



Characterizing Pelagic Seascapes

Submitted by: M. Kavanaugh, A. White, R. Letelier, Y. Spitz, S. Doney

Like landscapes, the surface ocean can be described as a spatially structured ensemble of environments or “seascapes” characterized by distinct patterns of physical forcing, which, in turn, support distinct ecosystems. Using a 13 year record of sea surface temperature (SST), photosynthetically active radiation (PAR), and chlorophyll *a* (chl-*a*) derived from remote sensing observations, we classified dynamic, hierarchical seascapes at monthly and interannual scales. Differences in microbial community structure within, between, and among seascapes were determined with data collected from the Hawaii Ocean Time-series (HOT) program (Station ALOHA; 22.75°N, 158°W), the Canadian Institute for Ocean Sciences (Line P and Station Papa; 50°N, 145°W), as well as cruises associated with the University of Hawaii and the University of Washington. In total, nearly 450 stations were included in the analysis (N=447), spanning 40 degrees in latitude (15-54.5°N), over 35 degrees of longitude (198-234°W) and 13 years (1998-2010).

Seascapes represent unique biogeochemical functioning and microbial communities (Figure 1). Seascape averages explained nearly 75 % of the variability in abundances in eukaryotes, 69% of *Synechococcus*, >60% of the variability in heterotrophic bacteria and >90% of the variation in *Prochlorococcus* abundance. At broad scales, community dissimilarity in the NE Pacific was driven by the presence or absence of *Prochlorococcus*, shifts in abundances of eukaryotic phytoplankton and gradients in SST and chl-*a*, however within-seascape drivers varied. The field of landscape ecology has been extremely valuable in providing a framework to understand how species distributions and biogeochemical cycles are affected by scale-specific patterns of environmental variability. The unique assemblage structure and forcing within dynamic satellite-derived seascapes represents a promising step toward developing an analogous framework for pelagic ecosystems.

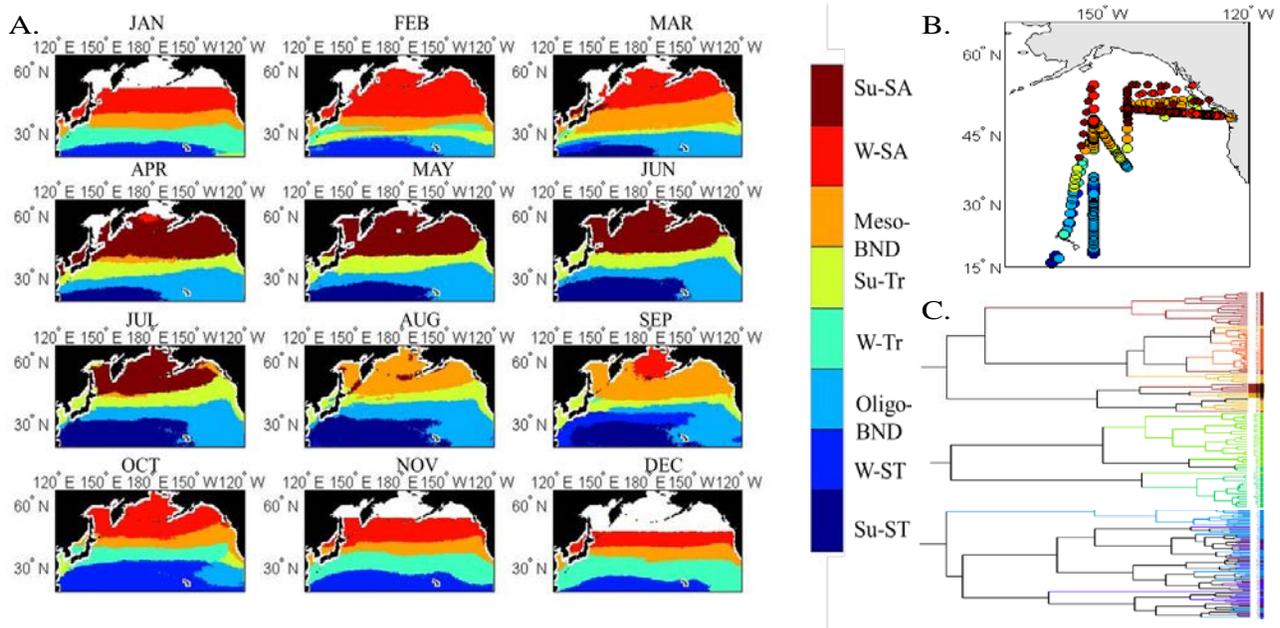


Figure 1. Seasonal evolution of North Pacific seascapes and similarity of microbial communities. A.) Eight seascapes characterize the majority of spatial and seasonal variability in chl-a, SST, and PAR as well as macronutrients and net primary production [2]. Summer subtropical (Su-ST), Winter subtropical (W-ST), Oligotrophic boundary (OB); Winter Transition (W-Tr); Summer Transition (Su-Tr); Mesotrophic boundary (MB); Winter Subarctic (W-SA) Summer Subarctic (Su-SA). B.) Sampling stations where flow cytometry samples were collected (1998-2009). C.) Nested cluster analysis of cytometric community structure using geometric scaling (Multiple response permutation procedure: $A_{stat}=0.7$, $p<0.0001$). Samples are color coded to correspond with seascape of collection location. Adjacency indicates similarity of community structure.



Highlight

Prochlorococcus Growth Rates

Submitted by: A. White and R. Letelier

In the summer of 2012, C-MORE conducted a "continuous" long-term field experiment at Station ALOHA to observe and interpret temporal variability in microbial processes, and the consequences for ecological dynamics and biogeochemical cycling. OSU researchers participated in this effort to characterize the growth response of various microbial groups to light. A series of novel experiments in collaboration with the Church and Karl groups led to a number of exciting results.

Using a photosynthetron approach (top right), water collected before dawn was incubated at 24-light levels and sampled for flow cytometry at three hour intervals for a 12-hr period. We then examined the cell-specific growth rate over time (0-6hr, 0-9hr, 0-12hr) as a function of growth irradiance (Right, middle). Here we present a subset of these results. In an experiment conducted on September 7th, we see that the maximum cell specific growth rates for *Prochlorococcus* decreased over the course of a photoperiod (from 0.78 over 6hr to 0.46 d⁻¹ over 12hr), although the half-saturation coefficient for growth (~ 12 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) remained constant. This half-saturation value is exceedingly low, suggesting that *Prochlorococcus* in the upper mixed layer of Station ALOHA are capable of maximal growth rates very soon in the daily cycle. These experiments were conducted a total of five times during the HOE-DYLAN occupation. We found that the half-saturation coefficients were relatively constant, although the maximal growth rates and the initial efficiency of light harvesting varied between sampling days (Right, bottom). These data provide insight into the circadian growth response of this ubiquitous cyanobacterium. Ultimately we will compare these findings to bulk productivity measurements to estimate the contribution of *Prochlorococcus* to community productivity.

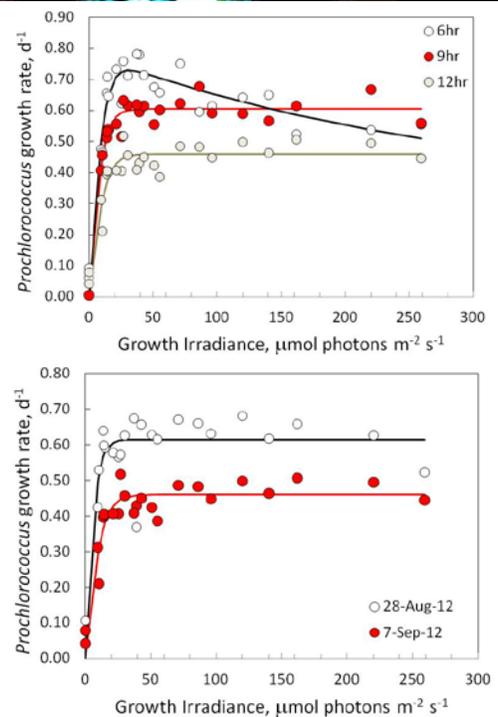


Figure 2. (Top) This experiment utilized a series of 24-well photosynthetrons to examine time-series of group-specific growth rates as a function of growth irradiance (Middle). Results for a single experiment where growth rates were calculated over 6 hr, 9 hr and 12hr showing decreases in maximal growth over time and (Bottom) Difference in light response curves for populations samples at different days (12hr).



Highlight

Plankton & Art

Submitted by: A. White

Every year, a science and art festival, da Vinci days (<http://www.davincidays.org/>), is held in Corvallis, Oregon. A centerpiece of this festival is an art exhibit with a revolving theme that brings together local artists to work on scientific subject matter. In 2012, 35 Oregon artists took up a call from The Arts Center of Corvallis for works based on Angelicque White's microscopy. A vast number of these images were collected on C-MORE cruises and are posted on the C-MORE microscopy webpage, where they have been used for outreach events, textbooks, scientific illustrations and other usages. Submissions for this call came from painters, fabric and glass artists, sculptors, potters and an expert in the ancient Japanese art of stencil dyeing. They comprised a show, *The Art of Plankton, Form Follows Function*.

A selection of artwork is shown in Figure 3, next page.

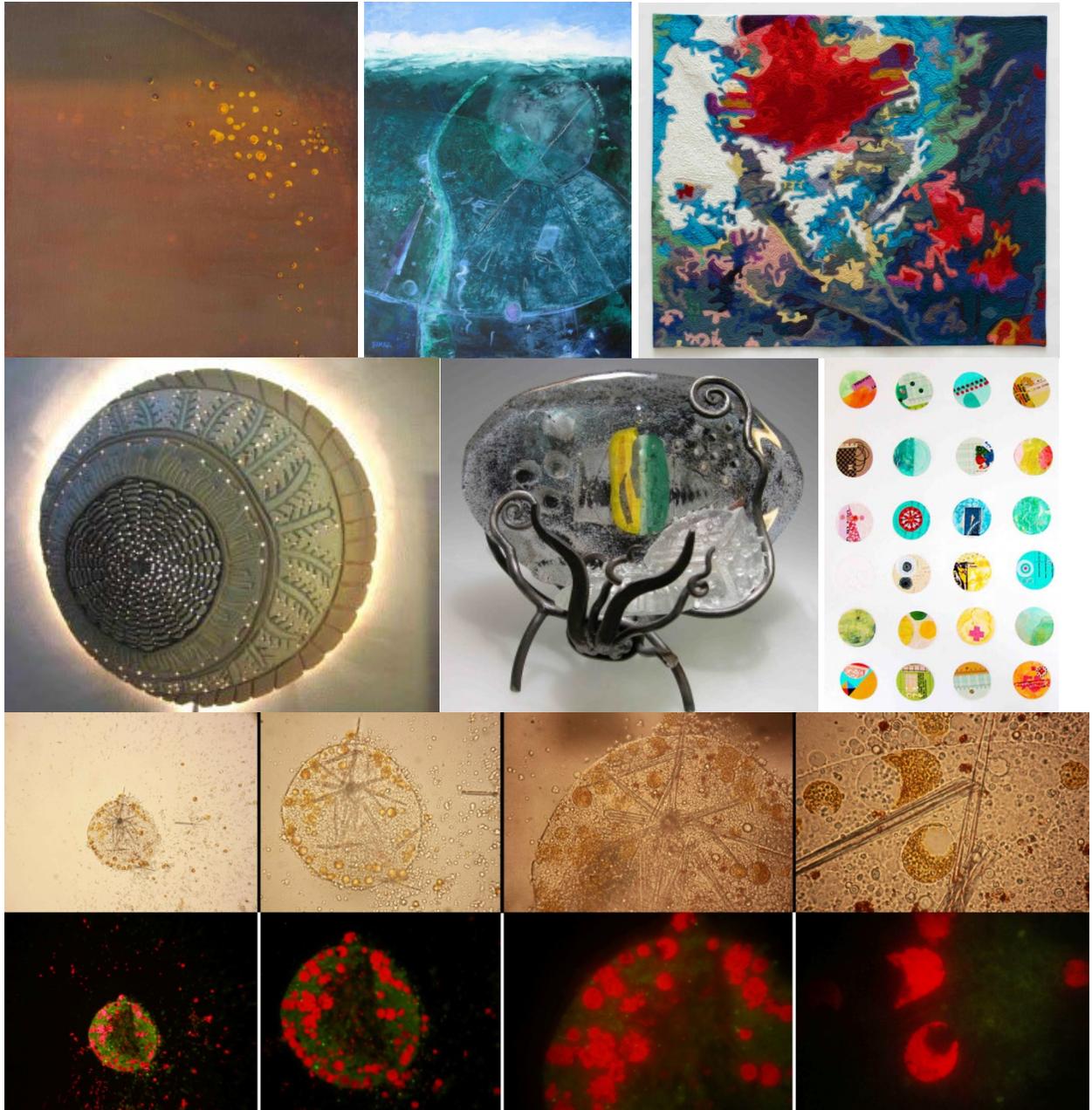


Figure 3. A selection of works from the 2012 The Art of Plankton, Form Follows Function show at the da Vinci Festival in Corvallis, Oregon. All artwork was based on C-MORE microscopy. From top, left to right, these include oil paintings, quilting, ceramics, glasswork, and mixed media. (Bottom) One of the images used as a basis for the exhibit.



Analysis of Chlorophyll Algorithms

Submitted by: J. Nahorniak, A. White and R. Letelier

An intriguing new satellite chlorophyll algorithm, the Color Index (CI) algorithm, was proposed by Hu and others in 2012 [1]. This algorithm may be less sensitive than the standard chlorophyll algorithms to errors introduced by imperfect atmospheric correction, stray light contamination, and instrument noise, yielding more accurate chlorophyll patterns and values, allowing for a larger number of pixels to be considered useful. As this algorithm is designed for low-chlorophyll waters, it is an ideal candidate for use in the waters off Hawaii.

For this study, satellite-measured radiances over the Hawaii region from the MODIS (Moderate Resolution Imaging Spectroradiometer) sensor onboard the Aqua satellite were used. Satellite chlorophyll was then derived from these radiances using both the new CI algorithm and the standard chlorophyll algorithm (OC3M).

