MICROBIAL OCEANOGRAPHY

CHALLENGES and OPPORTUNITIES
The specific and primary purposes are to perform research in the sciences and in mathematics, to disseminate the results obtained therefrom, all to benefit mankind.

Cover photo:
False color scanning electron micrograph of picoplankton from the Sargasso Sea. Cell dimensions range from <0.5 μm to >3 μm. Photo credit, Ed Delong. Colorized by Nancy Hulbirt, SOEST, University of Hawaii.

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CHALLENGES and OPPORTUNITIES

The Agouron Institute
INTRODUCTION

With the discovery of recombinant DNA technology in the mid-1970’s, came a tremendous set of new opportunities in biology and chemistry. The Agouron Institute (AI), a non-profit research organization, was formed in 1978 by John Abelson, Melvin Simon, and Joseph Kraut, all then professors at the University of California, San Diego, as a vehicle by which new research frontiers and technologies in biology and chemistry could be investigated in an expeditious manner free from what was perceived then as constraints in the University.

The first scientists working at the Agouron Institute in La Jolla came from the laboratories of the founders. The Institute commenced its activities with a small grant from the Office of Naval Research on the marine fouling problem — the beginning of a long-standing program in environmental microbiology. By 1982 the research program had expanded considerably and had obtained additional funding from the NSF and the NIH. A group of molecular biologists and chemists were collaborating to exploit new technology in which synthetic oligonucleotides were used to direct specific mutations in genes. A crystallography group had been formed and they were collaborating with the molecular biologists to study the properties of the altered proteins. These were among the very first applications of the new technology to form what is now the very large field of protein engineering. In addition a substantial group of computational chemists had formed which had the goal of predicting protein structure and the specificity of protein ligand interactions.

Early successes in the protein engineering and computational groups led to the idea that this technology could be used in the rational design of ligands that interact with and inhibit the activity of proteins, i.e rational drug design. In 1984 a commercial entity, Agouron Pharmaceuticals, was formed to exploit the potential of rational drug design. A commercial cooperation agreement was instituted between Agouron Pharmaceuticals and the Institute and in return the Institute received a major portion of the founding stock. Many of the molecular biologists, chemists and structural biologists shifted to the company and within a few years the two institutions moved in different directions with the Institute specializing in environmental microbiology and molecular biology.

The vision of rational drug design turned out to be a prescient one. Agouron Pharmaceuticals became a major biotechnology company and its first rationally designed drug, Viracept™ is still the leading HIV protease inhibitor. The use of protease inhibitors together with reverse transcriptase inhibitors in a multi-drug therapy regime has led to a dramatic decrease in deaths due to AIDS. In 1998 Agouron Pharmaceuticals was
sold to Warner Lambert that then merged with Pfizer. In the process, the endowment of the Institute increased substantially.

**AGOURON INSTITUTE AS A GRANTING AGENCY**

In 1997 the Board of Directors of the Institute adopted new strategies to continue the goal of making significant contributions in biology and chemistry. The Institute changed its mode of operation and explored making highly leveraged investments in basic and applied biology and chemistry. The decision was made to carry out a study program in several promising areas of research.

The study of prospects for the determination of the structures of supramolecular complexes was the first such effort. The results of our study have been published in a white paper entitled, “Supramolecular Assemblies: Current Technology and Resource Needs.” In 1998 we began awarding grants to institutions here and abroad to support research and facilities in this field. In addition to specific grants to principal investigators, we provided support for the cryo-electron microscopy facilities at three universities. We continue to fund a number of postdoctoral fellowships that are administered by the Jane Coffin Childs Memorial Fund for Medical Research (http://www.jccfund.org) and the Helen Hay Whitney Foundation (http://www.hhwf.org).

Our second study was in the field of Geobiology. In May 2000 we held a meeting in Pasadena, California. A summary of this meeting was included in a white paper entitled, “Geobiology: Current Technology and Resource Needs.” In 2002 we began support for a new international geobiology course (http://wrigley.usc.edu/geobiology) to provide interdisciplinary training to young scientists interested in this field. We also funded a multi-university project involving drilling deep cores (~1.5 km) in South Africa to provide new samples for study of the late Archean/Proterozoic time interval. These cores span the anoxic to oxic transition in Earth’s history (c.a., 2.7-2.2 billion years ago) and provide critical samples for the establishment of a chronostratigraphic framework for the region and permit the analysis of the paleobiological record.

Our third topic of study is in the area of Microbial Oceanography. In January 2004 we held a meeting in Del Mar, California. Participants of that meeting included: Gustaf Arrhenius (SIO), John Baross (UWash), Donald Canfield (U Southern Denmark), Penny Chisholm (MIT), Edward DeLong (MIT), Paul Falkowski (Rutgers), David Karl (UHawaii), Anthony Michaels (USC), Norman Pace (UColorado), Gabrielle Rocap (UWash), Edward Rubin (LBNL), Mitch Sogin (MBL), Edward Stolper (Caltech), Michael Surette (UCalgary), and Bess Ward (Princeton). In addition, Edward Penhoet, David Kingsbury and Dan Martin of the Gordon and Betty Moore Foundation, and Eric Mathur (Diversa) joined us. In November 2004, we met in Pasadena with Donald Canfield, Edward DeLong, Scott Doney (WHOI), David Karl, Andrew Knoll (Harvard) and Edward Stolper to discuss collaborative scientific efforts to develop thorough studies at specific ocean sites with the goal of comparing data at multiple sites. This report provides a review and recommendations for the field based on our meetings.
Microbial Oceanography is a relatively new subdiscipline that integrates perspectives of a wide variety of fields including Ocean and Earth sciences, Ecology, Evolutionary Biology, Molecular Biology and Microbiology. While the roots of Microbial Oceanography can be traced back to the discovery of the microscope in the 17th century, comprehensive investigations of the role of microorganisms in the sea have emerged only recently. This new interest in Microbial Oceanography resulted from both conceptual and technological advances that have provided the motivation and the tools for the creation and dissemination of new knowledge about the role of microorganisms in nature. Future challenges will be to link the biology of individual cells and populations to the structure of communities and the impacts of climate variability on ecosystem function. This scaling from genomes to biomes will require a new workforce of well-trained scientists and technicians, new observational and experimental approaches and — perhaps — new ecological theory. Hypothesis-driven field experimentation will emerge as an equal to careful laboratory-based analyses, and ecosystem scale (or larger) models will be needed to assimilate these new data sets into predictions of future states under altered climate forcing from greenhouse gas induced warming and related impacts. The contemporary challenges are grand, and these will provide exciting opportunities for the next generation of scientists.
It has been said that without a sense of the past, we have no future. This is especially true in science, because knowledge is cumulative. As its name implies, the field of Microbial Oceanography, as currently defined, has two major roots: microbiology and oceanography, in particular marine biology. In recent decades, the disciplines of microbiology and marine biology have been influenced significantly by physical/chemical oceanography, ecology, physiology and molecular genetics. Although the parent disciplines still exist, contemporary Microbial Oceanography can be viewed as an integration of knowledge to understand the physiological and ecological behaviors of the complex assemblages of marine microorganisms.

The earliest study of marine microorganisms can be traced to the Dutch lensmaker Antony van Leeuwenhoek in 1667. Others before him, dating back to Leonardo da Vinci in the 15th century, had employed magnifying lenses for the study of small objects. This line of investigation culminated with the publication of Robert Hooke’s monograph, *Micrographia*, in 1665. However, van Leeuwenhoek improved the art of polishing lenses of short focal length and with a single lens instrument was able to achieve a
magnification of 300-fold, enough to observe bacteria in seawater. Despite his impressive studies of “little animals,” there was no understanding at that time of the structure or phylogeny of microbes. This and other key information would not be known for nearly 150 years until the Prussian microscopist Christian Gottfried Ehrenberg (1795-1876) conducted detailed investigations of microorganisms, including culture studies of selected marine microbes. In 1828, he coined the term “bacterium” (from the Greek, meaning “little stick”), and in so doing separated them from other microorganisms. Ehrenberg traveled extensively to sample microbes from diverse habitats but his long odyssey with Wilhelm Hemprich through the Sinai Peninsula and into the Red Sea made a lasting contribution to modern science. He collected and characterized microbes from sediments, ponds and even the deep sea, and was the first to systematically describe fossil microbes (diatoms, radiolarians, forams, coccoliths) in rocks. Ehrenberg’s influential monograph, *Mikrogeologie*, published in 1854 marked the beginning of Micropaleontology as a scientific discipline and was an important precursor for the modern studies in Geobiology.

