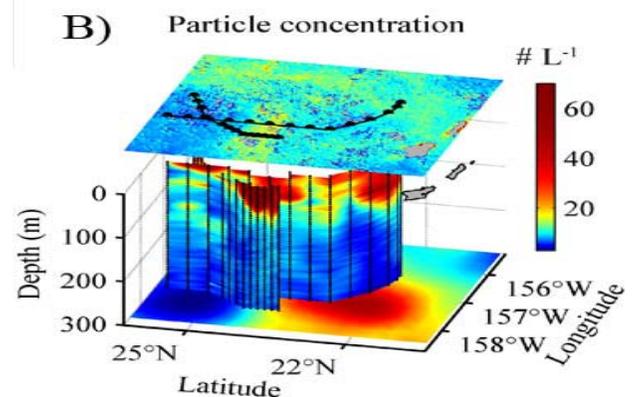
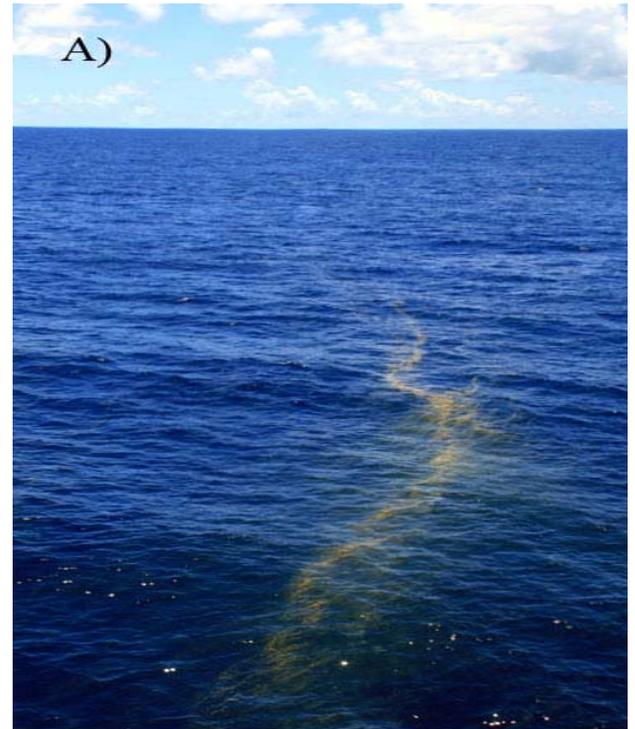




Highlight

Variability of Large Particle ($>100 \mu\text{m}$) Export Around Station ALOHA: The C-MORE OPEREX Cruise

The North Pacific Subtropical Gyre (NPSG) is characterized by low nutrient concentrations and low standing stocks of microorganisms. However, large cells and high surface phytoplankton concentrations are usually associated with late summer chlorophyll blooms in the region. These blooms are characterized by a wide range of scales, from sub-mesoscale ($\sim 2 \text{ km}$) streaks to mesoscale ($\sim 100 \text{ km}$) patches, and their triggering mechanism is unknown. The C-MORE-OPEREX cruise (Jul 31-Aug 14 2008) was designed to investigate the formation and evolution of these bloom around Station ALOHA and their impact on particle export. Low wind stress and high SST prior to the cruise favored the growth of the N_2 -fixing cyanobacterium *Trichodesmium spp* (Figure A). The study area was characterized by a mesoscale dipole feature with a cyclonic eddy to the north and an anticyclone to the south (Figure B). Two transects across these contrasting regions were performed at different resolutions (20 nm and 7 nm.). Although the mesoscale surface signal of the bloom was widespread, export production was localized. Optical measurements from an Underwater Video Profiler (UVP) and sediment trap samples revealed that the highest concentration of large particles occurred in the region between the eddies where particles were detected down to 300 m (Figure B). Using Lagrangian techniques, namely the Finite-Size Lyapunov Exponents (FSLE's) calculated from geostrophic velocities obtained through remotely-sensed sea surface height, it was found that the specific regions where particle export occurred were coincident with regions of large horizontal stretching. These Lagrangian coherent structures (LCS's) are barriers to horizontal transport. In addition, local horizontal density gradients are enhanced in regions of large stretching, hence prone to surface frontogenesis. By using an expression derived from the omega equation we obtain good agreement between frontogenetically-generated downwelling and particle export in the region between the two



A) Visible evidence of the presence of a *Trichodesmium spp.* bloom during the OPEREX cruise at the sea surface. B) Integration of satellite observation from MODIS (Chlorophyll-*a*, top layer) and AVISO (Sea Surface Height, bottom layer) and UVP measurements during the OPEREX cruise, showing the distribution of the large particle ($>100 \mu\text{m}$) relative to the biological and physical field.

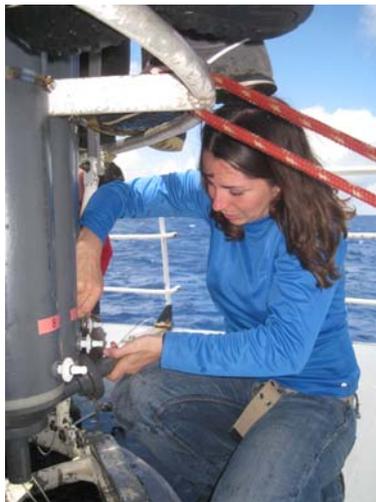
eddies. Our results suggest that the stretching of the eddy field produces submesoscale features that strongly modulate the export of organic material from the surface layers into the interior ocean. This has important implications for modeling the biological carbon pump and estimation of carbon sequestration.



Highlight

Linking Research and Education from Genomes to Biomes: A C-MORE Training Course in Microbial Oceanography

The Center for Microbial Oceanography: Research and Education (C-MORE) established and leads an annual summer training course in microbial oceanography for graduate students and postdoctoral researchers. The course, titled “Genomes to Biomes”, offers students opportunities to interact with and learn from many of the world’s leaders in the fields of oceanography, microbial ecology, and aquatic biogeochemistry. Each year since 2006, the University of Hawaii has hosted 12-16 emerging student leaders in Microbial Oceanography to participate in the 6 week course. The course is led and directed by C-MORE researchers Matt Church, Dave Karl, Mike Rappe, Grieg Steward, and Ed DeLong. The course receives substantial financial support from the Agouron Institute with additional funding from the National Science Foundation.



To date, nearly 60 students have participated in the course, including students from North America, South America, Europe, Africa, Australia and Asia. The full immersion course is designed to provide students with hands-on exposure to cutting edge tools used by microbial oceanographers, including the ability to analyze diverse types of data. In addition, daily interactive lectures and symposia provide students with opportunities to hone their critical thinking skills and gain exposure to contemporary topics in the emerging field.

A hallmark of the summer course is a 6-10 day research cruise aboard the R/V *Kilo Moana*, a 186 foot twin-hulled UNOLS vessel operated by the University of Hawaii. Among other themes, past cruises have studied the role of open ocean mesoscale eddies and fronts as physical processes controlling plankton ecology and biogeochemistry, a central research mission of C-MORE.

While at sea, students work in teams to learn new methods and carry out shipboard sampling. The cruise allows students to develop experience with field-based oceanographic research, including designing and implementing experiments and characterizing the physical, chemical, and biological properties that characterize the ocean as a microbial habitat. Results from the research course have been presented at 4 international scientific meetings, and one student-led peer-reviewed publication.





Highlight

C-MORE Sponsored 'Analysis of Microbial Sequence Data using ARB' Workshops

Today's ocean biogeochemistry research programs frequently necessitate students and scientists stepping beyond their standard 'toolbox' into research areas with new and less familiar methods. Learning new tools often comes with a steep learning curve, and the lack of available written support material for many methods provides an additional challenge.

In order to help scientists build their methodological skills, C-MORE recently sponsored a series of workshops focused on the analysis of microbial sequence data utilizing the specialized phylogenetics software platform ARB (Latin, 'arbor' = tree). The workshops were targeted at graduate students, post-docs and early career scientists and focused on presenting methodology for analysis of sequence data from the most common phylogenetic marker for marine microbes, the SSU ribosomal RNA gene.

During June - August 2009, three workshops were offered: two at the University of Hawaii-Manoa (UH-Manoa) and one at the Woods Hole Oceanographic Institution (WHOI). The 2-day workshops collectively brought together 45 participants from various institutions and departments that were engaged in diverse oceanographic research programs.

The workshops were organized and taught by Amy Apprill (post-doc, WHOI) with teaching assistance from Erin Banning (Ph.D. student, WHOI) and Megan Huggett (post-doc, UH).

During the workshops, each participant utilized the ARB software and was guided through tutorials using a practice dataset of marine bacterial SSU rDNA sequences. The workshop participants reported very positive experiences over the 2-days of learning, and most felt that they had mastered the skills necessary to independently analyze their own data.



The workshop materials (lectures, tutorials and practice datasets) are freely accessible on the web:

http://cmore.soest.hawaii.edu/education/grads-postdocs/arb_workshop.htm



Highlight

Eco-DAS: Ecological Dissertations in the Aquatic Sciences

Ecological Dissertations in the Aquatic Sciences (Eco-DAS) continues the series of symposia formerly known as DIALOG I-VII (Dissertations Initiative for the Advancement of Limnology and Oceanography), but takes that series in new directions. Key elements of the remodeled symposia include:

- Fostering sustained, cross-disciplinary interactions among the top new researchers in ecological oceanography and limnology, to the benefit of both the science and the researchers.
- Increasing the success rate of new researchers seeking funding to build their research programs, by familiarizing them with the diversity of available research opportunities, and introducing them to representatives of funding agencies with whom they will interact in future.
- Increasing the likelihood of professional success for these new researchers by:
 - Highlighting their initial work through high-visibility, open-access publication of a formal symposium proceedings volume, to be published in 2010
 - Focusing that volume on interdisciplinary and collaborative aspects of their work
 - Establishing mentoring relationships between participants and established scientists

Eco-DAS VIII was held on 11-16 October, 2008, at the East-West Center immediately adjacent to the University of Hawai`i, Manoa campus. Thirty-eight invited participants, 4 mentors and 4 representatives of federal funding agencies met for six days to present and discuss state of the art research in the ecology of aquatic ecosystems, to strengthen existing collaborations and form new partnerships, and to discuss the most challenging issues in aquatic ecology today. Following the symposium, participants prepared jointly written manuscripts submitted to the Symposium Proceedings, which will be published in Open Access early in 2010 by the American Society of Limnology and Oceanography.

Eco-DAS funding is provided by NSF with contributions from ONR, NASA and NOAA. Eco-DAS is sponsored by the **Center for Microbial Oceanography: Research and Education (C-MORE)**, the University of Hawai`i School of Ocean and Earth Science and Technology (SOEST) and the Department of Oceanography, and the American Society of Limnology and Oceanography (ASLO).

For more information, visit the Eco-DAS website at:
<http://cmore.soest.hawaii.edu/eco-das/index.htm>

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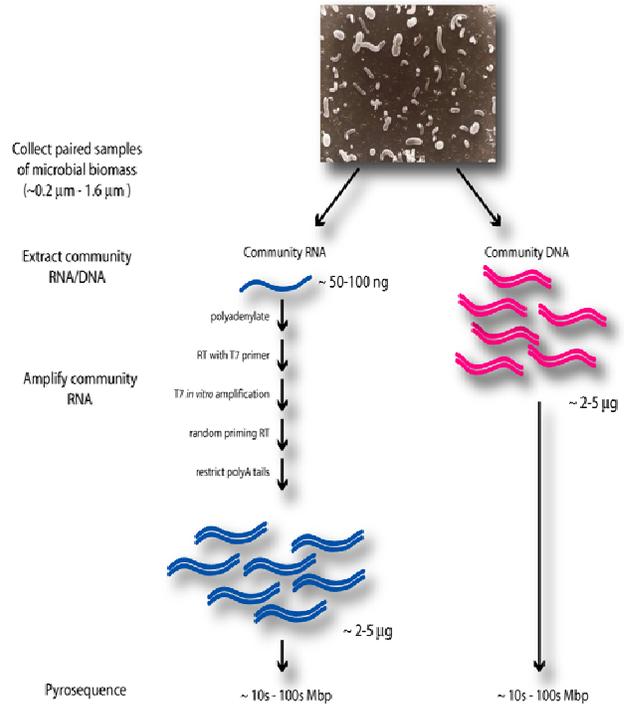


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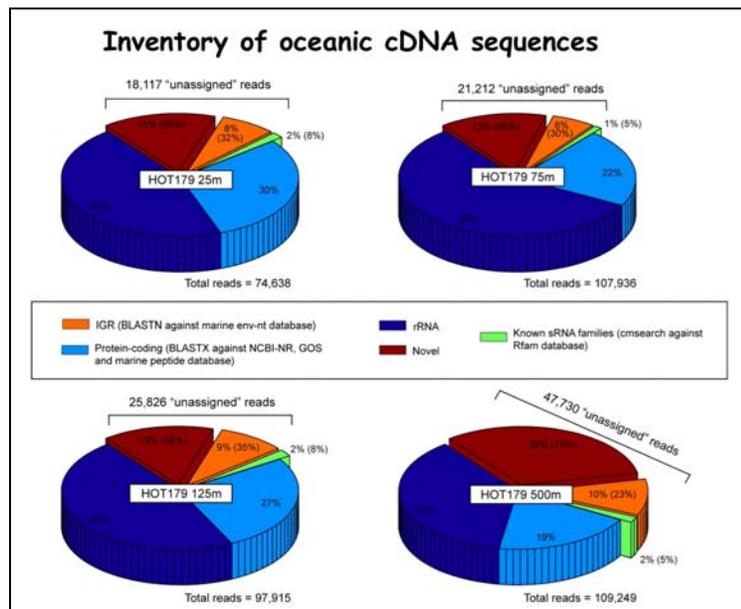
Leveraging Indigenous Microbial Biosensors to Measure Environmental Response and Variability

Recent efforts by a number of C-MORE Investigators (Chisholm, DeLong, Zehr) have established novel approaches for measuring and analyzing microbial gene expression in marine microbial communities, a new approach dubbed 'metatranscriptomics'. These methods are enabling a better understanding of the effects of natural and/or anthropogenic environmental changes on microbial community gene expression, population structure, and biogeochemical function. These data in turn will allow C-MORE scientists to harness naturally occurring microbial species to sense and predict environmental perturbation and change.

