Seasonality in Ocean Microbial Communities

Stephen J. Giovannoni and Kevin L. Vergin

Science **335**, 671 (2012);
DOI: 10.1126/science.1198078

Seasonality in Ocean Microbial Communities

Stephen J. Giovannoni* and Kevin L. Vergin

Ocean warming occurs every year in seasonal cycles that can help us to understand long-term responses of plankton to climate change. Rhythmic seasonal patterns of microbial community turnover are revealed when high-resolution measurements of microbial plankton diversity are applied to samples collected in lengthy time series. Seasonal cycles in microbial plankton are complex, but the expansion of fixed ocean stations monitoring long-term change and the development of automated instrumentation are providing the time-series data needed to understand how these cycles vary across broad geographical scales. By accumulating data and using predictive modeling, we gain insights into changes that will occur as the ocean surface continues to warm and as the extent and duration of ocean stratification increase. These developments will enable marine scientists to predict changes in geochemical cycles mediated by microbial communities and to gauge their broader impacts.

Seasonal dynamics in plankton communities emerge clearly in satellite observations of ocean color (*1*). At the largest scales, seasonal pulses of ocean surface chlorophyll can be discerned that are driven by dynamic, geographically variable, hydrographic processes that respond to day length. The magnitude of seasonality in the biosphere is so great that long-term measurements of atmospheric CO_2 reveal a rhythmic yearly imbalance in the ratio of carbon fixed by photosynthesis to carbon released by respiration. This ratio oscillates as carbon stored as plant biomass during the Northern Hemisphere summer is released again by respiration during autumn and winter.

Although marine microbial plankton contribute nearly half of gross yearly global photosynthesis, their contribution to the seasonal imbalance in the carbon cycle revealed by atmospheric CO_2 fluctuations is much less than that of land plants. This is because in the oceans, organic carbon production by phytoplankton is tightly coupled with its consumption by chemotrophic bacterioplankton, archaeoplankton, and protists (*2*). The catalysts of the ocean biological carbon cycle are dynamic communities of short-lived cells. Land plant biomass is about 600 Pg C (1 Pg = 10^{15} g), with turnover times of 15 years, whereas the biomass of marine phytoplankton is only about 2 Pg C, with a turnover time of 6 days.

Interest in the seasonality of ocean microbial communities overwhelmingly stems from the relationship between seasonality and global change. Disregarding complex hydrographic processes and weather, the main effect of warming on the oceans is to increase water column stratification (*3*), which occurs to some extent each year in temperate and subtropical seas as the ocean surface stratifies during the summer. Thus, studying seasonality can provide direct insights into how warming on broad scales can affect ocean microbial community functions. Although chlorophyll stocks and photosynthesis rates top the list of biological properties that are responsive to warming and stratification, concerns about rising atmospheric CO_2 have focused interest on the slight annual imbalance between photosynthesis and heterotrophy. This imbalance leads to net removal of atmospheric CO_2 and its long-term storage in the ocean (*4*). Biologically mediated ocean processes that remove carbon from the atmosphere are complex; they include sinking particulate organic matter (POM) and carbonates of biogenic origin, as well as the transport of dissolved organic matter from the upper euphotic zone (UEZ) into the upper mesopelagic (UMP) by turbulent mixing (*5*). One mechanism of carbon removal, sometimes referred to as "the microbial carbon pump," is the conversion of dissolved organic matter (DOM) to compounds that are recalcitrant to microbial oxidation (RDOM) and accumulate in the ocean (*6*, *7*). RDOM is a heterogeneous mixture that includes compounds, such as D–amino acids and lipopolysaccharides, that are found in microbial cell walls and membranes. The most abundant constituent of RDOM to be structurally characterized is carboxyl-rich alicyclic molecules (CRAM), a heterogeneous class of compounds that bear some structural similarities to terpenoids and may be partial oxidation products of sterols and hopanoids (*8*).

Single-celled microorganisms—the microbial plankton—are the main agents of ocean geochemical cycles. Dynamically changing communities of these organisms reduce and oxidize carbon, nitrogen, and sulfur. Ecological studies have used genetic markers for microorganisms to identify patterns of vertical stratification, latitudinal distributions, and variation with changing seasons. Powerful evidence of seasonality emerged from some ocean sites when genetic markers for microbial plankton diversity were used in oceanographic time series (*9*). One of the chief impediments to deciphering these communities is the difficulty of culturing their members, but this is being partially overcome by the development of methods for reconstructing genomes from fragments of community DNA or single cells taken from the environment (*10*–*12*).

Seasonality Is an Important Theme in Ocean Surface Ecology

Spring blooms of phytoplankton at mid- and high latitudes were the first manifestation of microbial plankton seasonality to be recognized, studied, and modeled (*13*). Several theories have been proposed to describe the physical, chemical, and biological interactions controlling the timing of vernal (spring) blooms of phytoplankton in seasonal seas. Sverdrup's critical depth hypothesis broadly serves as the conceptual foundation for understanding seasonal phytoplankton blooms. It predicts that blooms begin when the mixed layer shoals to the depth at which net phytoplankton growth exceeds net losses (*13*). An allied concept, the dilution-recoupling hypothesis, predicts the acceleration of phytoplankton growth in midwinter as mixing and dilution make grazing by protists less efficient (*14*).

Annual events at the Bermuda Atlantic Time-series Study (BATS) site in the western Sargasso Sea illustrate the principles of Sverdrup's critical depth hypothesis and the dilution-recoupling hypothesis (Fig. 1A). During the winter period of deep mixing, dilution of phytoplankton populations means less light, lower phytoplankton cell densities, and probably less efficient predation. Phytoplankton populations that are in the mixed layer as it shoals are exposed to higher light intensities, leading to pronounced phytoplankton blooms in the spring at higher latitudes. At lower latitudes, the picture is complicated by longer winter days and lower average nutrient (N and P) availability, which lead to increases in phytoplankton growth rates as soon as mixing moves N and P from the mesopelagic to the euphotic zone. The contrast between the critical depth hypothesis and the dilution-recoupling hypothesis illustrates two conceptual issues that broadly influence thinking about microbial plankton ecology: (i) To what extent are the compositions of microbial plankton communities determined by chemical and physical factors, such as macronutrient (N and P) flux and light, as opposed to being controlled by direct biotic interactions, such as predation by grazing protists and viruses? (ii) How do these processes vary between different ocean sites, or as climate change alters conditions?