Another important advance in our understanding of the microbial world was the formal classification of bacteria. In a comprehensive monograph published in 1867, the Prussian microbiologist Ferdinand Julius Cohn identified several unique groups of bacteria based on morphology, cellular structures, pigmentation and growth characteristics. He concluded that all bacteria belonged to the plant (not animal) kingdom and that they were related to algae.

The “microbiology root” of Microbial Oceanography had a major bifurcation in the late 19th century, a period that some refer to as the “First Golden Age of Microbiology.” The dominant scientific leaders of this time were Louis Pasteur (1822-1895) and Robert Koch (1843-1910). Their research included studies of infectious disease and pathology, vaccination and fermentation microbiology, and led to modern fields of medical and industrial microbiology. A hallmark of this era was the use of pure cultures. In his writings, Pasteur discussed the importance of the environment in controlling physiology and metabolism, and he expressed great interest in the nascent field of microbial ecology but time did not permit his full attention to these matters. At the diamond jubilee meeting of the American Society of Microbiology in 1974, René Dubos coined this dichotomy “Pasteur’s dilemma — The road not taken,” and rhetorically asked where the field of microbial ecology would be today if Pasteur had selected it as his primary research thrust. Instead, the discipline lay relatively dormant (at least in comparison to medical/industrial microbiology) for nearly 50 years. Towards the end of the 19th century, independent contributions by the Russian microbiologist Sergei Winogradsky (1856-1953) and the Dutch microbiologist Martinus Beijerinck (1851-1931) led to the development of the enrichment culture technique. This single advance in technology facilitated the discovery of chemolithoautotrophic metabolism (ammonium, sulfur and iron oxidation
linked to carbon dioxide fixation), nitrogen fixation and sulfate reduction, leading to a greater understanding of the role of microorganisms, especially bacteria, in global biogeochemical cycles. Beijerinck also was one of the first to recognize “soluble microbes” and coined the term virus to describe them. He also founded the Delft School of Microbiology which became an important center for research and training of the next generation of microbial ecologists.

The other major root of Microbial Oceanography, namely marine biology, can be traced back to Kiel, Germany and Plymouth, UK with the comprehensive studies of marine plankton by mostly European scientists, including Victor Hansen, Karl Brandt, Ernst Haeckel, Louis Agassiz, Anton Dohrn, among others. The world-wide oceanographic research voyage of the HMS Challenger (1872–1876) was a key benchmark that initiated at-sea studies of the distributions and abundances of marine organisms. Several influential marine laboratories were also founded during this period including the Stazione Zoologica in Italy (1872), the Sevastopol Biological Station in Russia (1872), the Plymouth Marine Laboratory in the United Kingdom (1888) and the Woods Hole Marine Biological Laboratory in the United States (1888). These facilities provided access to the sea and accelerated our understanding of marine microbiology and ecology. In 1894, the German biologist Bernard Fischer published his landmark treatise, *Die Bakterien des Meeres (Bacteria of the Sea)* which helped to stimulate similar investigations worldwide leading to modern integrated studies in the discipline of Microbial Oceanography. In 1931, while still a graduate student, Claude ZoBell was invited to join the staff of the Scripps Institution of Oceanography as one of that institution’s first marine microbiologists. According to published records, his responsibility was “to determine the extent to which bacteria are active in the open ocean and to assess their possible importance as geochemical agents.” While significant progress has been made in the intervening 75 years, these themes remain as the contemporary challenges of Microbial Oceanography collectively comprising the “Second Golden Age of Microbiology.”
The global ocean is grand, by any measure; it covers 71% of the Earth’s surface to a mean depth of 3.8 kilometers and comprises >95% of Earth’s probable living space (Fig. 1). Based on physical and chemical characteristics of temperature, salinity, pressure, dissolved nutrients, oxygen and light, the global ocean can be divided into distinct habitats that select for, or against, certain micro- and macroorganisms. As a result of these physical and chemical characteristics specific habitats can be identified, studied and compared — for example, rocky intertidal, coral reef and so on. Furthermore, the ocean is well-stratified in the vertical dimension with mostly horizontal exchange (Fig. 2). The ratio of biome width to depth exceeds 1000:1 in most of the major ocean basins. Even in the open sea, there are distinct water masses that are in constant motion and that can be traced horizontally and vertically to specific regions of formation. The global circulation rate, as deduced by non-conservative, chemical properties and radioisotopic tracers has a time scale of hundreds to thousands of years. Consequently, “young” and “old” regions of ocean water masses can be identified based on the time that the water was last in contact with the atmosphere. Due to unique topography and interactions with the atmosphere, certain areas “short-circuit” the mean circulation by serving as conduits for the more rapid delivery of nutrient-rich deep water to the surface of the sea. These so-called

**Fig. 1.** The world ocean covers 71% of Earth’s surface with the deep blue sea (regions seaward of the continental shelf) accounting for more than 60% of the total. With an average depth of 3800 m, the volume of habitable space on planet Earth is clearly dominated by marine ecosystems. Because microbes dominate the marine environment, they are directly or indirectly linked to most global processes and are largely responsible for the habitability of our planet. Adapted from ZoBell (1962).
“upwelling” regions occupy only approximately 1% of the surface ocean, but are important areas of solar energy capture through enhanced photosynthesis and the selection of relatively large algae and relatively short food chains thereby supporting some of the great fisheries of the world. The more usual condition is one of large expanses (90% of the global ocean) of oligotrophic (low nutrient, low biomass) habitats that select for very small primary producers, long and complex microbial-based food webs and relatively inefficient transfer of carbon and energy to higher trophic levels. These fundamental differences in physics result in ecosystems with different structures and dynamics, and dramatically different microbial assemblages that live within them (Fig. 3).

Marine habitats vary in both time and space over more than nine orders of magnitude in productivity, depth, temperature, salinity, nutritional regimes and other parameters.

Fig. 2. [Left] Pressure-temperature-depth relationships in the sea. Shown are typical distributions of temperature from tropics to poles versus water depth in the global ocean. Below a depth of approximately 2000 m, water temperature is relatively constant at about 3°C. Also shown is the depth dependence on hydrostatic pressure. Adapted from ZoBell (1962). [Right] Typical depth dependent relationships between nutrients, chlorophyll and rates of primary production in open ocean habitats. Rates of photosynthesis are dependent upon the availability of light and inorganic nutrients (nitrate, phosphate, trace elements). In most open ocean ecosystems, nutrients are scavenged to nearly undetectable levels by the activities of phytoplankton in the upper mixed layer. At greater depths, light becomes limiting for primary production and nutrients begin to accumulate at or near the bottom of the euphotic zone — usually defined as the 1% surface light depth. The distribution of chlorophyll a, the critical light-absorbing pigment for photosynthesis, is a result of a balance between light and nutrient availability — the two resource gradients for plant growth. The deep chlorophyll maximum layer is a characteristic feature in many open ocean habitats.
Fig. 3. [Top] Although any one section of the ocean’s surface looks very much like the next, the global ocean can be divided into unique biogeographical provinces based largely on distance from land, latitude and longitude, influence of wind and other physical forcing mechanisms, temperature and the rate of resupply of nutrients. The resultant provinces or biomes support unique biological communities from bacteria to top-level predators. Redrawn by Bill Li from Longhurst (1998). [Bottom] Since 1978 oceanographers have been able to estimate the concentration of chlorophyll in the near surface ocean using satellite-based remote sensing techniques. The global composite images emphasize the complex spatial structure of pelagic ecosystems. Repeat analyses reveal that the boundaries between major provinces are elastic, rather than fixed, and are internally dynamic with evidence for aperiodic phytoplankton blooms and shifts in community structure. Courtesy of SEAWIFS Project, NASA Goddard Space Flight Center and GeoEye.
magnitude of scale in each dimension (Fig. 4). Compared to terrestrial habitats, most marine ecosystems are out of “direct sight,” and, therefore, sparsely observed and grossly undersampled. The discovery and subsequent documentation of the oases of life surrounding hydrothermal vents in the deep sea in 1977 revealed how little we knew about life beneath the sea at that time (Corliss et al. 1979). Even today, new unexpected discoveries about the ocean frontier continue to be made, many of these involving marine microbes.