Using these new approaches collaborative inquiries by C-MORE chemists and microbiologists (DeLong, Repeta) are investigating the nature and turnover of dissolved organic carbon in the marine environment. The gene expression experiments point to a succession of microbial species, and specific pathways responsible for the turnover of this important component of the marine carbon cycle.

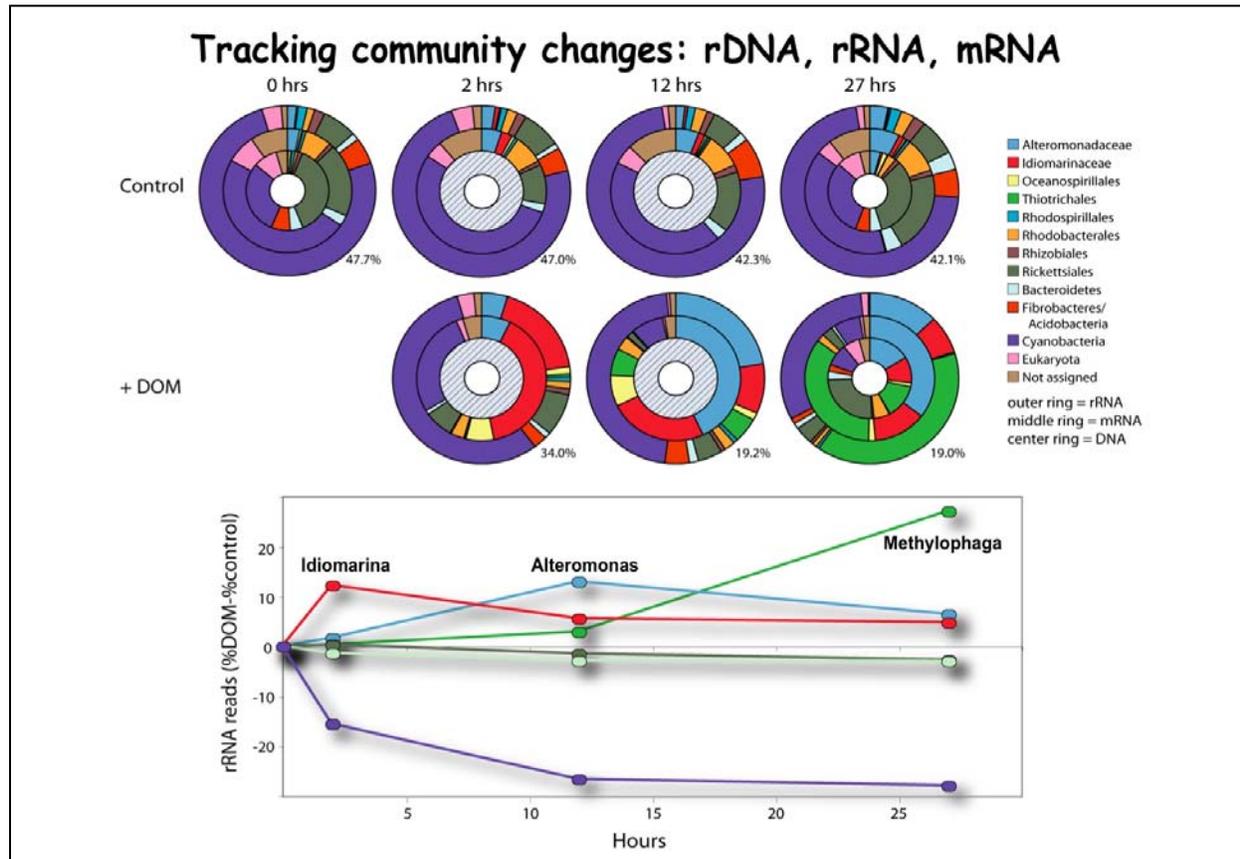


A flow diagram of generic procedures developed by C-MORE scientists for analyzing microbial community gene content and gene expression.



Distribution of ribosomal, protein coding and small RNAs revealed by metatranscriptomic methods

The application of new 'metatranscriptomic' methods by C-MORE graduate student Yanmei Shi and DeLong have also led to the discovery of new classes of regulatory molecules (called 'small RNAs') that are abundant and operational in native marine microbial species. Until the application of these new methods to native microbial plankton, these small RNAs had remained unknown to scientists. Now it has become clear that not only proteins, but small RNA molecules, play important roles in regulating gene expression in the environment.



Results from a perturbation experiment in seawater, tracking community composition, DNA content and gene expression.



Highlight

Ocean FEST: Families Exploring Science Together

After over a year of program development and field-testing, a new, intergenerational hands-on marine science program called Ocean FEST was launched in Hawaii in October 2009. Ocean FEST (Families Exploring Science Together) is a collaboration between The Center for Microbial Oceanography: Research and Education (C-MORE) and the Hawai'i Institute of Marine Biology (HIMB). These organizations, both based at the University of Hawai'i at Mānoa, are world-renowned research institutes that are equally committed to promoting high quality ocean science education. Our overarching goal is to interest Hawai'i's kids in careers in ocean science and related Science, Technology, Engineering, and Mathematics (STEM) fields through fun, hands-on activities.

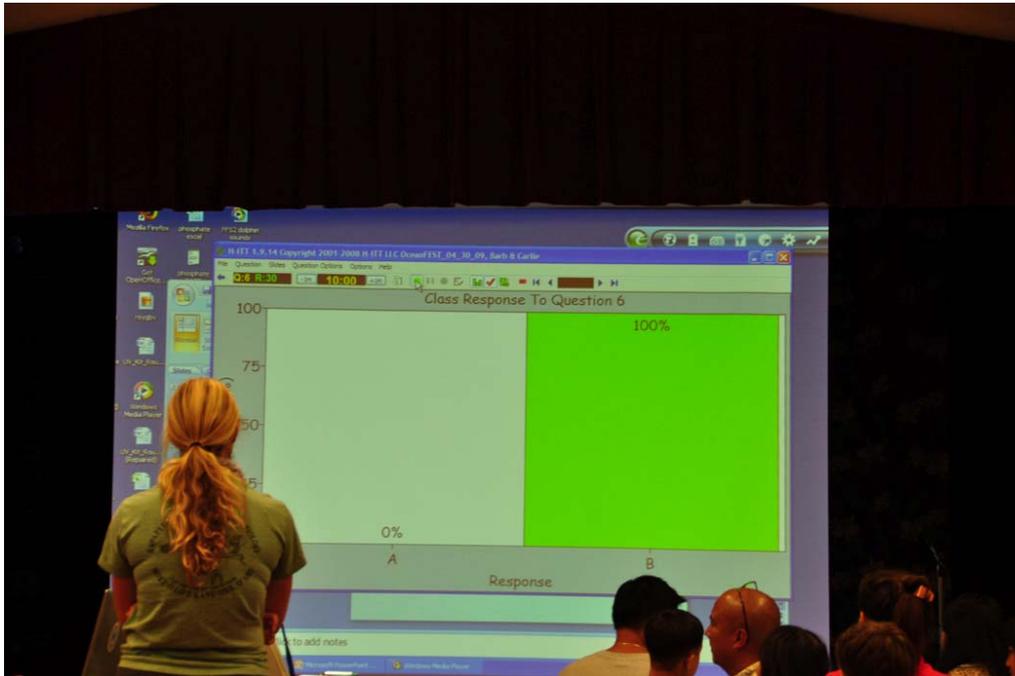
This three-year, state-wide program funded by the National Science Foundation (NSF) will offer twenty events annually held at public and charter schools and community event programs. Seed funding to conduct pilot events was provided by the State of Hawai'i (Act 111). Modeled after Art and Rene Kimura's highly successful space-themed family science nights, Ocean FEST engages students in grades 3–6 and their parents in hands-on science activities and demonstrations that explore the wonders of the ocean world. Ocean FEST is offered free of charge to participating schools; the two-hour program covers topics such as marine microbes, ocean acidification, and climate change. The program is preceded by a professional development component for teachers so that the activities can be brought into the classrooms as well.

Barbara Bruno, education coordinator for C-MORE and Carlie Wiener, Northwest Hawaiian Islands Research and Outreach Program Specialist at the Hawai'i Institute of Marine Biology, both of the University of Hawai'i at Mānoa, are the program founders and lead instructors. "We are very excited about this program; it gives both parents and students an opportunity to learn together about the importance of our oceans and the exciting career opportunities in marine sciences," says Wiener. Bruno adds: "We're especially interested in engaging students from groups that have been traditionally underrepresented. A local, diverse geoscience workforce in Hawai'i's next generation will be critical to tackling the urgent local problems posed by our rapidly changing environment."

All activities are aligned with Hawai'i 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. For more information on the program, please visit the Ocean FEST website:

<http://oceanfest.soest.hawaii.edu/>

Photos follow



Question 6 was “Are most microbes good or bad for us?” In the post-test given at the end of the program, 100% of respondents selected the correct answer (good).



Families explore the concept of density using Cartesian divers.



Families explore the effects of an acidifying ocean on corals and calcareous microbes.



In this activity, students explore the concepts of density, carbon dioxide production, and the formation of carbonic acid in the ocean.



Families make microbes that slowly sink through the water column.



A child with his "microbe"



Highlight

C-MORE Scholars Program

The Center for Microbial Oceanography: Research and Education (C-MORE) is a National Science Foundation Center dedicated to exploring the largely unknown and fascinating world of marine microbes, building partnerships between scientists and educators, preparing the next generation of microbial oceanographers, and facilitating outreach to the public at large.

Currently in its second year, the C-MORE Scholars Program provides undergraduates in the University of Hawai'i (UH) system the opportunity to participate in hands-on research experiences. They are mentored by either UH faculty members or scientists from federal/state agencies and the private sector. Although open to all students majoring in a field relevant to microbial oceanography, the program specifically targets students from traditionally underrepresented groups, especially Native Hawaiians and Pacific Islanders.

Three levels of awards ranging from \$2,000 to \$4,000 per semester are offered, depending on the skill and knowledge level of the applicant. Freshmen are generally awarded traineeships, in which they learn basic research concepts and computer and lab skills. Sophomores and juniors are eligible for internships, in which participants conduct research that allows them to see the “real-life” application of their coursework. Seniors can apply for the highest level of support (fellowships), in which they work on individualized research projects towards their senior theses. The C-MORE Scholars program supports 10 students each semester; approximately half of which are trainees.

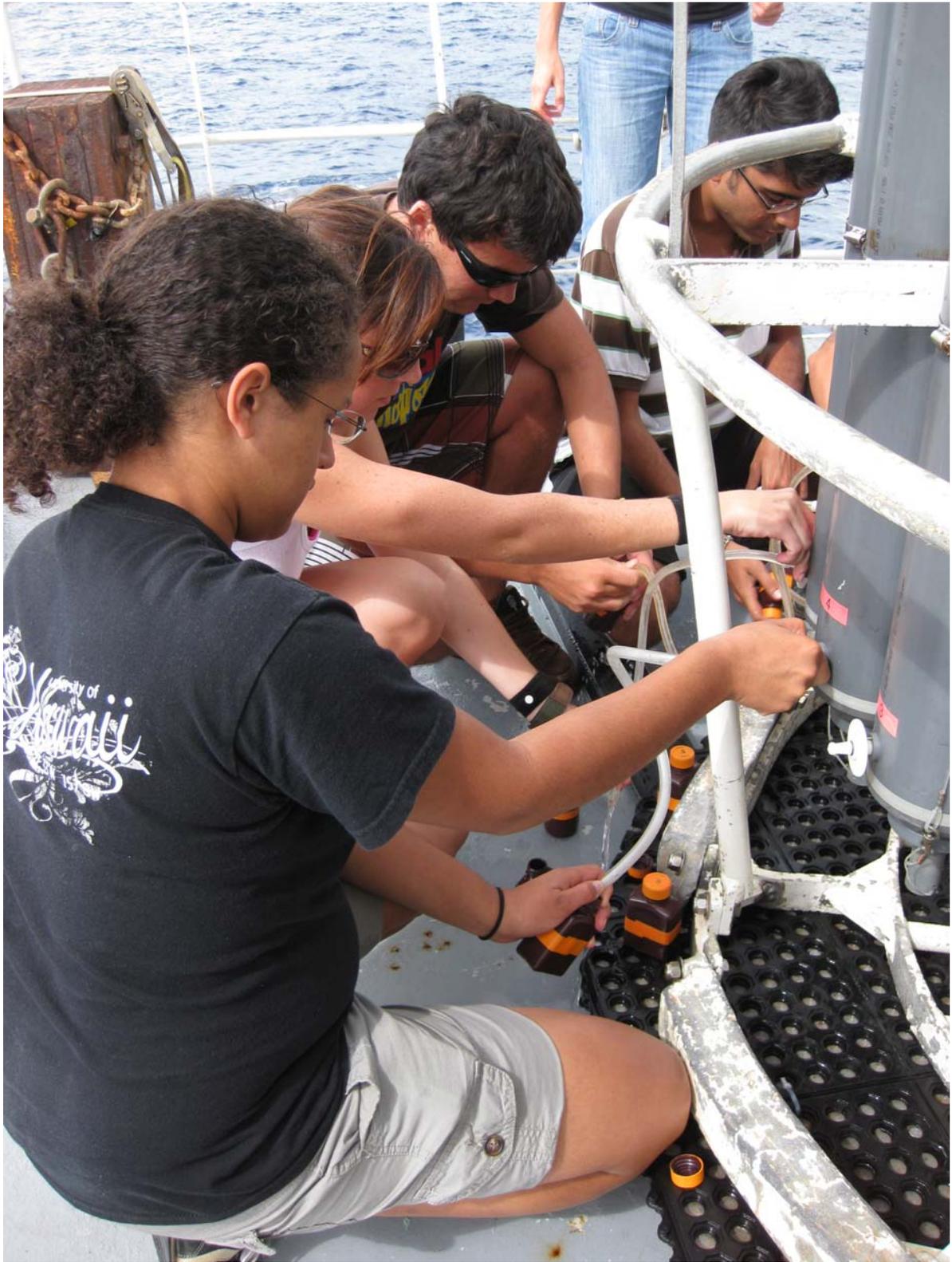
All C-MORE Scholars receive advising on applying for jobs and graduate school, free tutoring in math and science courses, professional development training, and cohort-building activities. They also have the opportunity to participate aboard research cruises. Scholars are expected to give back to their community: they actively participate in C-MORE's outreach activities throughout the academic year.