Preliminary analysis of the CI algorithm is showing promising results, one of which is highlighted here. Sunlight reflecting off clouds can cause elevated radiances nearby (otherwise known as stray light). If these high radiances are input to the standard OC3M algorithm, erroneous chlorophyll values are derived near the cloud edges. In standard processing, this is dealt with by placing a mask (about 3 pixels wide) around each cloud; chlorophyll values are not calculated for these masked areas. Unfortunately these masks remove a substantial number of pixels from the scene, particularly in areas frequented by scattered clouds like Hawaii. In contrast, we have found that chlorophyll derived from the CI algorithm appears to be unaffected by the presence of stray light. The direct result of this is that the CI algorithm can provide a more complete picture of the patterns of chlorophyll than the standard OC3M algorithm. Research continues regarding the accuracy of the derived CI chlorophyll values and their patterns.

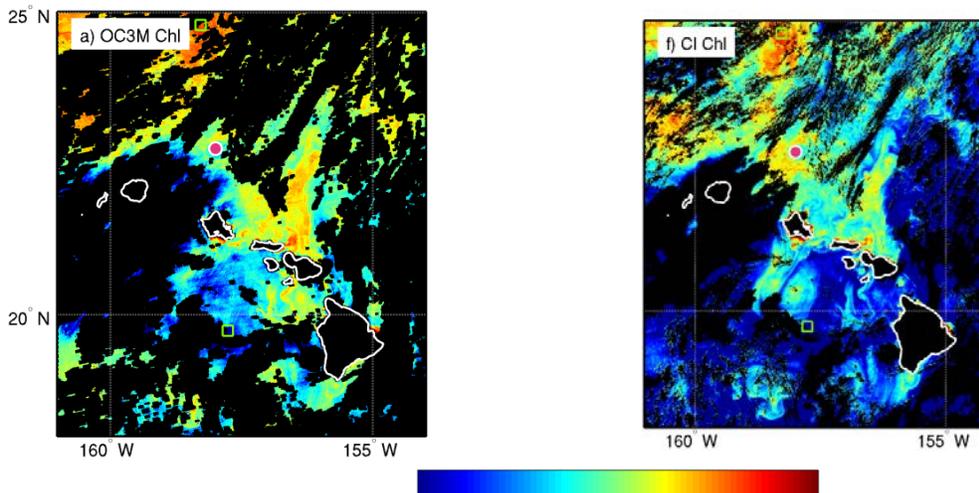


Figure 4. Normalized chlorophyll in Hawaiian waters from January 2, 2012: (Left) the standard OC3M product masked with the stray light flag, (Right) CI chlorophyll unmasked. The green squares mark two pixels used to normalize the figures to each other. The color scale ranges from blue (low chlorophyll) to red (high chlorophyll). Data were log-transformed before plotting. The pink dot marks the location of Sta. ALOHA. Note the more believable patterns in the CI image along the northeast edge of the large cloud over Kauai than in the OC3M image. [1] Hu, C., Z. Lee, and B. Franz, *Chlorophyll a algorithms for oligotrophic oceans: A novel approach based on three-band reflectance difference*, J. Geophys. Res., 117, C01011, doi:10.1029/2011JC007395 (2012).



Highlight

Metagenomics of Microbial Community Temporal Variation at Station ALOHA

Submitted by: Jessica Bryant, Matt Church, Dave Karl, and the DeLong lab

Microbial plankton are a central component of marine biogeochemical cycles. We have improved our understanding of the physiology and ecology of these organisms in recent years, but significant gaps in our understanding still remain. Notably, we still don't understand the extent to which microbial communities change across time, nor do we understand the environmental factors that drive temporal variation. To address these issues, microbial samples were collected across a 2-year period, alongside the ongoing long-term Hawaii Ocean Time Series (HOT) at the North Pacific Subtropical Gyre station ALOHA. MIT graduate student Jessica Bryant, Matt Church, Dave Karl, and Ed DeLong and his team at MIT are analyzing and interpreting these time series samples.

Microbial community DNA was analyzed by rRNA gene "tag" sequencing, as well as metagenomic sequencing and analyses. The results showed that microbial communities in surface water (25m) were less diverse, but more dynamic through time than communities in the mesopelagic (500m). For example, the mesopelagic samples had on average almost twice the number of taxonomic groups, as the surface samples but the standard deviation of mesopelagic samples was approximately 25% smaller.

We also explored correlations between microbial diversity, water chemistry, and meteorological data collected at the WHOI WHOTS buoy. At 25m, the number of unique taxa identified in a sample increased with increasing wind speeds. The chlorophyll a concentrations at 25m and the distance between the mixed layer depth and deep chlorophyll max (DCM) were both also correlated with average wind speed. In addition, generally at station ALOHA, pigment concentrations peak the DCM and microbial diversity at the DCM has been shown to be higher than at 25m. Together these results suggest that wind is driving mixing events that draw diverse microbial communities and algal biomass up from nearby the DCM into surface waters. It was an unexpected finding that microbial dispersal, rather than the chemistry of the water column or seasonal cycles, would be a major determinant of microbial diversity in surface water at station ALOHA.

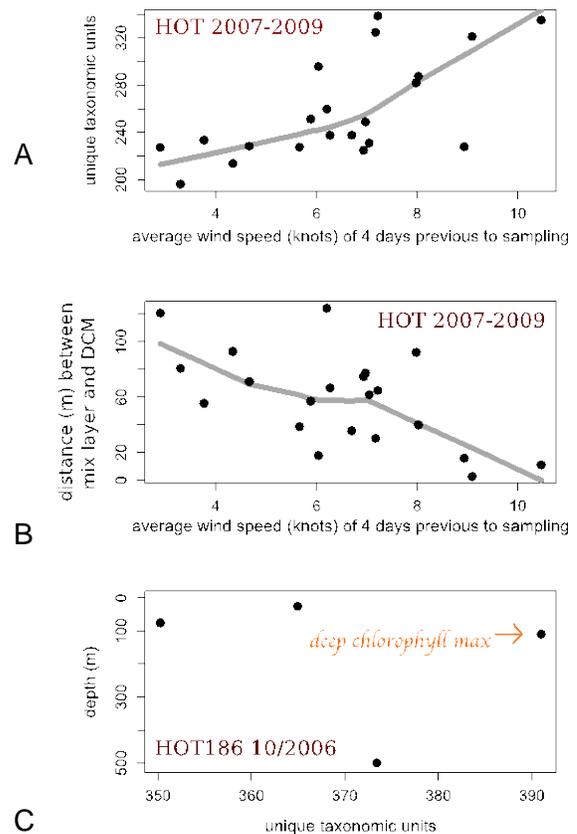


Figure 5. A) Diversity of microbial communities sampled between 2007-2009 plotted against the average wind speed during the 4 days before each sample collection. B) Distance across the water column separating the mix layer depth and the deep chlorophyll max at the time of microbial community sampling plotted against average wind speed. C) Microbial diversity at four points along the water column during the HOT186 cruise.



Highlight

Securing ocean benefits for society in the face of climate change

Submitted by: Scott Doney

Benefits humans rely on from the ocean–marine ecosystem services–are increasingly vulnerable under future climate. Scott Doney, a C-MORE co-PI, contributed to a recent paper on climate change impacts and adaptation strategies in *Marine Policy* (Ruckelshaus, Doney et al., 2013). Their analysis focuses on how three valued services have, and will continue to, shift under climate change: (1) capture fisheries, (2) food from aquaculture, and (3) protection from coastal hazards such as storms and sea-level rise. Climate adaptation planning is just beginning for fisheries, aquaculture production, and risk mitigation for coastal erosion and inundation. A few examples are highlighted, showing the promise of considering multiple ecosystem services in developing approaches to adapt to sea-level rise, ocean acidification, and rising sea temperatures. Ecosystem-based adaptation in fisheries and along coastlines and changes in aquaculture practices can improve resilience of species and habitats to future environmental challenges. Opportunities to use market incentives–such as compensation for services or nutrient trading schemes–are relatively untested in marine systems. Relocation of communities in response to rising sea levels illustrates the urgent need to manage human activities and investments in ecosystems to provide a sustainable flow of benefits in the face of future climate change.

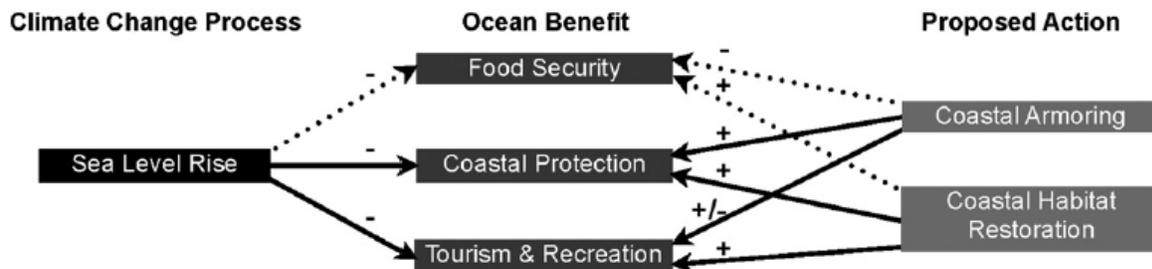


Figure 6. An illustrative example of how two potential adaptation strategies (coastal armoring and habitat restoration) to a climate change process (in this case sea level rise) affect three types of ocean benefits (food security, coastal protection, tourism and recreation). Type of impact indicated as either primarily positive (+), primarily negative (-), or mixed (+/-). Solid lines indicate stronger interactions than dotted lines. Although this represents a simplified view of these interactions and does not include other aspects such as cost-effectiveness over time, it illustrates the value of evaluating tradeoffs among policy options.

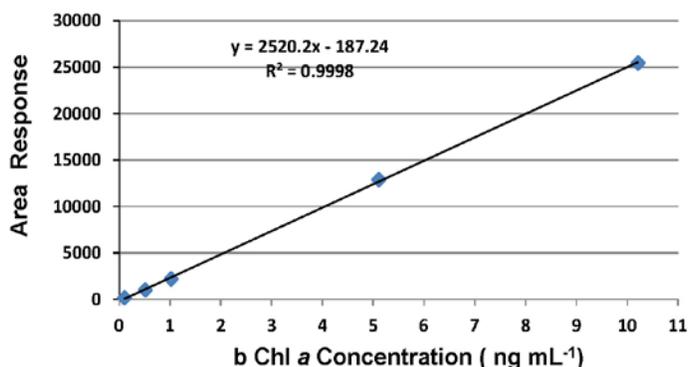
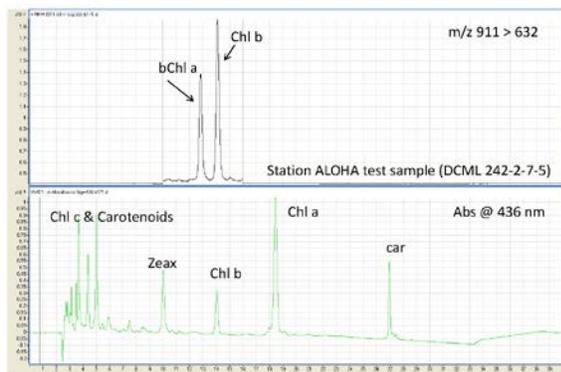


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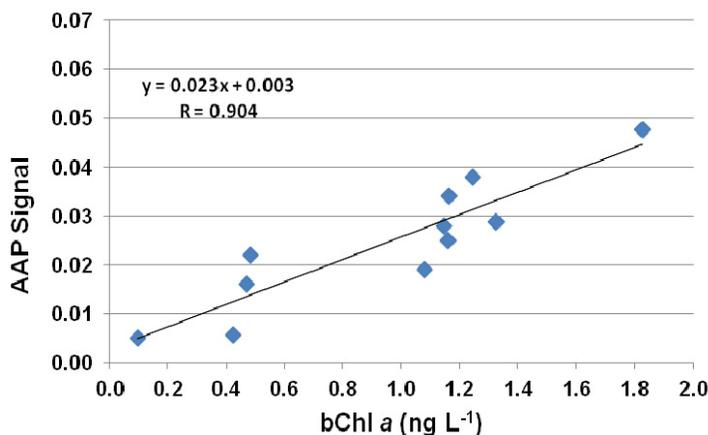
Measurement of bChlorophyll a by LC-UV/VIS-MS/MS and FRRF during HOE-DYLAN

Submitted by: Robert Bidigare

A long time-series of FRRF-derived bacteriochlorophyll a (bChl a) levels exists for Station ALOHA that includes a wealth of data collected during the HOE-DYLAN experiment. However, the FRRF measurements at Station ALOHA have never been validated with a direct measurement of bChl a concentration. We developed and validated a LC-UV/VIS-MS/MS method for the simultaneous determination of total chlorophyll a (TChl a) and bChl a concentrations (Figures 7-8-9). The method is linear over a wide range of bChl a concentrations (0.10–10.21 ng mL⁻¹, $R^2 = 0.9998$), and analytically precise, with a relative standard deviation (RSD) of 2.35%. The limit of detection (LoD) and limit of quantification (LoQ) values for this method are 0.013 ng μL^{-1} and 0.043 ng μL^{-1} , respectively, for a filtered sample volume of 2-L. Analysis of replicate bChl a samples collected at Station ALOHA yielded a CV of 2.3% (n = 4).



With the exception of one outlier (removed), an excellent agreement was obtained for paired measurements made during HOE-DYLAN 9. Concentrations of bChl a observed during HOE-DYLAN 9 averaged 1.16 ng L⁻¹ between 5 and 75 m (n = 13, ~1.0% TChl a) and 0.35 ng L⁻¹ between 125 and 175 m (n = 7, ~0.2% TChl a). We are in the process of analyzing bChl a samples collected on HOE-DYLAN 5 and 7. Once all samples are analyzed, the FRRF dataset can be used in conjunction with the paired sample results to provide quantitative bChl a data for Station ALOHA.