Fig. 4. Physical-biological interactions in habitats vary on multiple time and space scales ranging from seconds to decades and millimeters to entire ocean basins. Marine microbes are susceptible to, and respond to, many physical and biological forces though we are just beginning to gain a full appreciation for the multiple cause-and-effect processes. A major challenge in Microbial Oceanography is to sample and, ultimately, understand the ocean on a variety of time and space scales.
The life sciences and ocean sciences are currently poised at a remarkable and unique crossroads. More than ever before, the interdependencies that link evolutionary dynamics, biological processes, marine biogeochemical cycles and ocean and atmospheric chemistry are becoming critical issues for understanding the environment, and human activities in it. Studying the linkages between these hierarchies of complexity is becoming more tractable, in part due to the development of new technologies — ranging from genomic analyses of oceanic microbial communities en masse, to in situ ocean monitoring systems, to planetary productivity estimates gathered by Earth-orbiting satellites. These technological advances are relatively new however, and our understanding of the properties, dynamics and future trends of the world oceans is far from complete. While the physical and chemical properties of the sea are fairly well characterized, the biological ocean — by comparison — continues to withhold many secrets. In particular, the microbial ocean represents one of the single-most dominant and unexplored territories. Many new microbial species and processes, some described in this report, have been characterized over the past two decades. Still, a vast amount of evolutionary diversity, and by inference metabolic and physiological versatility, remains to be discovered and described.
Planktonic marine microbes, collectively present at billions of cells per liter, can grow at rates of one doubling per day or more, and are consumed at approximately equivalent rates. Consequently, day to day changes in the standing stocks are not nearly as great as the potential for change when and if environmental conditions are perturbed. Hidden within these population dynamics are fundamental lessons of gene regulation, environmental sensing and response, species and community interactions, and genomic plasticity and evolution. These processes in turn have global effects on fluxes of energy and matter in the sea, Earth’s atmospheric composition and global climate trends. Microbes are the perennial stewards of Earth’s geochemical cycles. They are Nature’s biosensors par excellence, and as Earth’s oldest inhabitants they represent the best available biotic recorders of evolutionary history. A convergence of microbiology, ecology, genomics, natural history and ocean science is now occurring, and it has the potential to be focussed in unprecedented ways through the lens of Microbial Oceanography. By working together, molecular biologists, microbiologists and oceanographers can now forge new world views that better describe the living ocean system. The questions, activities and goals described in this report, all aim in one way or another to catalyze this convergence.

Marine ecosystems are distinguished from their terrestrial counterparts, not only by their physical dimensions but also by their dynamic temporal response. The turnover time of carbon in the upper ocean is on the order of one week, compared to decades in terrestrial ecosystems (Falkowski et al. 2000). Because marine microbial assemblages track their environment very closely the climate-sensitive upper ocean may be thought of as a “sentinel ecosystem” for observing planetary changes. But the interactions between microbes and their environment are neither unidirectional nor linear. Chemical transformations mediated by marine microbes play a critical role in global biogeochemical cycles. In essence, biology — especially microbiology — affects the chemistry of the ocean, and in so doing helps to maintain the habitability of the planet as a whole.
Two emerging themes combine to make this a critical time to invest in Microbial Oceanography: a growing appreciation that human activities are having a profound impact on the biogeochemical cycles of the planet, and recent advances in molecular biology and genomics that have opened a new frontier for exploration of ocean ecosystems. Considering the growing impact of humans on the global environment, and application of new enabling technologies that decipher and monitor natural microbial communities, it is becoming increasingly possible to track microbes as living biosensors. While microbes have long dominated global biogeochemical processes, human activities now rival their impact. For example, unlike any other time on Earth before the industrial age, more nitrogen gas is “fixed” today by the Haber-Bosch process than is reduced by all extant biological nitrogen fixing processes combined (Galloway et al. 2005). This industrial scale synthesis of ammonia enriches the Earth and stimulates plant production, but also pollutes inland waterways and coastal marine ecosystems. Given the pervasive influence of humankind on extant global geochemical cycles, it is more critical than ever to understand microbial impacts on, and responses to, anthropogenic perturbations. Additionally, the microbial world harbors vast biochemical novelty, and contains within it many “green” solutions to complex technical problems associated with the burgeoning human population. The technologies are in hand, and the disciplinary integration and interactions are now poised to catalyze movement beyond the black box approach to understanding microbial processes in nature. The oceans represent one of the best systems to begin this endeavor, and promise large returns. One expected outcome is the provision of detailed knowledge of the inner workings of one of the largest, and biogeochemically significant biomes on the planet. Another deliverable is the development of new tools, methods and theory to understand complex microbial systems, and their influence on the physical, chemical and biological environment.

This report summarizes current questions of outstanding interest in the field of Microbial Oceanography, and delineates resource needs that are crucial to future progress in the discipline, all with an emphasis on a better understanding of the interaction between microbial communities, their activities and planetary processes.
We are in a time of unprecedented planetary change. The burning of fossil fuels since the industrial revolution has released enormous amounts of carbon dioxide (CO₂) previously stored in relatively inert reservoirs beneath the land and sea. Present concentrations of atmospheric CO₂ are higher than they have been in the past 420,000 years, and are predicted to continue rising (Fig. 5). As CO₂ increases in the atmosphere as a result of human activities, it will also impact the ocean through air-to-sea gas exchange. For example, direct measurements of the partial pressure of CO₂ (pCO₂) in the surface ocean at Station ALOHA in the North Pacific Subtropical Gyre (NPSG) has revealed a nearly 10% increase over less than 20 years (Fig. 5). This increase in pCO₂ has caused an acidification of the near-surface waters (Fig. 5) that could have serious ecological implications, particularly for those organisms that precipitate calcium carbonate or that are otherwise sensitive to change in environmental pH.

The living oceans play a critical role in the global carbon cycle. The coupling between the upper ocean and the atmosphere has resulted in increasing concentrations of dissolved CO₂ in surface seawater (Fig. 5). Formation and sinking of dense water at selected high latitude sites can effectively transport some of this CO₂ to the deep ocean, but this “solubility pump” accounts for only a small fraction of carbon exchanged between the surface and deep ocean (Fig. 6). Much more carbon is moved through the action of the “biological pump,” which begins with the growth of phytoplankton in the surface ocean. This newly fixed carbon is either locally respired back to CO₂ in the upper ocean or remains in a particulate matter form where it can sink out of the surface waters and be sequestered for various periods of time (up to millennia) in the deep ocean. Factors that influence the relative proportion of respired versus sequestered carbon include the availability of organic and inorganic forms of nutrients (nitrogen, phosphorus, silica and iron), the structure of the phytoplankton community, the rates at which...
Fig. 5. [Top] Concentration of carbon dioxide (CO₂) in Earth’s atmosphere over the past 450,000 years and a projection into the future. These data are derived from measurements of CO₂ gas locked into air bubbles preserved in a deep ice core from Vostok station, Antarctica. The red line in the main figure shows the recent impact of humans. Excess CO₂ is derived mainly from fossil fuel burning and other industrial processes. Adapted from Vostok ice core data published by Petit et al. (1999). The inset shows a more recent record of atmospheric CO₂ from the late Charles D. Keeling’s Mauna Loa, Hawaii air sampling program. [Middle] The increase in atmospheric CO₂ drives a corresponding increase in CO₂ dissolved in the surface ocean as shown by the 16-year data record obtained at the deep-sea Hawaii Ocean Time-series (HOT) Station ALOHA. The large seasonal oceanic variations are driven by changes in temperature and by the net production or decomposition of organic matter. For comparison to the atmosphere, the Mauna Loa data of C. D. Keeling are also shown. [Bottom] Systematic decrease in surface water pH at Station ALOHA based on HOT program data. The apparent “acidification” of the surface ocean is a direct result of the absorption of CO₂ from the atmosphere. If this trend continues, it will surely impact the distributions, abundances and metabolic activities of microbial assemblages and, therefore, have major implications for biogeochemical cycles including carbon sequestration.

HOT program data available at http://hahana.soest.hawaii.edu
phytoplankton are grazed or lysed by viruses, and the capacity of heterotrophic plankton to remineralize different forms of organic carbon. Many of these factors are controlled directly or indirectly by marine microbes so in effect they control carbon sequestration and impact climate. Understanding the microbially controlled biological processes in the marine water column will be important if we hope to develop a predictive capacity regarding the fate of carbon and associated bioelements in the sea.

Fig. 6. A schematic view of the ocean’s carbon pump showing the interactions between the surface ocean and the atmosphere above, and the surface ocean and the deep sea below. There are both physical and biological components of the ocean’s carbon pump; the latter involve a complex set of poorly constrained ecological processes. Microorganisms are key components of the ocean’s biological carbon pump because they largely control organic matter production and consumption in the sea. Permission to reprint, SCOR 1990.
The microbial ocean spans a broad range of size classes — referred to by oceanographers as femtoplankton (0.02–0.2 μm), picoplankton (0.2–2.0 μm) and nanoplanlton (2–20 μm). Diverse groups of microorganisms interact both within and between these size categories. Bacterial and archaeal picoplankton fix carbon and consume organic material, and eukaryotic microbes as small as 1 μm in size can be photoautotrophic, feed upon smaller particles, or both. Within these microscopic forests of the sea, complex biological and biogeochemical interactions play out on a daily basis (Fig. 7).