“This program is exciting and very rewarding,” said La'Toya James, 19, who is currently studying biology at UH Mānoa. Working in the lab of C-MORE investigator Grieg Steward, James is looking to better understand the role of the structure and functions of viruses in marine ecosystems. “I hope to acquire more lab techniques and knowledge about the viral communities and bacterial communities in Hawaii's waters. I want to be able to apply the knowledge and skills learned here to further my career.”

Biology major Ale'alani Dudoit, 23, notes, “This program will allow me to work in a lab under some of the best biologists and learn techniques that will help better me for graduate school. My ultimate goal from C-MORE is to gain a great hands-on experience that will help foster me into a better scientist.” Dudoit also appreciates the outreach requirement. “I would like to be able to get involved with the Hawaiian community...My goal is to give back to the community that once taught me.”

Highlights during 2009 include the first C-MORE Scholar hired at MBARI as a summer intern and a published paper in *Environmental Microbiology* co-authored by a C-MORE Scholar. Both of these students are Native Hawaiian students at the University of Hawaii (one female, one male).







Highlight

C-MORE Science Kits

In summer 2009, the Center for Microbial Oceanography: Research and Education (C-MORE) released an exciting set of six portable, educational kits that cover a range of marine science topics, including ocean acidification, coral bleaching, and marine debris. The kits provide teachers with innovative ways to integrate up-to-date oceanography content and technologies into their curriculum without requiring any previous training or specialized knowledge.

Each kit is self-contained and user-friendly: all supplies, lesson plans, handouts, background readings, and PowerPoint presentations are included. C-MORE Science Kits are designed for use with a range of grade levels and are aligned with both Hawai'i Content and Performance Standards (HCPS III) and the national Ocean Literacy Principles.

Each kit involved over a year of development and field-testing by C-MORE, in partnership with Hawaii's science teachers. C-MORE Science Kits were first field-tested with Hawaii's teachers in July 2008 and they have since received uniformly positive acclaim:

"These kits have certainly made life teaching environmental science easier."

– D. Weidman, St. Francis School

"[The Marine Debris Science Kit] is an easy kit to connect with my students' daily lives."

– S. Bright, Waianae High School

"[The Ocean Acidification Science Kit] was a dream come true for a science teacher."

– B. Bevacqua, Kalaheo High School

"It seems as if you've thought of everything to make it easy for teachers to use."

– A. Church, Punahou School

C-MORE science kits may be borrowed free of charge and are currently available to educators in California, Oregon, Massachusetts, and throughout the Hawaiian Islands. Interested teachers can find detailed descriptions of each kit and submit online requests to borrow them at: http://cmore.soest.hawaii.edu/education/teachers/science_kits.htm

Photos follow



Ocean Acidification Kit



Plankton Kit



Marine Mystery kit:



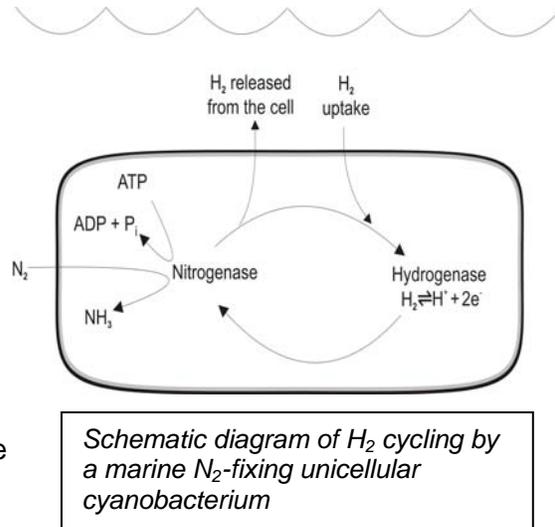
Marine Debris kit



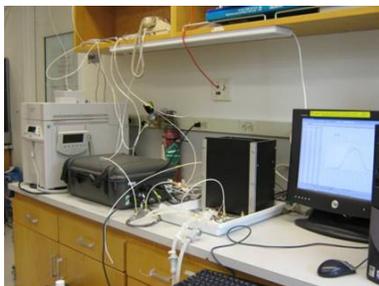
Highlight

The Marine Hydrogen Cycle

The production of H_2 during the fixation of N_2 by cyanobacteria is an obligate yet enigmatic process. To recuperate the loss of potential energy, diazotrophs re-assimilate the H_2 released from the cell via uptake hydrogenase (Figure). This balancing act between the production and consumption of H_2 plays out in the surface waters of the oligotrophic ocean. An imbalance in the process *i.e.* when the rate of production of H_2 exceeds consumption, contributes to the pool of dissolved H_2 in the surface waters. Concentrations of dissolved H_2 are super-saturated relative to atmospheric equilibrium and represent a potential energy source for either the diazotrophs who produce the H_2 or opportunistic microbes.



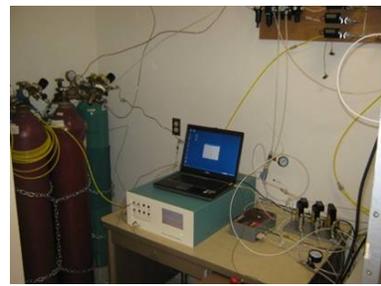
A series of collaborations between the laboratories of Dave Karl, Jon Zehr, Zbigniew Kolber and Matt Church investigated H_2 cycling by marine diazotrophs. Analysis of dissolved H_2 at Station ALOHA revealed concentrations were super-saturating all year round in the upper 100 m. To quantify the relative contribution of key diazotrophs, cultures of *Trichodesmium erythraeum* IMS101, *Crocospaera watsonii* WH8501, *C. watsonii* WH0002, and *Cyanothece* ATCC51142 were analyzed for H_2 production in conjunction with N_2 -fixation activity. Net production rates of H_2 varied over two orders of magnitude and revealed *T. erythraeum* IMS101 as the most prolific H_2 producer.



Measurements of N_2 -fixation were conducted using an on-line acetylene reduction assay.



Crocospaera watsonii WH8501 was grown in 2 L incubators that regulated light and temperature conditions. Measurements of pH, O_2 and fast rate repetition fluorometry were incorporated.



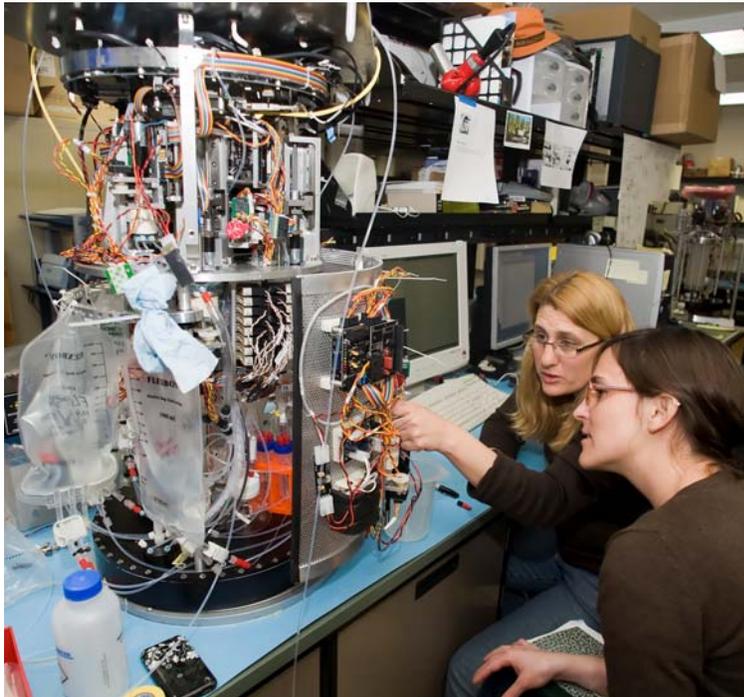
H_2 production was measured using a reduced gas analyzer connected to the cultures for on-line analysis.



Highlight

“Ecogenomic Sensors” add a new Dimension to Ocean Observing Systems

Molecular probe technologies are commonly used in the ocean sciences to identify specific organisms and reveal their genomic potential, but such work generally requires returning samples to a laboratory for analysis. “Ecogenomic sensors” were conceptualized as devices that would obviate the strict requirement for sample return by allowing for biomolecular analyses to occur autonomously, *in situ*. Researchers at the Monterey Bay Aquarium Research Institute, Lawrence Livermore National Laboratory, NOAA/National Ocean Service Marine Biotoxins Laboratory and partners comprising the Center for Microbial Oceanography Research and Education have given life to this concept through refinement and application of the Environmental Sample Processor (ESP). The ESP allows for utilization of DNA and protein array techniques along with quantitative PCR to sense microbial community structure and detect specific genes and gene products remotely within a distributed ocean observing framework. The instrument was recognized by R&D Magazine as one of the 100 most technologically significant products introduced into the marketplace during 2009, creating new ways for conducting long term environmental assessments and effecting water quality measurements. Development of the ESP was made possible by funding from NSF, NASA, and the Keck, David and Lucille Packard, and Gordon and Betty Moore Foundations.



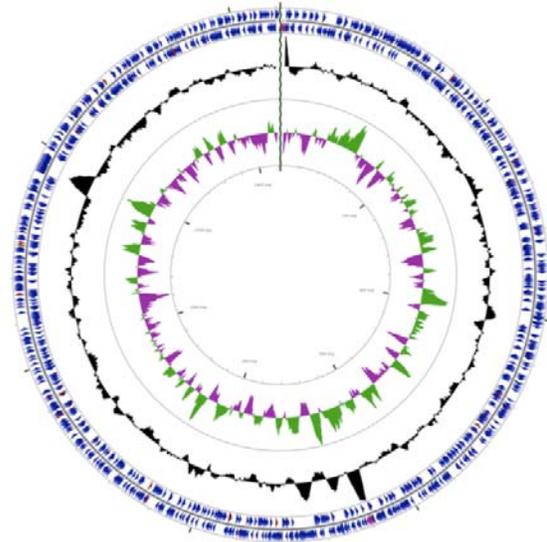
C-MORE affiliates Drs. Christina Preston (MBARI) and Julie Robidart (UCSC) work to incorporate quantitative PCR module into the ESP system (photo credit: Todd Walsh, MBARI).



Highlight

Open Ocean Nitrogen-Fixing Cyanobacterium Has Streamlined Metabolism

Researchers at the University of California, Santa Cruz and the NSF Science and Technology Center for Microbial Oceanography: Research and Education (C-MORE) have discovered an oceanic cyanobacterium with an unusual metabolism. This widely distributed cyanobacterium is important in nitrogen fixation—a natural nitrogen fertilization process that is performed only by Archaea and Bacteria, including cyanobacteria. Because this cyanobacterium remains uncultivated, genomic approaches were used to catalogue the major metabolic capabilities of the organism from a small number of cells sorted by flow cytometry. Previous results showed that the organism lacked a major component of the photosynthetic apparatus, suggesting that the organism had unusual metabolic properties. Recently, researchers at UC-Santa Cruz, working with new DNA sequencing techniques in collaboration with 454 Life Sciences Inc. (a Roche Company) and partially funded by the Gordon and Betty Moore Foundation, were able to completely sequence the genome of the cyanobacterium. Analysis of the complete genome uncovered more metabolic surprises, and showed that this cyanobacterium lacks many other fundamental pathways found in all other photosynthetic microorganisms. The study was made possible by the multidisciplinary support of C-MORE and access to the long-term oligotrophic ocean monitoring site, Station ALOHA, in the subtropical Pacific Ocean. The new genomic information has implications for the evolution of nitrogen-fixing photosynthetic microorganisms, but more importantly provides information on how nitrogen fixation by these, and other cyanobacteria, is controlled in the open ocean.



Complete genome sequence of novel uncultivated cyanobacterium from the Pacific Ocean, showing protein coding regions in blue, GC content relative to the mean (31%) in black, and GC skew in green (positive) and purple (negative). Credit: H. J. Tripp, University of California, Santa Cruz

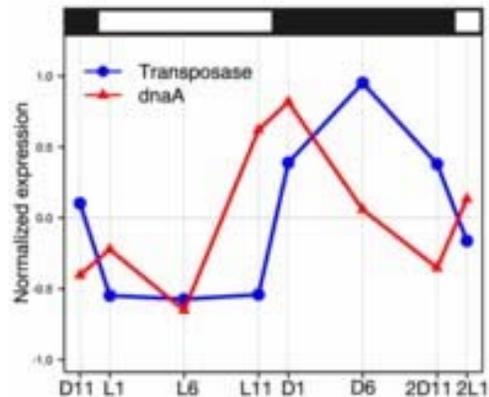


Highlight

Transposases Actively Rearrange Genomes and Drive Evolution of Oceanic Nitrogen-Fixing Cyanobacteria

Although viruses have been found to be the major agent causing genome evolution in many oceanic microorganisms, recent findings by researchers at the University of California, Santa Cruz and the NSF Science and Technology Center for Microbial Oceanography: Research and Education (C-MORE) show that transposases, small mobile genetic elements, play an important role in the genome structure and the unusual evolutionary strategy of the oceanic nitrogen-fixing cyanobacterium *Crocospaera watsonii*. *C. watsonii* strains are found in most tropical and subtropical marine basins, and the nitrogen provided by this cyanobacterium is a critical component of oceanic carbon and nitrogen cycles. A number of *C. watsonii* strains have been isolated from various global locations over the last three decades. For all genetic markers examined to date, these strains have nearly identical DNA sequences, even though there are significant phenotypic differences between these very closely related strains. The finding that these strains contained many mobile genetic elements, that are active (the genes are expressed), helps to explain how such highly conserved strains maintain genetic variability.