Highlight

Genetic diversity within a clade of unusual nitrogen-fixing cyanobacteria suggests adaptation to specific marine environments

Submitted by: D. Bombar, B. J. Carter, A. Thompson, P. Heller, J. P. Zehr

Atelocyanobacterium thalassa (UCYN-A) is a nitrogen-fixing cyanobacterium known for its global distribution in tropical- and subtropical seas, unusual streamlined genome, and symbiosis with a single-celled alga of the Prymnesiophyte class. Phylogenetic analysis of nucleotide sequences of the nitrogenase (*nifH*) gene shows branching of the UCYN-A group into at least three distinct clades, which have gone unnoticed so far since they appear almost identical on the amino acid level. The *nifH* gene of clade UCYN-A2 shares only 95% nucleotide similarity with UCYN-A, and more importantly, can be found in environments that clearly differ from oceanographic conditions at Station ALOHA, such as the relatively cold and nutrient rich coastal waters at the Scripps Institute of Oceanography (SIO) Pier in La Jolla, CA. We asked whether this *nifH* sequence variation corresponds to broader functional diversity by studying the metagenome of a UCYN-A2 population sampled from waters off SIO. Following proven techniques, we obtained a UCYN-A2 metagenome of 1.3 Mb after paired end Illumina sequencing of DNA from flow-cytometry-sorted cells. The most salient findings from this ongoing study are so far:

(1) Obtained contigs map to nearly the complete sequence length of the UCYN-A reference genome (Fig 10A). UCYN-A2 shares 99% 16s rDNA similarity, very similar basic genome features (31% GC content, 79% coding DNA) and functional gene content with UCYN-A, while the average nucleotide identity is only 86%, indicating niche differentiation. (2) UCYN-A2 also appears to lack PSII genes, Ru-BisCo, and TCA cycle components, suggesting that it can be grouped with UCYN-A on a functional and ecological level. In support of this, maximum UCYN-A2 *nifH* transcription occurs during the day (Fig. 10B), suggesting that indeed no photosynthesis-generated oxygen inhibits Nitrogenase. (3) While the identity of a picoeukaryote host of UCYN-A2 is still under investigation, we identified genes unique to UCYN-A2, including membrane transporters, cell-shape determining proteins, and hydrolases, possibly indicating special characteristics in the symbiont-host relationship of this clade. Our findings are important steps towards a better understanding of UCYN-A's symbiotic evolution, and the significance of these unusual symbioses in oceanic C- and N- cycling.

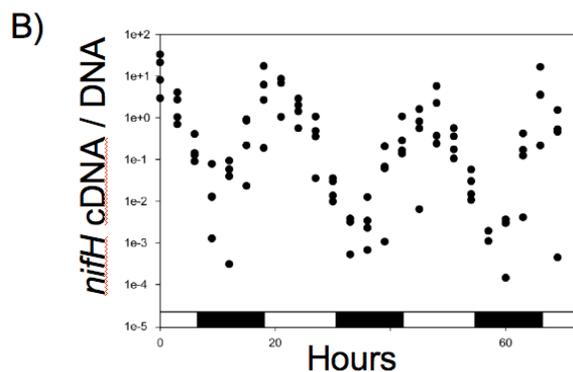
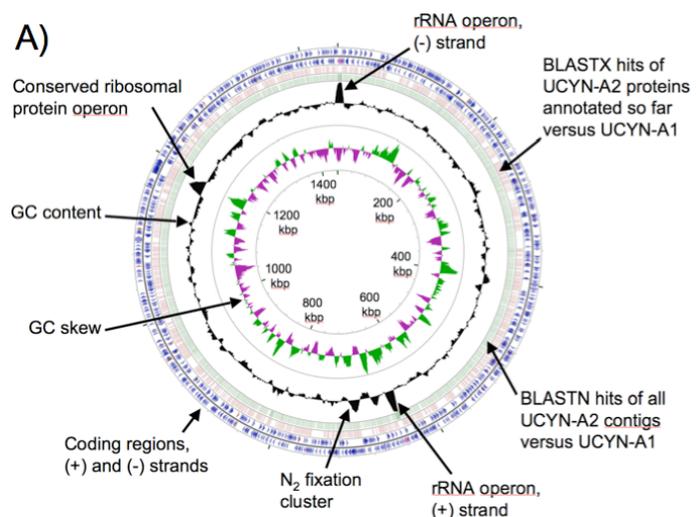


Figure 10. (A) The UCYN-A chromosome compared to the UCYN-A2 draft genome. Outer rings: Both strands of the UCYN-A genome with coding sequences in blue, tRNA in red, and rRNA in purple. Third ring: BLASTX comparison (E value $\leq 10^{-20}$, identity cutoff 80%) of UCYN-A2 proteins annotated from a first set of contigs that mapped to the UCYN-A1 reference genome. Fourth ring: BLASTN comparison (E value $\leq 10^{-10}$, identity cutoff 60%) of all UCYN-A2 contigs to the UCYN-A1 reference genome (annotation not finalized for all ORFs). Fifth ring (black): GC content. Sixth ring: GC skew (green = positive, purple = negative) of the reference genome. (B) UCYN-A2 *nifH* expression relative to *nifH* gene copies at 3-h intervals over a period of three days in waters off SIO Pier (black boxes indicate



Highlight

Isolation of a giant marine virus

Submitted by: C. Schvarcz, G. Steward

Viral infections of eukaryotic phytoplankton can have a large influence on carbon cycling in the ocean, but we still know very little about the biology and ecology of marine protistan viruses. One of the most exciting events in virology in the past decade was the discovery of giant viruses. These newly recognized viruses exceed the volume and genome sizes of most of the bacteria in the ocean. The largest isolates to date (400-500 nm diameter) infect freshwater amoebae. The first isolate shown definitively to have a marine host was a somewhat smaller virus (300 nm) that infects a heterotrophic marine protist. We have now isolated, for the first time, a giant virus (>300 nm) that infects a marine phytoplankter. The host of the virus is a heterokont alga in the genus *Florenciella* (Figure 11). This virus is not only physically large, but also appears to have the largest genome of any virus yet recorded (pending sequence confirmation).

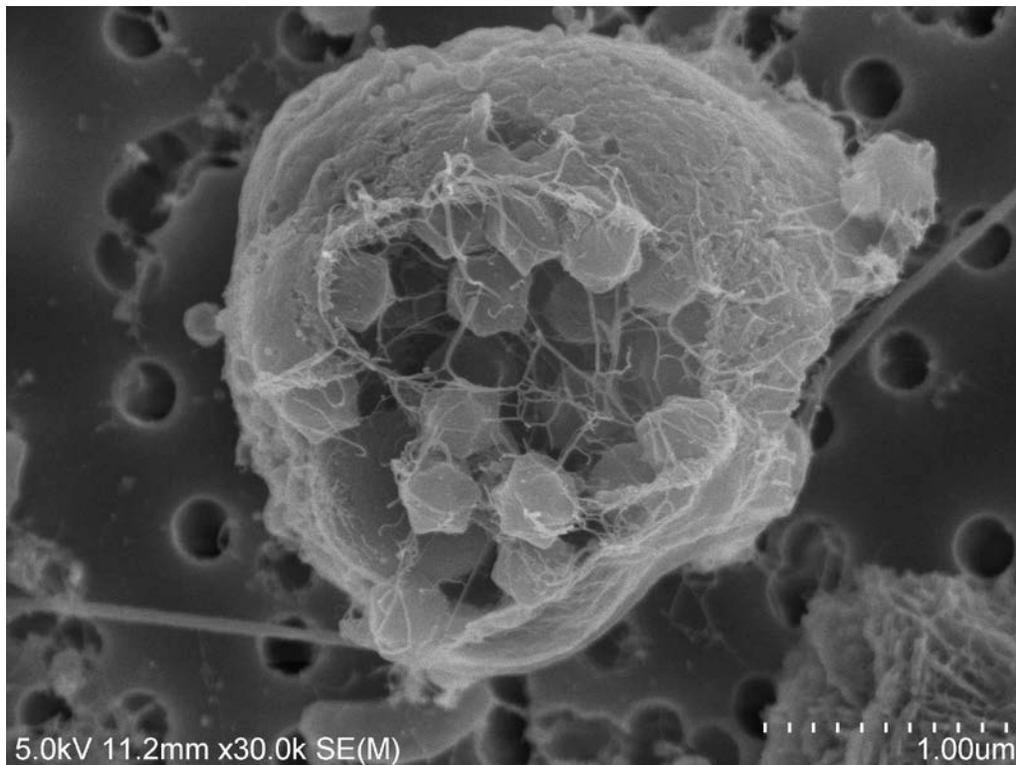


Figure 11. Scanning electron micrograph of a rupturing cell of *Florenciella* spp. revealing the viruses inside. Photo by C. Schvarcz



Highlight

The Hawaii Ocean Time-series completes 250 cruises to Station ALOHA

Submitted by: **Matthew Church**

On March 9, 2013 the Hawaii Ocean Time-series (HOT) program successfully completed its 250th research cruise to Station ALOHA. HOT was initiated in 1988 to document and describe time-varying changes to the open ocean waters of the central North Pacific. After a quarter of a century of sustained observations, HOT remains one of the most transformative oceanographic programs in the world. Through near-monthly, repeat sampling of the ocean waters at the field outpost Station ALOHA (22°45', 158°W), HOT research has identified long-term trends in ocean physics and biogeochemistry, and highlight important processes underlying ecosystem variability. The program's success in large part derives from dedicated efforts of a team of scientists and staff. Many of the contemporary core HOT staff have participated in more than 1/3 of the 250 cruises (Figure 12), and having such experienced, well trained people has proven invaluable to maintaining the high quality time series measurements.

CMORE benefits enormously from the HOT program. The robust temporally-resolved suite of HOT measurements has proven essential to formulating CMORE science. Logistical support enabled by HOT field and laboratory support continues to create new science and education opportunities. HOT cruises provide regular sampling opportunities for CMORE students, post-docs, and scientists. CMORE education programs routinely leverage the HOT program. For example, the HOT team hosts the CMORE Science Teachers Aboard Research Ships (STARS) program where high school teachers are given an opportunity to participate in a research cruise to learn about microbial oceanography.

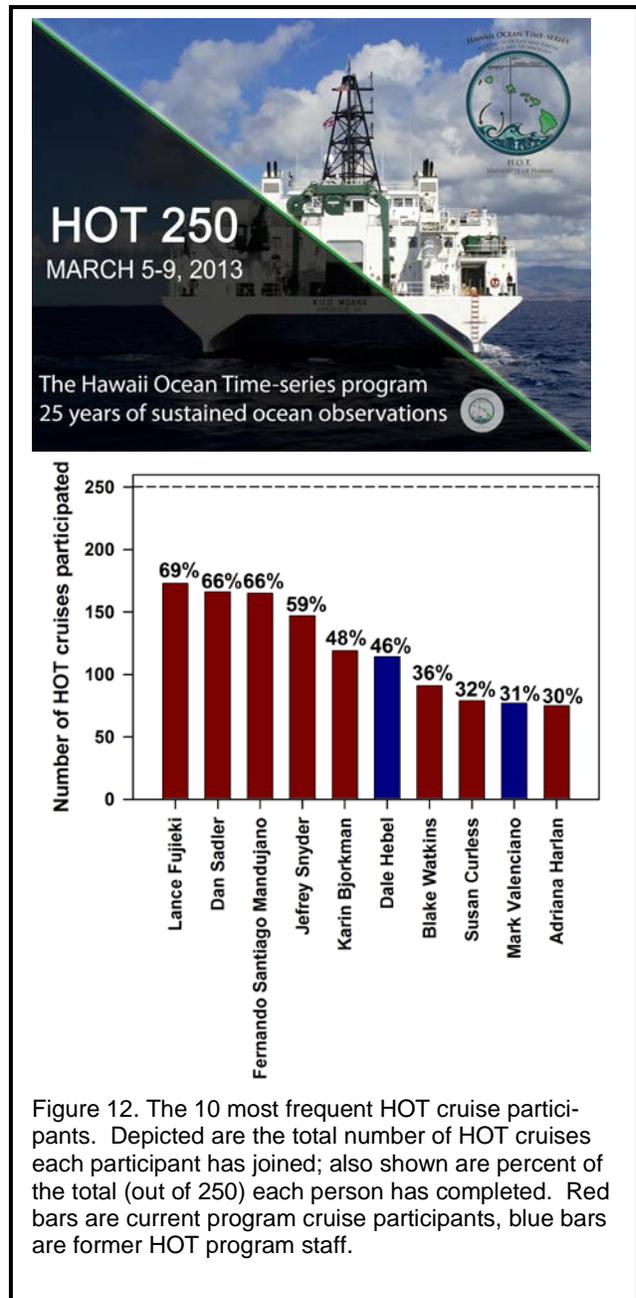


Figure 12. The 10 most frequent HOT cruise participants. Depicted are the total number of HOT cruises each participant has joined; also shown are percent of the total (out of 250) each person has completed. Red bars are current program cruise participants, blue bars are former HOT program staff.



Highlight

C-MORE at the EarthCube Early Career Strategic Visioning Workshop

Submitted by: Vanessa Michelou

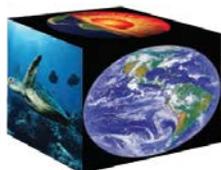
Vanessa K. Michelou in the Center for Microbial Oceanography was selected with input from NSF program officers as a leading early career scholar to be invited to an “Early Career Strategic Visioning Workshop” focused on the NSF’s EarthCube initiative. This is a ten-year initiative to develop the cyber-infrastructure for the earth sciences in order to better enable transformational science within and across disciplines – facilitating research on some of the most pressing challenges in Earth Science.

The workshop gathered 68 early career geoscientists, computer scientists, and others at the Geophysical Laboratory of the Carnegie Institution of Washington on 16-17 October 2012. The purpose of the workshop, which was supported by the U.S. National Science Foundation (NSF), was to develop a shared vision for success of the cyberinfrastructure necessary to support the next generation of earth science research. The workshop was invited to the Carnegie campus by the Deep Carbon Observatory, which recently launched a data science initiative that will spearhead fundamental changes in the conduct of earth science research.