Over the past three decades biological oceanographers have recognized the critical role of microbes in the ocean ecosystem. This period has been marked by a series of discoveries highlighting the importance of microbes in marine biogeochemical cycles and the important role of the extremely large, but poorly characterized, pool of dissolved organic matter (DOM) in the structure and function of marine microbial ecosystems. These include the formal description of microbial-based food webs (also dubbed the “microbial loop”; Fig. 8) and the discovery of ubiquitous and abundant marine cyanobacteria including Synechococcus (Waterbury et al. 1979) and Prochlorococcus (Chisholm et al. 1988) that dominate the photosynthetic activity of open ocean gyres. Other novel genera of cyanobacteria impact the nutrient stoichiometry of the sea through nitrogen fixation (Zehr et al. 2001). Molecular techniques have revealed the unexpected presence of Archaea in the temperate oceans (DeLong 1992; Fuhrman et al. 1992) and the existence of new eukaryotic lineages in the nanoplanlton (Lopez-Garcia et al. 2001; Moon-van der Staay et al. 2001). Viruses are acellular entities that obligately depend on the metabolism of their hosts for reproduction. They infect all three domains of life — Archaea, Bacteria and Eukarya — and so viral diversity may equal or exceed that in cellular life forms. Present in the sea at approximately ten times the concentration of cellular microorganisms, viruses are important in the biological dynamics of ocean systems. They have been implicated in phytoplankton mortality thereby impacting primary productivity. Viruses are also important in the lateral transfer of genetic material, acting as agents of evolutionary change, adaptive radiation and recombination. The diversity of virus genes and genomes, and their ecological roles are only now becoming apparent.
Fig. 7. Composite view of selected microbes that are found in different marine microbial assemblages. Shown are:
(a) scanning electron microscopic image of marine bacteria showing morphological diversity. Cell dimensions range from < 0.5 μm to > 3 μm. Photo credit, Ed DeLong.
(b) marine Archaea (green) stained with a fluorescent DNA probe, and viewed by epi-fluorescence microscopy. The green cells are approximately 1 μm in diameter. Photo credit, Ed DeLong.
(c) transmission electron microscopic thin section images of Prochlorococcus marinus, the most dominant photoautotrophic cell in the sea. Photo credit, Claire Ting and Penny Chisholm.
(d) Ditylum brightwellii, cell length ~30 μm photo credit Karie Holtermann and Ginger Armbrust; and scanning electron micrograph of an abundant marine diatom, Thalassiosira pseudonana, cell diameter ~10 μm. Photo credit, Nils Kröger.
(e) virus (cyanophage) that infect the numerically dominant oceanic phototrophs Prochlorococcus and Synechococcus. Photo credit, Matt Sullivan.
(f) brightfield (left) and autofluorescence (right) microscope images of the cultivated unicellular N₂ fixing cyanobacterium, Crocosphaera watsonii WH8501, which has recently been shown to be widely distributed in tropical waters around the globe. The approximate diameter of each cell is 5 μm. Photo credit, Jon Zehr.
Prokaryotic microbes, Bacteria and Archaea, are the biochemical dynamos of the sea. As a group, the prokaryotes contain the bulk of Earth’s physiological and biochemical diversity, accumulated over the nearly 3.8 billion years of their evolutionary history on Earth. They form the basis of marine food chains and food webs, from oligotrophic gyres to hydrothermal vents, recycle most of the oceans’ major nutrients and help maintain the global balance of atmospheric gases. The physiological and metabolic properties of most prokaryotes, however, are still largely unknown. Ongoing efforts to characterize the genetics, biochemistry, physiology and ecology of marine prokaryotes is a major challenge for Microbial Oceanography.

Members of the domain Eukarya play numerous important roles in marine food webs, and many marine Eukarya are also microorganisms. Small eukaryotes can be photosynthetic, heterotrophic or capable of both modes of nutrition (mixotrophic). Many lineages of eukaryotes are known only from their ribosomal RNA sequences, thus one can only speculate about how they make a living. The diversity of picoeukaryotic groups is only beginning to be appreciated, and there remains little doubt that it is still greatly undersampled.

The multiple deep independent lineages of marine eukaryotes may impact metabolic and biogeochemical processes in the sea. With different evolutionary histories, different lineages of eukaryotes may arrive at independent means for the reuse/salvage of “old” cellular technologies (ancient enzymes and metabolic pathways). These evolutionary choices influence the dominant enzymes (and hence metal cofactors) in each group. The net result is in influence on trace metal requirements and limitation in the oceans (Falkowski et al. 2004).

**Fig. 8.** Conceptual view of the upper ocean ecosystem with its component microbes, nutrient cycles and energy flow pathways from sunlight to carbon export. The left-hand side shows the production-intensive “food chain” from large eukaryotic algae to higher trophic levels, including fish. This contrasts with the “remineralization intensive” microbial loop that is supported both by solar energy and by the large pool of dissolved organic carbon (DOC). Reprinted by permission from MacMillan Publishers Ltd: from E.F. DeLong and D.M. Karl, Genomic perspectives in microbial oceanography. *Nature* 437:336-342, copyright 2005.
Current understanding of evolutionary and physiological diversity contained within marine microbial communities has advanced in part by use of molecular methodologies. The technique of extracting DNA from a sample, amplifying a gene of interest (often ribosomal RNA) using the polymerase chain reaction (PCR), and cloning and sequencing the resultant products is now a standard method for characterizing marine microbial diversity since its introduction two decades ago (Pace et al. 1986; Giovannoni et al. 1990). Extending this approach to genes involved in elemental transformations of interest, or to community RNA rather than DNA, has helped to define the functions carried out by these communities. More recently the increased speed and lowered cost of nucleic acid sequencing driven by the Human Genome Project, has ushered in the genomic era of oceanography and has helped to define the emergent “Second Golden Age of Microbiology.” Whole genome sequences of the prokaryotic primary producers Prochlorococcus, Synechococcus and the diatom Thalassiosira pseudonana have been completed, and those of many other ubiquitous marine microbes are in progress, including the nitrogen fixing cyanobacteria Trichodesmium and Crocosphaera, marine planktonic archaea, and the picoeukaryotic phytoplankter Ostreococcus. Chemo- and photoheterotrophs and photoheterotrophs like the ubiquitous SAR11 (Pelagibacter), a coastal heterotroph (Silicibacter pomeroyi), the pressure-adapted piezophile (Photobacterium profundum), the squid symbiont (Vibrio fischerii) and many other marine microbial genomes are also now completed, or in sequencing pipelines (Table 1; from DeLong 2005). The sometimes simple morphologies found in these microorganisms (Fig. 7) hide the genetic and physiological complexity that lies within.

The genome sequences of the unicellular marine cyanobacteria Prochlorococcus and Synechococcus have provided examples of how two “species” that thrive in similar marine habitats have evolved different gene complements that allow them to coexist with non-competitive, complementary functions (Dufresne et al. 2003; Rocap et al. 2003). Resource partitioning, including differential utilization of limiting nutrients, appears to be a key to reducing competition among these related species. This genomic microdiversity is a result of multiple ongoing processes, including gene loss, gene duplica-
tion, gene acquisition via lateral gene transfer and large scale genomic rearrangements (Fig. 9). The relative rates and significance of each of these processes in the wild remains a first order question. Certainly many instances of lateral gene transfer in the cyanobacterial genomes are associated with the “footprints” of phage (Palenik et al. 2003), and the recent discovery of photosystem genes encoded on cyanophage genomes provides further evidence for the role of phage in maintaining the dynamic genome and manipulating host gene regulation and function (Lindell et al. 2004).