The details of phytoplankton seasonality are continuously revealed as methods develop for monitoring populations of individual phytoplankton species. Flow cytometry studies of phytoplankton population dynamics in monthly samples at two long-term sites, the Hawaii Ocean Time-series (HOT; Fig. 1B), in the North Pacific subtropical gyre, and BATS (Fig. 1A), show that the unicellular cyanobacterium *Prochlorococcus* domi-

Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA.

*To whom correspondence should be addressed. E-mail: steve.giovannoni@oregonstate.edu

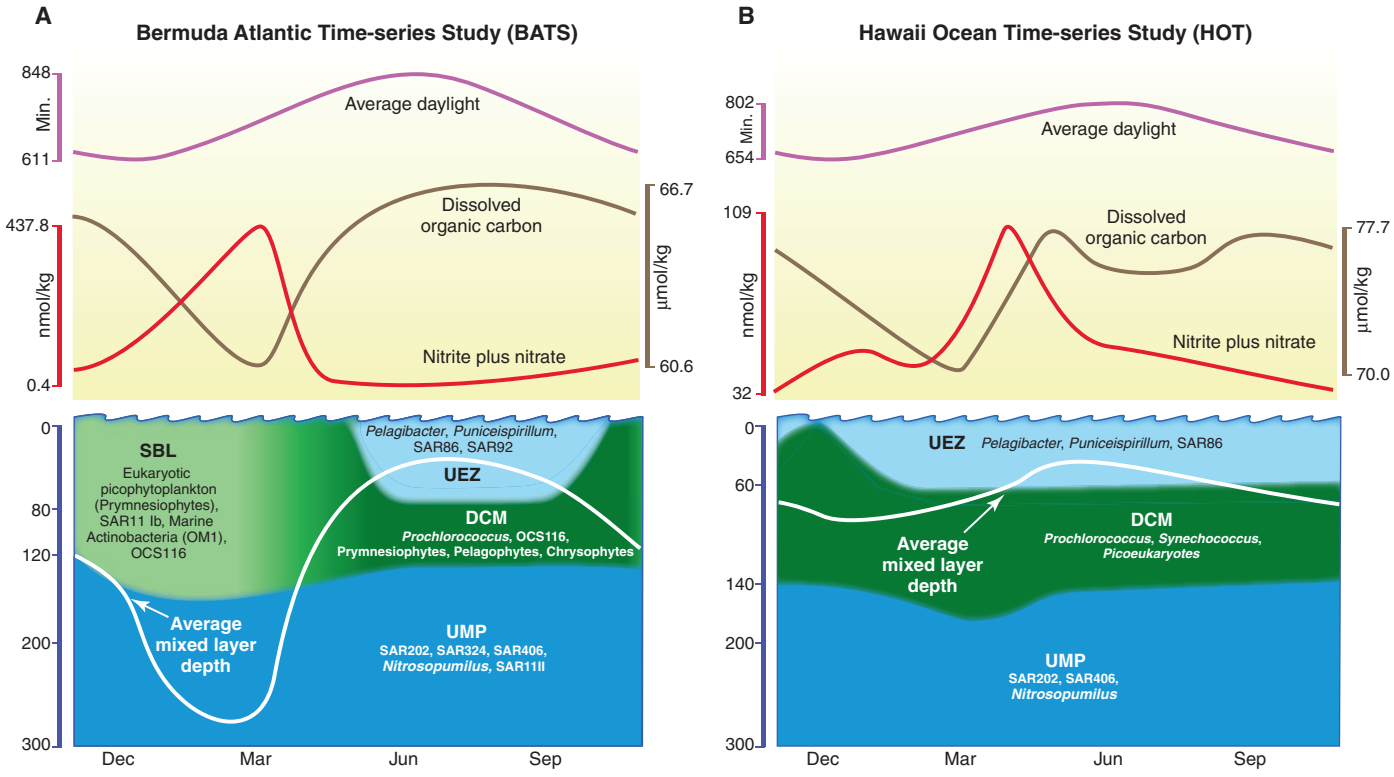

Fig. 1. Annual patterns of microbial community change in the surface layer (0 to 300 m) at BATS and HOT. (**A**) Four microbial communities have been resolved by their phylogenetic compositions. At BATS the euphotic zone community alternates between one dominated by eukaryotic phytoplankton in the winter and spring [spring bloom (SBL); light green] and a stratified pair of communities dominated by cyanobacteria in the summer and early autumn (see Fig. 2). Many important environmental variables change with these seasonal transitions: average monthly daylight (purple line), dissolved organic carbon (brown line), and nitrite plus nitrate (red line). At BATS, the SBL community begins developing late in autumn with the deepening of the mixed layer and intensifies in the spring as the mixed layer becomes shallow, exposing cells to higher incident light. The dilution-recoupling hypothesis predicts increasing phytoplankton populations due to relaxed predation during the early phase of the SBL. The critical depth hypothesis, which was developed to model events at higher latitudes, predicts phytoplankton blooms as sharply rising incident radiation causes increases in phytoplankton growth rates. Prymnesiophytes, marine Actinobacteria (OM1), OCS116, and SAR11 subclade Ib dominate the early SBL, but later, as the mixed layer shoals, other eukaryotic phytoplankton populations increase. As water column stratification increases and nutrients sink from the upper euphotic zone (UEZ; light blue), an oligotrophic community forms in the mixed layer that is dominated by *Pelagibacter*, *Puniceispirillum*, SAR86, and SAR92. During this period, a fourth community [deep chlorophyll maximum (DCM); dark green] forms near the chemocline. *Prochlorococcus* and the α-proteobacterium OCS116 (Fig. 2E) are characteristic members of the DCM community. The upper mesopelagic community (UMP; dark blue) is dominated by the distinct, early branching lineages SAR202, SAR324, SAR406, SAR11 subclade II, and *Nitrosopumilus* sp. (**B**) Less seasonal change in stratification of the water column at HOT results in less pronounced blooms of phytoplankton and more stable communities throughout the year. These communities resemble those found during summer stratification at BATS (*62*, *63*). There are other differences between BATS and HOT, including higher phosphate in the North Pacific. Values for dissolved organic carbon (brown line) and nitrate plus nitrite (red line) were averaged over the mixed layer and plotted on the same scale to emphasize their relative relationships. White lines indicate mixed-layer depth. Data were taken from the HOT-DOGS website (*64*) and the BATS website (*65*) and included years 2001–2009 (HOT) and 1996–2003 (BATS) averaged over the mixed-layer depth at station 2 (HOT).

