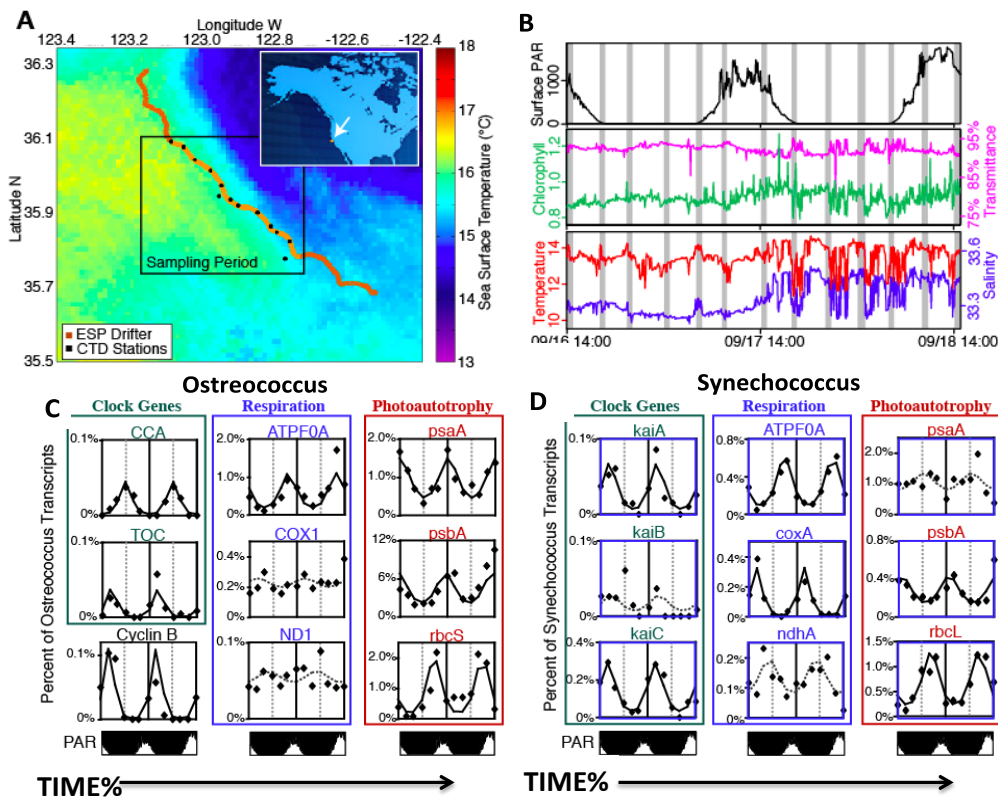


Highlight

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Time lapse community gene expression analyses by autonomous, LaGrangian sampling on the Environmental Sample Processor

Tracking marine microbial community structure and function on the appropriate temporal and spatial scales remains a major challenge for microbial oceanographers. Important events, like phytoplankton bloom and demise, occur on the order of days to weeks and are often missed by regular time series studies. In addition, sampling coherent microbial communities at fixed locations, where currents transport microbial assemblages laterally over time, is also difficult. To address these challenges, C-MORE collaborators developed and validated total community gene expression analyses using the Environmental Sample Processor (ESP) (Ottesen, Scholin, DeLong, et al, in prep.). In a first test the ESP was deployed in the coastal Pacific, with samples collected every two hours for 4 days (A and B). RNA was extracted from the samples, and was sequenced for total community DNA and RNA. Gene expression analyses of total microbial community RNA revealed regular diel patterns in the expression of specific genes in both eukaryotic (*Ostreococcus*) and bacterial (*Synechococcus*) phytoplankton (C and D). An even higher resolution test of the ESP for total microbial community gene expression analyses was conducted on the BioLincs cruise in the North Pacific Subtropical Gyre 2011 (data not shown).



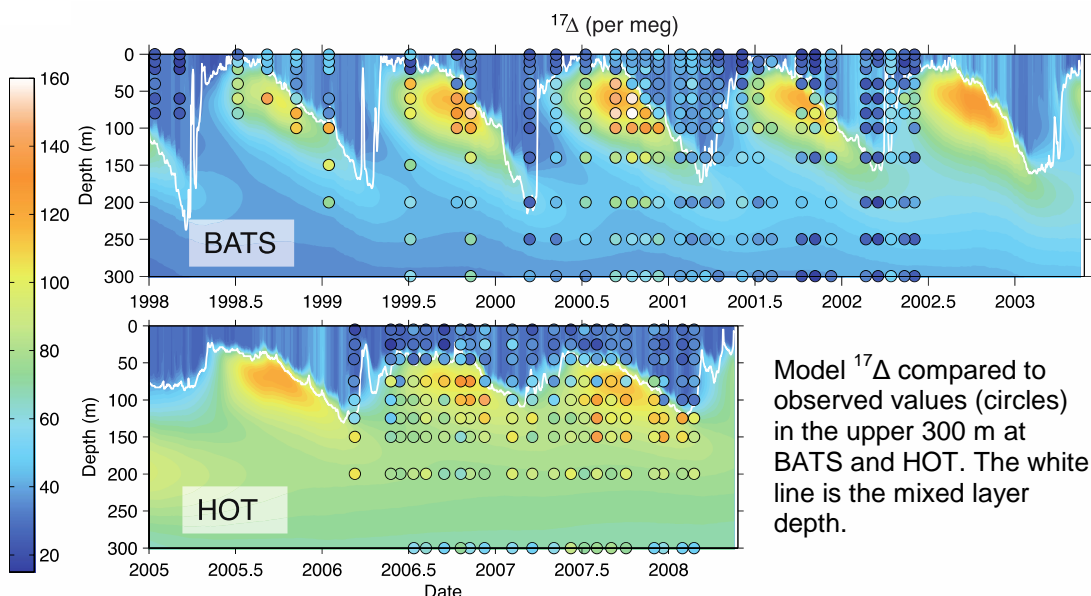
In total, the results show the feasibility and robustness of time-series gene expression profiles from complex planktonic microbial assemblages, and demonstrate the utility of the ESP for high resolution LaGrangian sampling of microbial populations.



Using models to test triple oxygen isotope estimates of ocean biological productivity near Hawaii and Bermuda

The triple oxygen isotope method is a novel geochemical technique that takes advantage of small oxygen isotopic signals in seawater dissolved O_2 to estimate rates of gross primary production of oxygen, a measure of biological productivity. ^{16}O atoms make up the vast bulk (99.8%) of all oxygen; two rare forms are ^{17}O (0.04%) and ^{18}O (0.2%). $^{17}O/^{16}O$ and $^{18}O/^{16}O$ ratios can be combined into a term $^{17}\Delta$ that is not affected by typical “mass-dependent” chemical reactions such as respiration. $^{17}\Delta$ varies depending on the source of the oxygen (Figure). Air-sea exchange injects atmospheric O_2 with low $^{17}\Delta$ (0 per meg) due to “mass-independent” photochemical reactions in the stratosphere; plankton photosynthesis generates O_2 with the isotopic composition of water and a high $^{17}\Delta$ of about 249 per meg. With some knowledge of air-sea exchange one can then back out the gross primary production from $^{17}\Delta$ field data

Ocean tracers such as $^{17}\Delta$ are advantageous because they average over noisy biological variability and do not require direct sample manipulations, which may bias traditional biological incubation methods. On the other hand, tracer methods often require simplifying assumptions about physical mixing. Researchers from the NSF Center for Microbial Oceanography: Research and Education (C-MORE), the University of Washington, and the Hebrew University of Jerusalem incorporated oxygen isotopes into a 1-D mixed layer model to assess biases in the $^{17}\Delta$ method at the Bermuda Atlantic Time Series (BATS) and the Hawaii Ocean Time-series (HOT). The simulations indicate that the standard interpretation of $^{17}\Delta$ data would overestimate mixed layer gross primary production by 60 to 80% due to entrainment of high $^{17}\Delta$ subsurface water but that this bias can be corrected for using models. The oxygen-derived gross primary productivity rates can then be combined with HOT and BATS ^{14}C -incubation data, which more closely match net primary production, to compute phytoplankton respiration. The model reproduces the observations best with a ratio of gross to net productivity of 2.6 (+0.9 -0.8) at BATS and 3.0 (+1.0 - 0.8) at the surface at HOT.



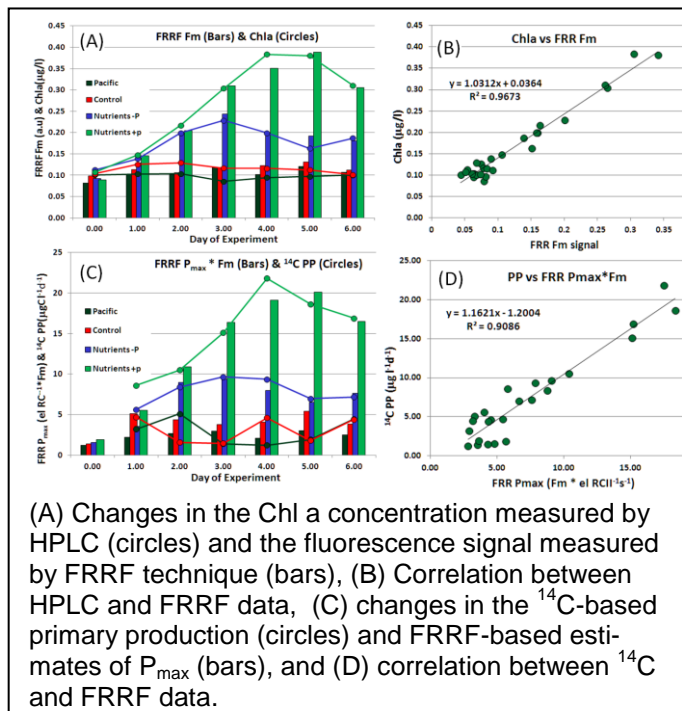
Model $^{17}\Delta$ compared to observed values (circles) in the upper 300 m at BATS and HOT. The white line is the mixed layer depth.



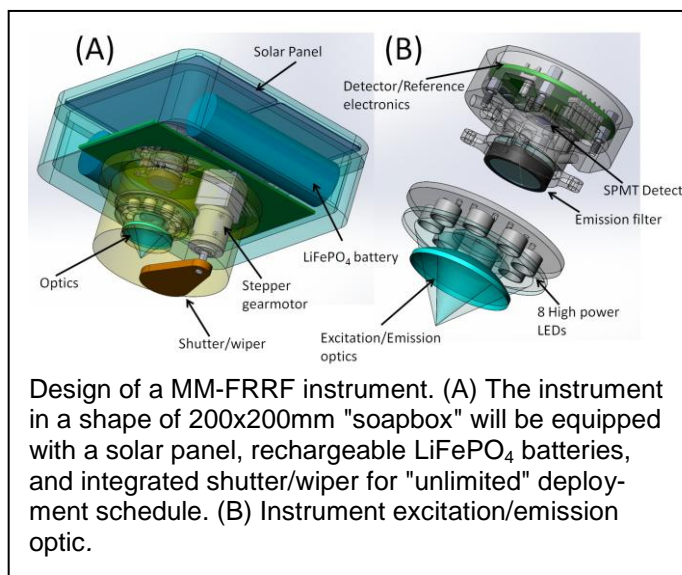
Highlight

Monitoring mesocosm experiments using Fast Repetition Rate Fluorometry

Fast Repetition Rate Fluorometry (FRRF) was used by Sasha Tozzi of the Kolber lab during the BAG-1 Open Ocean Mesocosm Deployment experiment, December 1-13, 2012. We monitored changes in biomass accumulation and photosynthetic performance in response to nutrient manipulations. A good correlation between HPLC pigments concentrations and FRRF fluorescence data was observed (R^2 of 0.97, A, B). Similarly good correlation was observed between ^{14}C based estimates of primary production and the FRRF-based estimates of P_{max} (R^2 of 0.91, C, D). The positive offset in the HPLC vs FRRF-based F_m signal indicates that some of the pigments recorded by HPLC analysis are not associated with the Photosystem II of the reaction centers. The negative offset between ^{14}C and FRRF-based P_{max} data indicate 10% to 15% respiratory losses. These data indicate that FRRF measurements can be used as a proxy for pigment concentration and primary production. As the FRRF measurements can be acquired at time intervals as short as 60 seconds, the mesocosm photosynthetic responses can be observed at high temporal resolution. To accommodate these measurements, we are designing a Mesocosms Monitoring version of the FRRF instrument, (MM-FRRF, at right). The wireless communication will allow continuous observation the mesocosm photosynthetic signatures from the research vessel. The MM-FRRF is equipped with four excitation wavelengths: 445 nm and 470 nm for eukaryotic algae and Prochlorococcus-specific absorption, and 495 nm and 545 nm for cyanobacteria-specific absorption to discriminate between these phytoplankton taxa based on organization of their photosynthetic pigments and signatures, and to assess their contribution to the bulk primary production.



(A) Changes in the Chl a concentration measured by HPLC (circles) and the fluorescence signal measured by FRRF technique (bars), (B) Correlation between HPLC and FRRF data, (C) changes in the ^{14}C -based primary production (circles) and FRRF-based estimates of P_{max} (bars), and (D) correlation between ^{14}C and FRRF data.



Design of a MM-FRRF instrument. (A) The instrument in a shape of 200x200mm "soapbox" will be equipped with a solar panel, rechargeable LiFePO_4 batteries, and integrated shutter/wiper for "unlimited" deployment schedule. (B) Instrument excitation/emission optic.