The genome of the type strain, *C. watsonii* WH8501, was previously draft sequenced. Using highthroughput DNA sequencing technology, UCSC researchers funded by the Gordon and Betty Moore Foundation and C-MORE obtained genomic sequence for a second strain, WH0003, which was isolated from another ocean basin, and is phenotypically distinct from WH8501. The subsequent comparison of these two draft genomes revealed unexpected genetic differences between the strains. Most striking was the fact that WH8501 has over five times the number of transposase genes found in WH0003. Additionally, most of the genetic variation between the two strains is not a result of the typical evolutionary mechanism of DNA mutation accumulation, but instead takes the form of genome rearrangements (e.g. inversions, insertions or deletions), many of which are observed near transposases. Microarray research, also being carried out at UCSC, provided significant support for this discovery. Whole genome expression profiles of WH8501, using microarrays, showed that an unusually large number of transposases are functional and expressed in a temporal pattern that coincides with DNA replication.



Transcription of one of transposase genes (blue, circles) and dnaA gene encoding the DNA binding protein (red, triangles) in C. watsonii WH8501 during 24 hours (light/dark). The expression values were normalized to the mean at all time points. The expression profiles for transposases and dnaA, which signifies initiation of DNA replication, suggested that transposition is most active during DNA replication at night in C. watsonii WH8501. The bar on top shows light and dark periods.

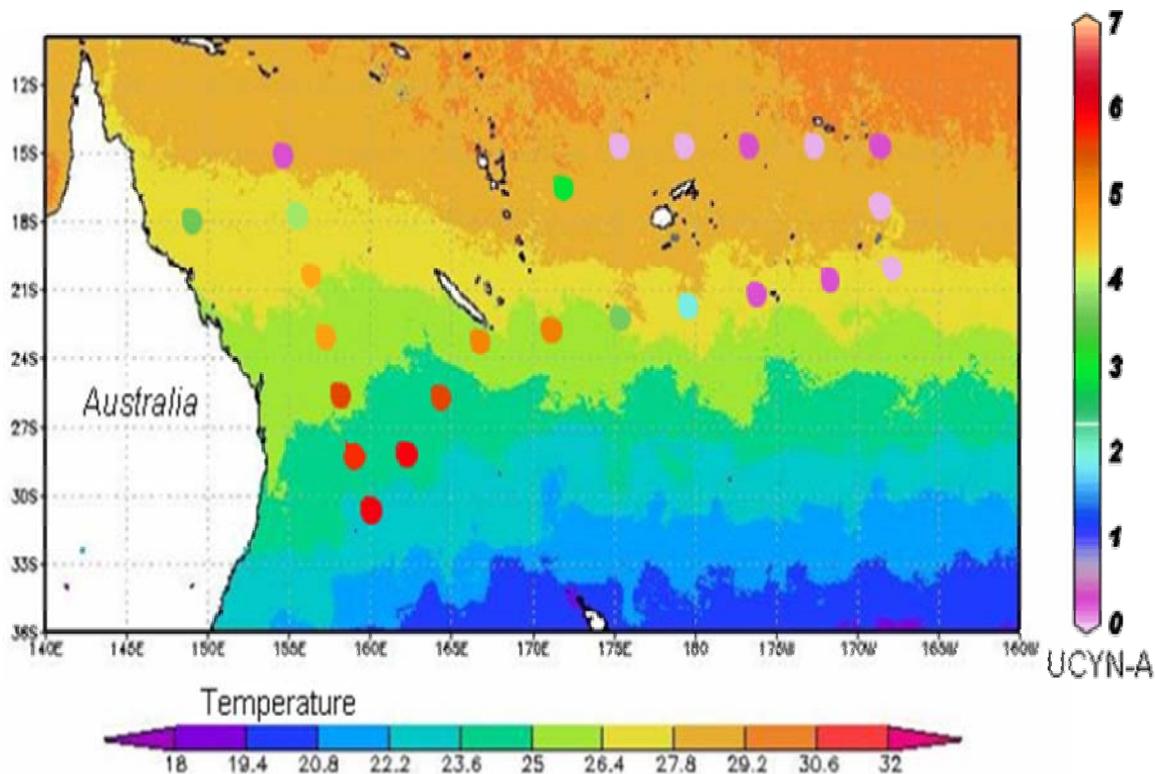
These discoveries suggest that transposases play an important evolutionary role in establishing the genetic variation seen in natural populations of these oceanic strains.



Highlight

Nitrogen-Fixing Cyanobacteria Have Different Ecological Niches in the Oligotrophic Ocean

A research team including researchers from the NSF Center for Microbial Oceanography (C-MORE) recently identified factors controlling niche specialization of two unicellular cyanobacteria known to play a major role in oceanic nitrogen fixation. Nitrogen fixation is performed by diverse groups of microbes, and is an important process in the open ocean where it converts atmospheric nitrogen to chemical forms that can be used for biological growth. Current oceanic nitrogen fixation estimates have large uncertainties partially due to the scarcity of biological information and field data for nitrogen-fixing microorganisms.



Distribution of one group (called UCYN-A) of unicellular nitrogen fixing cyanobacteria (determined from nitrogenase gene abundances and reported in $\log nifH$ gene copies L^{-1}) show that their abundances are highest in cooler waters in the South Pacific (surface water temperature shown in background color).

In a detailed survey in the tropical and subtropical South Pacific Ocean, the distributions of two different types of unicellular cyanobacteria were found to be distinctly different from each other and from that of the nitrogen-fixing *Trichodesmium*, a well-described, filamentous, bloom-forming cyanobacterium. The results show that temperature likely plays a major role in controlling distributions of the oceanic unicellular nitrogen-fixing cyanobacterial species, and that the UCYN-A cyanobacteria may fix nitrogen in cooler waters which has implications for the

distribution of global oceanic nitrogen. In a research study funded by the National Science Foundation and the Gordon and Betty Moore Foundation, microbial ecologists Pia Moisander and Jonathan Zehr from the University of California Santa Cruz in collaboration with researchers from the Georgia Institute of Technology, Monterey Bay Aquarium Research Institute, Oregon State University, and the University of California, Santa Barbara examined the distribution of unicellular nitrogen fixing cyanobacteria in relation to chemical and physical conditions. Since the microorganisms are not easily detected by microscopy, the distributions of nitrogen fixing cyanobacteria were determined by quantifying abundances of the *nifH* gene that encodes the enzyme that catalyzes nitrogen fixation. Temperature was found to be a major factor controlling species distributions and in particular one of the most abundant nitrogen fixers, an uncultivated unicellular cyanobacterium was found to extend into cooler waters than previously believed to support nitrogen fixer growth. High abundances of the unicellular nitrogen-fixers (the UCYN-A cyanobacterium) were observed at low temperatures that extend beyond growth temperatures of *Trichodesmium* (thought not to grow at temperatures less than 25°C) and in areas with elevated nutrients relative to typical oligotrophic waters. This finding suggests that nitrogen fixation may occur in wider regions of the open ocean than previously known by organisms that cannot be easily detected, which may be important in balancing global carbon and nitrogen budgets.

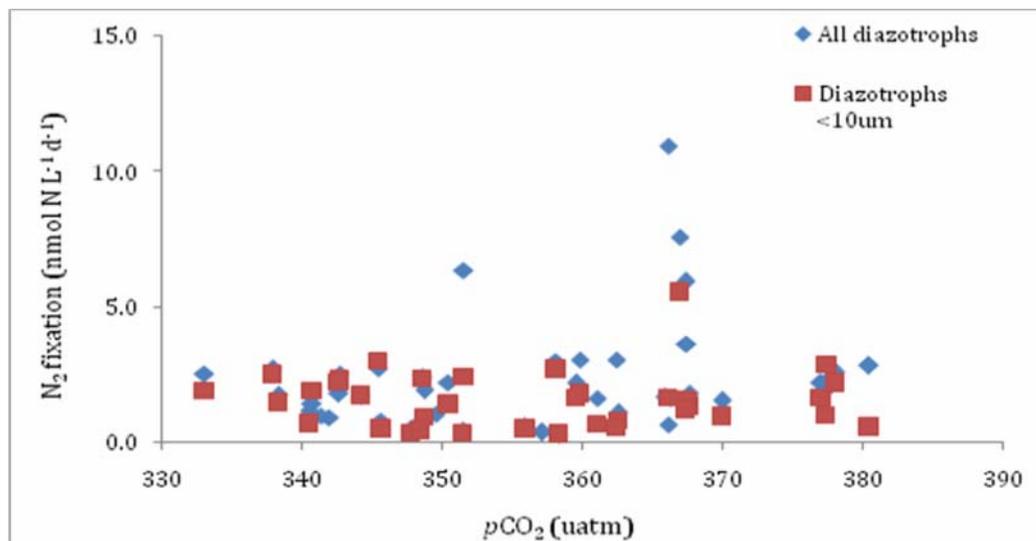


Highlight

Effects of CO₂ enrichments on marine nitrogen fixation

Human reliance on fossil fuel combustion continues to alter atmospheric and oceanic CO₂ inventories. Because CO₂ readily dissolves in the surface ocean, ocean pCO₂ demonstrates a long-term increase similar in magnitude to the rate that atmospheric pCO₂ is rising. Moreover, such changes in the ocean carbonate system have significantly decreased upper ocean pH. By the end of the 21st century, atmospheric pCO₂ is predicted to nearly double (from 350 to 750 μatm), with concomitant decreases in surface ocean pH of 0.3 units. To date, it remains unclear how such changes in the upper ocean carbonate system will influence ocean ecosystem structure and processes and how climate sensitive the various trophic components of the pelagic food web may affect marine elemental cycles in an increased pCO₂ world.

In a project led by C-MORE post-doc Daniela Böttjer (UH) and Ph.D student Donn Viviani (UH) as part of a collaborative effort between the Church lab at the University of Hawaii and the Letelier lab at Oregon State University, C-MORE scientists have begun studying how marine nitrogen fixing bacteria, referred to as diazotrophs, respond to changes in seawater pCO₂. Diazotrophs play a pivotal role in biogeochemical cycles since they provide a biological source of new nitrogen to large parts of the oligotrophic ocean, including the North Pacific Subtropical Gyre. Monthly measurements of diazotroph activity (nitrogen fixation rates) between 2004-2008 at Station ALOHA, the field site of the Hawaii Ocean Time-series (HOT) program, have revealed that a major fraction of daily nitrogen fixation appears attributed to diazotrophs <10μm in diameter (Figure). However, on some occasions nitrogen fixation is predominated by larger diazotrophs (most likely filamentous *Trichodesmium* spp. and diatom associated heterocystous cyanobacteria) and that such events tend to occur when pCO₂ levels exceed 360 μatm. These results suggest different groups of ocean diazotrophs may vary in their physiological responses to perturbation in CO₂ or other environmental variables such as temperature, light and the degree of water column stratification.



Relationship between surface ocean rates of nitrogen fixation and pCO₂ at Station ALOHA

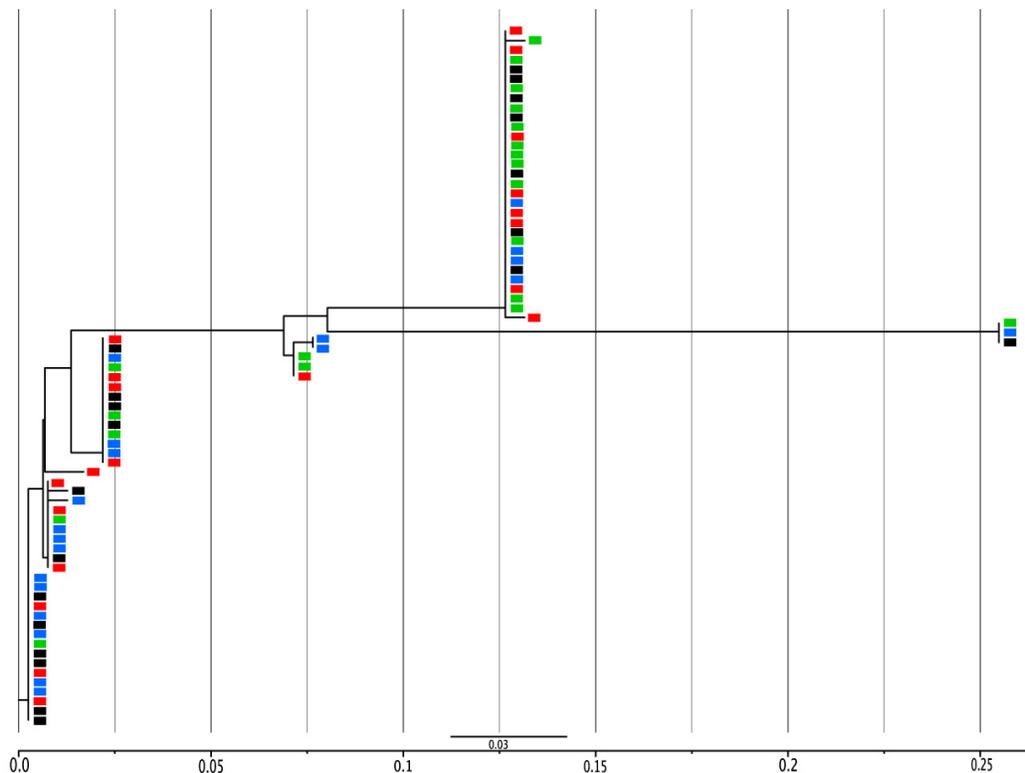
In addition to time series examination of the relationship between seawater carbonate system variability and rates of N₂ fixation, laboratory and in situ CO₂ enrichment experiments are being carried out that will enable quantification of how variations in seawater pCO₂ affect a) abundance, biomass, composition and fixation rates of natural assemblages of diazotrophs and, in addition, b) influence the carbon acquisition physiology, photosynthesis, and nitrogen fixation of different cultured cyanobacteria strains (*Trichodesmium* and *Crocospaera*).



Highlight

Persistent Populations of Marine Cyanophages at Station ALOHA

Researchers in the Steward laboratory (UH), including C-MORE post-doc Elisha Wood-Charlson and researcher Alex Culley, in collaboration with C-MORE investigator Matt Church (UH), are studying the population structure of viruses that infect cyanobacteria in the North Pacific Subtropical Gyre. Preliminary results indicate that viral phylotypes were present across physically distinct regions of the euphotic zone sampled during winter and summer months (Figure).



