

EXECUTIVE SUMMARY

>> THE AMERICAN ACADEMY OF MICROBIOLOGY CONVENED A COLLOQUIUM APRIL 8-10, 2005, IN SAN FRANCISCO, CALIFORNIA, TO FOCUS ON MARINE MICROBIAL DIVERSITY. MICROBIAL PHYSIOLOGY, ECOLOGY, GENETICS, OCEANOGRAPHY, INVERTEBRATE BIOLOGY, AND VIROLOGY EXPERTS DISCUSSED THE IMPORTANCE OF MARINE MICROORGANISMS TO LIFE ON THIS PLANET, THE BIOGEOGRAPHY OF THESE ORGANISMS, THEIR ROLES IN SYMBIOTIC RELATIONSHIPS AND PATHOGENESIS, THEIR METABOLIC CAPABILITIES, THEIR IMPACTS ON HUMANS, AND GOALS FOR RESEARCH, TRAINING, AND EDUCATION IN MARINE MICROBIOLOGY.

Marine microbes are uniquely important to life as we know it. Since life most likely began in the oceans, marine microorganisms are the closest living descendants of the original forms of life. They are also major pillars of the biosphere. Their unique metabolisms allow marine microbes to carry out many steps of the biogeochemical cycles that other organisms are unable to complete. The smooth functioning of these cycles is necessary for life to continue on earth.

Early marine microorganisms also helped create the conditions under which subsequent life developed. More than two billion years ago, the generation of oxygen by photosynthetic marine microorganisms helped shape the chemical environment in which plants, animals, and all other life forms have evolved.

A great deal of research on the biogeography of marine microorganisms has been carried out, but many unknowns persist, and more work is needed to elucidate and understand their complexity. It is now known that microorganisms live in every corner of the oceans. Their habitats are diverse and include open water, sediment, bodies of marine macro- and microorganisms, estuaries, and hydrothermal vents. By studying these habitats, scientists have developed a limited ability to predict the composition of marine microbial communities.

It has also been found that some marine microbes have more cosmopolitan distributions than others. Recent work has found that most of the ecological principles that apply to larger organisms can also be applied to microorganisms, including marine microbes, but there are exceptions. Almost every ecophysiological parameter in the oceans is thought to have an impact on the diversity of microbial communities.

Most of the direct interactions marine microorganisms have with larger organisms fall into one of two broad categories: symbiosis or pathogenesis. Beneficial microbial symbioses have enabled many invertebrate species to take advantage of habitats that would otherwise be unavailable to them. Invertebrates in these relationships may also enjoy the benefits of bioactive compounds microbes may produce to prevent biofouling or to ward off predators. Marine viruses are found in surprisingly high numbers in seawater, but it is likely that these populations are in equilibrium with their host populations.

The metabolic diversity of marine microorganisms allows them to assume many roles in the biogeochemical cycles that other organisms cannot complete. Marine microbes are also able to adapt to the many extreme environments in the oceans. As humans continue to alter the environment, climate change will inevitably impact marine microbial communities and the biogeochemical cycles in which they participate, but the exact nature of these impacts cannot yet be predicted. Human health relies on a number of critical equilibria that marine microorganisms broker, including the balance between viruses and their hosts in the oceans, the balances that keep harmful algal blooms in check, the processes that control nutrient concentrations in marine waters, and others.

The metabolic capabilities of marine microbes can be put to work in any number of biotechnology applications, including the manufacture of industrial products and energy production. Marine microbes are sources of novel bioactive compounds that may have application as pharmaceuticals. Potential applications for marine microorganisms in ameliorating environmental degradation also exist.

Innovative approaches in research, education, and training are critical for moving the field of marine microbiology forward. Modern research in this field should embrace the new tools of genomics and metagenomics, but not to the exclusion of other methods of discovery. Education and training in marine microbiology needs to be multidisciplinary. Arrangements that expose graduate students and postdoctoral scientists to laboratories that do work outside the students' immediate fields of focus should be encouraged.