The co-leads organizing the workshop, sought assistant professors (and some post docs or doctoral students) who might best provide input at the intersection of computer science and earth science – so that the cyber infrastructure will best enable the success of next generation leaders in the field

The workshop participants engaged in the highly interactive process of mapping their own career aspirations and considering how a robust cyberinfrastructure might enable them to tackle high-impact research questions and deliver education in new ways. Most participants were assistant professors, but post docs, doctoral students, and a few others also attended. The workshop invitees were selected based on their potential as emerging leaders in their respective fields.



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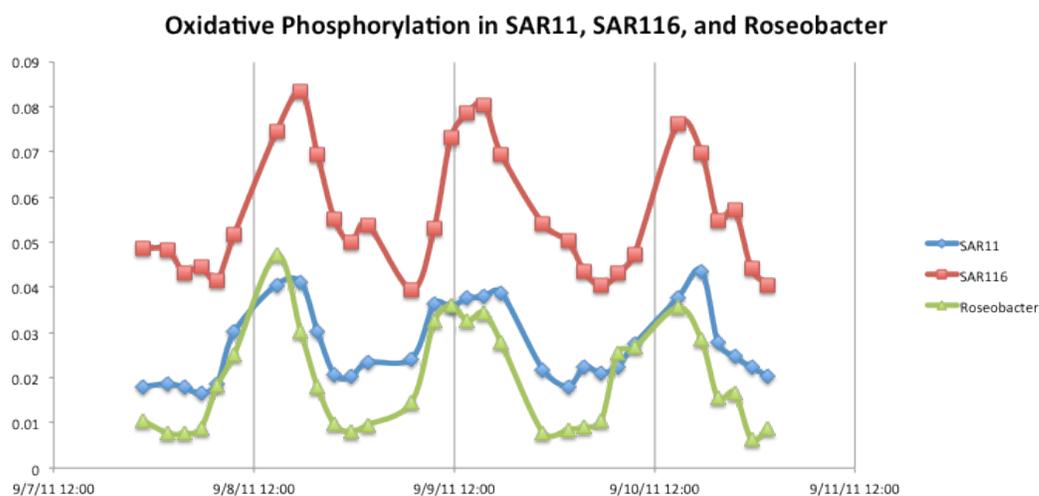
Lagrangian sampling of microbial gene expression at Station ALOHA

Submitted by: E. DeLong, L. Ottesen, C. Scholin

Microbial community transcriptome analyses has proven useful for assessing rhythmic gene expression associated with circadian cycles, as well as microbial community responses to environmental variation. To track microbial communities in space and time, the Environmental Sample Processor (ESP) can be deployed in a drifting configuration that permits Lagrangian sampling of coherent microbial populations. To better assess the daily activities of microbial communities at Station ALOHA, Chris Scholin and the ESP team, along with Liz Ottesen and Ed DeLong at MIT, collaborated on the BioLINC's cruise in a deployment of the ESP for a Lagrangian drifter experiment. Picoplankton samples were collected every two hours during a 3 day drift. Microbial community RNA was subsequently processed for metatranscriptomic sequencing, yielding a total of 62,048,204 transcript sequences. The most abundant taxa represented in the metatranscriptome included *Prochlorococcus*, *Synechococcus*, Rhodobacteraceae, SAR11, SAR116, SAR86, SAR324, SAR406 and Flavobacteriaceae.

Results and analyses to date have revealed the natural circadian rhythms within a variety of different phototrophic picoplankton types. As expected, the oxygenic phototroph *Prochlorococcus* exhibited strong diel cycling in the expression of genes associated with photosynthesis and carbon fixation. The anoxygenic aerobic phototroph (AAP) *Roseobacter* also showed circadian rhythms in its photosynthetic reaction center genes, but with peak synthesis occurring at night. Presumably, the AAPs reassemble the photosynthetic apparatus in the night for its repair or replacement, in preparation for the next photoperiod. Three different photoheterotrophs (SAR11, SAR116 and *Roseobacter*), all exhibited synchronous expression of oxidative phosphorylation genes, that appeared to be tuned to the light dark cycle (Figure 13, below). On the

whole, the Station ALOHA picoplankton community appeared to be much more highly regulated by regular light-dark cycle periodicity, than were similar communities examined in an ESP drifting experiment in coastal Monterey Bay.





Highlight

Fluorometric Assessment of Bacteriochlorophyll Concentration in the Water Column

Submitted by: Z. Kolber

Aerobic Anoxygenic Photoheterotrophic Bacteria (AAPBs) are present in the upper water column of most of the World's Oceans. Relatively low concentration, 1% - 5% of that of CHLa biomass, requires sophisticated techniques of HPLC pigment analysis or epifluorescence microscopy to quantify their abundance. Infrared Fast Repetition Rate (IRFRF) fluorescence technique, on the other hand, allows quantifying BCHLa biomass at picomolar concentrations, with just 2 ml of the water sample. Unfortunately, until now the IRFRF technique suffered from the fact that the BCHLa fluorescence measured at 880 nm is significantly contaminated by the red tail of CHLa fluorescence, leading to overestimation of BCHLa concentration. We have developed a new IRFRF approach to correct for this deficiency, where the fluorescence signal measured at 880 nm is deconvoluted with the fluorescence signal measured at 685 nm based on different saturation characteristics of these two signals (Fig. 14 A and B). This approach displays linear correlation with HPLC-based estimates of BCHLa, with r^2 of 0.75 (Fig. 14C). We have used this technique to measure BCHLa distribution in the water column during two cruises in summer of 2012 (KM1215 and KM1219), with total of 120 casts (Fig. 15E). We have also measured vertical distribution of excitation-resolved chlorophyll fluorescence at 445 and 470 nm related to picoeukaryotes, diatoms, and *Prochlorococcus* species, and at 505 and 530 nm related to cyanobacteria (Fig. 15 A, B, C, D). The depth of the DCM signal generally averaged at 130 meters, while the maximum of the bacteriochlorophyll signal was present at 70 to 100 meters. Such vertical shift toward the surface indicates higher preference for light in AAPBs compared with the oxygenic phytoplankton. Small functional absorption cross section, $40\text{-}60 \text{ \AA}^2$ (32-36 BCHLa/carotenoid molecules per reaction center) in AAPBs compared to $400\text{-}600 \text{ \AA}^2$ (~300 CHLa molecules per reaction center) in oxygenic phytoplankton provides partial explanation for higher light preference in AAPBs.

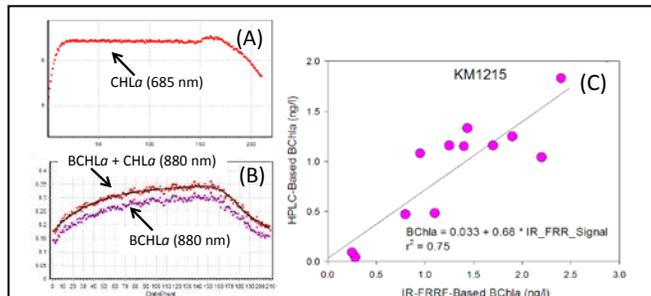


Figure 14. IRFRF technique for assessing BCHLa concentration. (A) CHLa signal measured at 685 nm; (B) BCHLa signal with a red tail of CHLa signal measured at 880 nm (red) and the reconstructed BCHLa signal (purple); (C) Intercalibration of the reconstructed BCHLa signal with HPLC-based estimates of bacteriochlorophyll concentration.

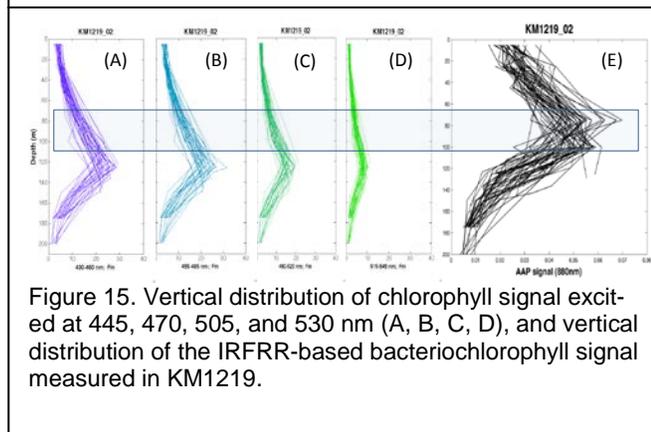


Figure 15. Vertical distribution of chlorophyll signal excited at 445, 470, 505, and 530 nm (A, B, C, D), and vertical distribution of the IRFRF-based bacteriochlorophyll signal measured in KM1219.



Highlight

The role of thiamine and other B vitamins at Station ALOHA and during induced blooms

Submitted by: D. Del Valle, S. Sañudo-Wilhelmy

As early as 1937 it was discovered that some flagellates require an external supply of thiamine (B_1) to grow. Since then, many species of phytoplankton have been found to have an obligate requirement for different B vitamins, and it has been proposed that in some oceanic regions the bioavailability of certain vitamins might limit primary production and have a control on the community composition of the phytoplanktonic community.

During summer and fall of 2012, C-MORE post-doc Daniela del Valle, in collaboration with Sergio Sañudo-Wilhelmy, induced blooms by addition of deep seawater (300m) to surface seawater (25m), in order to study the requirements of B_1 by different size fractions of the microbial community during different stages of a bloom. She also monitored the concentration of several other B vitamins (B_2 , riboflavin; B_6 , pyridoxine, B_7 , biotin; and B_{12} , cobalamin; samples yet to be processed) during these experiments with the aim of relating their changes in concentration to bacterial and eukaryotic abundance.

In these experiments, the abundance of *Synechococcus* increased up to 50X but this increase was not correlated to the uptake of B_1 in its corresponding size fraction (0.6-2 μ m), which is in agreement with genome analysis that shows that *Synechococcus* possesses the biosynthetic pathway for B_1 production. In contrast, the growth of large phytoplankton (>10 μ m) was highly correlated to the uptake of B_1 , which is in agreement with the lack of B_1 biosynthetic pathways in many diatoms.

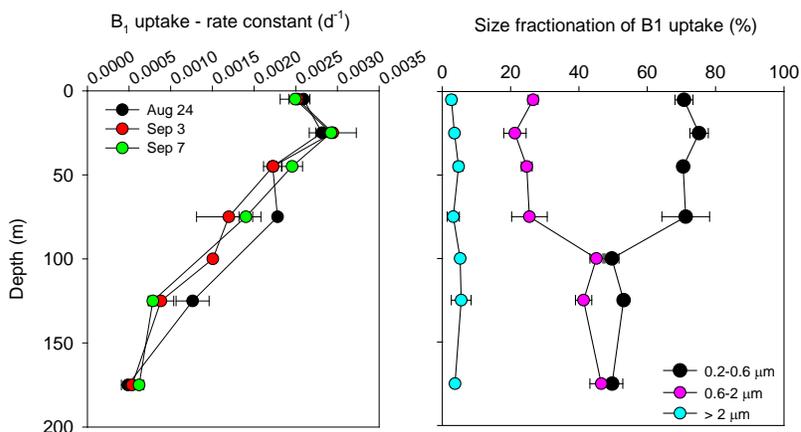


Figure 16 A: Depth profiles of turnover of B_1 at Station ALOHA.
Figure B: Relative contribution of different size fractions to B_1 uptake at Station ALOHA.

A complementary part of this work was also carried out at Station ALOHA to study for the first time the requirement of vitamin B_1 by natural communities during the summer, as well as the diel and seasonal cycling of the different B vitamins. At Station ALOHA the uptake of B_1 was slow under summer non-bloom conditions, with turnover times of ~ 1 year in surface waters (Figure 16 A). In the mixed layer, the uptake of B_1 was carried out mostly by the smallest fraction of the microbial community (<0.6 μ m) (Figure 16 B), despite the current paradigm being that most prokaryotes can synthesize their own B-vitamins. SAR11, an abundant and cosmopolitan clade, has been shown to be auxotrophic for B_1 and therefore might be responsible for an important fraction of the uptake in this size fraction.



Highlight

Methionine and dimethylsulfoniopropionate (DMSP) assimilation in the North Pacific Subtropical Gyre

Submitted by D. del Valle, R. Kiene and S. Sañudo-Wilhelmy

Sulfur is an essential nutrient that is found in two of the 20 amino acids and in other organic molecules, such as DMSP. The capability to reduce sulfate and assimilate it into cellular material was once considered to be an universal trait among aerobic marine bacteria. However SAR11, which is an abundant microbe in the NPSG, cannot reduce sulfate and therefore depend on the uptake of exogenous reduced sulfur to grow. Moreover, even if many organisms can reduce sulfate, they rather take it reduced from the environment in order to minimize energetic expenditures.

During HOE-DyLAN, C-MORE post-doc Daniela del Valle, in collaboration with Ron Kiene (USA) and Sergio Sañudo-Wilhelmy (USC), studied the turnover of two important S-sources for marine bacteria, DMSP and methionine. From previous work we knew that S from DMSP can satisfy up to 67% of the bacterial sulfur demand, however the role of methionine has never been studied.

Preliminary results show that methionine and DMSP uptake into macromolecules (i.e. proteins) follow a similar depth and diel pattern (see Figure 17). However, it was found that *Prochlorococcus* has a preference for S uptake from DMSP compared to methionine, while low and high nucleic acid content bacteria (LNA and HNA, respectively) are efficient at assimilating S from both substrates. During this study samples for methionine concentrations were taken, which will constitute the first direct measurement of this amino acid in the oligotrophic waters and will allow a direct comparison to DMSP in terms of their relative importance as S sources.