Highlight

Undergraduate research: Nitrogen fixation in heterotrophic bacteria in the Sargasso Sea

The goal of this EdVentures project was to involve student research to investigate microbial community composition in the oligotrophic waters of the Sargasso Sea, focusing on nitrogen fixation, while contrasting free-living and microzooplankton-associated communities. Samples were collected in conjunction of the BATS-Hydrostation time series sampling on R/V Atlantic Explorer over 7 days in August 2011, and additional samples were collected using a small boat in nearshore waters of Bermuda. A University of Massachusetts Dartmouth undergraduate student (Shane O'Hare) and graduate student (Katyanne Shoemaker) were involved in the research conducted in the Moisander lab. The students learned to conduct water column and zooplankton net sampling on the research vessel, and to collect samples for nucleic acid analyses, and the research continued at UMass Dartmouth during the academic year. The students collaboratively extracted the DNA samples and conducted PCR to amplify 16S rRNA and *nifH* genes from the samples, learned sequence editing techniques, and built phylogenetic trees with their sequence data. Clone library results show putative differences among the particle affiliated and free-living communities. Samples from zooplankton associations included sequences clustering with Vibrionaceae (16S), Rhodobacteriaceae (16S), Piscirickettsiaceae (16S), and Cluster III (*nifH*). 16S clones from the free-living fraction were affiliated with SAR11 cluster, Actinobacteria, Bacteroidetes, and unclassified gamma-Proteobacteria, and *nifH* clones were from *Trichodesmium* and the gamma-Proteobacterium 24774A11. Quantitative PCR to quantify diazotroph abundances is ongoing. Pyrosequencing fusion primers were used to amplify 16S rRNA genes from select water column and zooplankton associated microbial DNA samples and sent for one high throughput pyrosequencing (454) run, which is currently in progress.

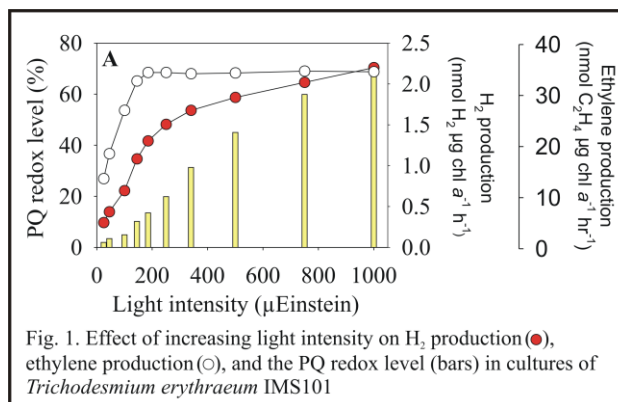


Hydrogen production from N₂ fixing micro-organisms: Studies at the molecular, cellular, and environmental scale

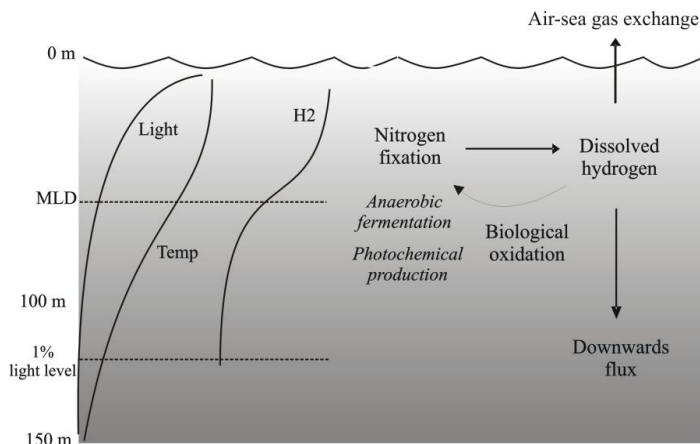
Over the past 3 years there has been growing research within C-MORE on the production of H₂ by N₂ fixing microorganisms, commencing with the study of laboratory-maintained cultures of *Trichodesmium* and *Crocospaera*. We provide an update on our current H₂ research at the (1) molecular; (2) individual, and (3) ecosystem level:

1. Quantify the *in vivo* stoichiometry of N₂ fixation under varying experimental conditions, using *Trichodesmium* as a model organism. The theoretical stoichiometry of N₂ fixation for nitrogenase predicts one molecule of H₂ is produced for every molecule of N₂ reduced, at the metabolic cost of 16 ATP molecules. Whilst the stoichiometry is often cited unequivocally, this ignores the fact that the reaction is still not unambiguously determined. In the absence of N₂, nitrogenase can still reduce protons to H₂, and the rate of H₂ evolution is thought to equal the total electron flux to the nitrogenase system. We are currently measuring gross H₂ production to provide an additional constraint for the quantification of nitrogenase.

2. H₂ production and consumption by diazotrophs. During exponential growth, laboratory cultures of *Trichodesmium* IMS101 produce H₂ at approximately 25 % of its N₂ fixation rate. This high rate of H₂ production relative to N₂ fixation is a direct consequence of fixing N₂ during the day-time as the supply of photosynthetically-derived energy and reductant decreases the need to re-assimilate the H₂ as an energy source. We also observed increases in net H₂ production under higher light intensity, in the presence of apparent N₂ saturation.



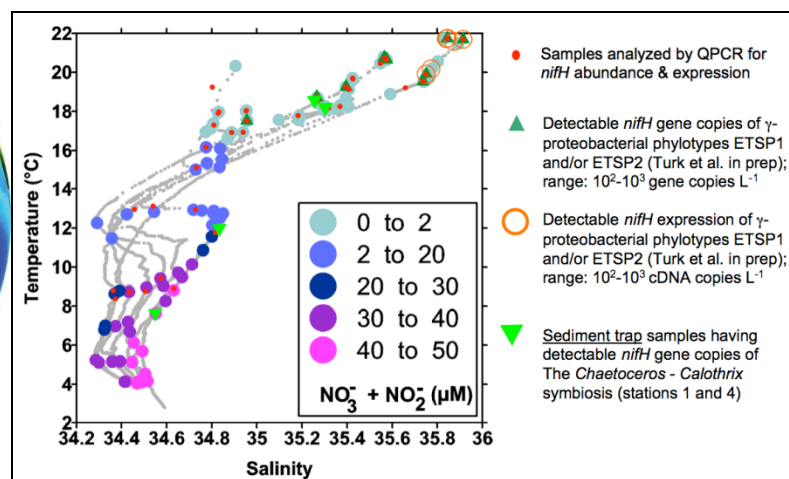
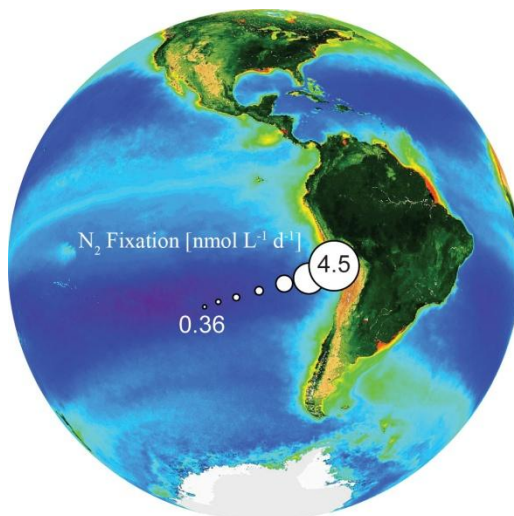
3. Unraveling H₂ cycling in the surface waters of the open ocean. The relationship between N₂ fixation and H₂ cycling was investigated during a C-MORE cruise in the North Pacific. The diel pattern of N₂ fixation varied from a maximum during night-time to a subsequent prevalence during the day-time. Dissolved H₂ uptake rates were equivalent on a molar basis to 10% of ¹⁵N₂ assimilation and 1% of C₂H₄ production. Overall, microbial consumption was the major sink for H₂ in the surface mixed layer, in contrast to sea-air gas flux and downward diffusion.





Nitrogen fixation in the South Pacific Gyre

In December of 2010, C-MORE scientists embarked on an expedition to the South East Pacific along a transect spanning the very high nutrient concentrations of the Chilean coast to the very low nutrient concentrations (oligotrophic) of the mid- South Pacific Subtropical Gyre (SPSG), near Easter Island. Near the coast, denitrifying bacteria are active and abundant in the deep, nitrate-deficient, oxygen minima zones (OMZ). Previous modeling and satellite studies have suggested that the nitrogen poor surface waters overlying and adjacent to the OMZ are hot-spots for N_2 fixation, however *in situ* verification of this suggestion have largely been lacking. A collaborative effort by UCSC, OSU and UH scientists sought to measure the abundance, activity and diversity of diazotrophic microbes along a gradient of microbial habitats. We applied a $^{15}N_2$ tracer approach to estimate N_2 fixation as per a recent methodological improvement to the classic method described by Joe Montoya. PCR and quantitative PCR (QPCR), targeting *nifH*, were utilized as measures of diazotrophic diversity and abundance. The most salient finding from these experiments are so far that: (1) Absolute rates of N_2 fixation were highest in the N-rich coastal surface stations (2); N_2 fixation rates were independent of light, suggesting that heterotrophic diazotrophs are most active; (3) In surface water samples, indeed the only diazotrophic microorganisms detectable by QPCR were γ -proteobacterial phylotypes; (4) However, in sediment trap material from 80, 150, and 500m on stations 1 and 4, we detected low *nifH* gene copy numbers of a symbiotic diatom-diazotroph association between *Chaetoceros* spp. and *Calothrix rhizosoleniae*, a heterocystous cyanobacterium. Given that sediment traps integrate vertical particle fluxes over time and space, our findings suggests that sporadic blooms of these associations can occur in the area, albeit they may be missed by surface sampling. Finally, while the relatively modest rates that we have recorded along the sampling transect are consistent with the few other studies in the region (i.e. volumetric N_2 fixation ranging from 1-5 $nmol L^{-1} d^{-1}$), the abundance of diazotrophs detected by PCR and QPCR appear insufficient to account for the measured rates. These results highlight the remaining mysteries in the N cycle.





Highlight

Phosphorus in our waters: An Edventures science kit

Phosphorus (P) is a required element for life; consequently, its availability may impact primary production rates as well as species distribution and ecosystem function. As an essential plant nutrient, phosphorus is predominantly used as a constituent of fertilizers for agriculture. Phosphorus is also used as a precursor for various chemicals used as flame retardants, pesticides, extraction agents, and water treatment. It is an important component in steel production, utilized in the making of special glasses and fine china, a component in some laundry detergents, baking powder, matchbook strikers, flares, and for military use in incendiary bombs and grenades. Much of the P from fertilizer and animal waste and from other anthropogenic sources enters surface waters and groundwater and these nutrient loads can stimulate large scale macroalgal and/or phytoplankton blooms in receiving waters. Nutrient enrichment in aquatic systems can cause diverse problems such as harmful algal blooms, anoxia, fish kills, loss of habitat and biodiversity, and other problems. Thus, identifying and understanding nutrient inputs and their effects on aquatic ecosystems are of critical importance to management and restoration efforts. In particular, educating the general public about the role of P in our aquatic environment and how human activity impacts natural cycles (including that of P) has implications for the creation of literate and environmentally aware citizens.

Given the elemental nature of P, C-MORE scientists Angelicque White and Adina Paytan have developed lesson plans and an activity kit (“Phosphorus in our Waters”) that can be used by educators to teach about water quality and nutrients in the environment. A three lesson unit has been prepared and includes all of the materials and instructions needed for teachers to lead a successful activity. These kits are slated to be tested by high school educators in the Santa Cruz vicinity and two kits will be ready for distribution for the 2012/13 school year. A portion of kit components are shown below. Given that P is an integrative research theme with investigators at each partner institute, this P-centric activity has the potential to be adapted to the needs of many within and outside of the core C-MORE personnel.



A subset of the components included in a single science kit: including a Vernier spectrophotometer, software, chemical reagents, pipettes and tubes for a standard curve.



Highlight

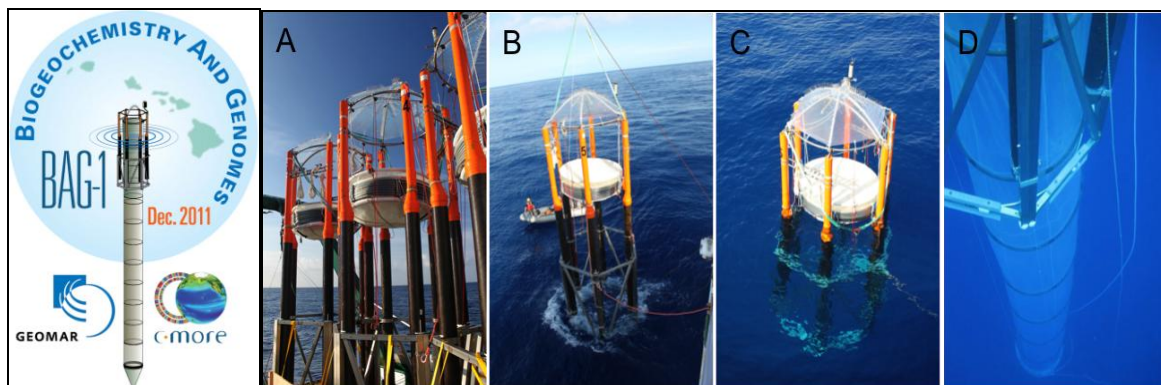
Biogeochemistry And Genomes (BAG-1): An open ocean mesocosm deployment off Hawaii

C-MORE and GEOMAR (Germany) partnered to conduct an open ocean deployment of three free-floating mesocosms (Fig. 1A-D). This collaboration provided the unique opportunity to not only test the feasibility of utilizing the mesocosms in the open ocean (the primary engineering objective), but also to examine the response of open ocean plankton assemblages to the addition of nutrients in different sized incubation vessels (the primary science objective). Our overall scientific aim was to study the response of ocean plankton to additions of nutrient mixtures containing NO_3^- , PO_4^{3-} , Si(OH)_4 , and trace metals, relative to responses to nutrient mixtures containing NO_3^- , Si(OH)_4 , and trace metals (+P vs. -P mixtures).