nates phytoplankton in the summer and autumn when the water column is most stratified (*15*). The larger cyanobacterium *Synechococcus* is more abundant in the winter at HOT and in the spring at BATS, and is nearer to the surface than *Prochlorococcus* (*16*). Photosynthetic picoeukaryotes [cell diameter <3 µm (*16*); 2 to 4 µm (*15*)] are most common in the spring, but at BATS the spring bloom is more pronounced, probably because of the deeper mixed layer established during winter (100 m at HOT versus 250 m at BATS) (*15*, *16*). Note that measurements of cell size obtained from light scattering enabled these studies to calculate carbon biomass; often this information is not available from studies that use environmental gene frequencies as proxies for cells. Similar patterns were observed in the Gulf of Aqaba, a deep, oligotrophic region of the Red Sea, where Cryptophyceae and Chlorophyceae were abundant in winter and *Prochlorococcus* was abundant in summer (*17*).

Phytoplankton are better understood than their nonphotosynthetic counterparts because pigments provide a means to track them, but seasonal patterns of phytoplankton populations are still only partly known for several reasons, including ongoing discoveries of new taxa. For example, a previously unrecognized group of eukaryotic phytoplankton that branches deeply in evolutionary trees, close to haptophytes, was recently described and named Rappemonads (*18*). As with nonphotosynthetic species, genetic data and time series are bringing more resolution to the study of phytoplankton. A recent analysis of BATS data relied on plastid 16*S* ribosomal RNA (rRNA) genes as genetic markers for eukaryotic phytoplankton. It revealed previously unseen seasonal patterns among photosynthetic picoeukaryotes, including a prominent bloom of prasinophytes, which are unicellular organisms related to green plants, in the winter, during mixing of the water column (Fig. 1) (*19*).

Bacterioplankton and archaeoplankton are largely responsible for the oxidative side of the carbon cycle, and also for oxidizing reduced forms of N and S of biotic origin. Time-series measurements of rRNA gene markers have led to remarkable progress in identifying annual patterns in their distributions. The wide use of rRNA gene markers for uncultured microbial diversity in the 1990s identified many new lineages of nonphotosynthetic microbial plankton and provided the

first evidence of patterns of microbial community variation between the open and coastal ocean, between the euphotic and mesopelagic zones, and with latitude (*20*). Some studies focused on specific populations—for example, in Antarctic coastal waters (*21*)—and fluorescence in situ hybridization (FISH) detected a shift from lower archaeal abundances in the summer to higher abundances in the late winter. Similarly, in the North Sea and southeast coastal waters of the United States (*22*), fewer *Roseobacter* clade cells were observed in the winter than during the summer.

Two large studies have used discriminant function analysis (DFA) and time-series analysis (TSA) to examine annual patterns in surface bacterial communities collected monthly for 4.5 years at the San Pedro Ocean Time Series (SPOTS) study site, in the coastal waters of southern California (*23*), and a 6-year study in the western English Channel (*24*). These studies revealed monthly turnover in community composition that recurred on an annual basis with remarkable precision, indicating that community composition is indeed determined by seasonal factors. Smaller studies have reached similar general conclusions; for example, a 2-year study off the New Jersey coast detected seasonal patterns of bacterioplankton communities with distinct clusters of samples separated by season for each year and a distinction between summer and winter communities evident in the entire data set (*25*).

Long ocean time series at BATS and HOT are providing insights into links between ocean conditions and microbial communities. Over the entire BATS data set, statistical ordination resolved three microbial communities—spring bloom (SBL), summertime UEZ, and UMP ("twilight zone")—and provided evidence of a fourth community found near the chemocline in the vicinity of the deep chlorophyll maximum (DCM) (Fig. 1A) (*26*). At BATS these communities, and their relationships to important environmental variables, change markedly during the annual transition from spring to summer conditions, as sinking POM carries nutrients such as N and P out of the UEZ (Fig. 1A). The phytoplankton community shifts from eukaryotic to prokaryotic dominance, and a suite of organisms adapted to the extremely low-nutrient, high-light conditions of the summer surface takes over (Fig. 2, A and B). Evidence from HOT indicates stable UEZ and UMP communities throughout the year and lesser seasonal expansions of phytoplankton, mainly associated with the DCM (*27*).

During stratified periods, the UEZ of the tropics and mid-latitudes is an extreme, high-light, low-nutrient environment. Studies at BATS and HOT (*26*) show that the UEZ community is dominated by the heterotrophic proteobacteria *Candidatus* Pelagibacter (SAR11 subclade Ia), *Candidatus* Puniceispirillum (SAR116; Fig. 2E), SAR86 (Fig. 2D), SAR92, and the cyanobacteria *Synechococcus* and *Prochlorococcus* (*15*, *16*). Genome-enabled research, including metaproteomics, has shown that *Pelagibacter* competes for labile DOM (LDOM) compounds such as amino acids, organic acids, polyamines, osmolytes (such as glycine betaine, dimethylsulfoniopropionate, and taurine), and one-carbon (C1) compounds (such as formaldehyde, methanol, and methylamine) (*28*). C1 metabolism potentially enables these organisms to demethylate osmolytes, as well as to use C1 compounds that are produced abiotically from DOM by photochemical reactions (*29*). The genome of the SAR116 isolate *Puniceispirillum marinum* indicates it can also oxidize a variety of LDOM compounds including C1 compounds (*30*). Unlike *Pelagibacter*, *Puniceispirillum* is motile, an adaptation that may give it a role in the geochemically important process of POM colonization and oxidation. *Pelagibacter*, *Puniceispirillum*, and SAR92 all have light-dependent proteorhodopsin proton pumps that appear to benefit cells by augmenting respiration as a source of energy when oxidizable substrates are scarce.