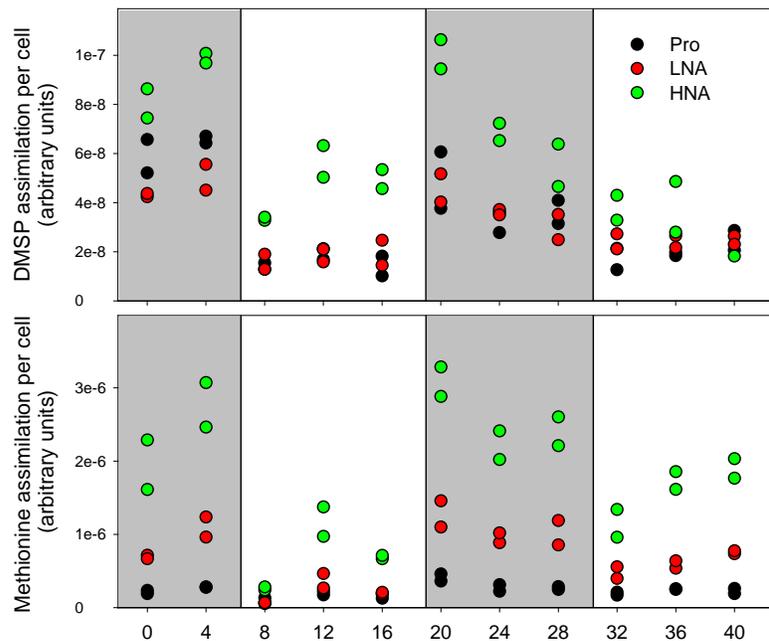


Figure 17: Assimilation per cell of (A) DMSP and (B) methionine during a 40-h cycle at Station ALOHA.



Highlight

C-MORE and Whyville: Informal microbial oceanography education in a virtual world

Submitted by: Sonya Dyhrman and Sheean Haley

Expanding literacy in microbial oceanography is an important C-MORE goal, one that CMORE has been particularly innovative in addressing. To bridge the gap between microbial oceanography research and science education, C-MORE has turned to a novel platform, the virtual world of Whyville (www.whyville.net). This internet-based world, launched by Numedea Inc. in 1999, serves children ages 8-15 and has over 7 million “citizens.”

The newest CMORE-sponsored activity in Whyville is a card game with a collection of microbe cards created by Sonya Dyhrman. The cards each highlight a different microbe (e.g. Prochlorococcus) and its attributes. They relate to the CMORE-produced Key Concepts in Microbial

Oceanography and help players learn about the size, abundance, and diversity of marine microbes. The game is refereed with a dimension

like “highest temperature” for the competition between 2 players. The microbe card that best matches the dimension wins the round. Since its release, 324,000 Whyville citizens have collected, traded and played games with the 35 microbe cards that each highlight a different microbe and its attributes. The card game is a unique complement to the hands-on C-MORE educational activities. The virtual platform promotes scientific inquiry in microbial oceanography to large numbers of children (well over 1 million and counting) without some of the obstacles encountered in the classroom. These standards-based activities are adaptable and can be refined by tracking assessment outcomes. Future activities can take advantage of the benefits of science education in a virtual world to illustrate other concepts in microbial oceanography.





Highlight

Physiological ecology and composition of *Trichodesmium* populations at station ALOHA

Submitted by: Sonya Dyhrman, Mónica Rouco, Harriet Alexander, Sheean Haley

Cyanobacteria in the genus *Trichodesmium* play a crucial role in the carbon and nitrogen cycle of the tropical and subtropical oceans. There are 6 defined *Trichodesmium* species that group into two major clades. *Trichodesmium* populations occupy a broader vertical distribution than previously thought, but the spatial and temporal distribution of the different *Trichodesmium* species is poorly understood. There are many physical and geochemical factors, including light, mixed layer depth, and nutrient availability (e.g., iron and phosphorus) that can influence *Trichodesmium* physiological ecology. During the HOE-DYLAN cruises CMORE scientists Sonya Dyhrman, Mónica Rouco, Harriet Alexander and Sheean Haley sampled *Trichodesmium* sp. at station ALOHA to investigate community composition and physiological ecology utilizing a molecular approach. For community composition, high-frequency sampling of *Trichodesmium* populations was performed from five different depths (5, 25, 45, 75 and 100 m) to investigate patterns of clade abundance with time and depth. A qPCR technique has been developed and employed in the lab and it is being used for the specific quantification of two of the most abundant

Trichodesmium clades. Initial analyses suggest that *Trichodesmium* is detectable, and largely from the *T. theibautii* clade. Building upon the community composition work, postdoctoral fellow in the Dyhrman group, Monica Rouco, is working to gain a better insight into the physiological ecology of *Trichodesmium* surface populations. *Trichodesmium* colonies from the surface were collected every 3 days for high-throughput metatranscriptome sequencing. The technique for the extraction of *Trichodesmium* total RNA and the enrichment of *Trichodesmium* mRNA has now been tested and optimized in the laboratory and is now ready for the processing of *Trichodesmium* surface samples from station ALOHA. Ultimately tracking community composition and metatranscriptomic signals will facilitate our understanding of the physiological ecology of this important genus.





Highlight

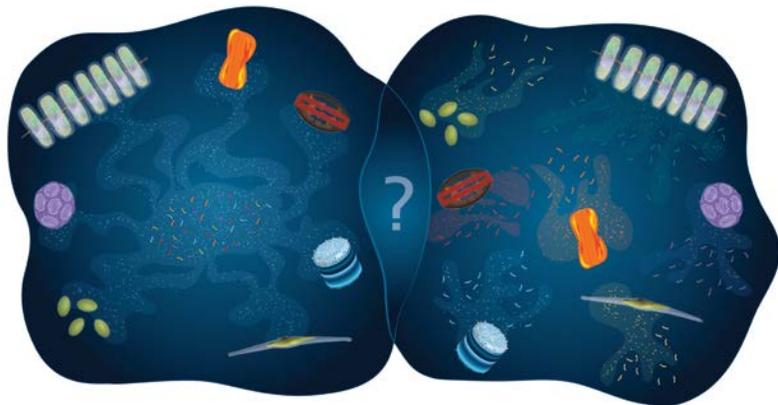
Metabolic profiling of a marine *Oceanospirillum* reveals elevated anabolism, decreased motility, and increased proteorhodopsin-based phototrophy in the presence of *Prochlorococcus*-derived dissolved organic matter

Submitted by: J.W. Becker, P.M. Berube, S.W. Chisholm, M.S. Rappé, D.J. Repeta, E.F. DeLong

A vast reservoir of Earth's organic carbon is almost exclusively accessible to marine microorganisms as nonliving material dissolved in seawater (DOM). Microbial uptake and allocation of DOM into catabolic and anabolic processes largely dictates whether nutrients and energy within DOM become available to higher trophic levels or remain inaccessible as recalcitrant DOM. Metabolic pathways involved in the consumption of DOM are therefore important to biogeochemical cycling, but are rarely incorporated into ecosystem models. We know very little about the physiology of DOM consumption or the structure of the microbial community that facilitates DOM degradation. To address this, C-MORE scientists from four different laboratories in three research institutions have teamed up to uncover new information regarding metabolic pathways and key organisms involved in microbial DOM cycling.

At the Woods Hole Oceanographic Institution and the Massachusetts Institute of Technology, putative identification of compounds produced and released by the marine cyanobacterium *Prochlorococcus* have been made using an untargeted metabolomic approach *via* ultra-high-resolution mass spectrometry. Information regarding the chemical composition of DOM produced by this abundant photoautotroph is providing new insight into the composition of labile DOM.

At the University of Hawaii, more than a dozen axenic strains of heterotrophic marine bacteria, including members of the SAR11, *Rhodobacterales*, and *Oceanospirillales* lineages were amended with DOM produced by five axenic cultures of marine phytoplankton to evaluate the effects of DOM sources on heterotrophic fitness. *Prochlorococcus*-derived DOM was found to be highly labile, increasing the growth rate and maximum cell abundance of nearly all heterotrophic bacterioplankton including an isolate from a novel family with the order *Oceanospirillum*.



Our research suggests that different marine phytoplankton do not produce similar DOM as shown here on the left, but rather each contributes unique components to the DOM pool, which are then preferentially utilized by specific heterotrophic taxa as shown in the scenario on the right. The chemical composition of marine DOM provides a link between autotrophic and heterotrophic microbial diversity in the sea. Image by Amy Caracappa-Qubeck, WHOI.

We then used transcriptomics combined with untargeted metabolomics to investigate the consumption of *Prochlorococcus* DOM by a marine *Oceanospirillum* (strain HIMB30). Transcriptional response of HIMB30 to the addition of *Prochlorococcus*-derived DOM revealed the use of carboxylic acid (tripartite ATP-independent periplasmic, or TRAP) transporters and sugar ABC transporters for DOM acquisition and the subsequent utilization of DOM in anabolic pathways including gluconeogenesis, the glyoxylate cycle, and the non-oxidative portion of the pentose phosphate pathway as well as for biosynthesis of cell membrane and cell wall material. Transcriptional analysis also revealed a decrease in the expression of genes related to flagellar assembly and chemotaxis and an unexpected increase in gene expression involved in proteorhodopsin-based phototrophy. Metabolomic analysis of *Prochlorococcus*-derived DOM revealed the putative presence of carboxylic acids, glucosides, and terpenoids that supported these gene expression signals. Future work will focus on the physiology of DOM consumption by other bacterioplankton to elucidate metabolic pathways employed by functionally diverse marine microbes pertinent to the cycling of marine DOM.



Highlight

Exploring DOC isotopic variability in the deep ocean- Have we been missing a large, abyssal microbial loop?

Submitted by: C. L. Follett and D. Repeta

Marine dissolved organic carbon (DOC) is a large, reactive carbon reservoir that mediates the oceanic microbial food web and interacts with the climate system on both short and long time-scales. Decades of global DOC measurements and a handful of DOC radiocarbon measurements have been synthesized into a model of DOC cycling that emphasizes production and rapid microbial cycling in the surface ocean and little to no production or consumption (cycling) at depth. Deep water (> 1000m) DOC concentrations are characterized by a slow decline along the trajectory of abyssal thermohaline circulation, and DOC radiocarbon values suggest a largely recalcitrant reservoir that cycles over millennial timescales. Microbial cycling of DOC in the deep ocean is thought to be a minor process at best. But is it?

Carbon isotopic content provides information on the DOC sources *via* ^{13}C and age *via* ^{14}C . Isotope measurements suggest a microbially sourced DOC reservoir with two distinct components, a modern fraction of labile DOC confined to the upper ocean, and an old fraction well mixed to all depths. However, such measurements cannot distinguish internal dynamics and fluxes. The possibility that deep ocean DOC might be a mixture of recalcitrant and labile carbon itself with vastly different cycling times has long been recognized, but as yet there are no approaches to testing this hypothesis.

We are exploring a novel approach to quantitatively assess the mass and radiocarbon values of different carbon fractions within deep sea DOC. Over the past year we have performed serial oxidation experiments using ultraviolet light that allow us to measure the radiocarbon value of DOC as it is being oxidized. Since different components of DOC have different oxidation kinetics, the approach allows us to look inside of the mixture of components that make up DOC. We find an unexpected isotopic diversity with DOC at Station ALOHA. Our results show diversity in both stable and radio isotopes at all depths, uncovering DOC cycling hidden within bulk analyses. We confirm the presence of young, labile DOC co-cycling with an isotopically older fraction in the upper ocean. However, our results show that isotopically young material constitutes 30% of the deep reservoir and is supported by a 1 Pg per year carbon flux, ten times higher than currently believed. The remaining 70% of the material turns over at an apparent time scale of 30,000 years, far slower than indicated by bulk radioisotope measurements. Collectively these results provide an unprecedented view (Figure 18) of the ways in which DOC moves through the marine carbon cycle, and suggest a large and active deep-sea microbial loop.

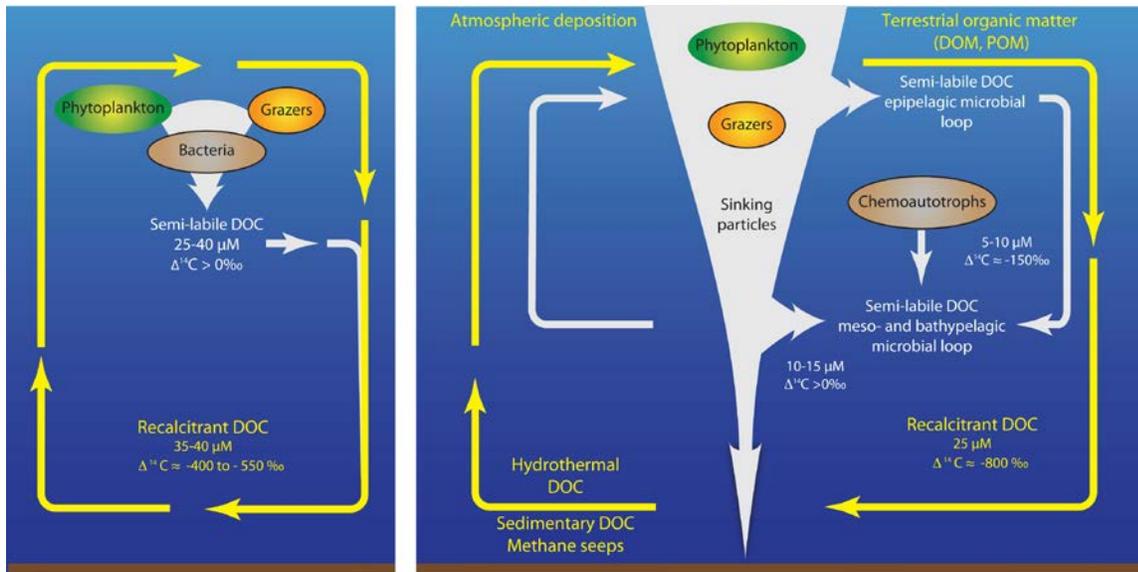


Figure 18. The old (left) and new (right) marine DOC cycle as suggested by our isotopic measurements. The current paradigm of DOC cycling (left) allows for DOC production and consumption in the upper ocean. The deep ocean acts as a closed system. Our isotopic measurements suggest that there is a large reservoir of post bomb DOC-14 in the deep ocean, suggesting a large pool of semi-labile DOC supported by the dissolution of sinking particles that cycles every few decades at most. There is a large, active microbial loop of DOC cycling in the deep sea. (Figure from *Follett et al. 2013; Nature, in revision*).