Participants included scientists, engineers, and a diving team from multiple institutions; the cruise departed Honolulu, HI on December 1st 2011 aboard the UH R/V *Ka'imikai-O-Kanaloa* (KOK), steaming south to the leeward coast of the island of Hawaii. Upon arrival, three mesocosms were successfully deployed and deck-board incubation experiments were set up. The mesocosms and deck-board incubation experiments were sampled daily to examine changes in nutrient and particulate matter concentrations (N, P, Si), photosynthetic pigments, biomass and rates of plankton production. Numerous samples have been analyzed and some first preliminary findings suggest:

1. The largest response of the phytoplankton community in the mesocosms was found when a nutrient mixture included PO_4^{3-} (biomass and production were 2-4 fold higher compared to the nutrient mixture lacking excess PO_4^{3-})
2. The response of the phytoplankton community in carboy experiments was different from that in the mesocosms. No large difference in phytoplankton biomass and production was observed between the -P and +P treatments. Instead, both were ~ 4 fold higher due to the addition of deepwater nutrients collected in 1000m from Station ALOHA.

We anticipate finalizing the remaining sample analysis within the next 1-2 month; in doing so, we hope to fully characterize how planktonic communities responded to the additions of nutrients and provide mass balances of numerous nutrient elements (C, N, P).



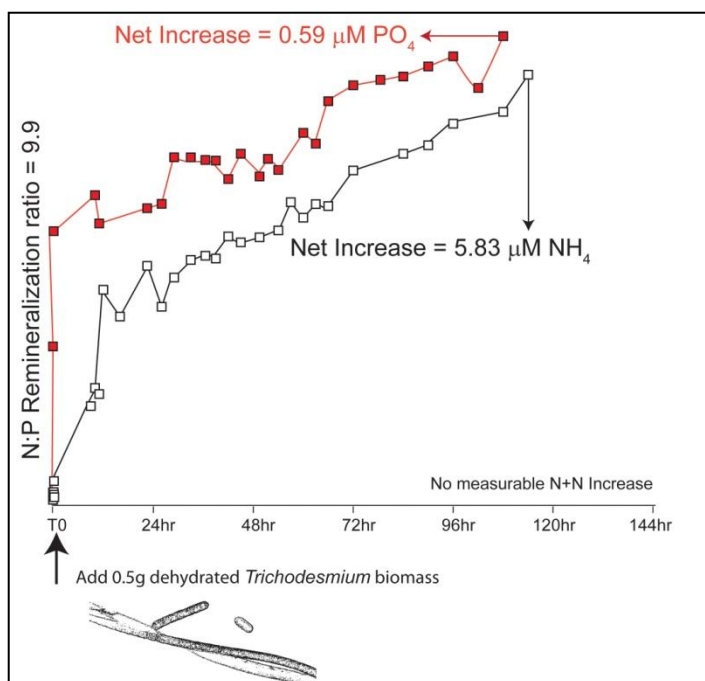
Photographs showing the 'BAG-1' logo and the mesocosms (A-D). (Photo courtesy K. Björkman)



Highlight

The rate and stoichiometry of particulate matter degradation: An experimental approach in the South East and North Pacific Subtropical Gyres

A series of exogenous addition experiments were performed in the North Pacific Subtropical Gyre in the boreal spring of 2011 and in the South Pacific subtropical gyre in the austral summer of 2010. These experiments, conducted by C-MORE scientist Angelicque White, sought to investigate the lability of particulate material generated by ecologically significant phytoplankton as well as the remineralization stoichiometry resulting from heterotrophic decomposition of organic matter. To address these objectives, particulate material was isolated from cultures of the diazotroph *Trichodesmium* strain IMS101, the cyanobacterium *Prochlorococcus* (MED-4), and the diatom *Thalassiosira*. This material was dried and the carbon, nitrogen, and phosphorus content was determined. At sea, water containing active heterotrophic bacteria was collected from below the surface mixed layer and aliquots of dried POM were added. Incubations were maintained in the dark at *in situ* temperature and remineralization was tracked over a period of roughly a week by measurement of the production of inorganic phosphate, nitrate+nitrite, and ammonium (a by-product of respiration). In collaboration with C-MORE partners on these cruises,



leucine uptake rates, alkaline phosphatase activity, DOM composition, and $^{32}\text{PO}_4/^{32}\text{ATP}$ uptake were measured. The figure to the left (note: different scales for PO_4 and NH_4) shows a subset of these experimental results: the time-course of PO_4 and NH_4 production over time following addition of dehydrated *Trichodesmium* organic matter. In sum, these experiments provide insight into the linkages between phytoplankton community structure and the inherent range of elemental composition and lability of photosynthetically produced organic matter and the stoichiometry of DON and DOP decomposition. Consistent with previous observations of preferential P remineralization, we also find that P is released rapidly and early relative to N.



Highlight

Diazotroph activity and community structure under different $p\text{CO}_2$ conditions at Station ALOHA

Human reliance on fossil fuel combustion and deforestation continue to alter atmospheric and oceanic carbon dioxide (CO_2) inventories. In large regions of the world's oceans, the partial pressure of carbon dioxide ($p\text{CO}_2$) in seawater is increasing at a rate similar in magnitude to rising atmospheric $p\text{CO}_2$. Such changes have also significantly decreased upper ocean pH. By the year 2150, atmospheric $p\text{CO}_2$ is predicted to almost triple (from currently ~ 385 to $1100 \mu\text{atm}$), with a concomitant decrease in surface ocean pH of ~ 0.5 units.

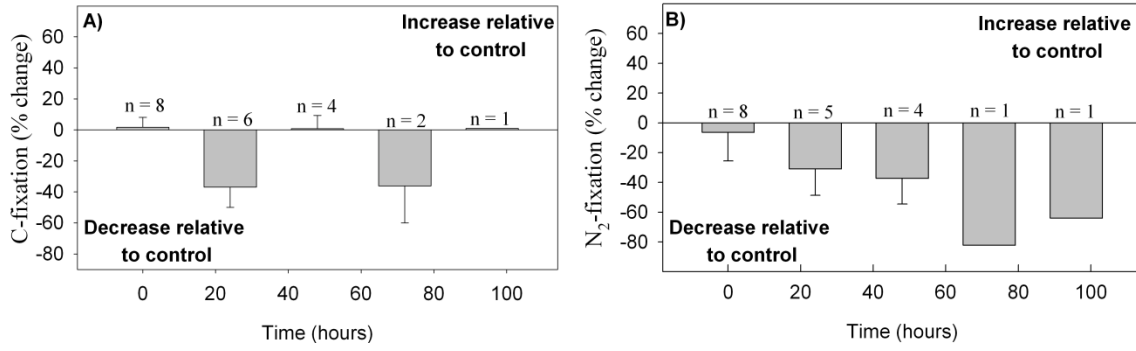
Biological N_2 fixation represents an important supply of new nitrogen (N) to the oligotrophic regions of the open ocean, supporting and regulating net plankton productivity and carbon export. In the North Pacific Subtropical Gyre (NPSG), supply of fixed N by N_2 fixation is estimated to be comparable to the delivery of N via mixing, upwelling and/or advection. Hence, ocean diazotrophs play pivotal roles as catalysts of biogeochemical transformations and it is fundamental to study their response changes in $p\text{CO}_2$ and pH.

Several laboratory-based studies have demonstrated that increases in $p\text{CO}_2$ (750 - $1000 \mu\text{atm}$) stimulates carbon (C) and nitrogen (N_2) fixation by cultivated diazotrophs such as *Trichodesmium* and *Crocospaera*. However, the response of natural assemblages of diazotrophs to predicted changes in the seawater carbonate chemistry remains still unclear.

As part of a collaborative effort between the Church Lab at the University of Hawaii and the Letelier lab at Oregon State University, we have been exploring the short-term responses of natural assemblages of diazotrophs to projected changes in the seawater carbonate chemistry through a series of incubation experiments (May 2010 – April 2011) at Station ALOHA, the field site of the Hawaii Ocean Time-series program ($22^\circ 45'\text{N}$, $158^\circ 00'\text{W}$). Rates of N_2 fixation and carbon (C) fixation have been measured coincident with determinations of nitrogenase gene abundances and transcription. Our results indicate:

1. Increased $p\text{CO}_2$ has little or no influence on rates of N_2 fixation and C-fixation relative to the ambient controls in the NPSG (Fig. 1A and B)
2. Unicellular diazotroph phylotypes dominated ($>95\%$) *nifH* gene abundances during all experiments
3. Diazotroph abundances and *nifH* gene expression remained largely unchanged during the experiments

To date, diazotrophs have been promoted as one of the few groups of microorganisms actually benefiting from elevated CO_2 . However, evidence for this has exclusively been based on results obtained from laboratory-controlled studies with cultured representatives. Our field-based study suggests that natural diazotrophic assemblages demonstrate complex responses to changes in $p\text{CO}_2$. Partly, these differences may be explained by the fact that field-based studies include a wide range of phylotypes that are physiologically diverse. Certainly, more research is needed until we can conclude whether diazotrophs are winners or losers in a high CO_2 world.



Figures: Changes in C-fixation (A) and N₂-fixation (B) in pCO₂ treated samples (1100μatm) relative to the control (~385μatm) expressed as % change.



Highlight

Around the microbial loop: Tracking dissolved organic matter from biological source to sink

A significant fraction of the planet's carbon is found in organic compounds dissolved in seawater. Longstanding questions regarding the composition and cycling of this dissolved organic material (DOM) are being tackled by a team of C-MORE researchers led by graduate student Jamie Becker representing five different laboratories spread over three research institutions.

At the Woods Hole Oceanographic Institution and in collaboration with the Massachusetts Institute of Technology, the composition and source of marine DOM is being investigated using pure cultures of various marine phytoplankton. To date, DOM from ten different phytoplankton strains has been isolated, characterized, and compared to evaluate potential connections between DOM composition and its biological source. The team has found that while all phytoplankton tested produce some DOM that is unique, the overall composition of phytoplankton-derived DOM appears to correlate well with phylogeny. This suggests that marine phytoplankton produce suites of DOM that mirror their genetic similarities and has implications for the structuring of microbial communities in the sea.

At the University of Hawaii, the consumption of marine DOM is being investigated using pure cultures of various heterotrophic bacterioplankton. DOM generated in the WHOI/MIT collaboration has been given to more than a dozen strains of heterotrophic bacteria in hundreds of combinations to evaluate the effects of this DOM on heterotrophic fitness. The team has found a variety of responses ranging from suppressed to enhanced growth, indicating that the consumption and lability of marine DOM depends both on the source of the material and on the identity of the consumer. Novel relationships between autotrophic and heterotrophic bacteria are being uncovered and potential model systems for DOM cycling are being identified.

At the Massachusetts Institute of Technology, the physiology of DOM consumption is being investigated by tracking the transcriptional responses of several heterotrophic bacteria to DOM additions. Two diverse strains of bacteria were tracked closely during a week of growth with and without the addition of DOM from a known phytoplankton source. The team hopes to gain new insight into the metabolic pathways employed by different bacteria during the consumption of marine DOM.





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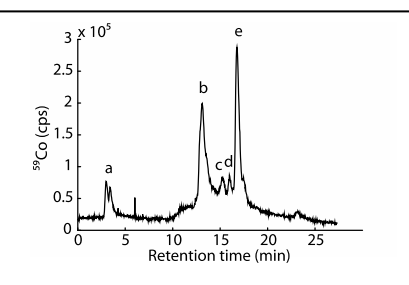
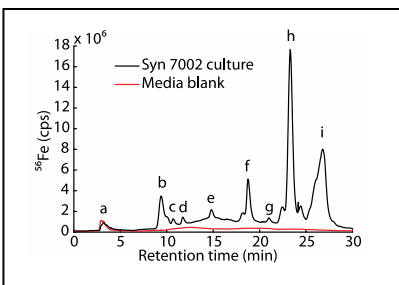
HPLC-ICP-MS characterization of organic ligands produced by marine microbes

Marine microbes produce organic ligands that influence the solubility and bioavailability of trace metals in the ocean. Determining the composition and biogeochemical role of these ligands is important for understanding the link between trace metals and marine ecosystems. We have recently developed a method to characterize these ligands by extracting them from seawater or culture media with a hydrophobic resin, separating them with high-performance liquid chromatography (HPLC) and measuring their metal content via multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS). Through a collaborative effort between CMORE laboratories at MIT and WHOI, led by Rene Boiteau and Jessica Fitzsimmons, this method has been used to investigate Fe and Co ligand production in cultures of marine cyanobacteria.

As a pilot study to verify that our HPLC-ICP-MS method can detect multiple strong trace metal ligands that are naturally produced in seawater, we analyzed media from a culture of *Synechococcus* sp. PCC 7002. Under low Fe conditions, this coastal cyanobacteria strain produces a suite of iron binding siderophores (synechobactin A-C) to facilitate uptake (*Ito et al.*, 2005). The ^{56}Fe chromatogram for the organic extract from *Synechococcus* sp. 7002 shows three large peaks with retention times of 26.7 min, 23.3 min, and 18.7 min (left figure, peaks f, h, and i) that correspond to synechobactins A-C. There are also a number of smaller peak (right figure, peaks b-e, g), which correspond to unknown Fe ligands that have not previously been detected.

We have also used this HPLC-ICP-MS method to demonstrate the production of strong cobalt ligands by *Prochlorococcus* strain MED4. A study by *Saito et al.* (2002) hypothesized that this *Prochlorococcus* strain may produce a cobalt ligand that enhance Co uptake *in vitro*. We analyzed the organic extract of a laboratory culture of *Prochlorococcus* strain MED4 by HPLC-ICP-MS and found that this strain at least 5 Co ligands (right figure). The peak at 17 min corresponds to cobalamin (vitamin B12), which is synthesized by this strain. The other peaks correspond to uncharacterized Co ligands that are released by *Prochlorococcus*. These may be precursors or degradation products of cobalamin, or some other ligand that is produced to facilitate Co acquisition.