Tree representing the diversity of a cyanophage marker at Station ALOHA.

Samples: **February 2007** (HOT-189) Red – surface, Black – DCM. **July 2007** (HOT-193) Green – surface, Blue – DCM (Each box represents a single sequence).

For this study, the researchers selected a cyanophage gene that appears to be cyanophage-specific, but which varies within the population because of host selection pressures. This gene was amplified, cloned, and sequenced to detect phylotypes present in samples from Station ALOHA, 100 km north of Oahu, Hawai'i. To date, samples have been analyzed from two Hawaii Ocean Time-series cruises (HOT; 189-Feb 2007, 193-July 2007) and from distinct regions of the euphotic zone - near surface waters and the deep chlorophyll maximum (DCM). When completed, this study will include data from up to 5 years of HOT cruises, with samples collected at approximately monthly intervals at ~8 depths in the upper 200m of water.

Results from this study will allow us to understand the dynamics of cyanophage populations in the N. Pacific subtropical gyre. In addition, these dynamics will be compared to the extensive biogeochemical and physical oceanographic data collected at Station ALOHA to look for environmental factors that may correlate with patterns in viral community composition.

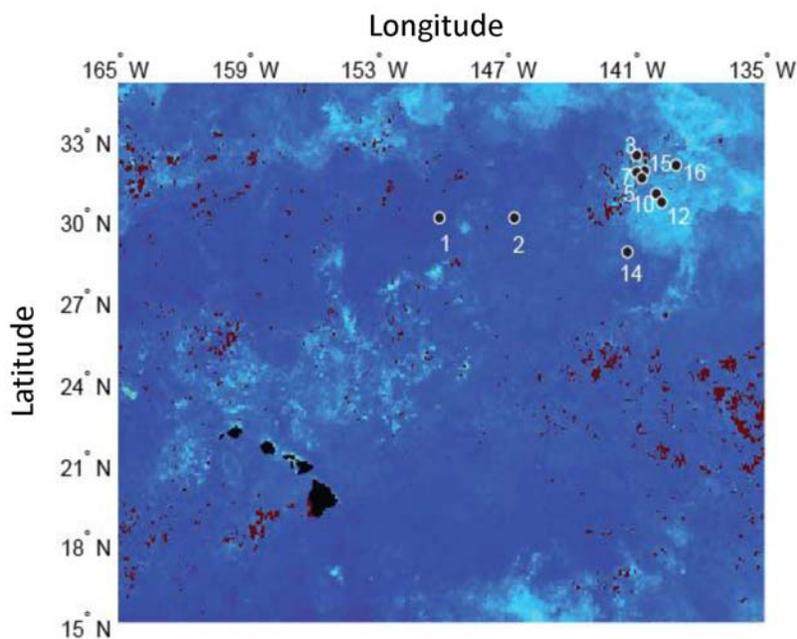


Highlight

Uptake of Dissolved Organic Phosphorus by Diazotrophs

Several research efforts over the past three decades have confirmed the potential use of dissolved organic phosphorus (DOP) by pelagic diazotrophs and suggested its potential role in supporting the growth of marine diazotrophs in regions where inorganic phosphorus is present in vanishing amounts. Most of these studies have focused on the bioavailability of P in molecules with a C-O-P monoester bond. However, recent work has presented strong evidence that marine diazotrophs are also able to cleave the more stable C-P bound found in phosphonates.

During a July 2008 cruise in the North Pacific Subtropical Gyre (NPSG) between 28°N and 32°N (Figure) C-MORE researchers Angel White and Ricardo Letelier measured rates of bulk carbon and nitrogen fixation in response to additions of inorganic phosphorus (P_i) and methylphosphonate (MPn). In 24-hr on-deck incubations, both experimental treatments (+ P_i and +MPn) displayed enhanced ^{13}C and $^{15}N_2$ fixation rates relative to control incubations to which no external P sources were added. No significant differences between P_i and MPn additions were observed when the treatments were compared across the full cruise transect. However, the ratio of $^{15}N_2$ fixation to ^{13}C fixation rates stimulated by P additions was highest at a series of stations bordering a region of elevated surface chlorophyll observed along the frontal zone of the NPSG (Station 5-7). In addition to confirm the rapid utilization of MPn by the diazotrophic assemblage, these results are suggestive of broad scale physiological P-limitation in diazotrophs within the study region.



Geographic location of stations sampled during June 2008 superimposed on the monthly averaged (June 2008) chlorophyll a concentration derived from MODIS-Aqua

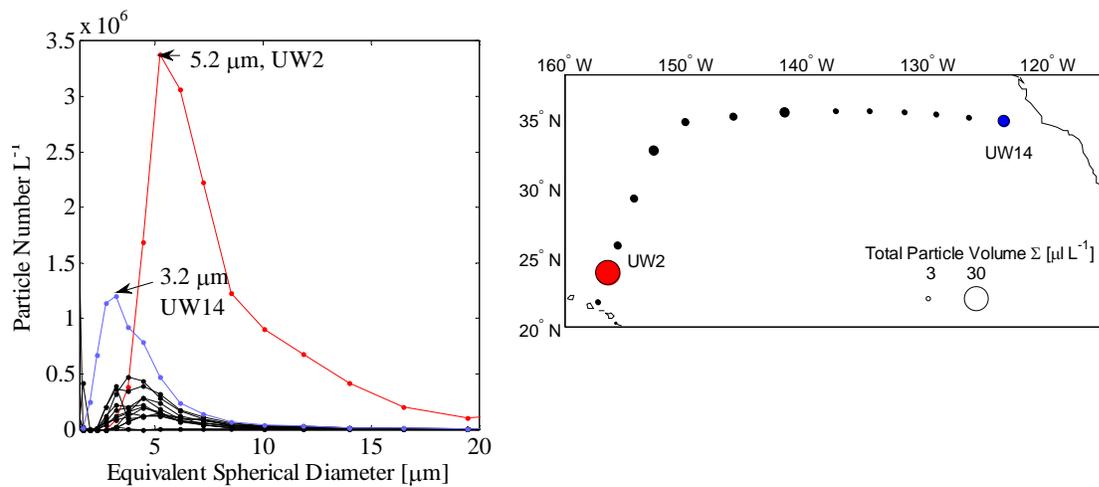


Highlight

Pacific Ocean Particles

As ocean color technology moves towards enhanced spectral resolution and the research community struggles to generate flexible parameterizations of phytoplankton physiology, there is an increased focus on relationships between community structure and productivity algorithms. While powerful, the traditional pigment-based (HPLC) or genomic approaches to the discrimination of phytoplankton community structure are hindered by the need for discrete measurements and time-consuming methodologies. Rapid, non-invasive, technologies for determination of phytoplankton productivity, elemental concentrations (particulate carbon), pigment composition and particle size distributions are robust complements to discrete measurements. Accordingly, C-MORE researchers Angel White and Ricardo Letelier have initiated *in situ* hyperspectral absorbance and particle size distribution measurements at Station ALOHA and aboard recent C-MORE cruises to complement ongoing multi-scalar measurements of primary productivity. Existing optics instrumentation for the HOT and C-MORE programs has been enhanced to include a hyperspectral absorption meter (WET Labs ac-s, 89 wavelengths from 350 - 750 nm), a hyperspectral radiometric profiler (Satlantic Hyperpro II) equipped with a two-channel fluorometer and backscatter meter, and a LISST-100X (Sequoia Scientific, USA) for laser-based determinations of particle size distributions in the 1-250 μm size range.

In the summer of 2008, as a component of the C-MORE SUPER (Survey of Underwater Plastic and Ecosystem Response) cruise, White deployed the LISST along a transect from Hawaii to California. Results from this cruise (Figure 1) revealed unexpected patterns: the mode of particle concentrations (number L^{-1}) was generally centered around 4 μm with one station characterized by a bloom of larger particles (mean diameter = 5.2 μm , underway station 2, UW2) and another showing a bloom of smaller particles (mean diameter = 3.2 μm , UW14). Microscope-based observations for UW2 revealed high concentrations of pennate diatoms while UW14 appeared to be dominated by *Synechococcus*. Combined with reports from the scientific literature, these results illustrate that the LISST is an ideal instrument for the acquisition of high quality, high-resolution, *in situ* particle size distributions even in the oligotrophic blue waters of the North Pacific. The novel combination of particle size spectra and ancillary instrumentation used routinely as part of C-MORE field efforts for the spectral deconvolution of phytoplankton pigments (ac-s) and productivity (FRRF), will allow unprecedented analysis of relationships between particle size, community composition and primary productivity. These efforts contribute to the C-MORE focus on instrument development and the overarching aim to better understand the chemical and biological factors governing microbial communities in the pelagic seascape.



(Left) Size distributions of particles collected from ship's flow-thru system (6m) along a transect from Hawaii to California in August of 2008. Most stations were roughly similar in relative particle abundance and size spectra with two exceptions, underway station 2 (UW2) and 14 (UW14) where particle abundance was enhanced and positive and negative shifts in size spectra were observed, respectively. **(Right)** Total particle concentrations at each station. Symbol area is proportional to total volumetric particle concentrations.

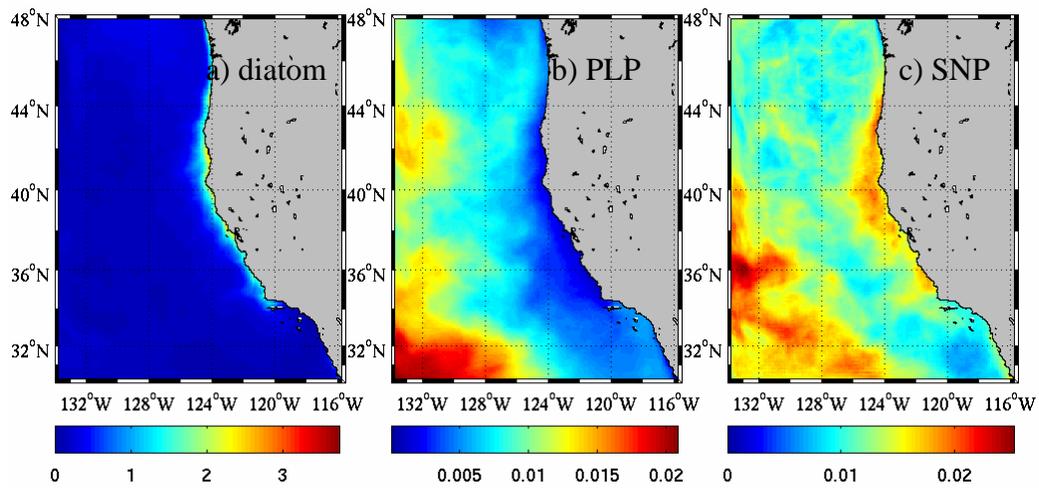


Highlight

Modeling Emergent Community Ecosystems within the California Current System

A new marine ecosystem modeling approach developed at the Massachusetts Institute of Technology was adapted by researchers at the University of California, Santa Cruz to improve predictions of coastal ecosystem phytoplankton dynamics. This novel ecosystem model differs from traditional modeling efforts by including tens of phytoplankton types, each with a unique response to environmental variables important for phytoplankton growth, such as light, nutrients, and temperature. The approach may better represent the many types of phytoplankton species found in nature than traditional models that include only a few phytoplankton groups with a limited range of environmental responses. Improvements in understanding the diversity and biogeography of different types of marine phytoplankton may ultimately improve predictions for nutrient cycling, oceanic CO₂ uptake, and provide information for fisheries management under current and future climate conditions.

Christopher Edwards, Nicole Goebel, and Jonathan Zehr at the University of California Santa Cruz in collaboration with Mick Follows of the Massachusetts Institute of Technology used this new modeling approach to predict the seasonal and spatial variability of phytoplankton in the California Current System (CCS). Results of this model agree with observed distributions of total phytoplankton biomass, with highest biomass in the high nutrient upwelling coastal waters and lowest biomass offshore where nutrients are low. Each of the individual phytoplankton were within one of four main types. Model results of these different types show predicted distributions of phytoplankton that fill different environmental niches. For example, high diatom concentrations thrive nearshore supported by high levels of nitrate and silicate associated with coastal upwelling, while low concentrations of small phytoplankton exist both nearshore and offshore. Small phytoplankton that dominate offshore were represented by *Prochlorococcus*-like phytoplankton (PLP), which are known to be abundant in low nutrient waters. Small non-*Prochlorococcus*-like phytoplankton (SNP) are more cosmopolitan, as demonstrated by their presence both on and offshore (see Figure). While these phytoplankton types and the majority of phytoplankton biomass are dominated by ~8 individuals, the remaining ~50 individuals are maintained at lower but non-zero levels. Many phytoplankton also exist at low concentrations in nature and could serve as an important reservoir of individuals that take the place of the dominant phytoplankton with changes in environmental conditions. The flexibility in the number and types of phytoplankton may be key for improving ecosystem model predictions in different global environmental change scenarios.



Five-year surface average chlorophyll concentrations (mg m^{-3}) of modeled (a) diatom, (b) Prochlorococcus-like (PLP), and (c) small non-Prochlorococcus-like (SNP) phytoplankton types. Note different scale bars.