INTRODUCTION: MARINE MICROBES AND EARTH'S HABITABILITY



>> MICROBIAL RESIDENTS OF THE WORLD'S OCEANS HAVE HELPED MAKE THE BIOSPHERE THE BUSTLING, THRIVING PLACE IT IS TODAY. ALSO, GIVEN THAT LIFE ON THIS PLANET MOST LIKELY BEGAN IN WATER, MARINE MICROBES MAY HAVE BEEN THE FIRST FORMS OF LIFE ON EARTH AND, HENCE, THE PROGENITORS OF ALL LIVING THINGS, A DISTINCTION THAT PUTS MARINE MICROORGANISMS IN A KEY POSITION IN THE SAGA OF EVOLUTION.



For millions of years after emergence of the first life forms, microbial life in the oceans influenced the planet's chemistry, altering the chemical balance of the oceans and atmosphere and introducing gradients of oxidizing agents (electron-scavenging) and reducing agents (electron sources).

Early microbes introduced molecular oxygen to the atmosphere, an accomplishment that set the stage for the evolution of plants, animals, and humans.

These microbe-induced changes introduced a new era of chemistry on the earth—one based primarily on redox chemistry, the shuttling of electrons from one molecule to another. Redox chemistry is now the basis of the balanced biogeochemical and climatological cycles that sustain life on this planet.

Marine microbes also carry out many of the steps in these biogeochemical cycles, making them the workhorses of the biosphere. The owners of a diverse portfolio of possible activities, marine microbes provide most of the planet's metabolic capabilities that keep elemental cycles in motion. The metabolic rates of marine microbial communities are also high. Although terrestrial organisms comprise the vast majority of the biomass on the planet (3,200 gigatons or more; that is, 10^{15} grams),

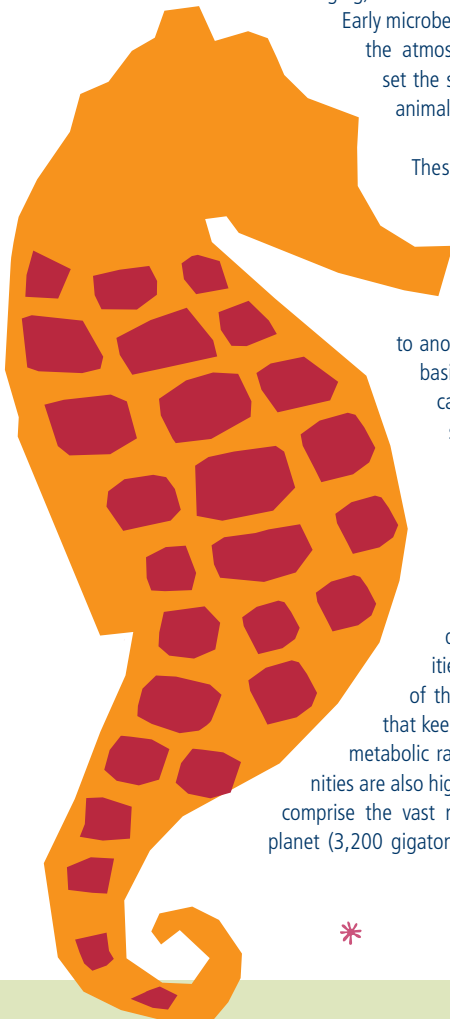
marine plankton (which weigh in at about 0.4 gigatons) carry out 45% of the total oxygen respiration on earth.

The ocean is filled with microorganisms that dwell there permanently and other microbes that have been carried there from terrestrial environments. In research on marine microbes, it is often necessary to arrive at a definition of what, exactly, a marine microorganism is. Any of a number of definitions can be used. A simple definition says marine microbes are just that—any microorganisms found in marine systems. However, this description does not exclude organisms that wash into the oceans from land and are not suited for growth in the marine environment.

An operational definition of marine microbes describes them as species that can grow and reproduce in the marine habitat. The possibility for growth can be determined using isotope feeding experiments, in which the organisms in question are monitored for growth on a diet of nutrients like those found in the marine habitat. The problem with this definition is that the reverse may not be true; a lack of growth does not necessarily indicate that a microorganism is not a contributor to the ecosystem. A dormant microbe found in seawater may be biding its time until conditions are right for its growth. A physiological definition identifies marine microbes as possessing adaptations specific to the marine environment. Under this description, marine microorganisms have precise physiological adaptations or even requirements for sodium.