A biochemical perspective on the oxidation of semilabile DOM (SLDOM) exported from the euphotic zone is slowly emerging from the studies of twilight-zone microbial communities. At BATS and HOT, these communities are similar in composition and relatively stable throughout the year (Fig. 1, A and B, and Fig. 2F) (*26*). Fluctuations in the twilight-zone microbial community at BATS have been observed during periods of organic matter export from the UEZ (*31*). Twilight-zone taxa are strikingly more diverse in their evolutionary origins than the UEZ community. The SAR202 clade of Chloroflexi (Fig. 2F), the SAR324 clade of δ-Proteobacteria, and the ammonia-oxidizing archaean *Nitrosopumilus* occur throughout the aphotic zone (*32*). Neither SAR202 nor SAR324 have been cultured, but several partial genomes amplified from single SAR324 cells have been reported recently (*8*, *33*). In one study, ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) and sulfur oxidation genes were identified, suggesting a chemolithoauto-

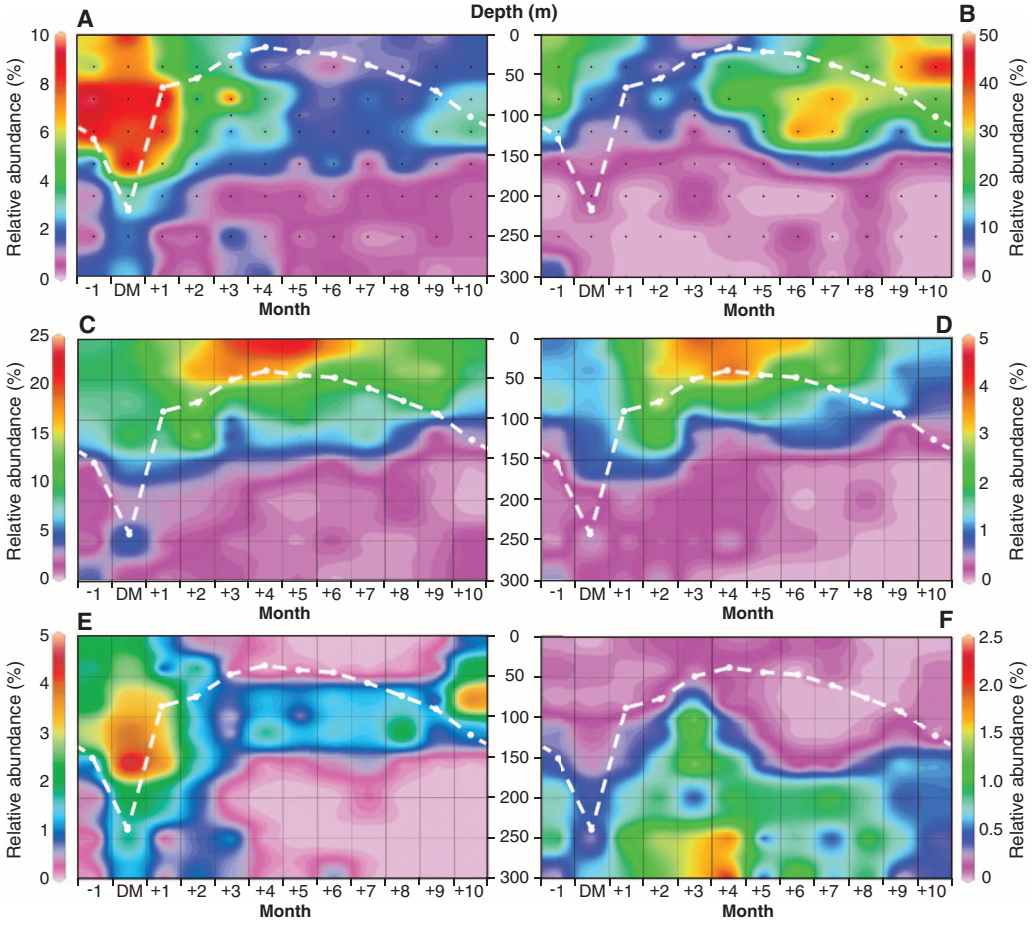

Fig. 2. (A to F) Ocean Data View (ODV) plots indicating average temporal and spatial distributions of microbial plankton at BATS. The graphs show relative abundance of (A) plastid 16*S* rRNA genes, (B) Cyanobacteria (*Prochlorococcus* and *Synechococcus*), (C) *Puniceispirillum*, (D) SAR86, (E) OCS116, and (F) SAR202. [Modified from (*19*, *26*)]

trophic lifestyle (*8*). Another SAR324 genome assembly contained 18 putative phytanoyl dioxygenases, which are predicted to catalyze the degradation of the lipid chain on chlorophyll a (*33*). The presence of motility genes in SAR324 genomes and direct microscopic observations suggest that these organisms swim toward and attach to sinking POM (*6*, *8*).

Microbial communities are known to vary across the ocean surface, differing between coastal and open ocean regions, with latitude, and in regions where mesopelagic waters upwell to the surface. Therefore, it is important to ask whether findings from a few well-studied sites can be extrapolated to broad geographical scales. Mounting evidence suggests that some themes of microbial community organization apply in different oceans: Most major microbial plankton groups are cosmopolitan, and their vertical relationships are similar across broad latitudinal transects (*34*). But there is clearly a complex relationship among latitude, upwelling, and seasonality that remains incompletely explored. A cruise in the boreal spring Atlantic from South Africa to the United Kingdom counted cells with FISH and found latitudinal variation in many of the most abundant groups of marine bacteria (SAR11, *Prochlorococcus*, Bacteroidetes, γ-Proteobacteria, and SAR202) (*34*). Other studies, using samples collected in all months across the world ocean, described latitudinal variation discerned through 16*S* rRNA gene phylogeny (*35*) and DNA fingerprinting (*20*).