Genomic technologies are also facilitating a better understanding of uncultivated marine microbes. By cloning and sequencing DNA directly from the environment, an unprecedented view on the natural microbial world can be obtained. Fairly large DNA inserts (up to 200 kb) cloned into bacterial artificial chromosomes (BACs) can represent 5-10% of an entire bacterial genome. BACs can be screened for the presence of selected genes or used to link phylogeny (via ribosomal RNA genes) to metabolic function (via a variety of known genes like carboxylase, nitrogenase or hydrogenase, to name a few). This helps to put a “physiological face” on otherwise anonymous phylogenetic groups detected in the marine environment. For example, the genomics-enabled discovery of new energy-generating microbes in bacterioplankton based on either bacterio-chlorophyll (Kolber et al. 2000; Kolber et al. 2001) or proteorhodopsin (Béjà et al. 2000; Béjà et al. 2001), illustrates the power of these approaches. These new insights into metabolic diversity challenge conventional views on the pathways and flux of carbon and energy within the ocean’s photic zone. Further, the whole genome sequences of

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Fig. 9. Cross comparison of gene contents of four closely related marine cyanobacteria including three physiologically distinct clades of Prochlorococcus marinus (MED4, SS120, MIT9313) and a close relative, Synechococcus. The values shown are numbers of common shared genes (blue shared by 2; magenta shared by 3; red shared by all 4), and those unique (black) to each organism. The bulk of the genome, 855 genes, are common to all 4 groups. Provided by Gabrielle Rocap.
### TABLE I. Phenotypic and genomic characteristics of some recently sequenced marine microbes

<table>
<thead>
<tr>
<th>Microbial Species</th>
<th>Habitat or Sample Origin</th>
<th>Phenotypic properties and/or genomic features</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thalassiosira pseudonana</em> (E)+</td>
<td>Surface water, plankton</td>
<td>Silicic acid metabolism, chitin buoyancy mechanisms, diverse nitrogen acquisition and metabolism, several energy storage and utilization strategies</td>
</tr>
<tr>
<td><em>Silicibacter pomeroyi</em> DSS-3 (B)</td>
<td>Coastal water, plankton</td>
<td>Lithotrophic energy metabolism (carbon monoxide, reduced sulfur compounds), DMSP metabolism, many amino and carboxylic acid transporters</td>
</tr>
<tr>
<td><em>Pelagibacter ubique</em> (B)</td>
<td>Coastal water, plankton</td>
<td>Has the smallest genome, the fewest genes, and the shortest average intergenic spacer region of any known free-living bacterium. Encodes and expresses proteorhodopsin.</td>
</tr>
<tr>
<td><em>Prochlorococcus marinus</em> MED4, SS120 (B)</td>
<td>Oligotrophic water, upper photic zone</td>
<td>High-light adapted, greater number of high-light-inducible proteins, photolyase genes, narrower range of nitrogen sources (nitrite reductase and transporter absent), fewer chlorophyll binding antenna protein genes</td>
</tr>
<tr>
<td><em>Prochlorococcus marinus</em> MIT9313 (B)</td>
<td>Oligotrophic waters, lower photic zone</td>
<td>Low-light adapted, fewer number of high-light-inducible proteins, no photolyase, wider range of nitrogen sources (nitrite reductase and transporter present), greater number of chlorophyll binding antenna protein genes</td>
</tr>
<tr>
<td><em>Synechococcus sp.</em> WH8102 (B)</td>
<td>Open ocean surface water</td>
<td>Wide range of nitrogen sources (organic, nitrate, nitrite, urea), amino acid transporters, swimming motility, more transporters than <em>Prochlorococcus</em></td>
</tr>
<tr>
<td><em>Photobacterium profundum</em> SS9 (B)</td>
<td>Decaying amphipod</td>
<td>Growth pressure optima &gt; 1 atm, polyunsaturated fatty acids in membrane lipids, differential gene expression at high and low pressure growth, trimethylamine oxide respiration, possible anaerobic amino acid fermentation</td>
</tr>
<tr>
<td><em>Vibrio fischeri</em> ES114 (B)</td>
<td>Euprymna scolopes light organ</td>
<td>Bioluminescence, specific association with squid <em>Euprymna scolopes</em>, many adhesions and toxin-like genes shared in common with other vibrio pathogens</td>
</tr>
<tr>
<td><em>Rhodopirellula baltica</em> (B)</td>
<td>Kiel fjord seawater</td>
<td>Derived absence of peptidoglycan, formaldehyde oxidation pathway, abundant sulfatases, high proportion of genes most similar to Eukarya</td>
</tr>
<tr>
<td><em>Desulfotalea psychrophila</em> Lv54 (B)</td>
<td>Permanently cold Arctic marine sediment</td>
<td>Grow below 0°C, sulfate-reducer, potential oxygen-reducing cytochromes</td>
</tr>
<tr>
<td><em>Idiomarina loihiensis</em> (B)</td>
<td>Mixed hydrothermal vent/ cold seawater (1296 m)</td>
<td>Amino acid fermentation, incomplete amino acid biosynthetic pathways, exopolysaccharide and biofilm formation, reduced carbohydrate metabolism</td>
</tr>
<tr>
<td><em>Pyrococcus horikoshii</em> (A)</td>
<td>Deep-sea hydrothermal vent</td>
<td>Aerobic, sulfur utilizing heterotrophic archaeal thermophile. Grows at temperatures up to 98°C</td>
</tr>
<tr>
<td><em>Methanosarcina acetivorans</em> (A)</td>
<td>Marine sediments</td>
<td>Versatile methane generating archaeon. Can grow on a wide variety of carbon and energy sources, reflected in its unusually large repertoire of metabolic genes.</td>
</tr>
<tr>
<td><em>Pyrobaculum aerophilum</em> (A)</td>
<td>Shallow coastal hydrothermal vent</td>
<td>Optimal growth at 0°C. Can grow in the presence of oxygen, and large amounts of sulfur.</td>
</tr>
</tbody>
</table>

+ Domain affiliation in parentheses. E, Eukarya; B, Bacteria; A, Archaea; See DeLong, 2005 for references
ubiquitous marine microorganisms such as Pelagibacter (Giovannoni et al. 2005; Table 1) are revealing the fundamental design plans of the tiny engines that drive biogeochemical cycles in the ocean.

Another approach for cultivation-independent microbial genome characterization is a variant of whole assemblage genome shotgun (WAGS) sequencing. Recent large scale sequencing efforts have extended this technique beyond a single culture to pools of community DNA (Tyson et al. 2004; Venter et al. 2004). Over 1.04 billion bases sequenced from 200 liters of surface Sargasso Sea water revealed 1164 discrete small subunit rRNA sequences, including 148 novel phylotypes (Venter et al. 2004). Many new genes never before seen were revealed by this massively parallel genomic approach. In another recent survey, extending from the sea surface to depths of 4 km in the Pacific Ocean (DeLong et al. 2006), ecological trends that differentiated deep-sea from surface dwelling marine microbial communities were revealed. The dawn of a new ecology, based on gene distributions in the environment, is beginning to emerge from such studies (Fig. 10). Variations on this theme, using linker ligation and subsequent amplification have also been used to generate shotgun libraries from native marine viral populations (Breitbart et al.)

Fig. 10. Patterns of gene distributions in microbial communities found at different depths at the Hawaii Ocean Time-series Station ALOHA. Characteristic sequences typifying upper water column microbes included those involved in photosynthesis, photo-damage repair, and motility. In deeper waters, genes facilitating attachment, as well as “jumping genes” (transposases) were more abundant. Larger datasets and evolving analytical methods will result in improved abilities to extract ecological information from gene distributions (DeLong et al. 2006). Reprinted with permission from DeLong, E. F. et al., Community Genomics Among Stratified Microbial Assemblages in the Ocean’s Interior. Science 311:496-503. Copyright 2006 American Association for the Advancement of Science.
However, for reconstructing complete genome sequences from complex natural microbial populations, no single approach — BACs or WAGS — has adequate resolving power independently. In the future, the most complete and reliable datasets will likely result from the combined application of a variety of new technologies. Single cell isolation methods such as flow cytometry, combined with rolling circle amplification strategies, represents an alternative for deconvoluting single genomes from complex populations for future applications. Whatever the approach, there is no doubt that genome sequences from both pure cultures and natural populations will continue to advance understanding of microbial processes in the sea (DeLong and Karl 2005).

Sampling is the first challenge in the study of marine microbes. Usually this requires the use of a large research vessel, as shown here. In the top image scientists are recovering a rosette of 24, 12 liter water bottles that have captured seawater containing microbes from the surface to the seabed. In the bottom image, a scientist is deploying an array of sediment traps that are designed to collect sinking particles and the microbes attached to them. Photos courtesy of SOEST, University of Hawaii.
Current questions in Microbial Oceanography range across all the scales of biological hierarchy. Some of the questions are more traditional and descriptive, but still very important and largely unanswered: e.g., What microbes live where? How do they differ functionally? What are their rates of growth and removal? What are their modes of dispersal and recruitment? What characterizes their specific environmental adaptations?
At the molecular and biochemical levels, questions revolve around fundamental mechanisms of physiological and metabolic versatility, including both energy acquisition and material flux. These molecular, biochemical and physiological data directly link into higher order questions related to biogeochemical cycling and ecosystem function.