In some cases, the distinction between true residents of the oceans and organisms that wash in from land is unimportant. Sometimes, exotic organisms (or even organisms that die once exposed to the marine environment) can play a role in the ecology of the oceans.

Regardless of the preferred definition, marine microbes hold a position of unique importance in the biosphere. They were the original form of life on earth and today marine microorganisms are a primary support for the biogeochemical cycles that continue to make life possible. A great deal of research has been carried out to elucidate the biogeography and metabolism of these organisms, but many unknowns persist. Uppermost on this list of questions is what effects human-induced changes will have on the services marine microbes perform for the planet. Research on marine microbiology must continue or accelerate in order to solve these problems.



>> CLEARLY, MARINE MICROORGANISMS ARE CRITICAL TO EARTH'S HABITABILITY, BOTH PAST AND PRESENT. UNFORTUNATELY, WE ARE ONLY BEGINNING TO UNDERSTAND MANY OF THE DETAILS ABOUT WHAT THEIR HABITATS ARE LIKE, HOW THEIR COMMUNITIES ARE STRUCTURED AND CHANGE OVER TIME, AND HOW THEY ARE DISTRIBUTED.

MARINE MICROBIAL HABITATS

Marine microbes continue to have a profound influence over the biosphere, but where, precisely, are marine microbes found? What are their various habitats like? How do you aseptically sample the habitats? These questions can be answered in a number of ways, based on the level of resolution that is of interest (see Table 1). The marine environment occurs on many scales, and there are many niche levels from which to approach a description of habitats. For example, a microbe floating in the middle of the Pacific Ocean could be described as "free-living," but this is no more or less accurate than the descriptors "pelagic" (meaning "open water," not sediment) or "within the Central Pacific gyre."

Perhaps the most important factor in defining marine microbial habitats is the distance over which these organisms interact with their environments. The habitat attributes that are apparent to the naked eye are usually less important to a marine microbe than the microscopic and submicroscopic facts, including concentrations of nutrients, the presence of gels and particulate matter, metal concentrations, light levels, pH, ultraviolet exposure and solar flux, temperature, oxygen saturation, and redox. Hence, the scale at which marine microbial habitats are most relevant is very small, but defining the boundaries of these habitats is difficult to accomplish in a controlled laboratory experiment and is even more difficult to define for a microbial cell embedded in the environment.

Microbial habitats in the oceans are influenced by an almost innumerable array of forces and factors, including salinity, currents, terrestrial inputs, and climate. Salinity is relatively constant in the open ocean, but is less stable in coastal areas. Ocean and seafloor currents have been shown to behave in ways other than previously thought, greatly affecting our understanding of transport processes in the deep sea. Terrestrial inputs create gradients of nutrients, pollutants, and other matter that affect habitats. Climate effects represent the largest scale of influence on microbial habitats. Temperature, precipitation, and wind (including windborne

TABLE 1>> EXAMPLES OF HOW THE MARINE ENVIRONMENT MAY BE DIVIDED INTO DIFFERENT MICROBIAL HABITATS

Criterion	Habitats
Presence of other organisms	Symbiotic Free-living Biofilm
Proximity to the ocean surface or sediments	Euphotic (0-150 m) Mesopelagic (150-1000 m) Bathopelagic (>1000 m) Benthos (sediments)
Concentration of nutrients and required growth substrates	Oligotrophic Mesotrophic Eutrophic

particulate matter) can each impact marine communities in a number of ways.



Importantly, marine microbes themselves exert influence on their habitats by consuming, producing, and sequestering a variety of compounds. Hence, in the oceans, gradients of materials important to micro- and macroorganisms alike are often controlled by processes carried out by microbes.

In the marine environment and elsewhere, interfaces tend to be hotspots of diversity and biological activity. Marine microbial habitats at interfaces include the air-water, water-sediment, water-ice, and host macroorganism-water interfaces. The sub-millimeter scale of physical and chemical variability in these habitats poses a serious challenge to studying interface habitats in detail.