Highlight

Organic ligands: linking microbes to metals

Submitted by: R. Boiteau, D.J. Repeta, J. Fitzsimmons, E. A. Boyle, S. W. Chisholm

We often think microbial production is limited by the input of nutrients to the ocean. In the case of many trace metals, the reverse is also true. Many biologically important trace metals, such as Fe and Co are largely insoluble seawater, but microbes have found a way to cope with this limitation by producing organic compounds that bind metals and keep them dissolved. These organic ligands determine how much Fe from dust, rivers and hydrothermal vents stays in solution as part of the standing stock of metals that is available to the microbial community. This observation leads to many important questions. How do organisms acquire iron from organic ligand complexes? Does the type of ligands impact the iron's bioavailability to particular organisms? Do microbes intentionally produce ligands that target only certain members of the community?

Before any of these questions can be addressed, it is important to understand the chemical nature and diversity of metal ligands that are produced by marine organisms. One approach is to grow cultures under iron limitation of marine microbes that are representative of environmentally important species, such as picocyanobacteria, and determine the types and diversity of ligands that they produce. This approach has been successfully used for decades to explore microbially derived metal-ligand complexes. Many ligands that form very strong complexes to iron and facilitate its uptake have been identified using this approach, and these model systems have helped to understand mechanisms of microbial iron uptake. However, with only one exception, no ligands have been identified in marine picocyanobacteria, and the use of ligands as an iron acquisition mechanism by picocyanobacteria has been questioned.

We believe the failure to find iron ligands in marine picocyanobacteria is due to a lack of sufficiently sensitive methods for their discovery. Using support from C-MORE, we have collaborated in the development of a technique for separating and detecting metal-organic complexes by HPLC-tandem inductively coupled plasma mass spectrometry (ICP-MS). With this method, culture media can be rapidly screened to determine the number and polarity of Co and Fe ligand complexes at sub picomolar detection limits. The resulting chromatograms (Figure 19a and 19b) show series of Fe or Co peaks that each correspond to a specific ligand. Six strains of *Prochlorococcus* have been tested so far. Our results suggest that all strains are capable of producing two to three different Fe ligands (Fig. 19a), yet no two strains produce the same suite of ligands. In contrast, every strain appears to produce the same set of three cobalt ligands (Fig 19b), the predominant one being cobalamin. By isolating and characterizing these ligands, we can determine whether they play a role in *Prochlorococcus* Fe uptake and whether they are important components of the Fe ligand pool in regions of the ocean where *Prochlorococcus* dominate.

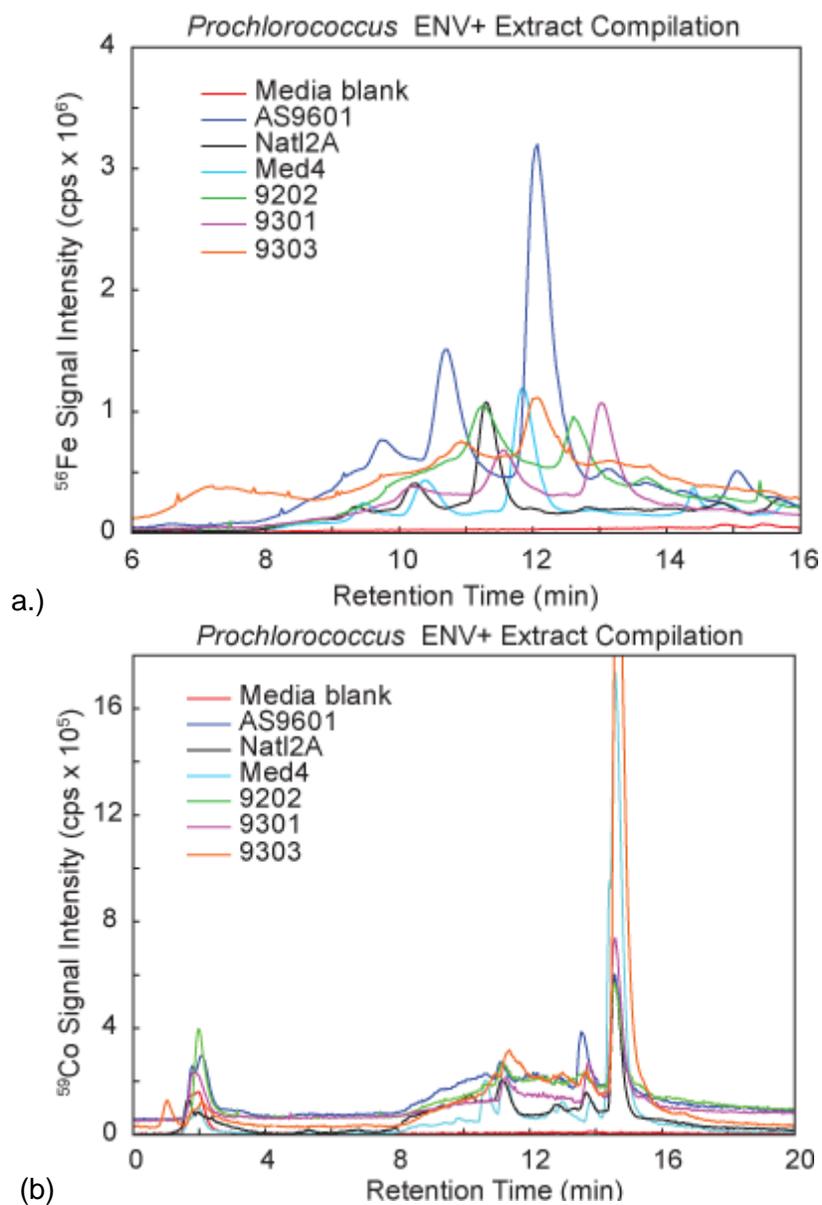


Figure 19: Compilation of HPLC-ICP-MS chromatograms of Fe and Co ligands in axenic *Prochlorococcus* cultures. **(a)** Most strains produce two or more iron ligands (indicated by the series of peaks), although no two strain produces the same ligand suite. **(b)** All strains produce the same suite of cobalt ligands. Cobalamin is the major Co ligand (peak at 15 minutes), but there are also two other Co ligands with retention times of 11 and 14 minutes. Med4 also produces a fourth ligand, with a retention time of 10.5 minutes.



Highlight

An Academic-Industry Partnership for Visualizing the Dynamics of Oceanic Microbial Communities

Submitted by: C. Scholin

Ocean ecosystems arise from a myriad of complex interactions that occur on multiple time and space scales, resulting in patchy distributions of biological communities and complicated population dynamics. Microorganisms are at the heart of that intricate and dynamic web. They mediate transformations of matter and energy that sustain all life in the oceans, and their fluctuations ultimately affect human health and prosperity. C-MORE researchers are investigating these linkages through a combination of approaches that include analyses of marine microbial gene content and expression. Advances in genomic technologies have led to great strides in our understanding of the biology and ecology of the “unseen majority”, and lent insights into how these important organisms respond to changing chemical and physical conditions. Expanding these investigations further is critically important, but is also very challenging from both a sampling and data visualization perspective.

C-MORE participants are exploring means for addressing this problem. New methods for collecting samples and assessing environmental conditions have been devised and applied, in turn allowing the team to observe microbial communities with much higher resolution than was possible previously (e.g., <http://www.mbari.org/news/homepage/2012/canon-fall/canon2012.html>). This trend is expected to continue as autonomous platforms that facilitate collection of microbial samples and provide concurrent chemical/physical measurements become increasingly available.

To address the data visualization problem, C-MORE partners at the Monterey Bay Aquarium

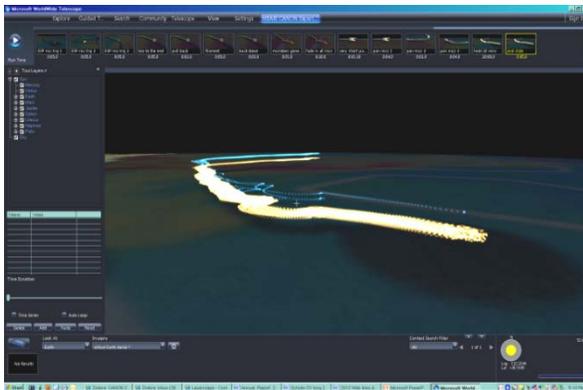


Figure 20. Layerscape representation the abundance of *Synecochoccus* (yellow) and *Crenarchaeota* (blue) as determined in real-time using the Environmental Sample Processor (ESP) deployed on a free-drifting platform along Central California in 2012. When viewed in Layerscape, the whirls are animated and reveal how the two groups evolve over the course of the drifter run.

Research Institute (MBARI) and MIT have been collaborating with a group at Microsoft Research that is developing a data visualization tool known as Layerscape (Figure 20). The focus of this work is to explore use of the Layerscape framework to merge space/time renderings of physical, chemical, and biological measurements, including visualizations of microbial gene expression and community dynamics. This work is in its earliest stages, but does demonstrate the potential of leveraging industry/academic partnerships to address a vexing ocean science problem. A preview of the data visualization effort can be found here:

<http://www.layerscape.org/Content/Index/756> .

Support for this work was made possible by funding from NSF, the David and Lucile Packard and Gordon and Betty Moore Foundations, and Microsoft Research.



Highlight

Nitrate utilization by *Prochlorococcus*: Evolution in action

Submitted by: P. Berube and S.W. Chisholm

The marine photosynthetic bacterium, *Prochlorococcus*, is intimately tied to life on this planet; it has an important role in the global carbon cycle and produces a significant proportion of the oxygen we breathe. Nitrogen is an essential nutrient for all cells, and it occurs in seawater primarily in the form of ammonia, nitrite, and nitrate. The latter is periodically very abundant, whereas ammonia and nitrite are found in low concentrations, but more easily assimilated by the cells. After it was first discovered, evidence showed that the *Prochlorococcus* strains in culture could not utilize nitrate, making it very unusual among the phytoplankton. Why would such a large standing stock of phytoplankton exist that could not use nitrate?

Recent discoveries have now shown that those early results were biased by the strains that had been isolated into culture. We now know that there are many *Prochlorococcus* lineages that can indeed use nitrate as a source of nitrogen, and *Prochlorococcus* populations in the wild have been shown to assimilate it. This knowledge has transformed how we conceptualize the role of *Prochlorococcus* in marine ecosystems. The question remains, however, as to how the different nitrogen-utilizing variants of *Prochlorococcus* have evolved.

Post-doc Paul Berube in the lab of C-MORE Principle Investigator, Sallie W. Chisholm, is addressing this question by examining the genome sequences of nitrate-utilizing strains of *Prochlorococcus* to understand the genomic context of the genes involved in nitrate assimilation. The nitrate assimilation gene cluster in one strain (SB, Figure 21 below) is nestled among genes shared by most *Prochlorococcus*, indicating that this strain has long possessed the ability to use nitrate. Another strain, however (MIT0604 in the figure below), has two copies of the nitrate assimilation gene cluster, both located in genomic islands near genes related to the integration of foreign DNA. This suggests that the nitrate-utilization trait is 'mobile' in wild populations of *Prochlorococcus*, and that these tiny cells have very dynamic genomes allowing them to readily adapt to changes in the marine environment.

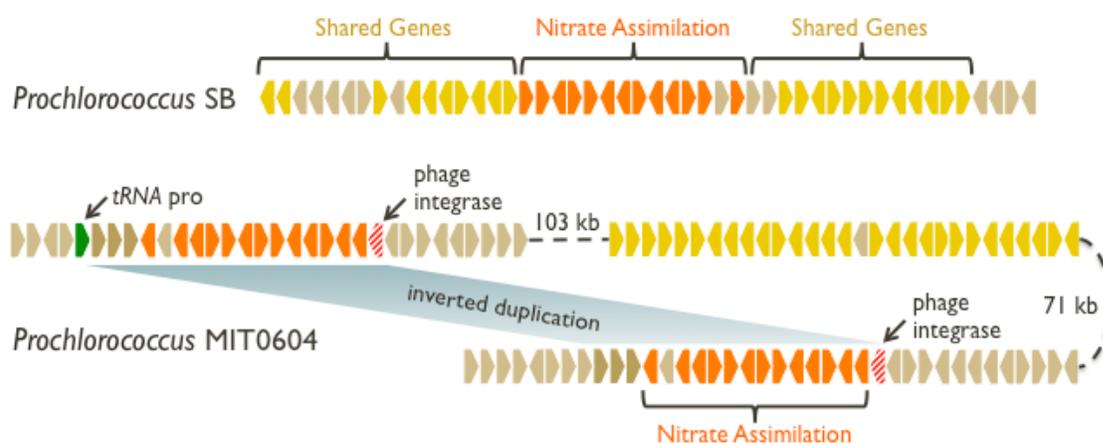


Figure 21. The genome context of nitrate assimilation genes from two closely related *Prochlorococcus*. Strain MIT0604 appears to have recently acquired these genes following a viral infection.