Lessons from SAR11

The SAR11 group of α-Proteobacteria (Pelagibacteraceae) are the dominant bacterial group in the oceans and have emerged as major players in all time-series studies (*24*, *31*, *34*, *36*–*39*). They illustrate three important problems that must be addressed before a global view of ocean microbial community seasonality can fully emerge: (i) the need for a standard system of measuring and describing microbial diversity, (ii) the need to attach ecologically meaningful functions to units of microbial diversity, and (iii) the need for more sampling. Molecular phylogenies indicate that the SAR11 clade evolved long ago and diverged into several major subclades [i.e., Ia (*Pelagibacter*), Ib, II, III, and IV] and probably into many minor ecotypes (*5*, *19*, *31*). "Ecotypes" are genetic variants that can be distinguished phylogenetically and by their differing spatiotemporal distributions in environments. The correlation between phylogenetic branching and spatiotemporal distributions of SAR11 is evidence that early in their evolution the marine environment was partitioned among SAR11 specialists (*37*, *39*). At BATS, SAR11 subclade Ib blooms in the spring mixed layer, coincident with phytoplankton, followed by subclade Ia (*Pelagibacter*) in the summer. Subclade II blooms with the remineralization of dissolved organic carbon (DOC) in the mesopelagic each spring. Some of these relationships emerged in early studies reliant on a few depth profiles, but others were not revealed until more extensive time-series data were used to examine seasonal change in spatial relationships.

There is no agreement in the field about schemes for classifying SAR11 or other taxa. Most workers use a sequence divergence threshold (*10*, *40*), but some emphasize phylogenetic structure (*26*, *39*). An unfortunate consequence of this is that it has been difficult to compare the data obtained in different studies to identify common patterns in the distribution of SAR11 diversity. But by summing all SAR11 into a single unit of diversity, some comparisons can be made. At HOT (*38*) and in the western English Channel (*24*), their relative abundance peaks in the winter, whereas at BATS, both relative abundance and cell numbers peak in the summer (*26*, *36*).

Oceanographers are concerned with geochemistry at broad scales, so microbial diversity classification systems also must be attached to the functional properties of cells to achieve their potential usefulness. The SAR11 subclades referred to above can diverge at 16*S* rRNA loci by more than 15%, which suggests multiple species and potentially complex variation in function. One theory is that ancient events represented by deep branches in phylogenomic trees correspond to evolutionary changes that retain their ecological impacts today. For example, this is true of the cyanobacteria (Cyanophyta), a lineage that includes all cells and organelles that photochemically split water to produce oxygen. The correlation between deep branches in SAR11 phylogenetic trees and spatiotemporal partitioning of the BATS environment also supports this theory. Alternatively, frequent lateral gene transfer provides a path for organisms to evolve into new habitats, potentially scrambling the ecological meaning of phylogenetic origins. Ample evidence indicates that this does occur; for example, phosphate acquisition genes are highly variable, and both *Prochlorococcus* and SAR11 have more phosphate acquisition genes at BATS than at HOT, presumably as a result of selection caused by less availability of P in the North Atlantic (*41*). Comparative genomics is expanding rapidly because of inexpensive next-generation sequencing, and we can expect powerful new studies that aim to identify genomic determinants of function that are associated with deep phylogenetic branches and distinguish them from variable genomic properties. It will be particularly interesting to apply this approach to the UMP community, which is home to anciently diverged clades of δ-Proteobacteria (SAR324), Chloroflexi (SAR202), Fibrobacteres/Acidobacteria (SAR406), and Archaea that directly or indirectly must play a role in remineralizing DOM.

Connecting Seasonal Communities to Geochemistry

Despite caveats related to variability between ocean provinces, observations of ocean surface microbial communities are making it possible to predict changes that are likely to occur as the oceans warm. Prolonged stratification means diminished seasonality, the spread of oligotrophic ocean conditions, and an expansion of the UEZ communities like those at HOT and BATS. The impact of such changes on phytoplankton productivity has received broad attention, but much less is understood about how seasonal changes in populations of nonphotosynthetic organisms might affect carbon sequestration processes such as the biological carbon pump and the microbial carbon pump, both of which are focused on the small fraction of organic matter that escapes oxidation by a gauntlet of microorganisms.

Despite increasing resolution of microbial community dynamics, as yet little information is available that connects community composition to variation in geochemical processes. One of the clearest examples of geochemical specialization is the ammonia-oxidizing archaean *Nitrosopumilus marinus*. Summer blooms of *Nitrosopumilus* coincident with maximal rates of ammonia oxidation to nitrate have been detected by FISH and quantitative polymerase chain reaction (qPCR) measurements of the *Nitrosopumilus* ammonia monooxygenase gene (*amoA*) taken biweekly at a North Sea coastal site (*42*). Although such findings help us to understand the biology behind the nitrogen cycle, so far they have not been of much use in geochemical models.

Examples that tie variations in microbial populations to seasonal geochemical data from the environment, like that of *Nitrosopumilus*, remain rare. One of the biggest challenges is the carbon cycle. For example, at BATS, DOC accumulates annually in the UEZ in the summer, subsequently oxidizing when winter storms mix surface water into the UMP (*43*). DOC export by mixing is important; it accounts for about half of oxygen consumption in the UMP. Why is a fraction of DOC refractory to oxidation at the ocean surface in the summer, but labile when exported to the dark, nutrient-rich environment of the UMP? DOM composition has been shown to change with depth, correlating with shifts in community structure (*31*, *44*). Is it the unique properties of organisms residing in the UMP, the chemical properties of the environment, or both that cause the refractory DOC to become labile? A BATS study to address these questions found that populations of the α-proteobacterium OCS116 (Fig. 2E), SAR11 subclade II, and the marine Actinobacteria (OM1) increase in the mesopelagic after spring mixing (*31*). Although this study showed that a unique mesopelagic microbial community responds annually to DOC export, it has not yet been possible—even with the availability of metagenomic data—to identify specific biochemical mechanisms that explain the change in susceptibility of DOC to oxidation when it is exported.