CHANGE OVER TIME

Microbial habitats change over many time scales—diel (daily), seasonal, decadal, and longer. Many of the changes induced by human activities can impact marine microbial communities and, in turn, can impact the ways by which those communities modulate the environment and climate. Temporal changes in marine microbial habitats can be illustrated by describing three disparate habitats: the central Pacific gyre, the Chesapeake Bay, and hydrothermal vents. The central Pacific gyre is an open ocean habitat that changes on a diel basis, but it has also exhibited changes over decades as





shifts occur between community domination by diatoms and by picoplankton. The Chesapeake Bay exhibits diel changes, marked seasonal changes, and profound decadal changes over the past couple of centuries as human activities have taken their toll. Hydrothermal vents exhibit both short and long periods of fluctuation, changing over the course of minutes, hours, and decades. This variability creates an ephemeral and unpredictable habitat for microorganisms.



In the coming years, if observed trends in greenhouse gas emissions continue, increasing concentrations of atmospheric carbon dioxide are expected to result in a pH decline of 0.3 in the oceans—a small number that signifies big changes. This would be an utterly radical transformation of the ocean habitat for microorganisms and macroorganisms alike. To illustrate, a similar pH shift in the acidity of human blood would result in acidosis and a painful death.

INTERACTIONS BETWEEN SEDIMENT-DWELLING AND PLANKTONIC MARINE MICROBIAL COMMUNITIES

In general, marine microbes in and near sediments interact with and intercept reductants diffusing from the sediments below and oxidants diffusing from the water column above. Conversely, planktonic microbes intercept carbon compounds from photosynthetic activities near the surface of the ocean and control the downward flux of nutrients to the sediments. The connections between subsurface and planktonic environments are probably greatest in zones where the sea floor is spreading. However, details of the interactions between the microbial communities of marine sediments and communities in the water column are not known—a clear gap in the current knowledge.

MARINE SEDIMENTS, BIOFILMS, AND EARLY LIFE

In some ways, the physical and chemical circumstances of marine sediments are thought to reflect those that nurtured the beginnings of life on this planet. Studying marine sediment and the life that exists there today could provide insight into early life:

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- >> Redox coupling may have been important to fostering formation of the organic molecules that propagated early life. Marine sediments harbor marked layering of redox potentials that enable extensive redox coupling.
 - >> Methane may have been one of the building blocks of early life, and there are numerous areas of the ocean floor where methane percolates up from the deep subsurface.

- >> Heat and pressure could have facilitated formation of early biological molecules, including amino acids. These conditions exist in parts of the sea floor that combine high depth with communication with the earth's hot core.
- >> Given the pivotal role of iron in the functioning of many enzymes, iron is thought to have been abundant where enzyme systems first evolved. Marine sediments are usually iron-rich environments.
- >> Before the dawn of oxygenic photosynthesis, the earth's atmosphere was anaerobic, a condition mirrored by subsurface marine sediments.

It can be argued that shallow microbial mats and biofilms are the most appropriate systems for modeling early life, as the high metabolic diversity and spatial separation of metabolisms of these arrangements closely reflect fossilized examples of early microbial communities. Microbial communities that dwell in marine sediments can be construed as biofilm or mat communities, given that these communities dwell on the surfaces of sediment particles (like biofilms) and can form thick accumulations of interacting cells (like microbial mats).

COMMUNITY COMPOSITION

Although intuitively apparent, it can be difficult to reach agreement on the precise technical definition of a microbial community. It is largely agreed that microbial communities are groups of microorganisms that interact and, together, accomplish more than those same organisms would separately. Communities are also influenced by common factors and/or by each other. However, the question of whether chemical interdependence, a form of interaction, is required among members of a community is more controversial. A continuum of interactions, ranging from obligate to minimal, is thought to exist among members of microbial communities; strict interdependence is not necessarily a requirement for the designation "community." Microbial communities, in which members interact, are distinct from microbial assemblages, in which members merely coexist.

PREDICTING THE COMPOSITION OF MARINE MICROBIAL COMMUNITIES

The particular physical and chemical conditions of a given marine habitat, including resource availability, select for distinct groups of microorganisms, and there is a certain amount of predictability in the character of the resulting community.



Mapping the microbial species onto the physical and chemical variability of marine habitats is becoming increasingly feasible in certain habitats and with certain well-described species. For example, the general distributions of two planktonic genera, *Synechococcus* and *Prochlorococcus*, are well-understood information that can be extrapolated to unknown planktonic communities. The presence of certain broadly-defined functional groups, such as nitrogen fixers or calcium carbonate producing microbes, can also be predicted.