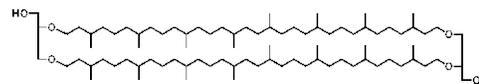
Highlight

New insights on planktonic archaeal lipids at Station ALOHA

Submitted by: S. Lincoln, E. DeLong, R. Summons, M. Church, B. Wai

Tetraether membrane lipids of archaea (at right) are ubiquitous in the world oceans, and their presence in the geologic record suggests that archaea have been active players in marine biogeochemical cycles for >

100,000 years of Earth history. These lipids have become useful tools in microbial ecology and paleoenvironmental reconstruction, but the factors influencing their diversity and distribution in the oceans remain poorly understood. In particular, the influence of archaeal community composition on the tetraether lipid pool has been underexplored.



a) Example of an archaeal tetraether lipid

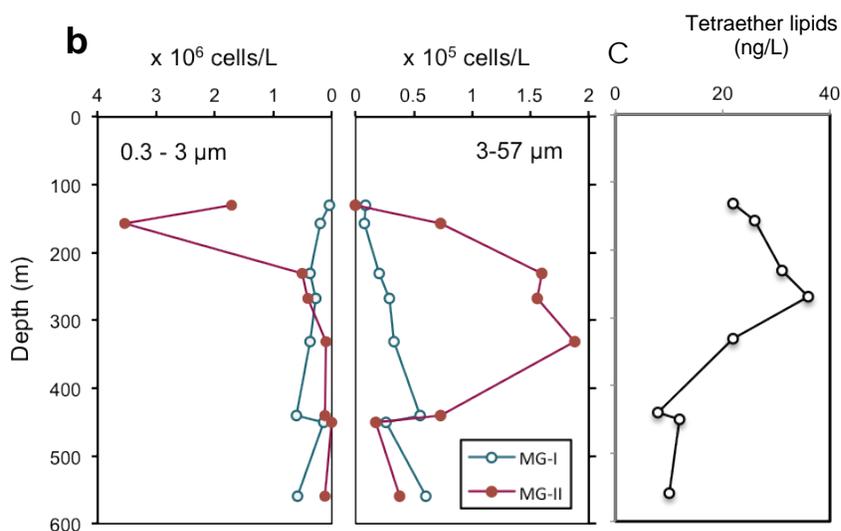
Marine Group I (MG-I) ammonia-oxidizing thaumarchaea and MG-II euryarchaea are the most abundant of the four archaeal phylotypes commonly detected in the oceans, but tetraether lipids have only been characterized in MG-I. C-MORE collaborators Matt Church and Brenner Wai, MIT geochemist Roger Summons, and DeLong Lab graduate student Sara Lincoln have now shown for the first time how archaeal community composition can influence tetraether lipid pools in the open ocean. On the BioLINCS cruise Sara used a submersible pump to filter more than 27,000 liters of seawater in the North Pacific subtropical gyre.

Sequencing of rRNA amplicons showed that the planktonic archaeal community was comprised exclusively of MG-I thaumarchaea and MG-II euryarchaea. When combined with MG-I 16S copy

numbers determined by qPCR, community composition data enabled inference of cell densities for both groups (Figure 22b). MG-II were more abundant than MG-I at depths <300-400 m, and were more highly represented in the larger size fraction. Tetraether lipids (Figure 22c; analyzed by HPLC-MS) peaked at 268 m and their profile more closely reflected that of MG-II than of MG-I.

The results suggest that MG-II not only contribute to tetraether lipid biosynthesis, but

may account for the bulk of archaeal lipids detected in the upper ocean. These findings have important implications for previous organic chemical interpretations of the significance of planktonic archaeal lipids in the water column, and their role in the sediment paleontological record.





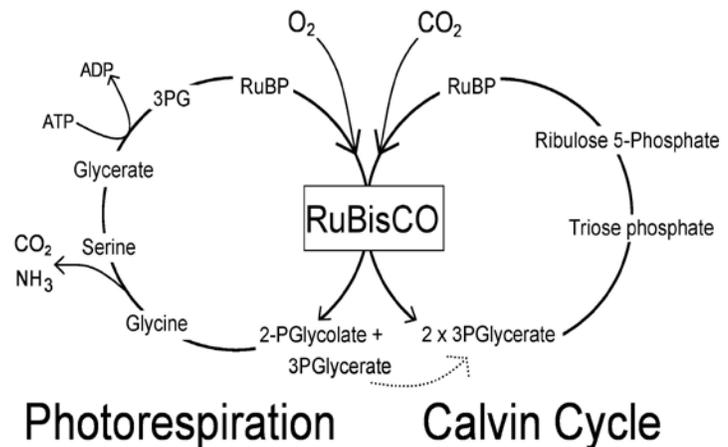
Highlight

Photorespiration

Submitted by J. Casey

Essentially all life on Earth begins with the “joining” of carbon dioxide to a five-carbon compound called ribulose biphosphate. Interestingly, the enzyme responsible for this transformation, known as RuBisCO, frequently misbehaves by instead joining oxygen to ribulose biphosphate, which initiates a chain of reactions ultimately resulting in a net *loss* of newly fixed carbon, a process known as photorespiration (illustrated at right). This deficiency, as it was long viewed, has been a particularly attractive target to improve crop yields and has thus been the subject of several

decades of search for functional bypasses. Recombinant techniques were used to produce modified plants called “photorespiratory mutants” with individual reactions removed or repressed, but none of the mutants were viable, pointing to perhaps a hidden alter-ego for the photorespiratory pathway. Indeed the view of photorespiration has changed in recent years to a favorable one, supporting the optimal functioning of central carbon metabolism, the balance of cellular energetics, and protection against harmful byproducts of photosynthesis. Since none of these roles have been evaluated in the marine environment, where roughly half of global photosynthesis takes place, C-MORE graduate student John Casey is investigating the ecological significance of photorespiration to the ocean’s most abundant algae, the picocyanobacteria and small photosynthetic eukaryotes. He is also studying some unintended roles that photorespiratory metabolism may play in the marine algae, including regulating the elemental composition of growth, and even providing a source of highly energetic carbon for bacteria. Just as has been demonstrated in land plants, he is designing experiments to describe the many faces of marine photorespiration under conditions that mimic important aspects of the chemistry and physics of the oceans predicted in the coming century.



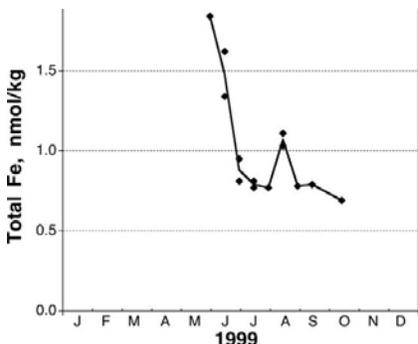
Credit: Rachel Purdon



Highlight

High-resolution trace metal surface sampling at HOT during HOE-Dylan

Submitted by: E.A. Boyle



Sampling for iron at HOT has been very sparse, with the only 10 samplings for filtered Fe samples collapsed on the annual cycle between 1994-2002 and a single biweekly time series for total Fe June-October 1999 (Figure 23) that shows a decrease of total (dissolved+particulate) Fe at the end of the Asian dust season.

←Figure 23: Total Fe in seawater samples collected by MITESS on Hale-Aloha mooring, April-June 1999, Boyle et al., 2005*):



During HOE-Dylan, the MIT group (Jessica Fitzsimmons, Abigail Noble, Gonzalo Carrasco, Simone Moos and Rick Kayser) were able to achieve daily surface sampling resolution for unfiltered, 0.4 μm filtered, 0.1 μm filtered, and 0.02 μm filtered Fe during much of the summer season (Figure 24). The Fe analyses of these samples will allow for an evaluation of the role of and response of Fe to biological processes and dust inputs (particularly through comparison to Th and Nd isotope data by Chris Hayes and Katharina Pahnke from samples collected on the cruise). We have also collected monthly euphotic zone profiles for total dissolvable Fe and 0.4 μm, 0.2 μm, and 0.02μm filtered Fe. A few deeper profiles will also assess variability of the Loihi hydrothermal plume at HOT.

← MITESS trace element vane samplers lined up to take a large volume trace element sample at depth.

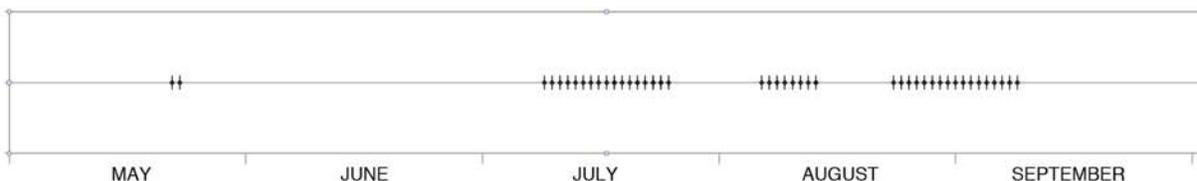


Figure 24: surface water samples collected for trace metal analysis during HOE-Dylan, May-Sept. 2012

*Boyle, Edward A., Bridget A. Bergquist, Richard A. Kayser, Natalie Mahowald (2005) Iron, Manganese, and Lead at Hawaii Ocean Time-series Station ALOHA: Temporal Variability and an Intermediate Water Hydrothermal Plume, *Geochim. Cosmochim. Acta*: 69:933-952.



Highlight

In situ collection and preservation of particulate filtered samples in a manner compatible with metagenomic & metatranscriptomic study

Submitted by: Craig Taylor

A stand-alone in-line filter system was developed in the laboratories of C. Taylor and V. Edgcomb in collaboration with McLane Research Laboratories for *in situ* collection and chemical preservation of filtered particulate samples (Figure 25). The Fixation Filter Unit (FFU) will have general application in microbial sampling and robotic tracer incubation studies. Fluids within the device are delivered by gravity and a density contrast between the preservative and medium being filtered, hence, requires no additional electromechanical devices for operation. The devices can, hence, be easily retrofitted into existing water samplers.

The FFU was specifically designed to preserve samples quickly (~30 sec), permitting collection of samples for subsequent gene function study. *In situ* preservation additionally eliminates one of the major uncontrolled variables in Niskin sampling for such studies, the sometimes long periods of time between sample capture & filtration/preservation on deck and potential for physiological response to changing physico-chemical conditions during sample retrieval to the ship (e.g., lighted samples suddenly exposed to darkness, pressure and perhaps temperature changes during retrieval, changes in redox conditions due to leaching of oxygen from Niskin bottle walls, etc.).

The FFUs are an integral part of a newly developed (C.Taylor, V. Edgcomb, McLane Research Labs) & tested (two cruises to Mediterranean Sea) robotic micro-laboratory for combined tracer incubation studies & microbial sampling (Submersible Incubation Device-*In Situ* Microbial Sampler (SID-ISMS)). Integration of these two capabilities within the SID-ISMS will allow combined rate measurements and associated community composition / gene function study of the catalyzing organisms.

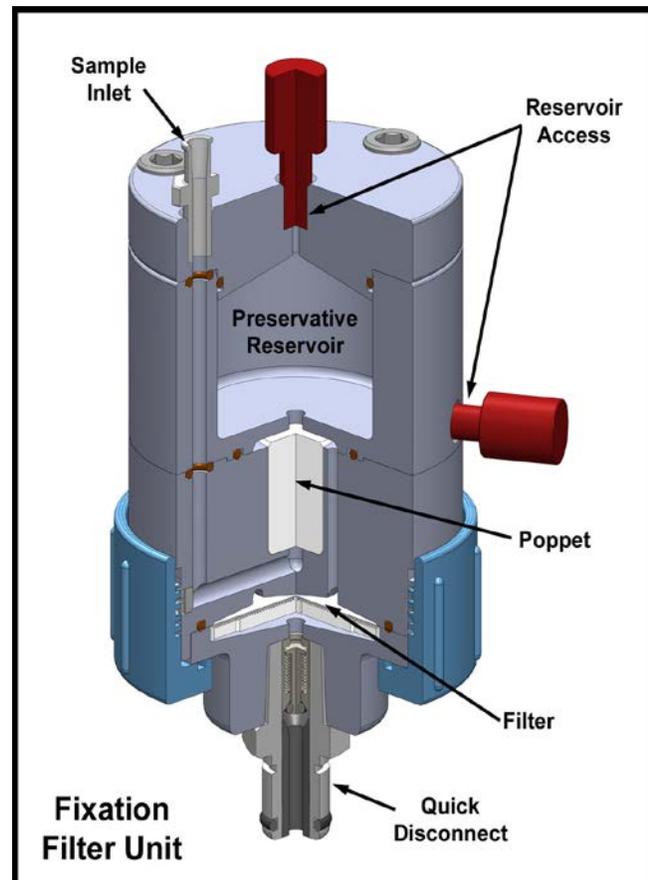


Figure 25. Fixation Filter Unit. Flow resistance between a negatively buoyant poppet and annular channel overcomes its weight and seals the only access to a preservative reservoir, preventing escape of preservative during the filtration process. When filtration is stopped, the poppet settles by gravity to its normally open position, allowing a dense preservative to flow from the reservoir (via laminar convection) onto the filter surface, preserving the retained particulate within ~30 seconds.



Highlight

C-MORE Scholars Program

Submitted by: Education Office

For more information, please contact Barbara Bruno (barb@hawaii.edu)

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, Scholars attend monthly meetings on career/professional development, participate in educational outreach and present their research results in an annual symposium.



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.

Since the C-MORE Scholars program was created in 2008, 51 students have participated and 20 have graduated. Seven program alumni are currently in graduate school, including two in the C-MORE graduate program (one of whom is Native Hawaiian). Of the 51 students who have participated in the program to date, 33% are Native Hawaiian, 8% Pacific Islander, 6% African American, 8% Hispanic, 6% Native American, 6% Filipino, 19% Asian, and 15% Caucasian.



C-MORE Scholars have had numerous notable successes. In 2013, two Scholars were promoted to Fellow: Paul Bump and Christina Johnson. Bump spent summer 2012 at Woods Hole's Biological Discoveries REU program at Oceanographic Institution and Fall 2012 at the Bermuda Institute of Ocean Sciences. He was recently honored with the Evo-Devo-Eco Networking internship award to conduct research on model organisms at a laboratory of his choice during Summer 2013. In addition, Bump recently participated in FameLab, a science communication competition, and was advanced to the national competition in April 2014 at the National Geographic Headquarters. Johnson, who in 2012 had won the Alan Church Environmental Steward scholarship, will soon enter a Ph.D. program at U. Southern California as a Tyler Doctoral Environmental Fellow.

C-MORE Scholars program webpage: <http://cmore.soest.hawaii.edu/scholars.htm>



Highlight

Diversity Training for C-MORE Graduate Students and Post-Docs

Submitted by: Education Office

For more information, please contact Michele Guannel (mguannel@hawaii.edu)

C-MORE is committed to engaging early career scientists in discussions examining the foundations of the lack of diversity in science. This commitment is reflected by the inclusion of diversity training in C-MORE's Professional Development Training Program for graduate students and post-doctoral researchers: <https://sites.google.com/site/cmoredprofdevtable/home/diversity>. Undergraduate and graduate students increasingly reflect a greater diversity in terms of race, ethnicity, gender, and disability status. In order for early career scientists to become effective leaders of upcoming generations of scientists, they need to be engaged in promoting inclusivity and addressing bias in all forms. To this end, the C-MORE Professional Development Training Program regularly supports diversity conferences for its members. Recently, C-MORE personnel attended two conferences on diversity across the partner institutions.