These results demonstrate the utility of our HPLC-ICP-MS for rapidly screening marine microbes for metal ligand production. The next step will be to collect the chromatography fractions containing the metal ligands and further purify them for characterization by nuclear magnetic resonance spectroscopy and soft ionization mass spectrometry. This work will contribute to a better understanding of metal speciation in the ocean and acquisition strategies that marine microbes use to compete for trace metals in regions where they are scarce.



Left: Separation of organic extract from a *Synechococcus* sp. PCC 7002 culture. Each ^{56}Fe peak (a-i) corresponds to one or more Fe ligand.

Right: HPLC-ICP-MS chromatogram of *Prochlorococcus* strain MED4 extract. Peaks a-d correspond to unknown Co ligands. Peak e likely corresponds to cobalamin.



Highlight

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Ecogenomic sensors make a global debut

Nearly two decades ago scientists and engineers conceived of developing a network of “ecogenomic sensors” – devices that would automate the detection and quantification of particular microorganisms, their genes and gene products as part of a larger earth and ocean observing system. Partners comprising the Center for Microbial Oceanography Research and Education (C-MORE) have played a leading role in realizing this vision through the development and application of the Environmental Sample Processor (ESP). To date, the ESP has been fielded in a variety of deep sea, coastal and open ocean settings. These tests have been unique in highlighting the emergent use of ecogenomic sensors for ocean research and monitoring, and have served to illustrate the vast potential for developing this new class of sensor further. However, the scope and duration of these deployments has been limited given the few ESPs available and the lack of trained personnel for operating them.



A distributed network of three ecogenomic sensors will operate spring 2012 through a collaboration between the Cawthron Institute, NOAA/NMFS, Woods Hole Oceanographic Institution, and the Monterey Bay Aquarium Research Institute.

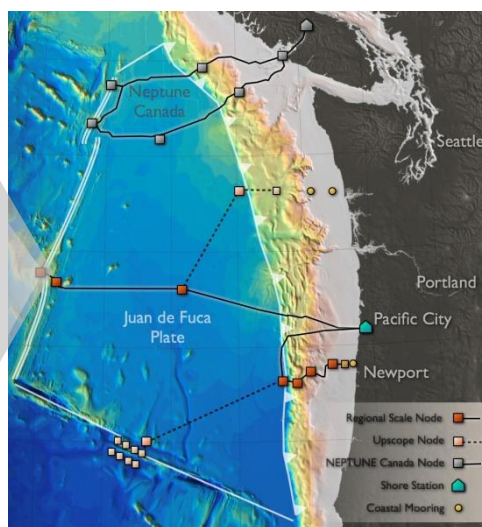
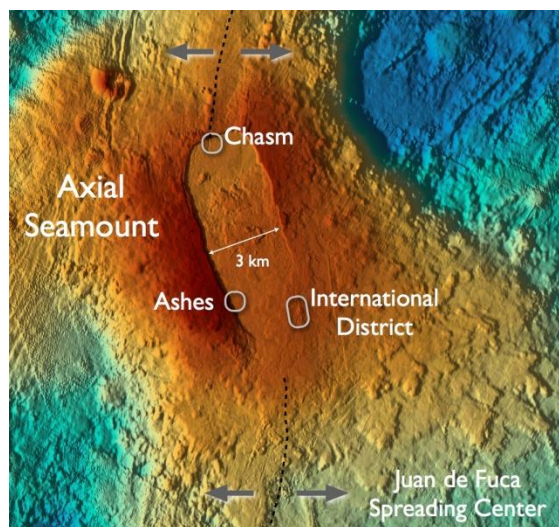
Recently, major strides were made in overcoming those limitations. Through commercialization efforts led by Spyglass Biosecurity and McLane Research Laboratories, the number of ESP systems has increased sharply with users now located both in the U.S. and abroad. Building on that trend, several ESPs will be fielded in different parts of the world during spring 2012 and will run simultaneously to demonstrate the feasibility of operating a “global” network of ecogenomic sensors as was envisioned long ago. One instrument will be located in a shellfish growing area in New Zealand and will be programmed to detect both harmful algal bloom (HAB) species and bacteria associated with sewage effluents. Sister devices also programmed to detect HAB species will be located in Puget Sound (WA) and in the Gulf of Maine (NH/ME). Information gleaned from these installations, along with a command and control capability, will be available in real-time via a web-based portal and wireless communication network. C-MORE, through the Monterey Bay Aquarium Research Institute, is coordinating this test as one element of the Center’s broader knowledge and technology transfer portfolio. Additional support for developing and testing the ESP was made possible by funding from NSF, NOAA, NASA, and the Keck, David and Lucille Packard, and Gordon and Betty Moore Foundations. This collaborative effort is being led by C-MORE scientist Chris Scholin.



Highlight

An ecogenomic sensor reveals deep-sea hydrothermal vent microbes in real-time

The advent of the Ocean Observatories Initiative (OOI) deep sea cabled sensing network will create unprecedented opportunities for deploying novel sensor systems in extreme environments that are often difficult to occupy with ship-based expeditions. The power and communications infrastructure afforded by the cable nodes will allow investigators to carry out interactive experiments and test hypotheses remotely *in situ via an internet portal, effectively making experimentation in the deep sea possible "24/7"*. Researchers at the Monterey Bay Aquarium Research Institute (MBARI), led by C-MORE scientist Chris Scholin, are exploring that potential through the development and application of the Environmental Sample Processor (ESP). The ESP employs molecular probe techniques to detect the abundance of microorganisms, their genes and metabolites in near real-time autonomously. Coupled with sustained environmental measurements provided by the OOI cable backbone, assessment of microbial community structure and function will soon be possible on temporal scales not achievable previously. In 2011, the MBARI team in collaboration with collaborators at Harvard and Caltech took a step toward reaching that goal by proving that the ESP could operate in a hydrothermal vent field in the caldera of Axial Seamount. Deployed to 1600m below the sea surface on a free-fall benthic lander, the instrument successfully used DNA probe array and quantitative PCR methods to reveal the fundamental differences in microbial community structure between the cold, "background sea water" in the vent field versus the hot fluids that were being expelled from the seafloor. This preliminary trial opens the door for longer duration runs once the cable network is fully installed and is operational. Design and construction of the deep-sea ESP was made possible by funding from NSF, NASA, and the Keck, David and Lucille, and Gordon and Betty Moore Foundations. Researchers from Harvard, Caltech, NOAA, Lawrence Livermore National Laboratory and the Center for Microbial Oceanography Research and Education (C-MORE) are contributing to its development and application.

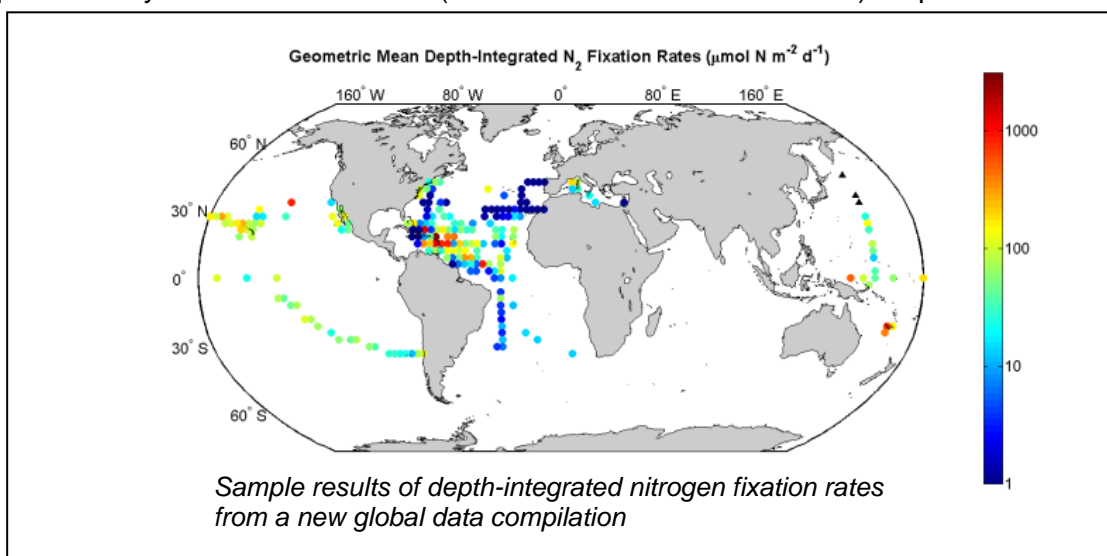




Highlight

Compiling a global dataset for marine nitrogen fixing microbes

A group of marine microbes called “diazotrophs” are able to fix di-nitrogen gas (N_2) into bioavailable nitrogen. Planktonic nitrogen fixers play an essential role in subtropical ocean gyres, providing a source of new bioavailable nitrogen into the otherwise nutrient poor surface waters. The last decade has seen a virtual explosion in new ocean field data on marine diazotrophs spanning a wide range of microbial taxonomic groups and including free-living unicellular and filamentous organisms as well as symbiotic organisms that live inside other plankton. Recently a group of researchers from the NSF Center for Microbial Oceanography: Research and Education (C-MORE) set out to compile a new, up to date global database on planktonic diazotrophs. They started out by collecting data from the C-MORE and Hawaii Ocean Time-Series (HOT) programs and by contacting colleagues around the country and the world; they also mined existing databases and collected data from the historical scientific literature. C-MORE postdoctoral investigator Ya-Wei Luo at the Woods Hole Oceanographic Institution led the effort with contributions from 46 collaborators. The resulting comprehensive database includes 3,536 nitrogen fixation rates, 5,191 cell-count based abundances, and 3,201 *nifH* gene-based abundances along with ancillary data on hydrography, nutrients, and chlorophyll. The nitrogen fixation rates and diazotroph cell counts, which are categorized into *Trichodesmium*, unicellular cyanobacteria, and heterocystous cyanobacteria, are being used to explore the environmental conditions that determine the spatial and temporal variations in nitrogen fixation and diazotroph community structure. The dataset also provides a valuable resource for testing and parameterizing a new generation of plankton functional group models that explicitly include diazotrophs. The database will be permanently stored at PANGAEA (doi:10.1594/PANGAEA.774851) for public access.



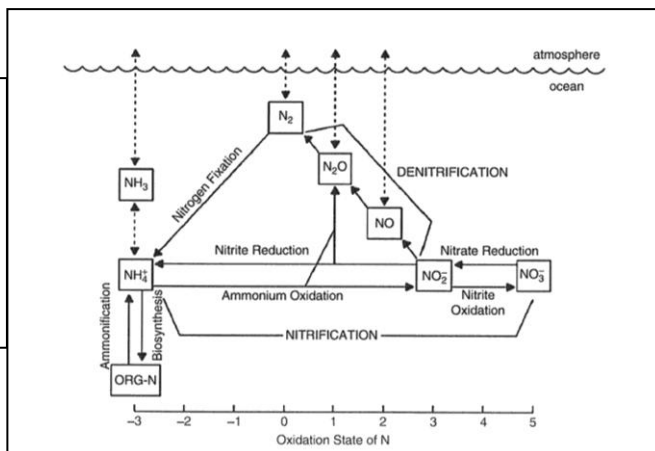


Highlight

Ammonium dynamics at Station ALOHA during the BioLINCS cruise

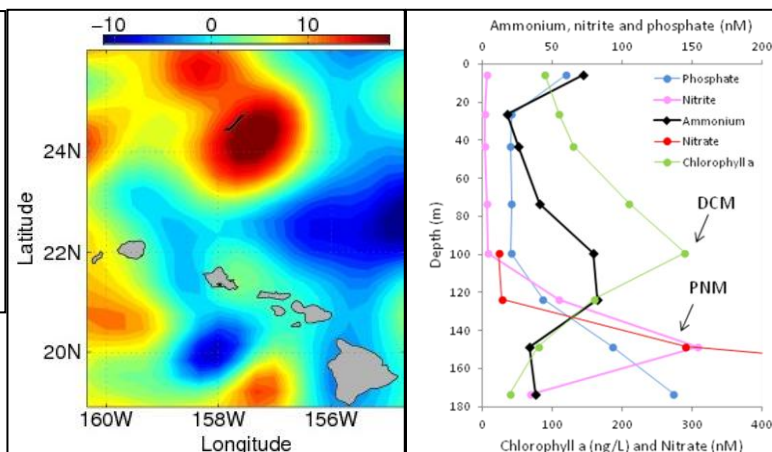
The North Pacific Subtropical Gyre (NPSG) is characterized by low nutrient concentrations in euphotic waters, nitrate being scarcer than phosphate. Under these circumstances, nitrogen fixing organisms are abundant, and primary production is mainly driven by regenerated forms of nitrogen, mostly ammonium (below). During the C-MORE cruise BioLINCS (6-21 September 2012), the distribution and dynamics of ammonium were studied at the NPSG.

Schematic representation of the marine nitrogen cycle, showing major pools and transformations. Ammonium (NH_4^+) is a key component involved in several pathways of the marine N-cycle. From Karl et al. (2008).



Usually, during the BioLINCS cruise, ammonium concentrations increased toward the near surface (5-25 m) and near the base of the euphotic zone (175 m) (below). Also, in 9 out of 10 profiles, an accumulation of ammonium was found at the depth of the chlorophyll maximum or below (coincident with the primary nitrite maximum). Except for the two last stations, we found that ammonium was inversely correlated with chlorophyll concentration, dissolved oxygen and light, consistently with a suggested decrease of ammonium uptake due to light-limitation. This light-limitation of ammonium uptake could be extended to nitrite and nitrate uptake too, as they were also positively correlated with ammonium concentrations. Ammonium was also highly correlated with the nitrogen to phosphorus ratio.