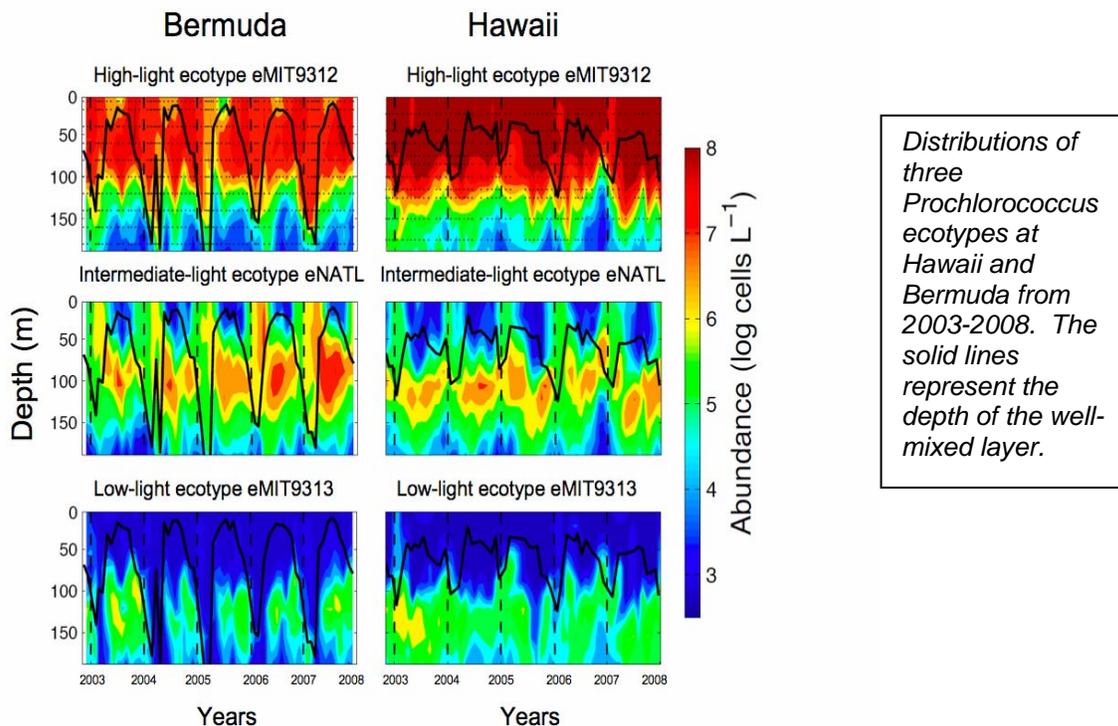
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A Five Year Chronicle of the Dominant Phytoplankter in the Pacific and Atlantic Oceans Reveals Remarkable Similarities and Differences Between Biomes

Rex Malmstrom and colleagues from the Chisholm Lab at MIT have recently synthesized a detailed five year record of the seasonal dynamics of *Prochlorococcus*, the phytoplankter that dominates tropical and subtropical oceans. Through a coordinated effort with the NSF-sponsored Center for Microbial Oceanography Education and Research (C-MORE) collaborators in the Hawaii Ocean Time-series (HOT) program, and with colleagues in the Bermuda Atlantic Time-series Study (BATS) program, they followed monthly changes in the abundance of different types of *Prochlorococcus* over five years in the waters around Hawaii and Bermuda. These studies revealed both striking similarities and differences in the population dynamics of *Prochlorococcus* in the two oceans.

Prochlorococcus, the collective, is composed of sub-groups, or “ecotypes”, whose members share common evolutionary histories and are adapted for optimal growth at different light and temperature levels. These differences in physiological optima can be seen in the depth distributions of the different ecotypes in the oceans. At the Pacific site, where seasonal changes are not as strong, the distribution patterns were maintained consistently over time, while in the Atlantic they were disrupted each year by winter mixing of the water column and later reestablished. One ecotype was shown to have an unusual light range tolerance in the laboratory, thus explaining its peaks in abundance during periods of deep mixing at both field sites (see eNATL group below).



Results from this study were compared to a more limited data set collected a decade earlier, revealing remarkable stability in the abundance and seasonal dynamics of the total *Prochlorococcus* population on decadal time scales in both oceans. This type of long-term record of a single phytoplankton group is unprecedented and will be useful, if continued, in diagnosing the response of ocean ecosystems to climate change.



Highlight

Metabolism in the Ocean's Most Abundant Phytoplankter is Highly Choreographed to the Daily Pulse of Energy

Ongoing projects in the Chisholm lab at MIT have sought to illuminate the genetic mechanisms underlying the highly coordinated metabolism of *Prochlorococcus* in the face of the pulsed supply of solar energy – its only energy supply – it receives each day. As part of the NSF-Center for Microbial Oceanography Research and Education (C-MORE), Chisholm and colleagues have been using *Prochlorococcus* as a model system for unveiling important couplings between microbes and biogeochemical cycles in the oceans. *Prochlorococcus* is the most abundant oxygenic photosynthetic organism on earth and a key component of oceanic carbon and nutrient cycling. The growth and cell division of *Prochlorococcus* cells is tightly synchronized to daily light/dark cycle in the ocean, and this production of new biomass accounts for a substantial fraction of global primary production.

Most recently, Jacob Waldbauer and Sebastien Rodrigue have measured how the levels of gene transcripts, and a significant fraction of all the proteins, in *Prochlorococcus* are choreographed in response to the daily light dark cycle. This work has extended our understanding from the gene transcription level by quantifying the abundance and periodicity of over 500 *Prochlorococcus* proteins during growth on a daily light/dark cycle. Strong oscillations in gene expression appear to be broadly damped at the protein level, and temporal offsets between the two suggest regulatory mechanisms that are important in determining the abundance dynamics of a number of biogeochemically-relevant proteins. These results shed further light on the complexities of inferring metabolic potential from measurements of gene expression in the wild.





Highlight

A Single Base Deletion in the Genome of a Marine Phytoplankter Confers Resistance to Damage by Ultraviolet Light

Marcia Osburne and colleagues in the Chisholm Lab at MIT have discovered a region of the genome of *Prochlorococcus* that regulates resistance to damaging UV light. Most surprising is that this UV-resistance is conferred by a single base deletion in the chromosome.

Prochlorococcus is the most abundant photosynthetic marine microbe found in the tropical and sub-tropical open oceans, responsible for up to 50% of the photosynthesis in these waters. In



the process of obtaining visible sunlight necessary to carry out photosynthesis some *Prochlorococcus* cells spend time near the ocean's surface, where UV radiation can damage or kill cells.

Osburne and colleagues isolated a mutant of a strain of *Prochlorococcus* that is much more resistant to the UV radiation present in sunlight than the wild type strain. The hyper-resistant strain contained a single base mutation in the region adjacent to two genes known to

produce enzymes that repair DNA damaged by UV light (photolyase and nudix hydrolase). The presence of the mutation affected the regulation of these two genes, causing the mutant strains to maintain high levels of the UV-repair enzymes all the time, as opposed to only when DNA damage is present.

Researchers hypothesize that these types of hyper-resistant cells are present in low-frequency in the oceans, and may have a slight growth advantage in populations occupying near surface waters. Analysis of global ocean genomic databases which contain pieces of DNA from *Prochlorococcus* may serve to confirm this hypothesis.

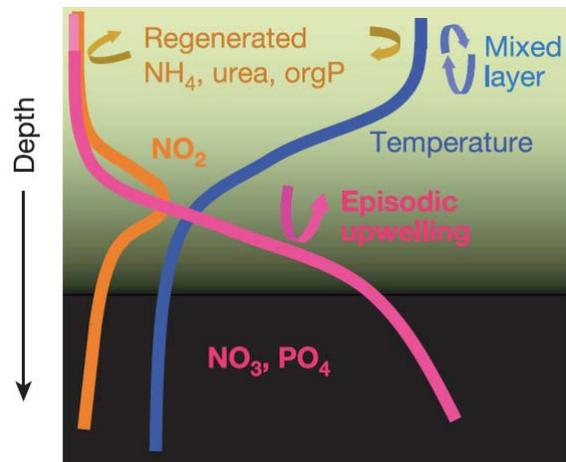
This research, which is supported by the NSF-Center for Microbial Oceanography Research and Education (C-MORE), invites analysis of the potential importance of UV light as a mortality factor for *Prochlorococcus* in the surface oceans. Furthermore it reveals that the streamlined *Prochlorococcus* genome has developed sophisticated mechanisms to survive intermittent exposure to high levels of radiation while carrying out primary production.



Highlight

Discovery of Widespread Metabolic Potential for Nitrate Assimilation by *Prochlorococcus* Sheds New Light on the Ecology of this Abundant Marine Phytoplankter

Researchers at UC-Irvine and MIT have discovered that *Prochlorococcus*, an abundant marine phototroph that supplies much of the fixed carbon in open ocean gyres, has the genes necessary for the assimilation of nitrate (NO_3^-). Nitrate is the most abundant nitrogen source in the open ocean and can often limit productivity. Previously, it was thought that *Prochlorococcus* could not use this highly oxidized nitrogen source which is brought up to the surface by periodic upwelling events. It was widely thought that the cells at the surface could only use the highly reduced nitrogen source, ammonium (NH_4^+), which is primarily recycled in the mixed layer.



Credit: Rocap and Chisholm et al., MIT

By analyzing the sequences of DNA fragments from the open ocean, C-MORE affiliate Paul Berube (MIT) and Adam Martiny (UC-Irvine) found compelling evidence that the genomes of some wild *Prochlorococcus* cells contain the necessary genetic components for nitrate assimilation. Importantly, these genetic signatures appear to be widespread in surface waters that are dominated by the abundant high light adapted ecotypes of *Prochlorococcus*. This discovery may significantly change models of nitrogen cycling in the oceans. In addition this metabolic capability provides researchers with a useful target for understanding how genetic microdiversity among *Prochlorococcus* ecotypes influences the microbial ecology of the open ocean.

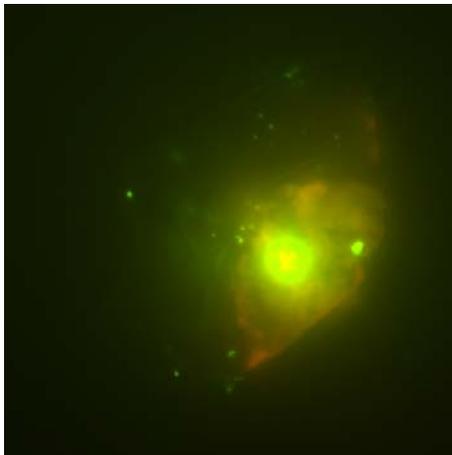
Through the support of the NSF Center for Microbial Oceanography: Research and Education (C-MORE), Berube and colleagues in the Chisholm Lab at MIT are in the process of further circumscribing the capacity for nitrate assimilation by *Prochlorococcus* and the role this plays in the oceanic nitrogen cycle and primary production. Methods have been developed to detect *Prochlorococcus* cells capable of nitrate assimilation in the wild, and several novel strains of these *Prochlorococcus* are now in culture. Their genomes will soon be sequenced. This data will further transform our understanding of the ecology of *Prochlorococcus* in the open ocean.



Highlight

Alkaline phosphatase activity and regulation in the North Pacific Subtropical Gyre

Alkaline phosphatase (AP) is one of several potential enzymatic facilitators of dissolved organic phosphorus (DOP) hydrolysis but it is the best studied and arguably the most important for nutrition, since it has the potential to hydrolyze a broad spectrum of DOP compounds and is produced by a majority of marine microorganisms. Under C-MORE leadership, scientists from The University of Hawaii and The Woods Hole Oceanographic Institution investigated the role of AP in the North Pacific Subtropical Gyre (NPSG). AP activity (APA) was measured at several stations in the between January 2008 and December 2009. The aims of this study were to investigate the P-status (i.e., P stress or limitation) and APA regulation capacity of picoplankton groups from the NPSG to better understand nutrient controls and stress or limitation responses of natural picoplankton populations; and to determine P-related responses to increased N supply predicted with future ocean climate interactions. APA was present at all stations and corresponds to the lower-range of values typically measured in oligotrophic environments. APA varied spatially, seasonally and with depth. APA was also assessed in a series of nutrient addition experiments in July 2008: nitrate plus ammonium (+N) or phosphate (+P), to study APA regulation and to evaluate the capacity of picoplankton organisms (i.e., in the 0.2-2- μm size range) to access the AP-hydrolysable fraction of DOP. The data indicated a primary limitation of the biomass by nitrogen. Both total (measured with a soluble DOP analog) and cell-specific (measured with the Enzyme-Labeled Fluorescence (ELF) Phosphate cell labeling method) APA were enhanced in the +N samples and reduced in the +P samples, suggesting that DOP is an important resource for picoplankton nutrition. Cell-free APA represented >65% of the APA in all samples but its contribution to total APA significantly decreased in the +N treatment as microbial biomass increased. In the +N treatment, <5% and up to 96% of the cells in the heterotrophic bacteria-enriched and picophytoplankton-enriched fractions, respectively, were ELF-alcohol-labeled after 5 d. Following N enrichment, the microbial assemblage shifted from cell-free phosphatase dominated under N limitation and P stress (i.e., physiological response) to picophytoplankton-based phosphatase dominated under P limitation (i.e., production or growth rate limitation). If, as predicted, the ocean evolves towards P limitation, DOP availability would become of major importance to sustain productivity.



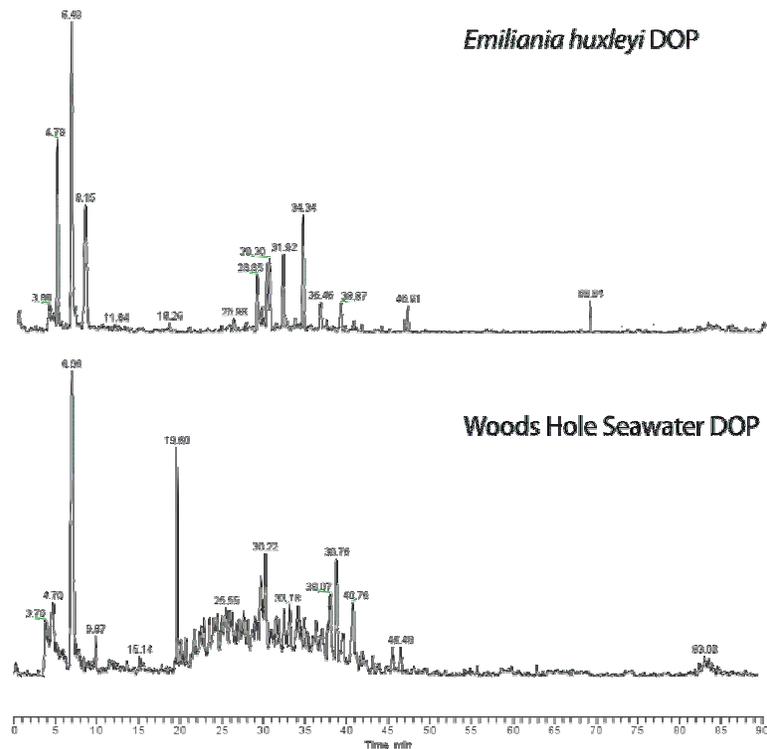
Composite image of an ELF-labeled Dinoflagellate taken during the C-MORE OPEREX cruise under the objective 100x. The ELF-alcohol deposits are represented in green while the chlorophyll a is represented in red.