At the population level, molecular approaches provide new opportunities for studying population genetics and genome evolution in action. A genome sequence is, in essence, a genetic snapshot in time. To fully understand “genomes in the wild,” they will need to be viewed within the dynamic environmental context — physical, chemical and biological. By studying genome variability in natural populations, one can move beyond the snapshots, to obtain a view of the motion pictures of genome evolution. These “feature movies” have the potential to reveal both qualitatively and quantitatively the specific details of genome microevolution — genetic drift, lateral transfer and adaptive radiation, their mechanisms, and their relative rates and contributions. Species interdependencies and cell-cell communication among sympatric microbes in situ are virtually unexplored topics of great importance.

A grand challenge is to link smaller scale characteristics to larger scale emergent properties — the quest to link genomes to biomes. Metabolic reconstruction of biogeochemical processes in different oceanic provinces represents part of this grand challenge that may soon be achievable. An ultimate goal is to interrelate the continuum from systems biology at the subcellular level, to systems ecology on global scales.

Single cells, individual species and the autecological dimension

The rapid rise in microbial genome sequencing provides a wealth of new information about the kinds of genes present in marine microbes. Using large genetic databases it is possible to create metabolic maps in silico and make ecophysiological predictions based on them. However, the annotation of a genome sequence is essentially a series of hypotheses about that organism’s potential. While the presence of conserved domains or sequence homology may provide clues about the function of a particular open reading frame (ORF), the function must also be confirmed in the lab. Furthermore, a significant portion of genes in fully sequenced genomes, generally between 30-40% of the total, are of unknown function. Elucidating the functions of these unknown ORFs will be a major challenge in
the coming decades. One example for how this may be accomplished comes from proteorhodopsin, whose function as a light-driven proton pump was demonstrated by expressing the functional gene in *E. coli*, and subsequent biophysical and biochemical characterization (Béjà et al. 2000; Béjà et al. 2001).

**Cellular response, environmental dynamics and gene expression**

The presence of a particular functional gene in a genome only suggests that the capacity to carry out that function is present, it does not provide information about if or when that gene is actually expressed in nature. In order to connect genomic data — i.e., the genotype — with biogeochemical processes and rates — i.e., the phenotype — measurements of *in situ* gene expression are needed. By designing primers based on known gene sequences it is possible to develop quantitative reverse transcriptase (RT)-PCR assays to measure the expression of specific genes in field populations. This approach is gaining in popularity and has already been applied to genes involved in cellular uptake and transformations of carbon (*rbcL*, *psbA*), nitrogen (*nifH*, *nirS*, *amoA*) and phosphorus (*pstS*), to name a few examples. The analysis of *in situ* gene expression can also complement studies based on specific *in vitro* enzyme assays or the uptake and incorporation of selected radio-labeled substrates to trace specific metabolic or biosynthetic activities.

One limitation of the single gene RT-PCR approach is that it pre-supposes which genes are important in specific ecosystem processes. Given the large percentage of genes of unknown function and our generally poor understanding of the regulation of any specific genes in marine microbes, it is likely that multiple target genes will be required for a more complete understanding of ecosystem processes. Methods for assessing the transcriptional profile of all genes in the genome simultaneously, such as microarrays, are being developed for selected marine microbes. Although whole genome arrays are now used predominantly in the laboratory to study gene expression, these methods will ultimately be adapted to study field populations *en masse*, enabling real time surveillance of population structure and physiological state over time and space. Proteomic analyses represent another technological advance that will extend studies of the variability of gene expression to the measurement of the expressed, functional proteins. There seems to be a nearly endless potential for future field studies.
Diversity, dynamics and evolution

The application of the “ribosomal RNA approach” has revealed a spectacular phyloge-netic diversity of microbial communities, yet quantifying the extent of this diversity is proving to be difficult. With the construction of larger clone libraries we are beginning to understand the species abundance curves in selected communities (Acinas et al. 2004), yet most ecosystems remain severely under-sampled. Novel ribotypes representing deeply branching lineages in the bacterial and archaeal domains continue to be found. In addition to the richness of community diversity (how many species are present) another important component is the even-ness of the distribution of the dominant microorganisms in a given habitat. Because of the potential biases associated with different efficiencies of DNA extraction and PCR, clone libraries are not able to address this important question. More quantitative methods, such as immunological or genetic probes, community genome hybridization using microarrays and quantitative PCR can be used to target abundances of specific groups. Still, the link between the phyloge-netic organization of a community and its biogeochemical function remains unresolved.

A key question remains to be answered:
Do communities with similar phylogenetic structure carry out similar biogeochemical functions? And if not, why not?

Cell-Cell and microbe-metazoan interactions — it takes a village

Many important biogeochemical transforma-tions are carried out by consortia of some-times unrelated microorganisms or in highly evolved symbiotic associations; studying a single species in isolation is often not enough to understand how biogeochemical pathways function. Undoubtedly microbial consortia, for example metabolic partnerships that form between Archaea and sulfate-reducing bacteria that drive methane oxidation in anoxic marine sediments, are common evolu-tionary and ecological themes. This should be reflected in the structure of naturally co-occurring microbial genomes and may represent an important element of lateral gene transfer and genome evolution. The occurrence of co-dependent metabolic symbioses might also be a major cause for the extreme difficulty of culturing any single organism of these complex multi-species con-sortia. Quorum sensing is a striking example of specific cell-to-cell communication.
Depending on the molecule involved it can be used for communication between members of the same species, or in a much more general manner. Such interactions are particularly important in the establishment of highly structured biofilms, but may also be common in more “dilute” habitats and possibly even in the open sea. Finally, microbial-metazoan associations are sometimes species-specific and mutually beneficial. If each animal species has one or more such specific associations (some coral species may have up to 100 unique prokaryotes associated with them), then the total number of microbial species may be much greater than previously estimated. This also has implications for “microbial extinctions” as metazoan biodiversity is reduced by habitat degradation and other processes.

**Mapping microbial community metabolism onto oceanic processes**

Microbes within different oceanic habitats can be viewed as being analogous to different organs in the human body, that together regulate homeostasis. In the case of marine microbial communities, a property of interest is the metabolic balance defined as the net biological flux of energy and matter in the sea. Light and nutrient availability, oxygen levels, pressure, temperature and many other physical and chemical factors influence the type and variety of microbiota and their metabolic activities — and hence the sum process of the ocean’s “organs.” High productivity, elevated respiration rates and rapid turnover are characteristic of the photic zone. In contrast, in the mesopelagic and oxygen minimum zones, slower recycling of organic matter and regeneration of deep water macronutrients is more typical. Just as metabolic maps of microbial species can be charted and displayed, metabolic maps of key genes and metabolic pathways that characterize ecosystems will begin to be available through the application of environmental genomics. These will drive the design and implementation of future field experiments in Microbial Oceanography.
Continuous observation of coupled physical, chemical and microbiological characteristics of the global ocean is an ambitious, perhaps unachievable, proposal. The ocean is large, relatively inaccessible and time/space variable. Ultimately the collection of comprehensive, time-series data sets from selected habitats that are representative of key biomes of the global ocean will be required and several new U.S. and international ocean observation programs are either in place or in various stages of advanced planning. The contemporary challenge of sustained observation will be met by an integrated ocean observing system based on research ships, deep-sea moorings, unattended drifters, gliders and other vehicles that periodically transmit their positions and data back to the shore-based laboratories via satellite. One of the more ambitious programs, involving 60 institutions from 22 countries, is the OCEAN Sustained Interdisciplinary Time-series Environment observation System (OCEANSITES) that will help to identify the most important biomes for sustained measurement and will assist with the distribution of the new data sets (Fig. 11, also see http://www.oceansites.org). A second, ongoing ocean observation program dubbed “Argo,” consists of a global array of nearly 3000 free-drifting profiling floats that measure the temperature and salinity in the upper 2000 m of the water column and transmit their data to shore-based facilities in near-real time (also see http://www-argo.ucsd.edu). In order for microbial oceanographers to benefit from this new wave of ocean research and from future infrastructural investments that are likely to be made, it is essential to refine existing microbiological and biogeochemical sensors and to develop novel approaches for continuous remote sensing of key variables that are likely to track environmental changes. These might include detection systems based on changes in microbial biomass or metabolic activity, or genomic-based sensors to assess changes in community structure or the presence/absence of key indicator species. Once the microbial detection systems are fully tested and ready to deploy, it will also be necessary to establish scientific collaborations to gain shared access to the observation platforms and to the complete data sets. Now is the time to begin the important work of promoting the interdisciplinary aspects of microbial oceanography in order to gain support from scientists in related disciplines of oceanography, ecology and engineering.