Sea level anomaly and track of the ship on 9 Sept (left), and vertical profiles (right) of ammonium (highlighted in black), chlorophyll *a*, nitrite, nitrate and phosphate. DCM: Deep Chlorophyll Maximum. PNM: Primary Nitrite Maximum.



The ammonium distribution and dynamics, together with other regenerated forms of nitrogen (i.e. dissolved organic nitrogen, including urea), will continue to be studied in C-MORE during the following year, with special emphasis in its interactions with the different microbial communities present at different depths of the water column at the NPSG.

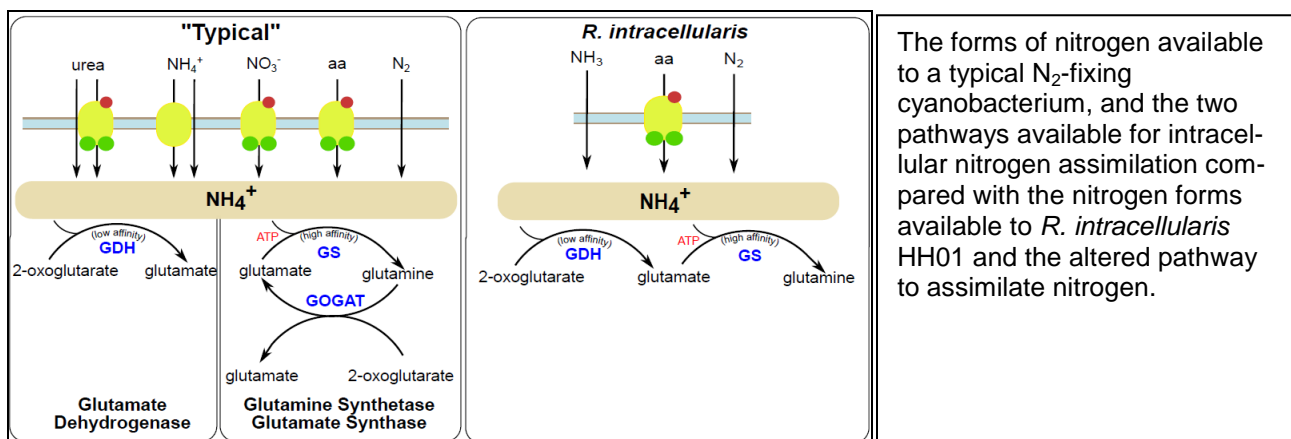


Highlight

Genome reduction in a diatom-associated endosymbiotic cyanobacterium redraws the conventional assimilation pathway for fixed N₂

In collaboration with Tracy Villareal (UT-MSI) and former CMORE members Rachel Foster (MPI – Bremen) and Jim Tripp (JGI), the genomes of two closely-related cyanobacteria associated with open ocean diatoms were sequenced. These symbiotic cyanobacteria supply the host diatoms with fixed N₂, and are an important source of new production and vital to carbon sequestration, but little else is known about these associations. We sequenced the genomes of *Richelia intracellularis* HH01, found within the frustule of the diatom *Hemiaulus hauckii*, and *Calothrix rhizosoleniae* SC01, an extracellular symbiont of the diatom *Chaetoceros* spp. The *R. intracellularis* HH01 genome is only half that of *C. rhizosoleniae* SC01 genome, suggesting the endosymbiont has undergone genome reduction from an ancestor with a larger genome. Nearly half of the *R. intracellularis* HH01 genome is non-coding, indicating the genome is likely still in the process of reduction. This reduction is likely caused by a symbiotic lifestyle with no free-living stage, and may continue evolving towards a N₂-fixing organelle, similar to the evolution of chloroplasts. *C. rhizosoleniae* SC01 may be in a much earlier stage of the same process.

The genome reduction in *R. intracellularis* HH01 is very apparent in nitrogen acquisition and assimilation pathways. The genome lacks the genes to assimilate several common nitrogen forms, ensuring N₂-fixation will continue even when other nitrogen sources are available. Furthermore, *R. intracellularis* HH01 is the only known cyanobacterium to not have a gene to encode glutamate synthase (GOGAT), which alters the pathway for assimilating N₂-derived ammonium. The modifications of the basic nitrogen metabolism machinery in *R. intracellularis* HH01 amplify N₂ fixation and streamline nitrogen transfer from symbiont to host.

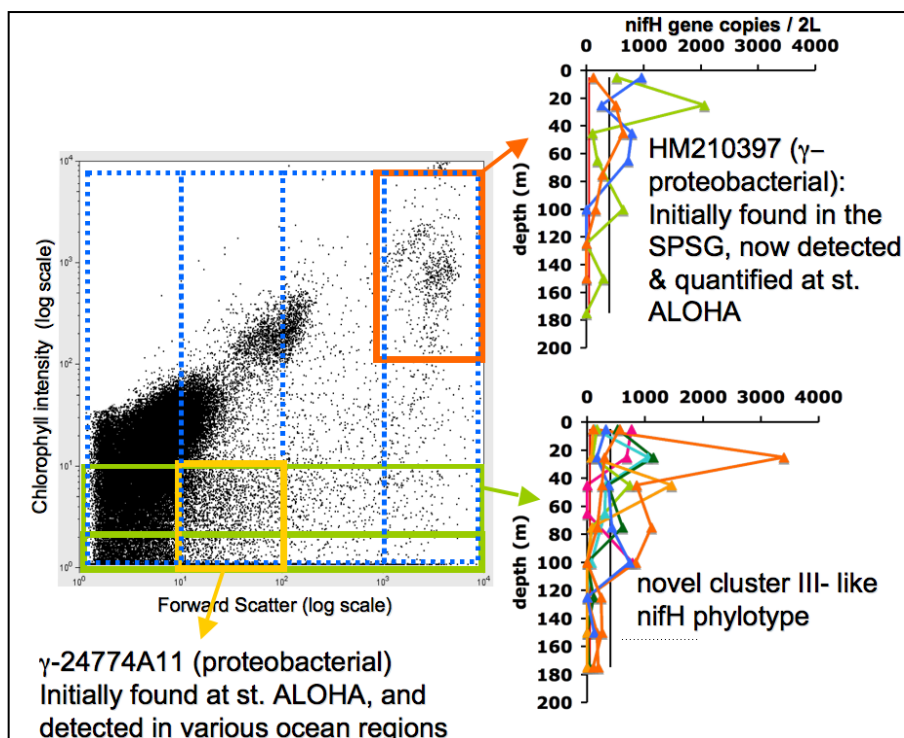




Highlight

New insights on non-cyanobacterial diazotrophs in the North Pacific Subtropical Gyre

In recent years, there has been increasing interest in the ecological significance of marine non-cyanobacterial, presumably heterotrophic N_2 fixing prokaryotes. Highly diverse nitrogenase (*nifH*) sequences clustering mainly with Proteobacteria are frequently detected or even prevalent in clone libraries and in libraries obtained from pyrosequencing of *nifH* amplicons. However, it is not trivial to distinguish between *nifH* sequences of real planktonic prokaryotes and sequences that possibly represent reagent contamination. Further, due to possible PCR bias it is not valid to infer ecological relevance from the relative abundance of certain *nifH* phylotypes in particular clone libraries. During the BioLINCS cruise in September 2011, a team led by C-MORE post-doc Deniz Bombar used flow cytometry to sort cells from a grid of sorting gates placed over cytograms of forward scatter vs. chlorophyll fluorescence, and subsequently *nifH* was amplified from these sorted cells using nested PCR with degenerate primers. We expected *nifH* sequences of real marine bacteria to be consistently detectable in distinct sort gates, while contaminant sequences should appear more randomly in various sorting regions. Some interesting first results from this are: (1) Phylotype γ 24774A11, a previously described and widely distributed oceanic *nifH* sequence, exemplifies the robustness of our initial expectation, given that this sequence was clearly constrained to a distinct sort region. (2) A novel cluster III-like (anaerobic bacteria) *nifH* sequence was found, which was also quantifiable in the water column at all BioLINCS core cast stations using quantitative PCR. (3) Other proteobacterial *nifH* phylotypes were recovered non-randomly as well, including a sequence previously found in the South Pacific Subtropical Gyre (SPSG). This novel approach will help to elucidate the role of non-cyanobacterial diazotrophs in nitrogen cycling in the North Pacific Subtropical Gyre.



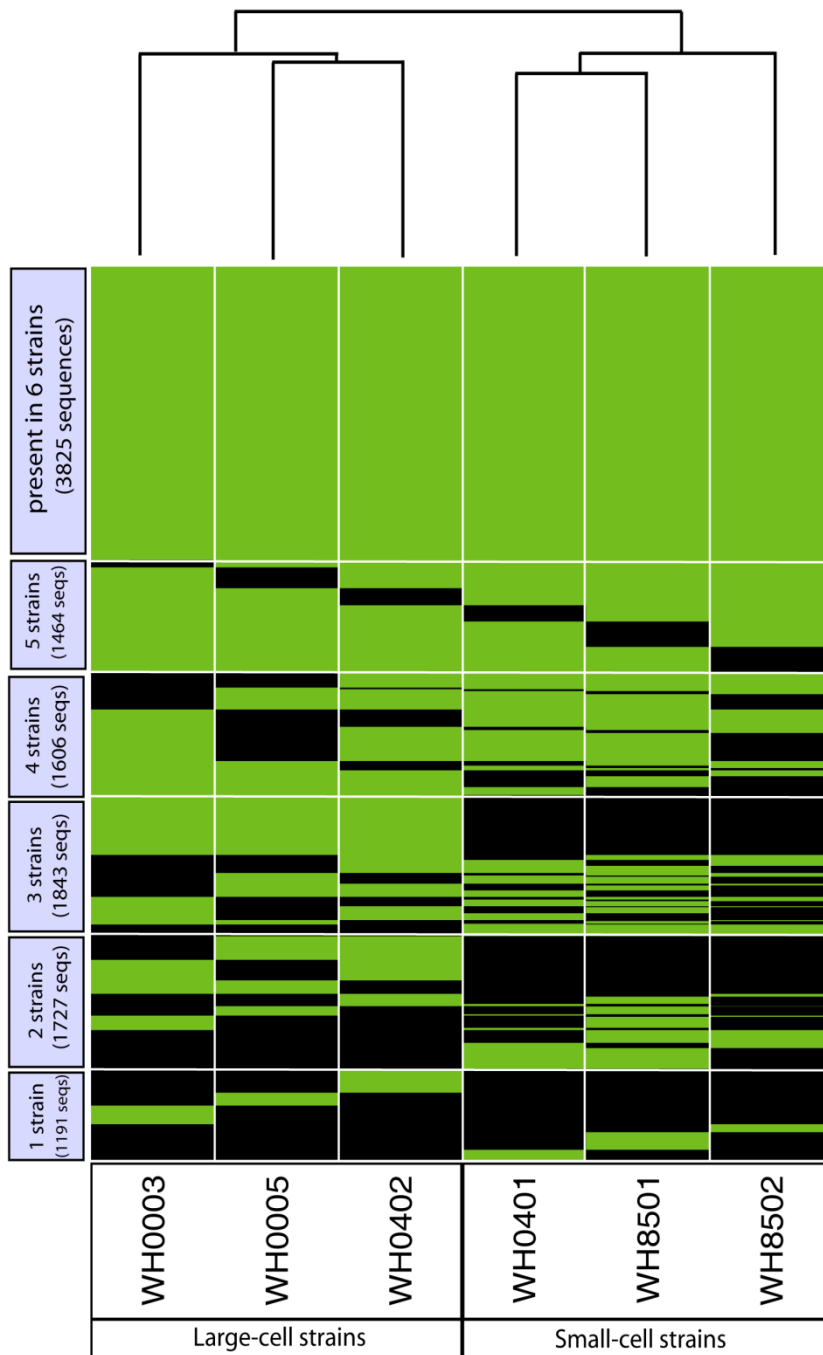


Highlight

Comparative Genomics of the N₂-fixing cyanobacteria *Crocospaera watsonii*

In the nutrient depleted surface water of the ocean's oligotrophic gyres, nitrogen (N₂) fixation is a key source of biologically available nitrogen for the phytoplankton community. *Crocospaera watsonii* is an abundant and widely distributed marine cyanobacterium that carries out a significant portion of such N₂-fixation. Because multiple strains have been cultivated, *C. watsonii* is also an important model organism for physiological and genetic studies of marine N₂-fixers. Cultivated strains fall into two phenotype categories based on observable differences such as exopolysaccharide (EPS) production and average cell size. Recent studies have shown that the two phenotypic groups are also distinguished by ecologically important differences in photosynthetic efficiency, phosphorous scavenging capabilities, and per-cell N₂-fixation rates. Despite this suite of physiological differences, early genetic comparisons targeted at one or a few genes found essentially no difference in DNA sequence composition between the strains. An explanation for this apparent conundrum was found through whole genome comparisons conducted by Shellie Bench (UCSC graduate student) in the lab of Jonathan Zehr (UCSC, C-MORE PI).

A recent publication by Bench describes the genomic comparison of two strains with alternate phenotypes, and showed that the vast majority (~80%) of the genomes were >99% identical at the nucleotide level. However, most of the remaining portions of the genomes were strain-specific (i.e. they showed no sequence similarity to any sequence in the other genome). These strain-specific sequences appear to be responsible for strain divergence in this species that otherwise maintains an unusually high degree of genomic sequence conservation. A subsequent analysis showed a similar pattern of genetic divergence by comparing the genomes of four additional *C. watsonii* strains (six total, three from each phenotype group). As part of this analysis, the open reading frames (ORFs) from all six genomes were grouped according to their presence or absence in each strain. Using the sequence similarity cut-off of >97% identity over >70% of the ORF, a set 11,635 non-redundant sequences were identified in the six genomes (figure 1). Over 90% of these ORFs were found in two or more strains, and nearly one third were present in all six strains. In addition, many more sequences were shared exclusively among strains of the same phenotypic group, as opposed to sequences shared across phenotypes. This suggested that, in *C. watsonii*, phenotype is more predictive of genetic similarity than similar time or location of strain isolation. This was supported by clustering based on the presence/absence pattern for ORFs in each strain (dendrogram in figure 1), and further supported by additional phylogenetic and statistical analyses. Finally, among sequences that were strain- or phenotype-specific, a number of ORFs were found with functions that could explain aspects of observed phenotypic differences, including exopolysaccharide biosynthesis genes and photosystem genes.