So far, we have focused on the interplay between seasonally changing microbial communities and geochemical cycles, but an alternative perspective is to consider, at the level of populations of organisms, how the environment selects. Ecologists refer to ordered patterns in community composition as nonrandom assembly. One cause of nonrandom assembly is environmental

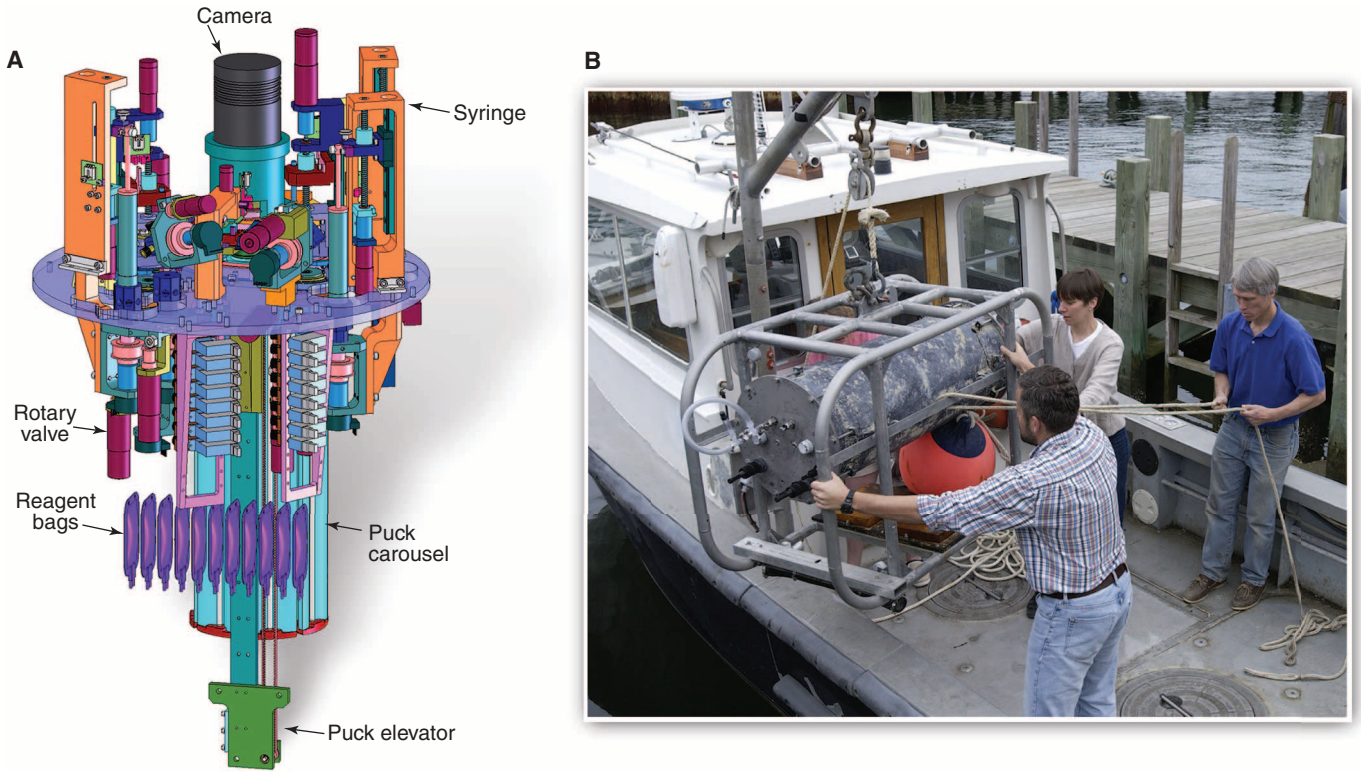

Fig. 3. (**A** and **B**) Autonomous devices, such as the Environmental Sample Processor [ESP; schematic illustration shown in (A)] and the FlowCytobot [deployment shown in (B)], were designed for in situ monitoring of microbial plankton. The ESP can conduct qPCR assays; the FlowCytobot can monitor phytoplankton by flow cytometry. In the future, devices like these may replace monthly cruises to ocean sites for the purpose of monitoring patterns of change in microbial communities. In a recent deployment, the ESP was used to monitor *Nitrosopumilus*, SAR11, and marine cyanobacteria 16*S* rRNA molecules. In parallel, qPCR was used to monitor the 16*S* rRNA genes from SAR11, *Nitrosopumilus*, and the large subunit RuBisCO gene (*rbc*L) from marine *Synechococcus* sp. during a 28-day deployment in Monterey Bay (*55*). [Images courtesy of Monterey Bay Aquarium Research Institute (ESP) and Tom Kleindinst, Woods Hole Oceanographic Institution (FlowCytobot)]

filtering—the selection pressure exerted on populations by abiotic factors. Nitrogen, phosphorus, and (to a lesser extent) iron have dominated oceanographic thinking about the environmental control of microbial populations. However, unusual nutrient requirements for trace metals, vitamins, and other organic growth factors, as well as allelopathic compounds that inhibit competitors and predators, are increasingly being studied to understand ocean microbial population dynamics (*45*, *46*). Growing evidence suggests that complex biotic interactions play a major role in shaping microbial community dynamics. Such interactions can be positive or negative; for example, the production of growth factors by one species, stimulating the growth of another, or growth inhibition due to predation, siderophore piracy, and other types of interference.

Patterns of variation caused by biological interactions were studied in the large SPOTS data set with methods designed to detect patterns in correlation tables (*37*). A follow-up study at SPOTS that included viruses, archaea, and protists provided stronger evidence that ocean microbial communities are highly interconnected ecological networks (*47*). Using methods familiar to systems biologists, SPOTS investigators detected a web of potential interactions between organisms, including interactions with abiotic factors. The DFA and TSA analyses include statistical support for interactions to examine delays of up to 6 months to find potential time-lagged interactions. Observing highly correlated community behavior is an important step toward understanding communities, but the process is far from complete and awaits work that will experimentally confirm patterns of connectivity and explain them with mechanisms. Very few examples of biotic interactions between microbial plankton can be demonstrated using live organisms, providing few opportunities to study underlying mechanisms experimentally (*46*). Validation is important because so many environmental factors in the ocean are functionally linked to seasonal changes in incident light. Hence, the concerted response of communities may include many organisms with unrelated biology that covary simply because abiotic factors covary, rather than because they are tied together by a specific interaction.