Highlight

Dissolved Organic Phosphorus: Isolation and Chemical Characterization in Seawater and Pure Cultures

Dissolved organic phosphorus (DOP) is likely a nutrient source for phytoplankton and bacteria that inhabit the vast expanses of phosphorus-depleted oligotrophic seas, but we remain largely unaware of the production, fate, or even the chemical composition of DOP. Using support from C-MORE, a team of WHOI investigators (Dan Repeta, Sonya Dyhrman and Travis Meador) are developing techniques to isolate P-containing organic matter from seawater and laboratory culture media in order to chemically characterize organophosphorus compounds. They selectively concentrate DOP components using solid phase extraction, then purify and characterize individual compounds to the molecular level using high pressure liquid chromatography coupled to tandem mass spectrometry. In pure cultures of the diatom *Thalassiosira pseudonana* and the prymnesiophyte *Emiliana huxleyi* distinct mixtures DOP compounds were observed, with molecular weights between 100-350 Daltons (Fig., top), while more complex mixtures of DOP with a wider range of molecular weights were found in natural and nutrient amended seawater samples (Fig., bottom). Comprehensive characterization of DOP in these samples by high resolution mass spectrometry and nuclear magnetic resonance spectroscopy is currently underway.



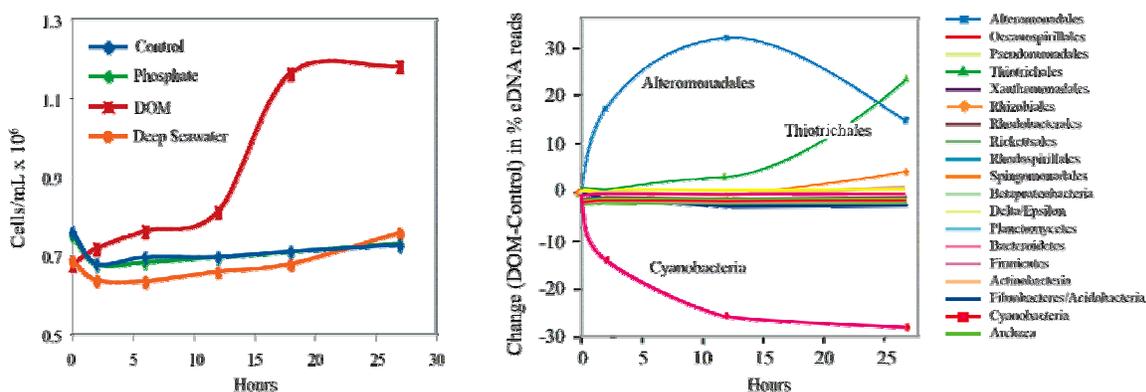
Separation of dissolved organic phosphorus compounds from a laboratory culture of *Emiliana huxleyi* (top) and nutrient amended Woods Hole seawater a two-week incubation (bottom). In the figure, each peak within the trace represents a different DOP component. Pure algal cultures produce a complex mixture of more than two dozen organophosphorus compounds, while environmental samples have an even more complex mixture with > 100 components due to the multiple sources and sinks for DOP.



Highlight

A Coupled Genomic, Transcriptomic, and Organic Geochemical Approach to Elucidate the Mechanisms and Environmental Sensitivity of Dissolved Organic Carbon Transformation by Marine Microheterotrophs

One goal of C-MORE is to better characterize and quantify the specific molecular species, microbial activities, metabolic pathways, and control mechanisms responsible for the cycling, turnover, and degradation of marine dissolved organic matter (DOM). Microcosm field experiments performed by a team of WHOI and MIT scientists on a 2007 C-MORE cruise indicated that several specific groups of bacteria, particularly Alteromonadales and Methylophaga work together in succession and synergy to catalyze the degradation of naturally occurring semi-labile DOM (Figure). Further, metatranscriptomic analyses indicated that by 24 hours post-DOM addition, the biochemical pathways for both dissimilatory and assimilatory single carbon compound degradation (e.g., methanol) were highly expressed, as indicated by transcript abundance. To model the initial stages of DOM degradation, Dan Repeta, Ed DeLong and their colleagues have been using *Alteromonadales* species closely related to the prevalent species in marine waters and seawater microcosm experiments as model systems. They have found that *A. macleodii* strains are capable of growth on semi-labile DOM as the sole carbon source, and respond at DOM concentrations found in seawater, with cell yields that correspond to cell numbers found in the environment. To further investigate the metabolism of Methylophaga bacteria, a new, sensitive method for measuring the concentration of methanol, a likely substrate, in their DOM amendment experiments was developed and tested. The new analytical approach couples selective enzymatic oxidation of methanol to formaldehyde with subsequent analysis of a fluorescent formaldehyde complex by high-pressure liquid chromatography. Using this method, the team found very high concentrations of methanol to be associated with our DOM concentrates and we are now exploring the potential production of methanol from DOM by *A. macleodii*.



Changes in cell numbers and microbial community transcript composition in response to nutrient and DOM amendments. The left panel shows the near doubling in cell numbers over 27 hr in response to the addition of semi-labile DOM addition. Transcriptomic analysis (right panel) was used to track changes in the relative amounts of transcripts derived from the most abundant taxonomic groups. We postulate that the growth of Methylophaga (*Thiotrichales*) bacteria near the end of the experiment was stimulated by single carbon compounds (methanol, formaldehyde) produced by the catabolism of DOM-derived methyl sugars by *Alteromonadales*.

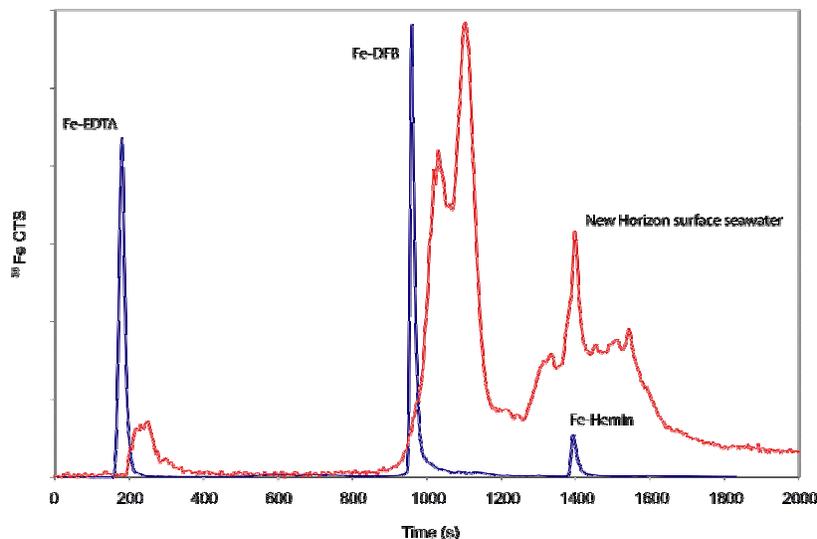


Highlight

Biogeochemistry of Iron-Organic Ligands in Seawater

Iron is an essential element in many key biochemical processes, including respiration, nitrogen fixation, nitrate reduction, and chlorophyll biosynthesis. Over large areas of the ocean, extremely low iron concentrations select for species with low iron requirements or very efficient uptake mechanisms, thereby regulating microbial community structure, and often limiting primary production. Iron speciation measurements show that >99% of dissolved iron is complexed by organic ligands of unknown composition. These ligands form very strong ferri-organic complexes that help stabilize Fe (III) in seawater, increasing its concentration to levels that can sustain microbial growth. Ligands therefore play an important role in the marine iron cycle and microbial biogeochemistry.

Over the past year Dan Repeta (WHOI), Ed Boyle (MIT) and their colleagues have worked to develop an approach to isolate and identify the organic ligands that bind dissolved iron in seawater. Since iron-organic complexes are embedded in a much larger matrix of marine dissolved organic matter, their focus has been to selectively detect only that organic matter associated with iron. To accomplish this, they coupled high-pressure liquid chromatography (HPLC), a technique used to separate complex mixtures of organic matter into pure compounds, with inductively coupled mass spectrometry (ICP-MS), a technique which can selectively measure iron. Using this approach the team has successfully separated and analyzed a mixture of commercial iron-ligand (Fe-L) complexes (Fe-EDTA, Fe-DFB, Fe-Hemin, etc.) to demonstrate the sensitivity, selectivity, chromatographic properties, and stability of the system. Although many analytical issues remain to be solved, they also processed samples collected from the subtropical North Pacific Ocean (Fig.) and found a mixture of Fe-Ls with a broad range of polarity, but with several distinct components that they have now targeted for further chemical characterization.



HPLC-ICP-MS of Fe-L standards (blue trace) and iron ligand complexes extracted from a surface seawater sample collected in the subtropical North Pacific Ocean.

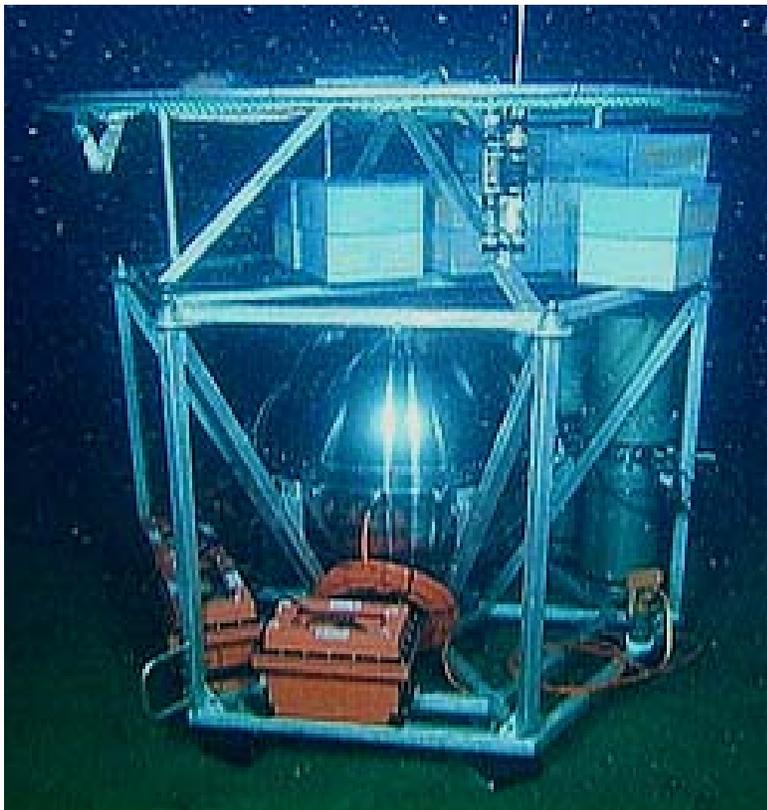
Finally, in September of 2009 a natural Fe-L addition experiment was performed at Station ALOHA in collaboration with Ed DeLong's laboratory at MIT and Mak Saito's laboratory at WHOI. The researchers added natural Fe-L concentrates to seawater samples to determine if they would stimulate production. A "no addition" and an "inorganic Fe spike" served as negative and positive controls, respectively. DeLong's group is using transcriptomics to monitor iron uptake responses in the plankton community, and Repeta's group is measuring changes in Fe-L composition and amount over time. Sample analysis will continue throughout 2010.



Highlight

DNA Probe Technology Goes Live in the Deep-Sea

The advent of the Ocean Observatories Initiative (OOI) has created unprecedented opportunities for deploying novel sensor systems from coastal to open ocean biomes. Researchers at the Monterey Bay Aquarium Research Institute (MBARI) are exploring that potential through the development and application of the Environmental Sample Processor (ESP). The ESP employs molecular probe techniques to autonomously assess the abundance of microorganisms, their genes and metabolites in near real-time. Coupled with sustained environmental measurements provided by the OOI backbone, assessment of microbial community structure and function will soon be possible *in situ* on spatial and temporal scales not achievable previously. To date, tests proving the feasibility of this approach were largely limited to coastal waters. In 2009, the MBARI team proved for the first time that the ESP could also be operated for extended periods at depth. Deployed to 900m on a free-fall benthic lander, the instrument successfully used the same DNA probe array and sample archival functionality as that proven to work in shallow waters, revealing time varying alterations in microbial community structure as a function of changing environmental conditions. The instrument ran as a stand alone system as well as networked via the MARS cabled observatory, the benthic node which serves as a test bed for the OOI's regional scale observatory. Design and construction of the instrument was made possible by funding from NSF, NASA, and the Keck, David and Lucille Packard, 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.



The ESP deployed at 900m as seen through the ROV Ventana ahead of installation on the MARS cabled observatory in Monterey Bay, CA (photo credit: MBARI).



Highlight

Regenerated Biogenic C:Fe Depends on the Iron Concentration of the Upper Ocean: Data from the Tropical North Atlantic

Although it has not been demonstrated, ocean biogeochemical models usually assume that biogenic uptake occurs at a fixed "Redfield" C:Fe ratio of about 300,000:1 (a value that is consistent with the water column profiles in the northeast Pacific and Pacific sector Antarctic waters) and that this value is maintained during microbial degradation of sinking biogenic matter. A team of researchers led by Ed Boyle (MIT) have mapped out the concentration variability of Fe in the oxygen minimum zone of the tropical North Atlantic and find that the regenerated Fe (as determined from Fe:AOU) is consistent with a significantly lower C:Fe ratio of about 100,000:1. They attribute this Fe enrichment to the significantly higher levels (0.6 +/- 0.3 nM) in surface waters of this region caused by Northwest African dust deposition; the organisms in this region engage in "luxury" Fe uptake and the resulting lower C:Fe ratio of sinking biogenic particulate is transferred to the oxygen minimum zone by quantitative microbial release of the low C:Fe created in the euphotic zone. This evidence shows that current ocean biogeochemical models must be modified to take this effect into account.



Seagoing research team led by Ed Boyle (center) prepare metal-free sampler for deployment.