Presence/Absence of all ORFs in six *C. watsonii* genomes.

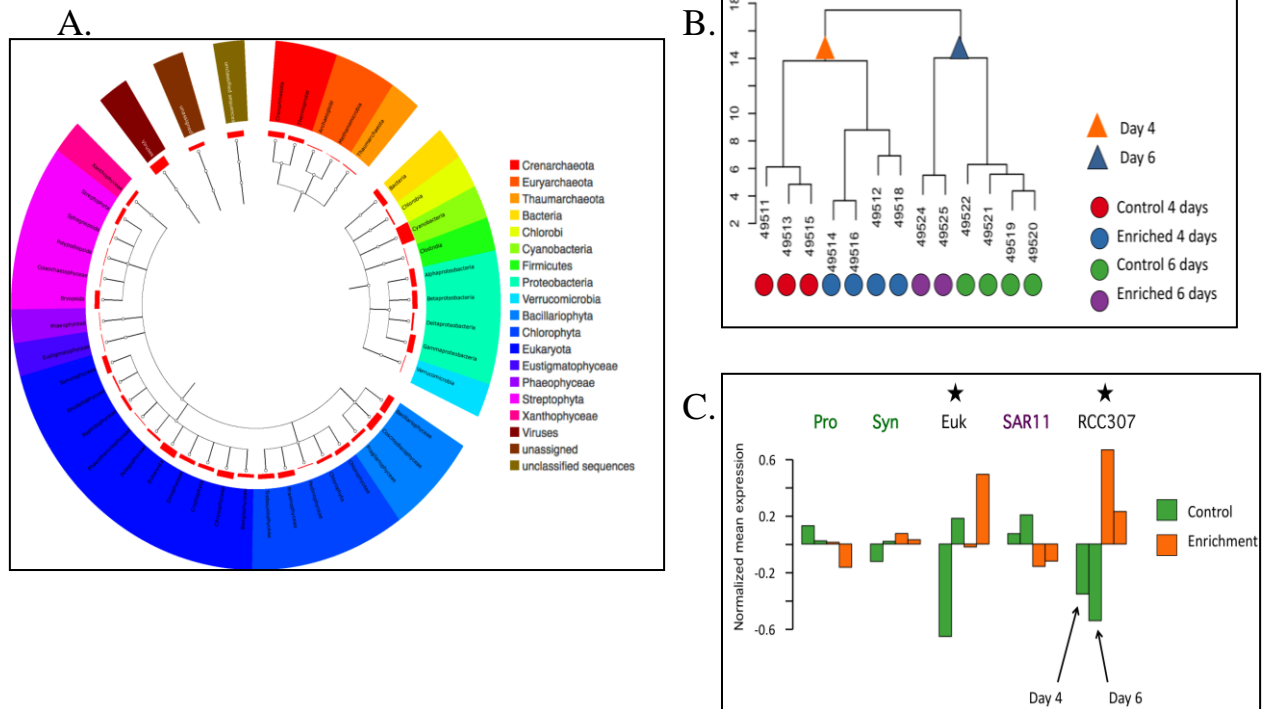
Presence (green) or absence (black) of 11,635 sequences that represent all ORFs in the genomes of six strains. Each strain is represented by the column above the strain names, and each row represents one sequence. Rows are grouped by the number of strains in which the sequence is found, and the total number of sequences in each category is listed on the left. The dendrogram above the columns is based on the presence/absence pattern for all 11,635 rows.



Highlight

High-density oligonucleotide microarray is a promising tool to study ocean biogeochemistry from a microbial view

Ocean biogeochemical processes are largely driven by diverse microorganisms, and to understand and predict how ocean may change in the near future due to global climate change, it is necessary to interrogate microbial communities at a high resolution in space, time, and depth. Genes and gene expression, markers of phylogeny or ecologically relevant processes, are powerful tools used to study the diversity and activity of natural microbial populations. As a result of a collaborative effort (MicroTOOLS, 2010), we have developed an assay that targets 22,000 genes in key marine microorganisms. This assay is based on a microarray technology that uses DNA hybridization to oligonucleotide probes complementary to genes of interest and linked to a solid surface at high density. The genes of interest were selected based on the knowledge and expertise of the MicroTOOLS collaborators, and included genes for photosynthesis, carbon fixation, carbohydrate and nitrogen metabolisms, stress responsive genes, as well as genes for cell cycle, replication and transcription. The nucleotide diversity for target genes was obtained from marine metagenomic and metatranscriptomic studies available in public repositories. The key microbial groups targeted on the microarray included important primary producers *Prochlorococcus* and *Synechococcus*, abundant heterotrophic alpha-proteobacteria clade SAR11, nitrogen fixing cyanobacteria such as *Trichodesmium*, *Crocospaera*, eukaryotic phytoplankton groups, including diatoms, haptophytes, and green algae, and viruses (Figure A). The microarray design and the assay are available to scientific community. We have successfully used the microarray to analyze the microbial community response to nutrient enrichments in poorly studied regions of the South-West Pacific (BiG RAPA cruise). Despite the absence of sequence data originated directly from that region by the time of microarray design, we were able to detect expression of ~2,000 functional genes from the main representatives of marine microbiome. In long-term experiments in S. Pacific, time of incubation had the strongest effect on the community followed by the effect of enrichment with deep water (Figure B). Deep-water enrichments with high concentrations of nitrate stimulated the activity of eukaryotic phytoplankton (Figure C) particularly later in the incubation, as indicated by the significant increase in transcription of genes encoding RuBiSCO (*rbcL*). Transcription of genes originating from a *Synechococcus* RCC307-like spp. was over-represented in enrichments as well, while the total transcriptional activity of *Synechococcus* spp. did not change significantly. Genes indicative of phosphorus limitation were among the highly transcribed genes from *Synechococcus* RCC307-like spp. indicating that nitrate-assimilating microbes consumed the available phosphate. Thus, the use of the microarray allowed to obtain information on a microbial shift at high resolution with detection of species that are not usually targeted by conventional assays (PCR). This microarray can help understanding how biogeochemical cycles are controlled in the oceans and provide a tool for detecting biological shifts corresponding with global environmental change.





Highlight

C-MORE at the National Science Teacher's Association (NSTA)

Representing C-MORE science, education and outreach, Sonya Dyhrman from WHOI attended the 2012 NSTA meeting March 29-April 1, 2012. In addition to a keynote about microbial oceanography, Dyhrman participated in hands-on activity demonstrations as part of a day-long special event hosted and organized by the COSEE Network.

With over 10,000 teachers in attendance and many exhibitors, this was both a learning experience for Dyhrman and a valuable way to promote the C-MORE key concepts in Microbial Oceanography. Many C-MORE key concept brochures were distributed, along with reprints of the article in *Science and Children* about the Artistic Oceanographer Program as well as post-cards advertising the program and C-MORE. Feedback was very positive and Dyhrman made several connections with teachers who serve primarily underrepresented groups, who are interested in partnerships and bringing school groups to Woods Hole for the interactive Science Days Dyhrman has been running using components of the Plankton kit and Outreach box.

12:15–1:15 PM COSEE Luncheon

The Oceans and Human Health—From Australia to Antarctica: The Benefits and Detriments of Marine Microbes and Their Linkages to Our Daily Lives

(By Invitation Only)

309/310, JW Marriott



Sonya Dyhrman, Woods Hole Oceanographic Institution, Woods Hole, Mass.
The Oceans and Human Health—From Australia to Antarctica: The Benefits and Detriments of Marine Microbes and Their Linkages to Our Daily Lives

Much of the U.S. population lives along the coast and many of us are acutely aware of the importance of the ocean for food, transportation, recreation, and more. Often called the “unseen majority,” billions of marine microbes are working behind the scenes to keep the ocean functioning the way it does. The effects of these microbes are both beneficial and, in some cases, detrimental to the oceans and human health. With examples from her research around the globe, Dr. Dyhrman will discuss the benefits and detriments of marine microbes and their linkages to our daily lives.

Visit www.cosee.net for more information about this event.





Highlight

Microscopes in Middle Schools Project

As part of its wide-ranging education program, the Center for Microbial Oceanography: Research and Education (C-MORE), based at the University of Hawai'i at Mānoa, is partnering with the State of Hawaii to distribute digital video microscopes and related supplies (valued at \$1775) to Hawai'i's public middle schools statewide.

The oceans are of vital importance to life on Earth: they regulate climate, provide food and oxygen and cycle essential chemical elements and compounds. Marine microbes are the drivers of these ocean processes. For example, phytoplankton (plant-like plankton) form the base of the marine food web and produce over half the oxygen that we breathe. However, phytoplankton and other microbes can be difficult to conceptualize because they cannot be seen without specialized equipment.

So, how do you get students interested in learning about something they can't even see? One way is to start with something they can see! If you go up one step in the food web from phytoplankton, you'll find zooplankton. Zooplankton are a diverse group of organisms that feed upon smaller phytoplankton or other zooplankton, or are parasites. Zooplankton can be easily caught with plankton nets and studied right from shore with microscopes. Connecting a video microscope to a computer allows for whole-class viewing and discussion.

The Microscopes in Middle Schools project equips Hawaii's public middle schools (most of which are minority-serving institutions) with video digital microscopes, plankton nets, identification guides and related supplies through funding leveraged from the State of Hawaii's Fostering Inspiration and Relevance through Science and Technology (FIRST) Pre-Academy program. Through a Memorandum of Understanding with the Hawaii Department of Education (DOE), C-MORE trains DOE middle school science teachers in microscope use and relevant curriculum. DOE awards professional development credit (which count toward "highly qualified teacher" status) to teachers who attend the C-MORE teacher-training workshop.

From December 2011 to April 2012, four workshops were held on Oahu, Maui, Big Island and Kauai, all led by C-MORE marine science educator Jim Foley. Thirty-four schools participated and 27 schools have already received the equipment. As Hawaii has 54 DOE middle schools, we are approximately at the midpoint of this project. In addition to educating Hawaii's middle school teachers and students, this project also enables C-MORE undergraduate and graduate students to gain valuable outreach experience while assisting at the teacher professional development workshops.





Highlight

Ocean FEST (Families Exploring Science Together)

Ocean FEST, an intergenerational hands-on marine science program, is a collaboration between the Center for Microbial Oceanography: Research and Education (C-MORE) and the Hawaii Institute of Marine Biology (HIMB). Our overarching goal is to interest Hawai'i's kids in careers in ocean science and related STEM fields through fun, hands-on activities. The program is targeted at underrepresented groups and operates with leveraged funding from NSF's Opportunities for Enhancing Diversity in the Geosciences (OEDG) program.



Ocean FEST engages students in grades 3–6 and their parents in hands-on science activities that explore the wonders of the ocean world. The program uses the marine environment as a “hook” to explore important science concepts and conservation topics. Developed for Native Hawaiians and Pacific Islanders, the program emphasizes marine science and conservation for island communities. Program themes include ocean properties, climate change and sea level rise, coral reef ecosystems, marine microbes and marine science careers. The evening program begins with the introduction of the scientific method through an activity that encourages inquiry and scientific thinking. Subsequently, the ocean and its microbes are explored through a series of hands-on activities in the context of the scientific method and current environmental issues. Activities are designed so students can see how globally important issues (e.g., climate change and ocean acidification) have local effects (e.g., sea level rise, coastal erosion, coral bleaching) which are particularly relevant to island communities. Using hands-on examples, demonstrations and authentic research data, students and their families come to understand how climate change and the ocean are inextricably linked.



All activities are aligned with Hawaii Content and Performance Standards. They are also aligned with two of C-MORE's key education goals: Increasing literacy in microbial oceanography and broadening participation in geosciences among underrepresented groups. The program is preceded by a professional development component for elementary schools science teachers, who serve as teaching assistants during the event.

Established in 2009, Ocean FEST has just completed its third year. To date, over 60 events have been held in Hawaii, reaching over 11,100 participants. A detailed evaluation using a pre- vs. post-survey comparison showed that participants (students and parents) reported positive gains both in content knowledge and attitudes towards ocean science careers. This program enables students and their families to recognize that ocean science is not scary or difficult, but fun and exciting, and something everyone can participate in.

For more information, please visit the Ocean FEST website: <http://oceanfest.soest.hawaii.edu/>



Highlight

Professional Development Training Program (PDTP)

Science Communication Module

C-MORE in partnership with COMPASS organized two workshops on Science Communication at MBARI (May 2011) and MIT (Nov 2011). These workshops train graduate students and post-docs to distill their research into a language that is free of jargon and accessible to a general audience. After the training, participants are asked to produce a communication product based on their research, such as a magazine article, press release, podcast or a blog.

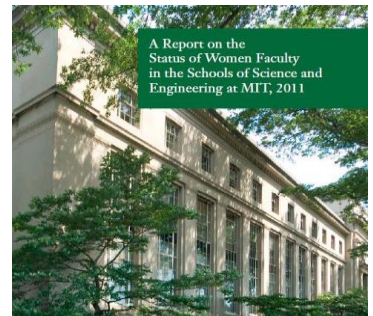


<https://sites.google.com/site/cmoredprofdevtable/home/science-communication>

Diversity Module

Two videoconferences on diversity were attended by C-MORE personnel across the partner institutions. The May 2011 videoconference discussed two papers on racial and gender bias. A key "take-home" message was that we all have biases and we need to recognize them in order to ensure fairness. Participants seemed surprised to learn that there's a body of literature of double-blind experiments showing that women have to be significantly better than men to get the same treatment. The October 2011 focused on the MIT faculty equity pay study. It was co-led by Penny Chisholm, one of the study's authors. At C-MORE Hale, many people lingered after the conference ended, continuing discussions in small groups.