Nonetheless, organisms that have evolved in an environment where abiotic factors provide a strong driving rhythm do have the opportunity to coevolve. Insights emerged from the genome streamlining theory, which explains the small size of genomes in some abundant groups of marine bacteria, such as *Prochlorococcus* and *Pelagibacter* (*48*), as an adaptation that enables these cells to use limiting resources efficiently in oligotrophic regions of the oceans. For example, unusual requirements for reduced sulfur compounds (*49*) and glycine (*50*) have been described in *Pelagibacter*, indicating that during the evolution of its small genome, the biosynthesis of some essential compounds was "outsourced" to other members of the microbial community. Complex nutritional requirements caused by genome reduction can lead to increased food web connectivity and less versatility in cells, which could explain why so many abundant taxa of marine bacteria are challenging to cultivate. The cultivation of other abundant organisms that also have reduced genomes and unusual nutritional requirements will determine how broadly genome streamlining theory can be applied to explaining patterns observed in ocean surface plankton ecology.

Food web connectivity caused by nutritional dependence and other types of interactions has important consequences for predicting ecosystem responses to change (*51*). Microbial plankton communities are sensitive to perturbations, one class of which, referred to as "bottle effects," result in rapid changes in microbial community composition when water is collected and confined (*52*). The stability of microbial plankton communities is directly relevant to a host of environmental concerns, where interest focuses critically on whether environmental functions are maintained in response

to changing inputs. There is some good news, albeit incomplete, on this topic, from the 2011 Gulf of Mexico oil spill disaster, where the microbial plankton community showed an unexpected capacity to convert petroleum to carbon dioxide (*53*).

Outlook

Seasonal patterns of microbial community turnover have emerged prominently in satellite observations of chlorophyll from different ocean provinces, but as yet there is no consensus whether the same patterns occur at different sites, or about the factors that control community composition. Next-generation sequencing technology is playing an important role in the expansion of ocean microbial diversity measurements (*11*, *40*). To identify generalities that apply across systems, researchers will first have to reach a consensus about how to classify microbial diversity. To overcome the obstacles of ship expenses and weather, which impede collection of time-series data, scientists are testing a new generation of instruments that can be left in the ocean to monitor microbial communities in situ (Fig. 3) (*54*, *55*). Oceanographers will be particularly interested in using these data to develop a better understanding of how latitude, weather, pollution, nutrients, pH, and other factors alter the rhythms of microbial communities. Moving from descriptively assessing ocean surface microbial community structure to understanding factors that determine community structure and predicting how communities will respond to changing inputs is the long-range goal of most workers in the field (*9*). The best illustration of this idea is the Darwin Project, a marine ecosystem model that predicts the distribution of *Prochlorococcus* ecotypes according to physiological traits (*56*).

Metagenomics and allied technologies, including single-cell genome amplification (*11*, *30*) and population sorting (*12*), are leading to progress in associating uncultured microorganisms with functions, and a growing list of microbial plankton taxa can be grown and studied in laboratories (*30*). But so far, no comprehensive metagenomic studies of ocean seasonality have been published. Limited by data and methods, studies still have difficulty identifying anciently evolved genome characteristics that might link major branches of microbial plankton evolution with distinct functional roles in the environment (*57*). This goal is important because microbial diversity is dauntingly complex, making it imperative to find principles that simplify.

Highly resolved information about patterns of microbial community turnover has the potential to provide important refinements to models of geochemical cycles. For example, the partitioning of organic carbon among structured microbial communities that vary in seasonal patterns has been linked to the microbial carbon pump (*5*, *31*). But current models rarely take transitions in community structure into account because of insufficient information about the geochemical properties of organisms. One of the impediments to progress is the difficulty of determining geochemical function from genome information alone, without passing through a labor-intensive period of laboratory investigation for each organism. Another is the difficulty of resolving geochemical processes at finer chemical scales—that is, breaking broad chemical classes down to specifics. Current efforts to identify specific components of dissolved organic nitrogen (DON) and dissolved organic phosphorous (DOP) illustrate the challenges. Only recently was it understood that a fraction of DOP is in the form of phosphonates that have C–P bonds and thus are harvested by pathways that differ from those used to assimilate P from typical phosphate esters (*58*). A variety of new tools are emerging that provide more highly resolved chemical information. Some use high-performance liquid chromatography or mass spectroscopy to resolve complex mixtures of molecules at high resolution (*59*), to measure specific molecules with high sensitivity and precision (*60*), or to track the isotopic composition of molecules with either naturally occurring or introduced isotopes (*61*).

Some microbial communities, such as those that dominate the low-nutrient, high-irradiance environment of the euphotic zone in ocean gyres, are more accessible, have been more heavily sampled, and are at a more advanced stage of study. If the methods of systems biology are going to be successfully expanded to encompass communities of interacting microorganisms, it seems likely that early milestones will emerge from further studies of the stratified, upper euphotic zone community. Some potential advances that might require highly integrated approaches include the description of adaptations that determine success in this extreme environment, the refinement of geochemical cycles to finer scales of molecular specificity, and the identification of factors and interactions that control community structure.

Understanding fundamental aspects of microbial plankton biology, such as seasonal processes, may be important to understanding ocean responses to long-term changes, such as global warming, as well as short-term impacts, such as pollution events. It is important not to underestimate the complexity of this problem: A large-scale, long-term effort will be needed to understand seasonality and other dynamic aspects of ocean microbial communities, and to gauge their resilience to environmental perturbations.