Most marine microbial communities are not yet fully described, however, so it is difficult, if not impossible, to predict the species-level composition of a marine microbial community. Hence, it is often possible to predict the functions in place in a given marine microbial community, but it is seldom possible to predict the genera or species present.

The scale and level of resolution of the inquiry into a marine microbial community are important factors in predictability. Communities can be unpredictable on a small scale (1 meter) but predictable over larger scales (kilometers). The level of resolution often determines the results of a study on community diversity. Deep, exhaustive sampling can reveal much greater diversity and complexity than shallow sampling. Predictability in marine microbial communities that is based on 16S rRNA genes may not be corroborated by further work at the genome sequence level. Genome sequences usually reveal much greater and, currently, unpredictable diversity.

The biome concept may be useful in predicting the composition of microbial communities. By this reasoning, the energy inputs into an ecosystem are evaluated, and the role this energy plays in defining the attributes of the microbial community is examined.

Alternatively, a microbial community may be structured by an evolutionary history that prevents the spread of that community into new environments or, conversely, prevents the entry

and establishment of new species into that community. If a community's history dictates its composition, it would be very difficult to predict the structure of the community from transitory features like nutrient status and temperature.

Certain tight associations, like the one between the bacterium *Vibrio angulata* and the alga *Ulva*, can allow researchers to use the presence of one species to predict the presence of a partner species. Recent studies have shown *Ulva* propagules will not establish themselves on a surface in the absence of chemical signals from their biofilm partner, *V. angulata*. Hence, if *Ulva* is detected in a biofilm, it can be assumed that *V. angulata*, too, is present.

In seeking to better predict the structure of marine microbial communities, there is a need to know more about possible keystone species—organisms that may be present at low or high numbers but perform indispensable functions for the community.



MICROBIAL DISTRIBUTION IN THE OCEANS

The issue of whether marine microbial species are either cosmopolitan, or are more provincial and limited to certain geographical areas, was raised decades ago. The question endures today because of the bearing it has on the conservation of biodiversity. If marine microbes are not cosmopolitan, then does the international scientific community need to act to preserve the microbial diversity harbored in endangered habitats?

Current evidence indicates that most marine microbes are not cosmopolitan, but, instead, are restricted to specific habitat types or geographic locations. However, there are a few examples of truly cosmopolitan organisms, including the deep-sea marine group I archaea. As new, higher resolution technologies become available, further research may show other microorganisms to be more widespread than previously thought.

Free-living marine microbes may be more cosmopolitan than symbionts, biofilm-associated microbes, and others. Extinction is a real possibility for symbiotic microbes since they are dependent on the survival of their host and many, many species of marine macroorganisms are currently endangered.





It is essential to note that the number of representatives of a given group is not necessarily linked to the importance of that group in the functioning of the community. Common organisms may not play a critical role in the dynamics of a given community despite their numbers, and organisms that only muster 0.1% prevalence, like nitrogen fixers, can be of pivotal importance. Consequently, it is not known whether cosmopolitan microorganisms like *Synechococcus* are common because they are essential to their communities or because they are weedy individualists that can survive in a wide spectrum of environments.



One observation that appears to support the idea that marine microbes are largely cosmopolitan is the establishment of novel microbial communities in the wake of a disturbance. Often, the new communities are dominated by microbial types that were previously present in low or undetectable numbers.

The long-standing question of how to taxonomically divide microbes creates some confusion in discussions about cosmopolitan distributions. A working definition of an appropriate taxonomic unit is needed to address research in microbial distribution in the oceans and elsewhere.

Millimeter-scale analyses of marine habitats may help to reveal the distribution variability of marine microbes over very small spatial scales.

OUTSTANDING QUESTIONS ABOUT MARINE MICROBIAL DISTRIBUTION

A number of outstanding questions about marine microbial distributions remain to be addressed. These include:

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- >> What is the ecological relevance of the functional gene diversity observed in marine microbial communities? Does the diversity have an impact on ecosystem function or long-term stability?
 - >> Are changes in microbial communities predictive of changes in environmental function?
 - >> The long-term variability of marine microbial communities is poorly understood. Can intermittent sampling, like taking community snapshots, address this question or is continuous sampling necessary to evaluate community composition shifts?