A videoconference in June 2012 focused on the recruitment and retention of individuals with disabilities (IWDs) in Science, Technology, Engineering and Mathematics (STEM) fields. C-MORE partnered with the Pacific Alliance for Supporting Individuals with Disabilities in STEM Fields Partnership. The conference was organized by C-MORE and facilitated by Pacific Alliance Coordinator Kiriko Takahashi and Disability Specialist Steve Brown, both of whom are based at the University of Hawaii (UH) Center on Disability Studies. The conference

revealed that instructors of postsecondary students greatly influence students' perceptions of self-efficacy, which is critical to retention of individuals with disabilities in STEM fields. This take-home message emphasized the potential for early career scientists to create an inclusive and supportive environment for all, hold, through their roles as teachers and mentors,.

In March 2013, a STEM-focused colloquium at the University of Hawaii entitled "Women as Scientists" was co-organized and well-attended by C-MORE members. Four of the six speakers were C-MORE personnel who volunteered in response to a request for speakers from the colloquium organizer, Dr. Jennifer Small, the only female faculty member in the UH Department of Meteorology. Speakers shared their personal stories of gender and ethnic bias, as well as struggles and tips to balance family and career responsibilities. C-MORE speakers also shared summaries of the current status of women in science, drawing heavily from recent studies and articles on the subject in major science journals (e.g., the March 2013 Nature special section on gender equity in STEM). Approximately one-third of the audience included C-MORE graduate students and post-docs. As a result of this colloquium, C-MORE members are beginning collaborations with other UH representatives on future outreach to middle school girls, as well as incorporating family balance issues into upcoming C-MORE diversity workshops.

Women as Scientists

Join successful Female Scientists as they share their stories and answer YOUR questions.

What is it like to be a scientist?
 Why did you want to be a scientist?
 How do you balance Work and Family?
 Are you treated differently as a woman?

Speakers:
 Marguerite Butler, PhD - Biology
 Olivia Negro, PhD - Oceanography
 Vanessa Michelon, PhD - CMORE
 Patricia O'Hagan, PhD - Health Science
 Christine Shulze, PhD - Oceanography
 Michele Guannel, PhD - CMORE Educator

Support Provided By:
Diversity & Equity Initiative

When: Tuesday March 19th
 Where: POST room 126
 Raffle for PRIZES! Time: 5:00-7:30 pm
 Light Refreshments Provided



Highlight

Professional Development Workshops on Careers and Networking

Submitted by: Education Office

For more information, contact Sean Jungbluth (seanpj@hawaii.edu)

Organization and Motivation

The C-MORE Professional Development and Organizing Committee (PDOC) organized a series of career and networking workshops focused on producing leaders in the next generation of microbial oceanography. Given the paucity of academic job openings and the overabundance of PhD-level scientists, information about alternative scientific careers is important to disseminate. In the present era of increasing connectedness and communication among the global scientific community, focused training is needed to help early career scientists build and develop their professional scientific networks. Four workshops were funded by a C-MORE EDventures grant to the PDOC to focus on these aspects of professional development.

Career Workshops

Three local workshops were held at selected C-MORE partner institutions (i.e. MIT, UCSC, UH) and showcased a broad range of disciplines relating to marine sciences (e.g. academia, education, government, biotechnology, environmental consulting, funding agencies, business). Fourteen speakers participated with ~65 students, post-docs, and early career scientists (mostly C-MORE affiliates) in attendance. Speakers were invited to give presentations in which they discussed their careers, experiences, and some of the “behind-the-scenes” aspects, like what they didn’t like about their jobs and how well graduate school prepared them for certain duties (e.g. administrative tasks), as well as answer participant-generated questions. As indicated by a survey, participants commented favorably upon the workshops. For example, one participant shared that it “was great to hear from such a diverse group of speakers. It introduced me to jobs I didn’t know existed...”



Networking Workshop at ASLO 2013

A Networking Workshop was held in conjunction with the ASLO 2013 Aquatic Sciences Meeting, the first C-MORE-sponsored event at a national meeting. The goals of this workshop were to 1) enhance the communication and networking skills of early career C-MORE scientists, 2) create a foundation for potential future collaborations among these scientists, and 3) equip these scientists with skills helpful for exploring collaborations outside of C-MORE. Participants discussed research interests and research questions one-on-one and in small groups, allowing interactions among almost all of the participants during the workshop. Twenty-four participants consisted of graduate students, post-docs, and faculty/staff from multiple C-MORE institutions (MIT, WHOI, UCSC, MBARI, and UH). A survey indicated that the networking workshop was well received. One participant commented, “I have to say that I was expecting this workshop to be less productive, as other networking workshops normally are. However, I am leaving with a very great sensation of having a very productive day and have built potential future collaborations.”





Highlight

Bacterioplankton means and extremes in the surface to bathypelagic zones of the North Pacific subtropical gyre

Submitted by: Darin H. Hayakawa, Alexander Eiler, Matthew J. Church, David M. Karl, and Michael S. Rappé

The structure and dynamics of marine bacterioplankton communities are fundamental properties of seawater environments that impact a variety of system services, including the biogeochemical cycling of all major nutrients. In this study, a combination of 16S rRNA gene-based approaches including massively parallel pyrotag sequencing of the V6 hypervariable region were used to characterize the structure of bacterioplankton communities from surface waters to a depth of 4000 m in the water column of a representative station in the oligotrophic North Pacific subtropical gyre monthly over a 4-year period. Genomic DNA from 359 discrete bacterioplankton samples were acquired from November 2004 to January 2009.

Distinct stratified communities were observed (Figure 26), with a major separation in communities around the euphotic boundary. These communities were dominated by only a small number of specific taxa that displayed very little temporal variation. Weak seasonality was observed only within surface bacterial communities that coincided with the regular fluctuation of rare phytoplankton. These findings define the mean state in the taxonomic composition of stratified North Pacific subtropical gyre bacterioplankton communities, and can act as a baseline for monitoring future change in the anthropogen.

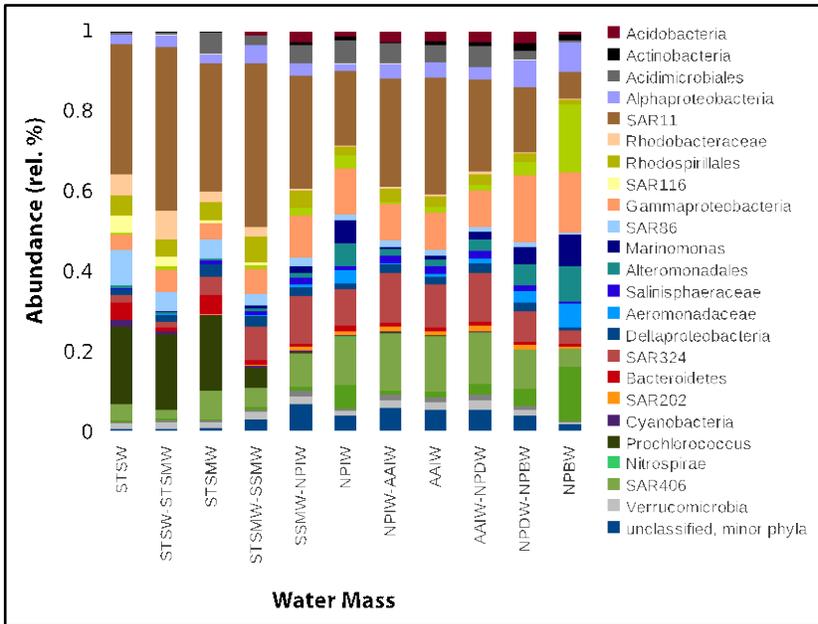


Figure 26. Mean bacterioplankton community structure throughout the water column at Station ALOHA, binned by water mass. STSW – subtropical surface water; STSMW - subtropical salinity maximum water; SSMW - shallow salinity minimum water; NPIW – North Pacific intermediate water; AAIW – Antarctic intermediate water; NPDW – North Pacific deep water; NPBW – North Pacific bottom water.



Highlight

Nutrient limitation of the utilization of dissolved organic matter by heterotrophic bacteria at station ALOHA

Submitted by: Sandra Martinez-Garcia for the Karl lab

Bacterioplankton communities play an essential role in the flow of energy and nutrients through planktonic food webs. Overall, more than 50% of the organic carbon synthesized by marine primary producers is channelled through heterotrophic bacteria. Multiyear time series analysis at station ALOHA suggest a net accumulation of DOC over the past 20 years and also an annual pattern of increased accumulation during summertime.



HOE-DYLAN cruises were performed on board RV Kilo Moana

During HOE-DYLAN 9 C-MORE Post-docs Sandra Martinez-Garcia and Daniela del Valle performed a series of incubation experiments using natural microbial communities from station ALOHA in order to investigate the utilization of organic matter by heterotrophic bacteria.

Four microcosm (4.5 L) nutrient addition experiments were carried out with additions of ammonium (N), phosphate (P) and glucose (C) in different combinations. A variety of bio-

logical variables were monitored during the experiments: heterotrophic bacteria abundance, respiration and leucine uptake. No response was observed after C or C+P additions. However positive responses were observed after N+P and stronger positive responses were found after C+N and C+N+P (Fig. 27). The results obtained demonstrated a primary limitation of heterotrophic bacteria by N and a secondary limitation by organic carbon. The addition of phosphorus did not promote any changes in heterotrophic bacteria abundance.

Future research will investigate the role of different microbial communities, predation pressure and natural DOM availability.

Heterotrophic bacteria abundance response to nutrient additions

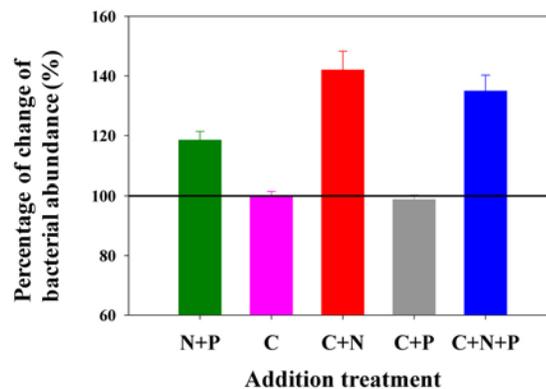


Figure 27. Average response of heterotrophic bacterial abundance to nutrient additions, represented as the percentage change of treatments compared to controls.

Dissolved organic matter (DOM) availability to heterotrophic bacteria at station ALOHA

Submitted by: Sandra Martinez-Garcia for the Karl and Repeta lab teams

Several factors have been proposed to control the utilization of DOM by heterotrophic bacteria: microbial community structure, limitation by inorganic nutrients and also the composition of the DOM. In a parallel investigation on inorganic nutrient limitation of utilization of DOM by heterotrophic bacteria CMORE post-doctoral researcher Sandra Martinez-Garcia reported a primary limitation of heterotrophic bacteria by nitrogen and a secondary limitation by organic carbon.



Set of filtration capsules used for the preparation of the seawater cultures.

During HOE-Dylan 9 Sandra Martinez-Garcia in a collaboration with Daniela del Valle and Daniel Repeta performed a series of long time incubation (25 days) experiments using seawater cultures of natural heterotrophic bacteria populations from station ALOHA in order to investigate the availability of natural occurring DOM. The experimental design included mixture of surface seawater (a 0.2 μm -filtrate from 25 m) with heterotrophic bacteria inoculums (0.65 μm -filtrate) from 25 and 125 m in a dilution factor of 9 to 1. Addition treatments included ammonium (N), phosphate (P), glucose and natural DOM concentrated from ALOHA surface waters. Several biological variables were monitored during the experiments: heterotrophic bacteria abundance, respiration and leucine uptake. Heterotrophic bacteria responded quickly to the addition of organic plus inorganic nutrients (Glucose+N+P and DOM+N+P). Smaller positive responses were found when organic matter was added alone (Glucose and DOM treatments), suggesting inorganic nutrient limitation. Bacterial cell numbers also increased in the negative control (dilution only), suggesting a probable important role of predation. Glucose and DOM yielded similar responses suggesting that natural DOM is bioavailable for these populations (Fig.28).

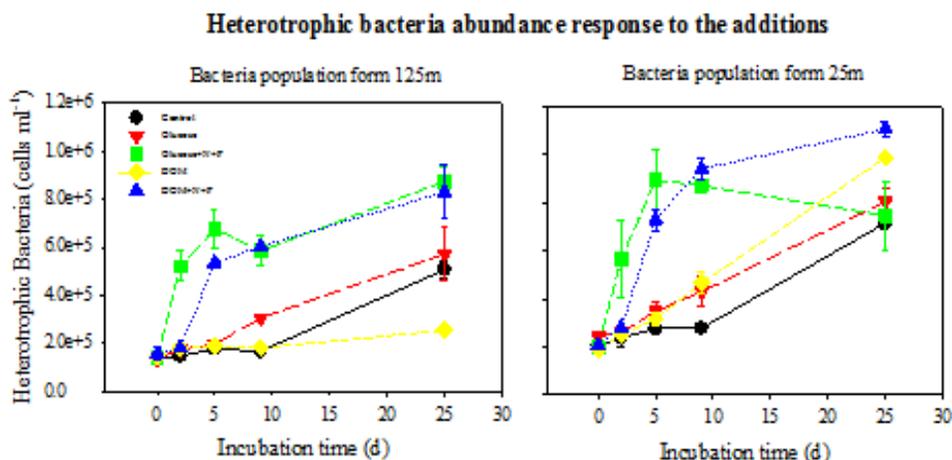


Figure 28. Time course of the mean heterotrophic bacteria abundance (cells/ml) in experiments with heterotrophic bacteria from 125m and 25m.