While many of the broad features of carbon and nitrogen turnover are known, most of the details remain elusive. In many biomes, the mechanistic details of carbon and energy flux in the sea are not known. New evidence suggests, for instance, that planktonic crenarchaeota may be responsible for dark CO₂ fixation (Wuchter et al. 2003) fueled by aerobic ammonium oxidation (Venter et al. 2004; Konneke et al. 2005). New metabolic processes like anaerobic ammonia oxidation (anammox) may be very important for recycling of nitrogen in the oxygen poor zones found in productive coastal regions (Dalsgaard et al. 2003). In these areas, it now appears that a new class of microbe (related to Planktomyces) can combine NH₄⁺ and NO₂⁻ to form dinitrogen gas in the absence of oxygen. These unusual anammox microbes possess a chemically impervious organelle.
Fig. 11. The beginning of a ‘new wave’ of global ocean observations that will lead to new opportunities in Microbial Oceanography. Today more than 60 ocean time-series stations are providing an unprecedented view of life in the sea. The OceanSITES program (http://www.oceansites.org) involves more than 60 institutions in 22 countries and serves as a scientific clearinghouse for time-series information about the ocean. Their website provides an interactive OceanSITES map (shown above) that can be used to identify specific sites, the scientists involved in each experiment and a catalogue of the data sets that are available from the 64 (as of March 2006) multi-disciplinary surface and sub-surface stations including individual moorings and lines established to measure transport at key locations. Some, but not all, of these stations are equipped with microbiological/chemical sensors. The development of novel tools for remote detection of microbial processes is a major challenge for the future.
surrounded by novel ‘ladderane’ lipids, that helps contain the highly reactive hydrazine intermediate that results from the anammox reaction (Fig. 12). Anammox-catalyzing microbes may greatly influence the flux of nitrogenous macronutrients in productive waters, and have been recently observed in the Black Sea and in Namibian and Chilean coastal habitats. The details of their dynamics, relative distributions and ecological importance are still largely a mystery.

It has been suggested that in vast oligotrophic areas of the world ocean, microorganisms may consume more carbon than is locally produced through the process of photosynthesis. If it is true that much of the world ocean is net heterotrophic, then current models of the ocean’s carbon cycle and pathways and rates of organic matter delivery to the open sea required to sustain the excess respiration will need to be re-evaluated. Two major impediments in resolving this fundamental question are the size of the habitat, relative to our ability to sample it in time and in space, and its temporal variability which makes integration and extrapolation difficult and imprecise. Even our current conceptual metabolic model based on photosynthesis and respiration may not accurately capture the emergent metabolic complexity in the marine microbial world. The continuum of metabolic strategies between pure photoautotrophy and pure heterotrophy means that field measurements of the net balance in ecosystem metabolism are not straightforward (Fig. 13). New forms of photoheterotrophy based on bacteriochlorophyll and proteorhodopsin complicate our quantitative analyses. The extent to which phytoplankton are capable of using

**Fig. 13.** A current view of the complex relationships between sunlight, biological energy production and dissolved organic matter (DOM) in the open sea. Recent discoveries of new marine bacteria include all three modes of photosynthesis: oxygenic photosynthesis (OP), anaerobic anoxygenic photosynthesis (AnAnP) and aerobic anoxygenic photosynthesis (AAnP), as well as two other potentially important light-driven processes, rhodopsin-based (RH) and phytochrome-based (PC) interactions that involve both light and DOM. Together, these light-driven processes, as well as others not shown here, sustain and control the flow of external energy into the global ocean. Reprinted by permission from MacMillan Publishers Ltd: from D.M. Karl, Hidden in a sea of microbes. *Nature* 415:490-491, copyright 2002.
organic forms of nitrogen and phosphorus is beginning to be appreciated. Characterizing the processes by which organic material is remineralized, the types, concentrations, rates and genes involved are important topics for the future.

**Comparative community genomics and ecosystem analyses**

One promising new direction for studying natural microbial assemblages is to conduct systematic comparative analyses ranging from genotype to phenotype. For example, intersite metagenomic comparisons, some containing billions of base pairs of genetic information, have enormous potential for Microbial Oceanography. What remains to be seen is the extent to which biological and ecological information can be extracted from metagenomic datasets. What genes typify selected planktonic ecosystems? Do shared gene sets amplify our predictive powers for determining ecosystem function, trends and responses? Can specific adaptations be predicted simply by the presence, absence or variation of genes along physical, chemical and biological gradients in the environment? Can lateral gene transfer be “caught in the act”, along gradients of environmental selective pressures? The newly emerging perspectives from environmental genomic approaches cut across traditional disciplinary boundaries, and may help form an important bridge in the quest to link systems biology with systems ecology.

From the perspective of the ecosystem, comparative studies across productivity or nutrient gradients, for example, can provide information about community structure/function, genome selection and metabolic regulation. For example, ectoenzymatic activity surveys across entire ocean basins (Fig. 14)

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**Fig. 14.** Cross-ecosystem comparison of in situ physiological potentials of the respective near-surface microbial assemblages as measured by the activities of two different enzymes: β-glucosidase (BGase; an enzyme that degrades polysaccharides) and leucine aminopeptidase (LAPase; an enzyme that degrades proteins). The potential rates shown are maximum velocities (Vmax) in units of nmol substrate per liter per day. The graph clearly shows that microbes near the equator have 3-4 orders of magnitude greater reactivity towards polysaccharides than either Station ALOHA (22°45’N, 158°W) or Southern Ocean microbes. Reprinted by permission from Christian and Karl (1995). Copyright 1995 by the American Society of Limnology and Oceanography.
reveal selected regions where either protein metabolism or carbohydrate metabolism appears to dominate. Although the microbial loop probably plays an important role in marine ecosystems in all climatic zones, there appears to be a significantly greater dependence on microbial processes in subtropical and tropical marine environments compared to higher latitudes due to the combination of low concentrations of nutrients, low turbulence and a shift in the size spectrum of primary producers towards picophytoplankton. Community structure matters; e.g., the ability of the ecosystem to assimilate allochthonous nutrients or anthropogenic pollutants, including carbon dioxide, is determined largely by the species present and their abilities to capture and transform energy and sustain element cycles and export. By studying the structures and dynamics of microbial communities in planktonic ecosystems with very different physical and chemical characteristics, common features as well as contrasting metabolic strategies can be observed. Ultimately, these cross-ecosystem comparisons might result in a more general mechanistic understanding of microbial processes in the sea.

Towards a general theory and overall research prospectus for Microbial Oceanography

Marine ecosystems are variable, complex adaptive systems. These different systems may have emergent properties, that cannot be predicted based on individual components, yet result from their complex interdependencies and interactions. The elemental composition of the microbial assemblage (dubbed ecological stoichiometry) is one example of an emergent property of marine ecosystems. Another characteristic of complex systems is that many interactions are non-linear with thresholds for response to change, and complex feedbacks across different scales of time and space. This implies that simple correlations do not exist, thereby precluding predictions of future states for key properties at the present time. Nevertheless, a significant body of ecological theory has been developed to explain the complex phenomena observed in plant and animal communities and this needs to be extended to marine microbes. Recent progress in this area shows that microbial communities in forest soils follow the same taxa-area law that applies to larger metazoans. This central tenet of ecology suggests that larger biomes will be able to support a higher number of taxa, and this was found to be the case for microbes whether a “taxa” was considered to be 95, 97 or 99% identical in the 16S rRNA (Hughes et al. 2006). If this is a more general rule, applicable to marine microbes, remains to be seen, but this is an important question because of the expansive size of many marine biomes, especially open ocean gyres. It is certainly plausible that microdiversity in habitats may result in increased genetic and physiological microdiversity, yet the scale at which marine microbial communities are routinely sampled may be inappropriate for observing these relationships.
The oceanographic research vessel Kilo Moana at its homeport in Honolulu, Hawaii.
Photo courtesy of SOEST, University of Hawai'i.
Resource needs