- >> Are there keystone species in marine microbial communities that are critical to a given function? How can scientists identify those organisms?

FORCES AT WORK IN MAINTAINING STEADY STATE IN MARINE MICROBIAL COMMUNITIES

Many marine surface waters maintain steady populations of approximately 10^6 bacteria and 10^7 viruses per milliliter, a condition that may be determined by nutrient limitations and predation controls enforced by protists and viruses. It is thought that, in some cases, microbial population numbers are kept at about an order of magnitude below the carrying capacity of the habitat—presumably by predation. It is thought that viruses may coexist with their hosts, helping to structure communities and diversity, but some studies have shown that the removal of viruses from a system has no effect on bacterial abundance and community structure. Protists, on the other hand, are thought to play a more antagonistic, less discriminating role in steady state maintenance of bacterial cell abundances by consuming many different types of bacterial prey.

MACROECOLOGICAL THEORY AND OCEAN MICROBES

It only became feasible to test ecological theory as it applies to marine microbial communities within the last 10 years, after basic questions about the abundance and species distribution of marine microorganisms had been answered. In general, ecological concepts like predation, competition, and diversity appear to be applicable to marine microbial communities, but particular problems related to scale, food webs, the species concept, and the traditional focus on biochemical and geochemical sciences within marine science preclude broad application of ecological theory to these communities.

The small scales relevant to microbes are often not addressed adequately by traditional macroecology. Also, the structure of food webs in microbial systems is likely to take on a very different form than those described by traditional macroecology.

The species concept poses a big stumbling block for applying ecological theory in marine microbial systems. The grouping of organisms into clusters of species is the basis of most ecological theory, but most microorganisms do not fall neatly into species categories because of their ability to reproduce asexually. It may be that genes or genomes are more appropriate taxonomic units than species for modeling in microbial systems.

To a certain extent, the biogeochemical and geoscience focus of marine science has prevented robust integration of eco-

logical and evolutionary theory into marine microbiology. Rigorous inclusion of ecological science within classical oceanographic science is needed to satisfactorily address contemporary challenges in marine microbiology. This will require extensive dialogue between theoretical ecologists, evolutionary scientists, physical and chemical oceanographers, and marine microbiologists.

With man-made pressures on the world's natural systems growing annually, a thorough grasp of the ecology of all environments, the oceans included, will be necessary to mitigate damages and manage our natural resources for the benefit of future generations. Because of their short generation times and relatively simple physiology, microorganisms have been and will continue to be powerful model systems for testing ecological theories.

FACTORS THAT IMPACT MARINE MICROBIAL DIVERSITY

The list of factors that impact marine microbial diversity is not a short one. Nearly every measurable physical, chemical, and biotic variable in the marine environment has been found to increase, decrease, or otherwise alter microbial diversity. See Table 2 for a list of several of the more important factors.

For the most part, the extent to which each of these influences actually operates in the environment and the contexts in which they are important remain to be determined.

Climate change, which will be felt by marine microbial communities as changes in ocean temperatures, will undoubtedly alter the diversity of communities in unforeseen ways. Climate change should be considered a major top-down controller of microbial communities.

Pollution, including nitrogen inputs due to anthropogenic nitrogen fixation, also impact marine microbial diversity (see

section on Humans and Marine Microbes). Anthropogenic nitrogen inputs to the oceans now comprise about half the total nitrogen inputs to the oceans, a circumstance that has resulted in vast dead zones in coastal areas and an increased incidence of harmful algal blooms.

INTERACTIONS BETWEEN MARINE MICROBES AND MARINE MACROORGANISMS

Most marine ecosystems are fueled by the regeneration of nutrients—processes mediated by marine microorganisms. This is the most fundamental service provided by marine microbes; they are responsible for the cycles that sustain all living things in the oceans.

Microbes also play more direct roles in the health of corals and other marine organisms. For example, corals die when the bacteria that live on their surfaces are removed, although the mechanism behind this observation is not known. Also, bacteria associated with squid eggs have been shown to protect the eggs from fungal infection. Biofilm bacteria are known to broadcast attraction cues that affect the settlement of invertebrate larvae in those biofilms.