<https://sites.google.com/site/cmoredprofdevtable/home/diversity>



Outreach Module

C-MORE students and post-docs at all partner institutions actively participated in numerous outreach events and trainings. Events ranged from large Open Houses and Ocean FESTs to smaller classroom-sized events and science fair mentoring. The C-MORE Outreach box and C-MORE Science Kits were used to disseminate key concepts in microbial oceanography to K-12 students, teachers and the general public.

<https://sites.google.com/site/cmoredprofdevtable/home/outreach>

C-MORE Professional Profile web pages

C-MORE now offers graduate students and post-docs (and others) the opportunity to have professional profile web pages linked through the C-MORE website. These web pages can be used to showcase scientific and academic accomplishments, publications, participation in education and outreach activities, and photo and video galleries. A total of 18 C-MORE personnel have developed profile pages during this reporting period.





Highlight

C-MORE Scholars Program

The C-MORE Scholars program provides hands-on, closely mentored research experiences for University of Hawai'i (UH) undergraduates who are interested in ocean and earth science-related careers. Students, especially underrepresented students such as Native Hawaiians and Pacific Islanders, from all UH campuses are encouraged to apply.

Three levels of awards (listed below) are offered, depending on the student's skills, knowledge and experience. All Scholars receive guidance and help from a mentor who is an ocean or earth scientist. In addition to conducting research, all Scholars attend monthly meetings on career/professional development, participate in educational outreach and present their research results at the end of the year.

Level I: Traineeship. Trainees are new to the program and do not have previous research experience. They can be at any academic level. Trainees receive close mentoring in order to learn basic laboratory and computer skills, research methods and science concepts.

Level II: Internship. Interns are typically juniors or seniors who have taken numerous science classes. They may have previously worked as a trainee or have had other research experiences. Internships help students apply what they learn in the classroom to ocean and earth science research and careers.

Level III: Fellowship. Fellows are seniors who have been interns for at least one year. They work independently on a research project, such as a senior thesis or honors thesis. Fellows also serve as role models to the other Scholars.

As of April 2012, 42 students have taken part in the Scholars Program. Students have progressed from community college to university, graduated with their Bachelor's Degrees, and entered graduate school. Two former scholars (Thomas, Wai) are currently in the C-MORE graduate program at UH. During this reporting period, C-MORE Scholars have had several notable successes. Three current or former Scholars (Frazier, James, Thomas) co-authored a peer-reviewed publication. Frazier graduated in December and will enter UH Hilo's masters program in the Fall. James was initiated into Phi Beta Kappa. Bulseco will graduate in May 2011 and enter a Ph.D. program at U Mass in the Fall. Two Scholars (Bulseco, Kao) presented posters at national conferences, and Kao won the best undergraduate poster prize at the UH Tester Symposium. Christina Johnson was awarded the 2011 Alan Church Environmental Steward Scholarship. Lani Johnson received an Undergraduate Research Opportunities Program award. Two Scholars (Bump, James) were accepted into prestigious REU programs at WHOI and MIT, respectively, for summer 2012.

For additional program information: <http://cmore.soest.hawaii.edu/scholars.htm>





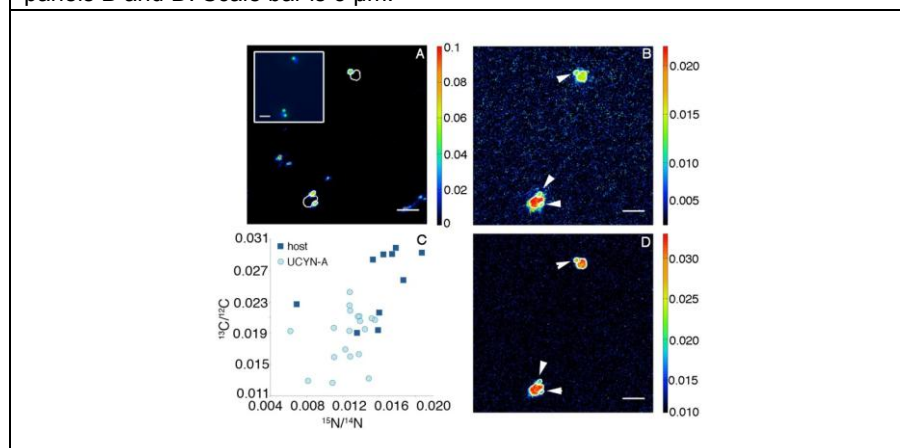
Highlight

Novel unicellular diazotroph is symbiotic with a single-celled eukaryotic alga

In nitrogen (N) limited environments, symbioses between nitrogen-fixing (diazotrophic) Archaea and Bacteria and photosynthetic eukaryotes are an important strategy for nitrogen acquisition. Recently, an unusual cyanobacterial diazotroph (UCYN-A) was discovered and hypothesized to be involved in an obligate symbiosis because of its reduced genome size (1.44 Mbp) and the absence of essential pathways (notably photosystem II and carbon fixation genes). The UCYN-A genome did have the genes required for nitrogen fixation, so we hypothesized that it would provide fixed nitrogen to a symbiotic partner in exchange for fixed carbon but evidence for this partnership did not exist.

We tested this hypothesis through a collaborative effort between members of the Zehr Lab (CMORE) and the Max Plank Institute for Marine Microbiology by experiments and sampling at Station ALOHA through the Hawaiian Ocean Time Series (HOT) program. First, using flow cytometric cell sorting, we screened distinct phytoplankton populations and discovered UCYN-A among the photosynthetic picoeukaryotes (PPE) (1-3 μm diameter). Next, by a single cell approach, we identified the UCYN-A partner cell as a prymnesiophyte most closely related to *Braarudosphaera bigelowii*, a calcareous phytoplankton present in the fossil record. To measure transfer of nitrogen and carbon between UCYN-A and its partner, in a novel experimental approach, we coupled flow cytometric cell sorting to halogenated *in situ* hybridization nanometer scale secondary ion mass spectrometry (HISH-SIMS). We performed incubations with $^{15}\text{N}_2$ and

Microscopy and elemental composition of two UCYN-A partner cells and their associated UCYN-A(s). **(A)** $^{19}\text{F}/^{12}\text{C}$ (HISH) labeling of UCYN-A. Inset displays labeling of the same UCYN-A cells by CARD-FISH (green) and DAPI staining of partner cell nucleus (blue). **(B)** $^{13}\text{C}/^{12}\text{C}$ incorporation, **(C)** Relative enrichment of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ in 10 selected partner cells and their associated UCYN-A(s), and **(D)** $^{15}\text{N}/^{14}\text{N}$ incorporation. UCYN-A cells are indicated by white arrows in panels B and D. Scale bar is 3 μm .



^{13}C -bicarbonate, sorted the photosynthetic picoeukaryotes, located UCYN-A by HISH, then measured ^{15}N and ^{13}C incorporation in UCYN-A and the attached partner cells. We observed UCYN-A (sometimes in pairs) attached to its partner and show that enrichment of labeled nutrients was high in both cells (Figure), demonstrating exchange of N and C between UCYN-A and its prymnesiophyte host.

The implications of this collaborative effort are many. Prymnesiophytes were not known to participate in symbioses, thus, this work presents a new aspect to the ecology of these abundant primary producers and presents effective methods for pursuit of other planktonic symbioses. If

the UCYN-A partner is calcifying like *B.bigelowii*, it may be possible to identify geologic time-periods that were conducive to nitrogen fixation by comparing abundance of the UCYN-A partner to paleoceanographic records. The association of UCYN-A with a picoeukaryote also highlights the enigmatic absence of N₂-fixing plastids in evolution since N₂ fixation is an energetically expensive oxygen-sensitive reaction, and nitrogenase is an ancient enzyme.



Highlight

Improving N₂ fixation methods used in the oligotrophic open ocean

Accurate measurements of N₂ fixation in the marine environment are important to help resolve upper water column biogeochemical cycling. In the past year, Sam Wilson and Daniela Böttjer, postdoctoral scholars in the laboratories of Dave Karl and Matt Church conducted a comparative assessment of N₂ fixation field measurements of acetylene reduction (AR), a proxy for N₂ fixation, together with measurements of ¹⁵N₂ tracer assimilation in the oligotrophic North Pacific Subtropical Gyre. Methodological improvements were made to both the AR assay and ¹⁵N₂ assimilation technique, with a number of associated benefits:

1. We demonstrated that it is possible to conduct the AR assay on samples of oligotrophic seawater, with no preconcentration of the microbial biomass using a mercuric oxide bed as the detector instead of the more commonly utilized GC-FID. It is now possible to obtain an estimate of nitrogenase activity whilst at sea.
2. Improvements to the ¹⁵N₂ tracer technique resulted in decreased discrepancy between estimates of global N₂ fixation based on field measurements and indirect geochemical calculations. The addition of ¹⁵N₂ in dissolved form resulted in an overall 2-6 fold increase in rates of ¹⁵N₂ assimilation compared to the more common method of adding ¹⁵N₂ as a gas bubble. Taking this underestimation into account, measured surface N₂ fixation rates at Stn ALOHA are more in agreement with estimates of N₂ fixation derived from indirect approaches.
3. We showed that the ¹⁵N₂ enriched seawater can be prepared prior to its use at sea with no detectable loss (<1.7 %) of dissolved ¹⁵N₂ during 4 weeks of storage. This is both time-efficient and will facilitate conducting N₂ fixation rate measurements at sea.

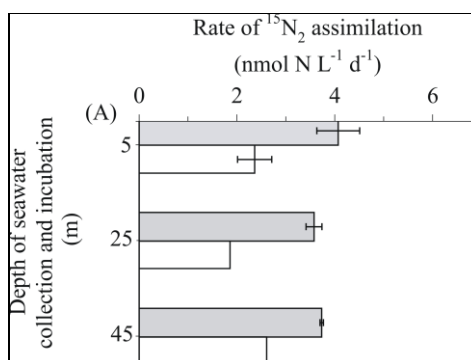


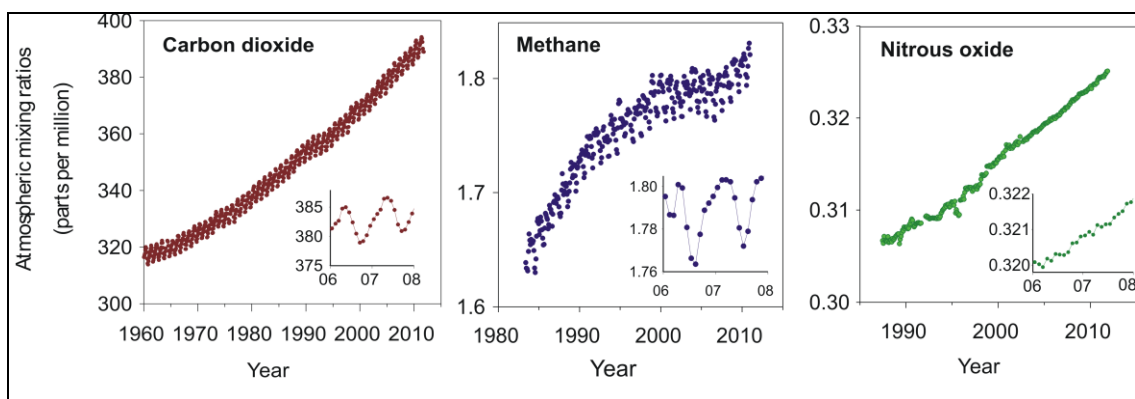
Photo showing the production of ¹⁵N₂ enriched seawater and a figure depicting the resulting rates of ¹⁵N₂ assimilation when the ¹⁵N₂ tracer is added in dissolved (gray bars) or as a gas bubble (white bars). The seawater samples were incubated *in situ* on July 20 at Stn ALOHA



Highlight

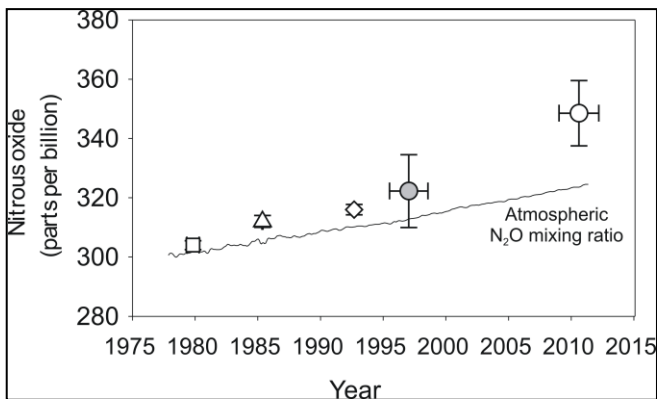
Oceanographic time-series measurements of the greenhouse gases nitrous oxide and methane

Global monitoring stations report that atmospheric concentrations of N₂O and CH₄ are increasing at rates of 0.25 and 0.4 % per year, respectively (below). The increase in atmospheric greenhouse gas mixing ratios has resulted in a global scientific appeal to better constrain and understand the sources and sinks of greenhouse gases at the Earth's surface. The global oceans are identified as a source of both N₂O and CH₄ to the overlying atmosphere, contributing 1.8-5.8 Tg N₂O yr⁻¹ and 4-15 Tg CH₄ yr⁻¹.