Highlight

C-MORE Scientist Scott Doney at the Copenhagen Climate Summit

Scott Doney, an expert in the ocean's carbon cycle and C-MORE's theme IV (Ecological Modeling, Synthesis and Prediction) team leader recently attended the UNFCCC Conference of Parties (COP15) in Copenhagen, Denmark in December 2009. Scott gave two talks to policy makers, media, scientists and the representatives from a variety of NGOs. The first, "Economic and Social impacts of Ocean Acidification" was part of "Ocean Day" at the summit. A second invited talk, "Ocean Carbon Uptake and Ocean Acidification" was given at the U.S. Center in a NOAA-organized session on Oceans and Ecosystems in a Changing Climate. He was also featured on YouTube (see below).



*The Department of State video is available at:
<http://www.youtube.com/user/statevideo#p/c/DE1718C16040788A/93/BNKjzYTMtBs>
(Right) C-MORE investigator Scott Doney with NOAA Administrator Dr. Jane Lubchenco*



Highlight

C-MORE Hale: The Future is Today

In 2008, the University of Hawaii administration announced their intent to construct a new wing to the existing Biomedical Sciences building. This addition, dubbed C-MORE Hale, (“at the house of C-MORE”) will provide 28,000 sq. ft. of office, research, education and outreach space and will be used exclusively in support of the C-MORE mission. In late 2008, the administration selected Group 70 International, Inc. as the architect and decided to move forward with a plan for a design-build package. C-MORE faculty and staff worked closely with the Group 70 team to begin the hard work of planning their new home. C-MORE Hale is planned to be a LEED Gold rating (minimum) building, complete with photovoltaics and a green roof. On 6 April 2009, it was announced that the University of Hawaii had completed the sale of revenue bonds on Wall Street. Proceeds from the series 2009A bond sale will be used to fund several capital projects across the UH system, including \$22.5M designated for C-MORE Hale. The funding arrived just as the invitations for the groundbreaking were being mailed. On 15 April 2009, ground was broken for the eventual construction of C-MORE Hale. The list of distinguished guests included U.S. Senator Daniel K. Inouye, NSF Director of the Office of Integrative Activities Lance Haworth, UH President David McClain and UH at Manoa Chancellor Virginia Hinshaw. Within a few weeks of groundbreaking, site work began. As of the end of December footings, underground utilities and final design work have all been completed, and the walls are starting to appear. Construction is expected to be completed in Fall 2010 with final certification and occupancy planned for November 2010.



Groundbreaking, 15 April 2009



C-MORE Hale rendering



*31 December 2009
C-MORE project coordinator
Steve Poulos inspects progress*



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C-MORE Hale

C-MORE Hale Groundbreaking: 15 April 2009

On Wednesday 15 April 2009, ground was broken at UH Mānoa for a \$22 million building to house C-MORE labs and offices. It will be one of 17 National Science Foundation centers of science and technology nationwide, and the only one in Hawai'i. US Sen. Daniel Inouye noted that when he started his college career, ambitious students wanted to go elsewhere to get a top educational experience. "When I got into this business, I said to myself, "We're going to change that," he said, "And this is a demonstration of that change. Now ... this is the place to look into oceanography. This is the place where the experts reside."



Daniel K. Inouye
 United States Senator for Hawaii

Thank you to the participants and attendees of the groundbreaking ceremony, with special thanks to University of Hawai'i President David McClain, UH Mānoa Chancellor Virginia Hinshaw, US Senator Daniel Inouye (above), C-MORE Director Dave Karl, NSF-OIA head Lance Haworth, and Kahu Kordell Kekoa! Read more about in the *Honolulu Star-Bulletin*. (Photo courtesy of Sam Wilson; click on it to see the full image.)



To view a video of the ceremony, click on the image above of Sen. Inouye, or [click here](#) (requires RealPlayer; the video may take a few moments to download, depending on your connection). This video was directed and produced by Eric Grabowski.



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New lab to house ocean studies

By Craig Gima

POSTED: 01:30 a.m. HST, Apr 16, 2009

The University of Hawaii at Manoa broke ground yesterday on a \$22 million research building, the first new laboratory space to be constructed on campus in a decade.



JAMMI AQUINO | AQUINO@STARBUCKET.COM
 Kahu Kordell Kekoa supervised the wielding of the molo yesterday during the ground-breaking ceremony for the University of Hawaii's Center for Microbial Oceanography Research and Education at the Manoa campus.

The building will house the Center for Microbial Oceanography, Research and Education, or C-MORE, and the research of UH oceanographer and microbiologist David Karl.

Karl's research, focusing on the smallest life forms in the ocean, has brought in more than \$48 million to the university through his three decades at Manoa.

The building will be one of 17 National Science Foundation centers of science and technology across the nation.

U.S. Sen. Daniel Inouye praised the new center, noting that when he started his college career,

ambitious students wanted to go elsewhere to get a top educational experience. "We're going to change that," he said.

In 2006, the National Science Foundation awarded a \$19 million, five-year grant to UH and other universities for the C-MORE project to conduct research into marine microorganisms, including what role they play in climate change and sustaining life on Earth. Part of the grant will also pay for educational outreach efforts and to try and motivate students in underrepresented groups to study science and engineering.

The cost of constructing the new building will come from research funds, not taxpayer money. Earlier this month, the university issued \$100 million in bonds for construction projects and to acquire student housing to be paid back by research grants, student housing fees, bookstore revenues and other sources.

The new construction also comes as the university is updating its estimate of needed repair and maintenance for existing buildings.

Last year, the university estimated that its aging facilities needed \$351 million for repairs and upgrades of electrical, air conditioning and other systems and that the backlog was growing by about \$60 million a year.

The state Senate is proposing to spend \$118.6 million next year on repairs and health and safety projects at UH and could appropriate more money for 2011, while the House is proposing to spend \$154 million over two years.

"For us, a major priority is to kind of work down the backlog," said Sen. Jill Tokuda, Senate Higher Education Committee chairwoman. "I think this will hopefully help stimulate the economy as well."

Federal stimulus funds could also help the university, although the \$35 million available must be split between UH and the state Department of Education.

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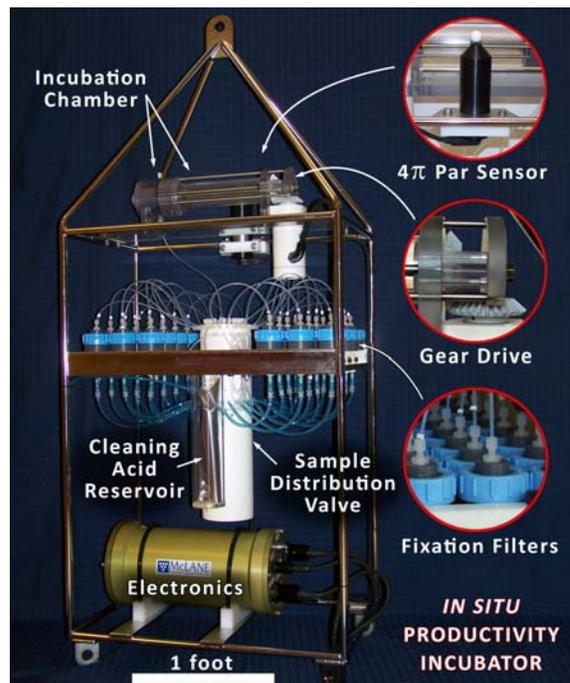
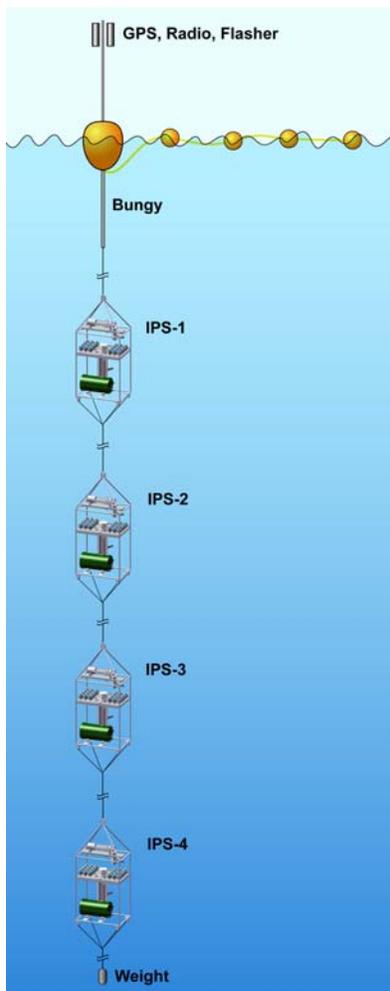


Highlight

In situ Productivity Sampler (IPS)

C-MORE investigator Craig Taylor (WHOI) has designed a smaller, less expensive Time Series-Submersible Incubation Device (TS-SID) for in situ studies of the metabolism of marine microbes. Four units recently built at McLane Research Laboratories will permit tracer incubation studies to be conducted on a broader spatial scale or at multiple depths to provide depth-dependent measures of microbial activity (e.g., depth-integrated primary production). In the IPS the 50-port Sample Distribution Valve not only directs incubated sample to the appropriate Fixation Filter Unit (device with no moving parts that will chemically preserve the filtered incubated samples), but also controls the introduction of tracer, via multiple plumbed in tracer-containing coils, and biofouling control operations, via acid contained within a flexible plastic bag; effectively an in situ micro-laboratory. The IPS will collect & preserve up to 24 incubated samples.

The IPS might typically be deployed from a surface spar buoy so that *in situ* incubations can be conducted independently of the ship for the duration of an entire cruise. Initial application of the IPS will be to obtain simultaneous measures of depth integrated ^{13}C -primary production and ^{15}N -nitrogen fixation but other tracers can be used as well.



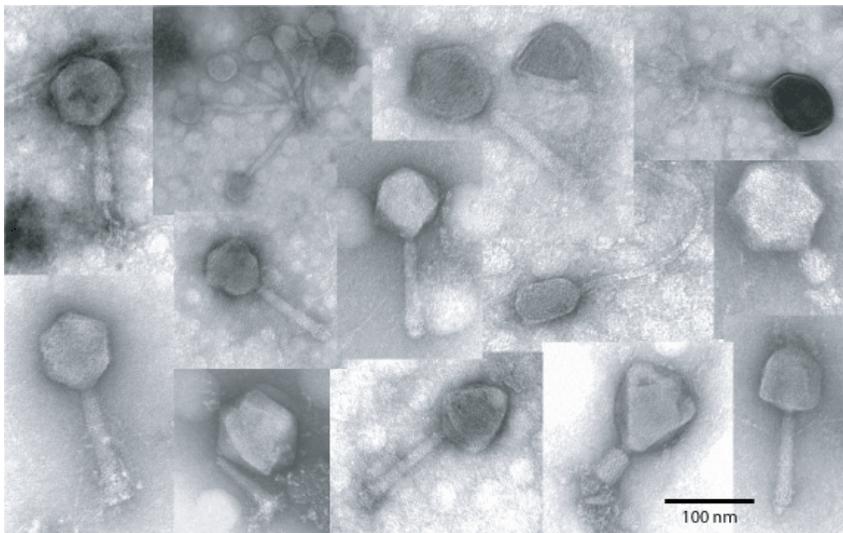


Highlight

Isolating Uncultured Viruses

Lurking amongst the tens-of-thousands of microbial cells in a drop of sea, lake, or pond water are ten times as many viruses. Viruses constantly sculpt microbial communities through actions that are either destructive (cell lysis) or creative (sharing of genetic information). Despite their pervasive influence, most aquatic viruses remain uncharacterized because cultivating them remains a major challenge.

Members of the Marine Viral Ecology Laboratory at the University of Hawai'i at Manoa, led by Grieg Steward, have taken a new approach to the problem by using fractionation instead of cultivation to isolate viruses for further study. They have shown that the physical and chemical differences among viruses in a complex natural assemblage can be used to separate viral populations from one another using chromatographic methods. Application of this approach will enable researchers to study the morphology, chemical composition, and genome of essentially any virus in the environment without requiring that it first be domesticated.



A few of the many morphologies of viruses that can be found in natural water samples (photo credit: Jennifer Brum).



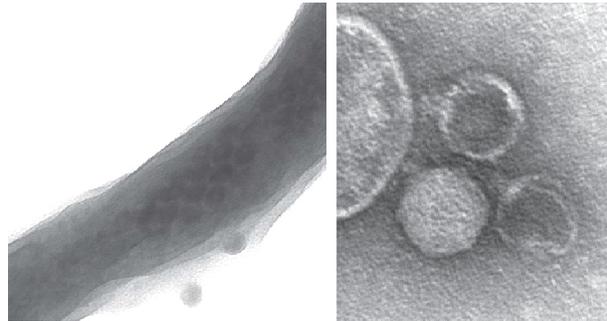
Highlight

Isolation of Novel Marine Virus

Researchers in the Center for Microbial Oceanography: Research and Education (C-MORE) have recently collaborated to isolate and partially characterize a bacteriophage that infects a coastal bacterium in the OM43 clade. The bacterium (strain HIMB624) and the virus were isolated from coastal waters of Hawaii. The virus is approximately 40 nm in diameter, has a 38 kb dsDNA genome, and appears to belong to the Family *Podoviridae*, which are bacteria-infecting viruses having short tails.

Bacteria in the OM43 clade constitute a large fraction of coastal bacterial communities, but are challenging to grow in culture. Genomic and physiological studies indicate that members of this clade are methylotrophs, capable of growth on simple compounds such as methanol, methylamine, and formate as their only source of carbon. The high abundance and widespread distribution, specialized diet, and extremely small genome of these bacteria make them particularly interesting subjects for further

ecological investigation. Given the importance of phages in mediating both mortality and evolution of bacteria, having a OM43 phage-host system will provide an important new dimension to future research in this abundant group of bacteria. This work was a collaboration between members of the laboratories of Michael Rappé and Grieg Steward at the University of Hawaii. Graduate student Darin Hayakawa propagated the phage and post-doctoral researcher Alex Culley conducted the morphological and molecular analyses.



Electron microscopy reveals viruses adsorbed on the surface and accumulating inside a cell from an infected culture of the bacterium HIMB624 (left) and three virions with short tails (right). Photo credit: A. Culley, UH