Grand challenges

Resource requirements fall into several broad categories: human resources, technological and infrastructural needs and the financial resources that supply and sustain them. Microbial Oceanography is an important new field with global relevance. The excitement of fundamental discovery and the inextricable linkages between marine microorganisms and Earth’s habitability need to be widely publicized in order to attract the best and the brightest of tomorrow’s scientific leaders. The demand for multi-disciplinary education and transdisciplinary data syntheses may require the development of a new curriculum and an educated workforce to teach it. With regard to financial resources, we should encourage and foster public-private partnerships and other non-traditional vehicles to support the primary research objectives outlined in this report. In the U.S., following World War II, a system of taxpayer-supported oceanographic research began with the establishment of the Office of Naval Research in 1946 and the National Science Foundation in 1950. This infusion of public funding for basic research along with the post-war growth of the academic fleet of research vessels provided new opportunities in addition to those provided by private foundations (e.g. the Rockefeller Foundation generously supported marine research in the first half of the 20th century) and corporation-sponsored, mostly applied, research (e.g., petroleum industry support of selected marine microbiological research projects). The scope and importance of the contemporary research prospectus for Microbial Oceanography demands new funding partnerships and international collaborations. Many of the research questions are so grand that reasonable progress can only be achieved by the formation of large coordinated teams of scientists working collectively toward a common goal.
Elucidating the structure and functioning of marine microbial communities requires many different areas of expertise from physical oceanography and organic chemistry to molecular biology and bioinformatics. Currently, there is only one location in the world — the Max Planck Institute in Bremen, Germany — where students can receive a Ph.D. degree (or equivalent) in “Marine Microbiology.” Even then, the training lacks exposure to the broad spectrum of disciplines that is necessary to tackle many contemporary and future challenges in Microbial Oceanography. The establishment of a summer course to provide an intensive interdisciplinary experience for senior graduate students, postdoctoral fellows and junior faculty represents a major and positive response to this challenge (http://cmore.soest.hawaii.edu). This is a significant first step in the establishment of a novel curriculum, perhaps leading to a specialized graduate degree, that could be replicated at major research universities worldwide. Additionally, the establishment of a postdoctoral fellowship program would provide specialized training across traditional academic disciplines and would facilitate the recruitment of qualified individuals, largely because of the unique challenges. The human resource needs for Microbial Oceanography are significant; successful recruitment of top tier scientists will ultimately determine the progress towards the stated goals.
Development of new methods

New technologies

Progress in Microbial Oceanography is limited, in part, by methodology and instrumentation that are required to obtain novel data on the characterization, distribution, metabolic activities and interactions of marine microbial assemblages. Because natural microbial communities are diverse but dilute, many of the “traditional” methods used in other subdisciplines of microbiology are inappropriate or ineffective when applied to marine ecosystems. Furthermore, current analytical capabilities are inadequate to measure many compounds at environmentally relevant concentrations and scales. There is a need for a focus on novel, high-sensitivity detection methods as well as miniaturization of current technologies to enable real-time data collection at high temporal or spatial resolution (mounted on a buoy, an ocean drifter or on a commercial vessel of opportunity). We recommend support for the development of novel technologies to fractionate heterogeneous field samples based on a variety of criteria for downstream analyses (isotopic, gene sequencing, proteomics) and the ability to conduct these analyses on very small quantities of material, ideally single cells (Lidstrom and Meldrum 2003).

Ocean surveillance and remote ocean observation

The field of oceanography is poised to initiate a fundamentally new phase of observation, one that relies heavily on the use of autonomous, remotely controlled instrumentation including fixed-position cabled observatories, mobile platforms and novel surveillance systems. This new age of ocean observations is driven by our need to detect environmental variability and climate change. Because microorganisms are so critical to the chemical state of the sea and the planet overall, it is imperative that microbe sensor technology and instrument development not lag behind other subdisciplines like geophysics and physical oceanography which currently enjoy the luxury of having commercial, off-the-shelf instruments available for their use. If microbial ecogenomic and metabolic sensors are not included in these field studies, microbial oceanographers will have missed a unique opportunity to collect high frequency, long-term observations at key locations. This opportunity is time sensitive because plans are already well underway for the development of several decade-long observation programs.
Database management and data synthesis

New databases and community reporting standards will need to be developed to take advantage of the large amounts of sequence, expression and proteomic data from the ocean, as well as the corresponding environmental data and many types of metadata. For example, the current structure of GenBank is not geared towards storing repeated occurrences of identical sequences in clone libraries, yet this information is critical to assess questions of species richness/evenness and adequacy of sampling. Perhaps even more important, environmental data collected during sampling are not currently linked with sequence information. In addition, upon completion of a genome project it is not standard to deposit sequence assembly files containing the raw sequence reads in their assembled state. This information is necessary to determine the degree of allelic variation between the two paired chromosomes of a eukaryote (Armbrust et al. 2004) or microdiversity within closely related cells of the same species that would be assembled into a single contiguous DNA sequence in an environmental shotgun sequencing project (Venter et al. 2004). Without this information, the scientific community is without access to a significant portion of the information generated by most sequencing projects, a missed opportunity to explore questions of community diversity, ecology and biogeochemistry. The field of Microbial Oceanography needs to establish a database that is unique and responsive to its needs and to the objectives of the discipline. To be most effective, the data which could be centralized or distributed, should be available to everyone free of charge in a common format. In addition, the scientists need to develop new tools for searching, synthesizing and integrating individual data sets so that meaningful conclusions can be drawn from complementary studies. It would also be desirable to develop openly accessible tools for data assimilation and construction of relevant ecosystem-based metabolic models for a variety of time and space scales.
Support for specific research projects

**Focused multi-investigator projects**

Microbial oceanographers could take a page from a geology book and agree on a single site to examine with a variety of approaches. These studies could be aimed at one or more existing ocean time-series sites to place the novel ecogenomics data into a larger framework of physical and biogeochemical investigation. Another project could focus on the intensive comparison of two different sites with similar chemistries. Are the same microbial communities found at each site? Or the same sets of genes apportioned among different phylogenetic lineages?

**Development of model systems**

Our understanding in the fields of microbiology, genetics and developmental biology has been profoundly influenced by the development of model systems that can be manipulated and studied from a variety of perspectives. The combined impact of many research teams working on the same system can result in accelerated progress compared to the division of this effort among multiple organisms. While no single organism can represent all of the phylogenetic or physiological variability in the sea, efforts should be made to identify a handful of model microbes that are amenable to intensive study.

Potential targets should include both single species as well as model consortia that could provide broadly applicable insights into the formation and maintenance of stable multispecies systems.

**Culture collections and intensive efforts to grow previously uncultivated organisms**

The existence of isolated cultures of marine microbes is of primary importance for exploring physiology, metabolism and gene expression in response to environmental conditions. Yet the maintenance of culture collections is often difficult to fund explicitly in research proposals. Similarly, while the majority of environmental microbes are not cultivable on traditional nutrient-enriched media, recent breakthroughs demonstrate that the cultivation barriers are not insurmountable (Rappé et al. 2002). However, such efforts are time consuming, and not without risk, and thus deserve a special category of support.
anammox: A microbial process catalyzed by specific bacteria, that converts ammonia in oxygen depleted environments to dinitrogen gas.

BAC: Bacterial Artificial Chromosome. A DNA cloning vector developed for the Human Genome Project. These vectors are capable of archiving and stably maintaining very large fragments of DNA greater than 200 kilobases.

biogeochemical cycle: The naturally occurring conversion of elements in nature, that usually requires a series of oxidations and reductions catalyzed by complex communities of different organisms. Each suite of organisms relies on another for provision of a given element (nitrogen, carbon, phosphorus, sulfur) in a specific oxidation state, that serves as its electron source or sink.

biological pump: The conversion of CO₂ to organic carbon, and its subsequent sinking and burial in the ocean, that effectively “pumps” carbon from the atmosphere to the deep-sea.

carbon sequestration: Any processes that collects atmospheric CO₂, and converts it to forms that separate it from the atmosphere. Carbon sequestration can be a purely biological process such as plant-associated CO₂ incorporation, or human-engineered.

cyano bacterium: Any bacterium that contains chlorophyll a and is capable of oxygen-generating photosynthesis.

genome: All the deoxyribonucleic acid (DNA) within a given cell that contains the complete set of instructions for synthesis and replication of that organism and its genes.

Haber Bosch process: A chemical process for producing ammonia from dinitrogen gas and hydrogen, under conditions of high pressure and temperature. Used industrially worldwide to chemically produce plant fertilizer.

heterotroph: An organism that derives cellular energy and carbon from organic material.

metagenomics: The sequencing and functional analyses of genomes or large DNA fragments recovered from collective microbial communities en masse.

microbe: A cellular organism not visible to the naked eye, generally smaller than 0.2 mm.

nitrogen fixation: A biological process catalyzed by bacteria and archaea in which dinitrogen gas from the atmosphere is converted to ammonia, and subsequently incorporated into organic matter.

photoheterotroph: An organism that derives cellular energy from light, and carbon from organic material.

photoautotroph: An organism that derives cellular energy from light, and carbon from CO₂.

phytoplankton: Photosynthetic algae or bacteria that are found free-floating in water bodies such as lakes or oceans.

plankton: Free-floating organisms found in the water column in lakes and oceans.

virus: Small infectious particle, usually composed of nucleic acid and protein, that replicates inside of cells using the host biosynthetic machinery.
The literature cited is not intended to be exhaustive, but simply represents a sampling of the current state of the field.


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