Atmospheric mixing ratios of CO₂, CH₄, and N₂O measured at Mauna Loa Observatory (ESRL, NOAA). Data are shown for the entire time series measurement for each trace gas species. The insert plots show concentrations over a 2 year time period (2006-2008) to highlight the natural seasonal variability in the atmospheric mixing ratios.

To investigate the response of the oceanic environment to the increasing atmospheric concentrations of N₂O, data was compiled by C-MORE post-doc Sam Wilson from the multiple oceanographic cruises passing through the North Pacific Subtropical Gyre (NPSG) over a 30 year period. The compiled time series dataset for N₂O reveals that alongside the atmospheric increase in N₂O mixing ratios (from 301 ppb in 1979 to 325 in 2011), the surface mixed layer seawater concentrations of dissolved N₂O have increased from an average of 304 ppb in 1979 to an average of 351 ppb in 2011.



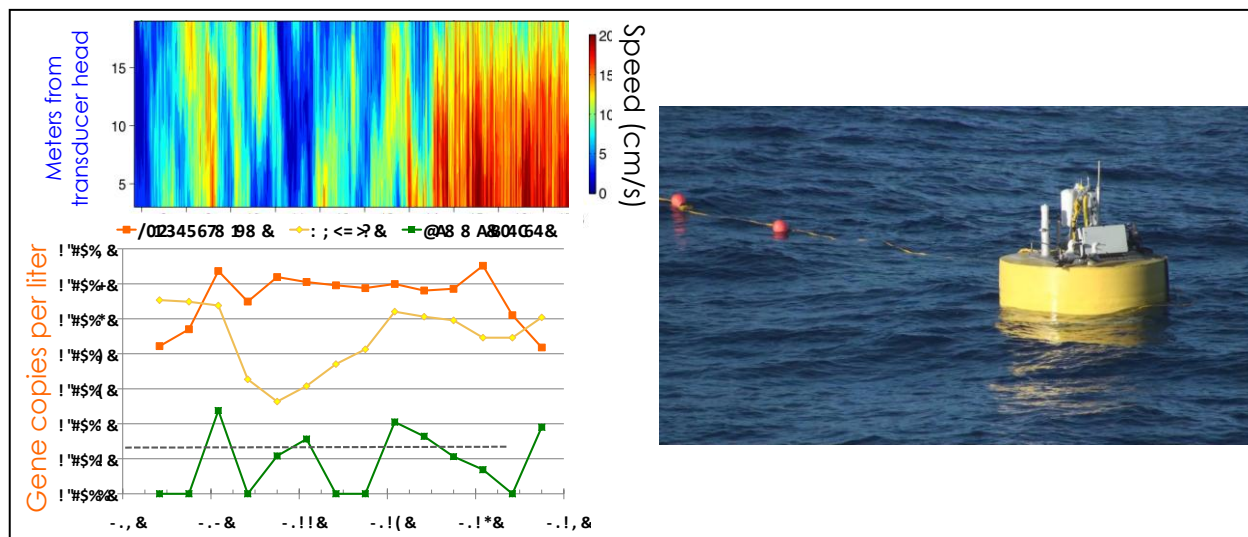


Highlight

Autonomous examination of nitrogen fixation activity and population dynamics on drifter platforms in the North Pacific Subtropical Gyre

A team from the University of California, Santa Cruz, the Monterey Bay Aquarium Research Institute, the Woods Hole Oceanographic Institute and the University of Hawaii led by C-MORE post-doc Julie Robidart participated in a biogeochemical study in the nutrient-limited North Pacific Subtropical Gyre. Nitrogen fixing bacteria fuel productivity in the open ocean by converting nitrogen gas (N_2) to a nutrient that can be assimilated by other forms of life. Several drifting instruments were deployed in parallel at the periphery of an anticyclonic eddy, for the first-ever analysis of the distributions and activities of the nitrogen fixing population *in situ*. Microbial abundances were measured with quantitative PCR on the Environmental Sample Processor (ESP) and nitrogen fixation rates with the Submersible Incubation Device (SID). Anticyclonic eddies are often described as nutrient-limited, but we measured highly variable nutrient concentrations in surface waters, and statistically significant increases in two populations of nitrogen fixers in more nutrient replete waters.

Despite the expectation of decreased patchiness due to the drifter sampling scheme (where instruments move with the microbes), we found tremendous patchiness of nitrogen fixing populations in the eddy periphery (2-3 orders of magnitude change over 32 hours and 22 km). Low but variable nitrogen fixation activities, as measured with the SID and on-deck incubations, reflect the community heterogeneity. Though estimating basin-scale nitrogen budgets in light of this extreme spatiotemporal variability in the abundances and activities of nitrogen fixing populations may seem daunting, most organisms demonstrated relatively consistent correlations with the physical and/or chemical environment: correlations that were strongest when the drifter was best tracking the current. As one of the first of its kind, this study emphasized the utility of drifter sampling and we conclude that understanding the relationship between microbial populations and environmental factors is key to forecasting biogeochemical change in future climate scenarios.



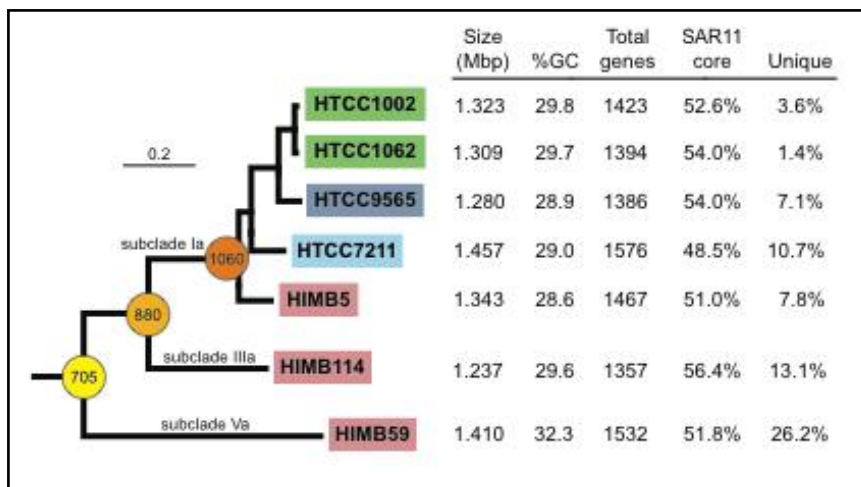


Highlight

Genome evolution within the SAR11 marine bacterioplankton lineage

A group free-living marine bacteria known as SAR11 are one of the most abundant organisms on Earth, at times making up over 50% of the microbial cells inhabiting surface seawater environments. Despite their ubiquity in the global ocean, until recently only a handful of closely related SAR11 strains had been brought in to culture in the laboratory and investigated for traits that contribute to their tremendous success. While much has been learned from studying the physiological and genomic characteristics of these isolates, broadly extrapolating the resulting observations to other members of the group has not been straightforward because common diversity indices used for bacteria show SAR11 to be highly diverse. A group of researchers led by C-MORE scientist Mike Rappé sought to broadly investigate the pan-genome of SAR11 – that is, conserved and variable features of genome structure and gene content – in order to explore shared characteristics that may contribute to the dominance of SAR11 in marine systems, as well as those that may contribute to phenotypic differences between SAR11 strains. By comparing seven SAR11 genome sequences, including representatives from two of the group’s most divergent lineages (below), we found that selection for efficient cell replication in the planktonic marine environment has been a continuous pressure during the evolution and diversification of this ancient clade, resulting in small, streamlined genomes with unprecedented constraint in genetic content.

We compared the genomes of seven isolates that span three major lineages, or subclades, of the SAR11 clade (at right). The presence of a low GC (28.6-32.3%) content genome that is exceptionally small for a free-living microorganism (mean genome size of 1.354 ± 0.07 million base pairs) is a unifying characteristic of the SAR11 clade. As such, the genomes code for only 1357-1576 genes.



By examining the SAR11 pan-genome, we identified a conserved core of 705 shared genes, representing 48-56% of the total gene repertoire per strain. When compared to the core genomes of other prokaryotes, the high degree of conservation of the SAR11 core genome is extraordinary, deviating dramatically from previous observations regarding relationships between gene conservation and nucleotide sequence similarity. Not surprisingly, we found the core genome to contain a high proportion of genes coding for proteins involved in housekeeping functions and central metabolism, but also discovered core genes and pathways thought to be related to survival in the natural environment. For example, a high affinity iron uptake system was common to all SAR11 genomes, suggesting that the ability to cope with the low levels of iron and iron limitation, as is often observed in the open-ocean, is an important trait. Similarly, conservation of a mechanism for harvesting light energy via proteorhodopsin (and associated genes) demonstrates an important adaptive role for this func-

tion across the SAR11 clade. A requirement for a reduced form of sulfur for growth also appears to be a core feature of SAR11, which foregoes the ability to use readily available but oxidized sulfur (in the form of sulfate) in seawater.

Modeling the SAR11 pan-genome reveals an open gene pool for SAR11 that increases in size when new SAR11 genomes are added. This represents a significant genetic reservoir that this group could exploit for adaptive purposes. Similar to gene content, gene order (synteny) is also conserved between SAR11 strains, with core genes grouped in blocks and variable genes scattered throughout the genomes. A hypervariable region bounded by ribosomal RNA genes found in all seven SAR11 genomes may constitute a kind of test-bed for genetic novelty in otherwise relatively gene content-constrained genomes.

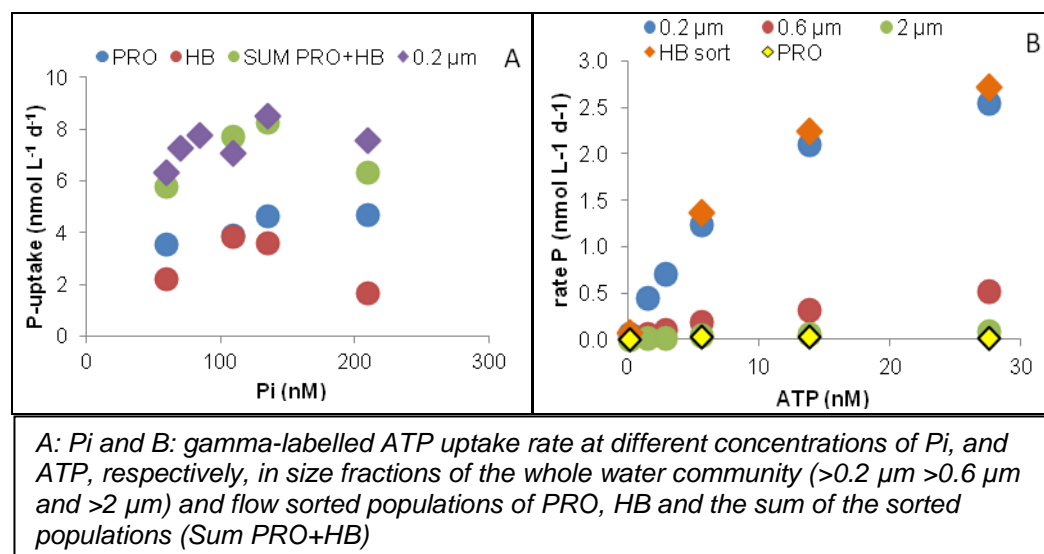
We find that variability in carbohydrate, amino acid, phosphorus, and sulfur transport and metabolism are traits that are probably linked to the adaptation of different SAR11 lineages to specific ecological niches. For example, a subset of SAR11 strains appears able to acquire phosphorus from organic sources via phosphonate transport and utilization genes. In addition, predicted genes for the transport and demethylation of the reduced organic sulfur-containing compound dimethylsulfoniopropionate (DMSP) are missing in one genome completely, implying that mechanisms for meeting reduced sulfur requirements are variable within SAR11. Similarly, while some central features of one-carbon metabolism are widely distributed within the SAR11 clade, considerable variation in one-carbon metabolism pathways is evident.

The results of our study reveal that, in spite of high apparent diversity in SAR11 populations by common metrics of diversity, gene content between SAR11 strains from highly divergent lineages and geographical habitats is extraordinarily conserved compared to other groups of bacteria or archaea. Thus, selective pressure for genome streamlining has not only led to small SAR11 genomes, but has also constrained the genetic content of these organisms across significantly large phylogenetic distance.



Microbial group specific phosphorus compound utilization in the North Pacific Subtropical Gyre

Using radiolabeling techniques coupled with size fractionated and flow cytometry cell sorted samples, a C-MORE team led by Karin Björkman and Solange Duhamel investigated the group specific phosphorus (P) uptake by *Prochlorococcus* (PRO) and non-pigmented picoplankton (assumed to be mostly heterotrophic bacteria, HB) in samples from the North Pacific subtropical gyre. We studied microbial response to a range of concentrations of inorganic phosphorus (Pi) and adenosine 5' triphosphate (ATP) sorted PRO and HB revealed that their Pi uptake was approximately the same, hence these groups appeared to be equally competitive for Pi (Fig. A). The HB were by far superior competitors for P derived from ATP and the PRO signal represented < 2% of the uptake by HB (Fig. B). These results imply that resource partitioning of P sources between the smallest phototrophic bacterium PRO and other HB may be an important niche separating strategy in oligotrophic environments.



Flow cytometry sorted group uptake rate measurements also allowed us to demonstrate that PRO but not HB Pi-uptake was enhanced by ambient light. ATP utilization by PRO was also enhanced in the light, both for the uptake of the terminal Pi ($[\gamma\text{-}^{33}\text{P}]\text{ATP}$) and of the adenine ($[2,8\text{-}^3\text{H}]\text{ATP}$) moiety. This could be the result of secondary uptake of Pi and/or adenine after ATP cleavage by HB, since they were responsible for most of P uptake from ATP (Fig. B). Our results suggest that phototrophs may use excess light energy to transport Pi and possibly adenine (and adenosine), a feature that may influence bacteria-phytoplankton competition and microbial communities structure. Two manuscripts detailing these results are currently in review.