References and Notes

1. www.youtube.com/watch?v=sQvYvlonu7Y
2. M. J. Behrenfeld *et al.*, *Nature* **444**, 752 (2006).
3. J. M. Lyman *et al.*, *Nature* **465**, 334 (2010).
4. D. A. Hansell, C. A. Carlson, D. J. Repeta, R. Schlitzer, *Oceanography* **22**, 202 (2009).
5. C. A. Carlson *et al.*, *Deep Sea Res. II* **57**, 1433 (2010).
6. F. Azam, *Science* **280**, 694 (1998).
7. N. Jiao *et al.*, *Nat. Rev. Microbiol.* **8**, 593 (2010).
8. B. K. Swan *et al.*, *Science* **333**, 1296 (2011).
9. D. K. Steinberg *et al.*, *Deep Sea Res. II* **48**, 1405 (2001).
10. D. B. Rusch *et al.*, *PLoS Biol.* **5**, e77 (2007).
11. T. Woyke *et al.*, *PLoS ONE* **4**, e5299 (2009).
12. M. L. Cuvelier *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 14679 (2010).
13. H. U. Sverdrup, *J. Cons. Perm. Int. Explor. Mer.* **18**, 287 (1953).
14. M. J. Behrenfeld, *Ecology* **91**, 977 (2010).
15. M. D. DuRand, R. J. Olson, S. W. Chisholm, *Deep Sea Res. II* **48**, 1983 (2001).
16. L. Campbell, H. B. Liu, H. A. Nolla, D. Vaulot, *Deep Sea Res. I* **44**, 167 (1997).
17. T. Al-Najjar, M. I. Badran, C. Richter, M. Meyerhoefer, U. Sommer, *Hydrobiology* **579**, 69 (2007).
18. E. Kim *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 1496 (2011).
19. A. H. Treusch *et al.*, *ISME J.* (2011).
20. J. A. Fuhrman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 7774 (2008).
21. A. E. Murray *et al.*, *Appl. Environ. Microbiol.* **64**, 2585 (1998).
22. A. Buchan, J. M. González, M. A. Moran, *Appl. Environ. Microbiol.* **71**, 5665 (2005).
23. J. A. Fuhrman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 13104 (2006).
24. J. A. Gilbert *et al.*, *ISME J.* **6**, 298 (2011).
25. J. D. Nelson, S. E. Boehme, C. E. Reimers, R. M. Sherrell, L. J. Kerkhof, *FEMS Microbiol. Ecol.* **65**, 484 (2008).
26. A. H. Treusch *et al.*, *ISME J.* **3**, 1148 (2009).
27. A. Eiler, D. H. Hayakawa, M. S. Rappé, *Front. Microbiol.* **2**, 140 (2011).
28. J. Sun *et al.*, *PLoS ONE* **6**, e23973 (2011).
29. K. Mopper *et al.*, *Nature* **353**, 60 (1991).
30. H.-M. Oh *et al.*, *J. Bacteriol.* **192**, 3240 (2010).
31. R. M. Morris *et al.*, *Limnol. Oceanogr.* **50**, 1687 (2005).
32. E. F. DeLong *et al.*, *Science* **311**, 496 (2006).
33. H. Chitsaz *et al.*, *Nat. Biotechnol.* **29**, 915 (2011).
34. M. Schattenhofer *et al.*, *Environ. Microbiol.* **11**, 2078 (2009).
35. T. Pommier *et al.*, *Mol. Ecol.* **16**, 867 (2007).
36. R. M. Morris *et al.*, *Nature* **420**, 806 (2002).
37. J. A. Fuhrman, J. A. Steele, *Aquat. Microb. Ecol.* **53**, 69 (2008).
38. A. Eiler, D. H. Hayakawa, M. J. Church, D. M. Karl, M. S. Rappé, *Environ. Microbiol.* **11**, 2291 (2009).
39. C. A. Carlson *et al.*, *ISME J.* **3**, 283 (2009).
40. M. L. Sogin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 12115 (2006).
41. M. L. Coleman, S. W. Chisholm, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 18634 (2010).
42. C. Wuchter *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 12317 (2006).
43. C. A. Carlson *et al.*, *Limnol. Oceanogr.* **49**, 1073 (2004).
44. S. J. Goldberg, C. A. Carlson, D. A. Hansell, N. B. Nelson, D. A. Siegel, *Deep Sea Res. I* **56**, 672 (2009).
45. E. M. Bertrand *et al.*, *Limnol. Oceanogr.* **52**, 1079 (2007).
46. A. Vardi *et al.*, *Science* **326**, 861 (2009).
47. J. A. Steele *et al.*, *ISME J.* **5**, 1414 (2011).
48. S. J. Giovannoni *et al.*, *Science* **309**, 1242 (2005).
49. H. J. Tripp *et al.*, *Nature* **452**, 741 (2008).
50. H. J. Tripp *et al.*, *Environ. Microbiol.* **11**, 230 (2009).
51. T. Gross, L. Rudolf, S. A. Levin, U. Dieckmann, *Science* **325**, 747 (2009).
52. B. M. Fuchs, M. V. Zubkov, K. Sahm, P. H. Burkill, R. Amann, *Environ. Microbiol.* **2**, 191 (2000).
53. T. C. Hazen *et al.*, *Science* **330**, 204 (2010).
54. R. J. Olson, H. M. Sosik, *Limnol. Oceanogr. Methods* **5**, 195 (2007).
55. C. M. Preston *et al.*, *PLoS ONE* **6**, e22522 (2011).
56. M. J. Follows, S. Dutkiewicz, S. Grant, S. W. Chisholm, *Science* **315**, 1843 (2007).
57. G. C. Kettler *et al.*, *PLoS Genet.* **3**, e231 (2007).
58. S. T. Dyhrman *et al.*, *Nature* **439**, 68 (2006).
59. B. P. Koch, K. U. Ludwichowski, G. Kattner, T. Dittmar, M. Witt, *Mar. Chem.* **111**, 233 (2008).
60. E. B. Kujawinski *et al.*, *Environ. Sci. Technol.* **45**, 1298 (2011).
61. J. D. Neufeld, M. Wagner, J. C. Murrell, *ISME J.* **1**, 103 (2007).
62. Y. Shi, G. W. Tyson, J. M. Eppley, E. F. DeLong, *ISME J.* **5**, 999 (2011).
63. T. D. Mullins, T. B. Britschgi, R. L. Krest, S. J. Giovannoni, *Limnol. Oceanogr.* **40**, 148 (1995).
64. http://hahana.soest.hawaii.edu/hot/hot_dogs/interface.html
65. http://bats.bios.edu/bats_form_bottle.html

Acknowledgments: We thank M. Behrenfeld, C. Thrash, and K. Halsey for critical comments. Supported by a grant from the Marine Microbiology Initiative of the Gordon and Betty Moore Foundation and NSF Microbial Observatory grants MCB-0237713 and OCE-0802004.

10.1126/science.1198078