TABLE 2>> FACTORS THAT IMPACT MARINE MICROBIAL DIVERSITY	
Factors	
> Turbulence	> Salinity
> Light	> pH
> Temperature	> UV and Solar flux
> Nutrients	> Presence of macro-organisms, such as invertebrates and macroalgae
> Surfaces and interfaces	
> Redox potential	
> Metals	



SYMBIOSIS AND PATHOGENESIS IN THE OCEANS

>> MANY DIRECT INTERACTIONS BETWEEN MARINE MICRO-ORGANISMS AND MACROORGANISMS CAN BE CLASSIFIED UNDER ONE OF TWO CATEGORIES: SYMBIOSIS OR PATHOGENESIS. IN BENEFICIAL SYMBIOSES WITH INVERTEBRATES, BOTH THE MICROBE AND THE INVERTEBRATE BENEFIT FROM THE RELATIONSHIP. THIS TYPE OF WIN-WIN RELATIONSHIP IS ALSO CALLED MUTUALISM. IN PATHOGENESIS, THE MICROBE TAKES ON THE ROLE OF PREDATOR, SEEKING TO EXPLOIT AND POSSIBLY DESTROY THE HOST.

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SYMBIOSES WITH INVERTEBRATES

A number of marine invertebrates, including species of corals, sponges, squids, shipworms, and others, are associated with unique species of bacterial and/or archaeal symbionts. Symbiosis between a microbe and a marine invertebrate affords the microbe shelter, nutrients, and possibly a route for reproduction and dispersal, and offers the host a variety of benefits. In some cases, one invertebrate species may be host to many, possibly hundreds, of unique microbial species, a detail that has important implications for marine microbial diversity considering the fact that about 1,000 coral species and more than 5,000 sponge species populate the oceans. The diversity of marine symbionts is an understudied field.

The diversity of marine microbial symbionts and the closeness of symbiotic relationships may be determined by the mode of transmission employed by the invertebrate hosts. Transmission is accomplished either by "horizontal" or "vertical" means. Studies have found that horizontally-transmitted symbionts, which are dispersed in the environment and picked up by invertebrates, are more taxonomically diverse than vertically-transmitted symbionts with respect to the internally transcribed spacer (ITS) regions of their ribosomal DNA. (The ITS region is variable in length and sequence and can be used to identify and establish relatedness between microorganisms.) The reason for this difference may be the fact that

vertical transmission, passing symbionts from generation to generation through the gametes, more easily allows the transmission of identical clonal organisms between members of an invertebrate species. The full genome sequences of both vertically- and horizontally-transmitted marine symbionts are currently being studied and will probably shed more light on the diversity of these organisms.

Vertical transmission is likely to lead to a close symbiosis between the microbe and its host, since inheriting a symbiont from one's forbearers affords few opportunities for host- and symbiont-switching. In horizontal transmission, the host is more likely to acquire symbionts appropriate for the particular location where the host settles down.

It is possible that in the early stages of a new symbiotic relationship, horizontal transmission of a symbiont can be used effectively, but as the relationship becomes tighter and more necessary to the survival of the microbe and its host, transmission must shift to the vertical mode, via the gametes. Symbionts have been known to dispose of those parts of their genomes that are redundant within the protective confines of the host. Giving up part of its genome can render a microbe less fit for survival outside the host, making vertical transmission necessary for the survival of the symbiont.

In studying marine symbionts *in situ*, it can be difficult to separate the symbionts from the pathogens and transient populations of microbes that may also be found in and around an invertebrate. Phylotype-specific probes that preferentially detect microbes with high concentrations of RNA compared to those with lesser concentrations can be used for this purpose.

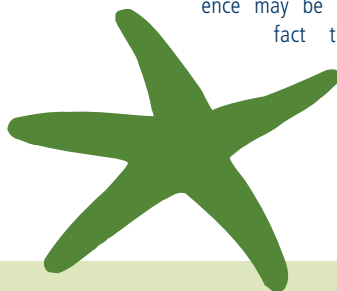
Symbiosis and the Invasion of New Environments

Symbioses with bacteria and/or archaea have enabled some marine invertebrates to exploit habitats that would otherwise be unavailable to them. Hydrothermal vents, for example, represent an extremely inhospitable environment to the unprepared tube worm, but the tube worms that thrive in these sulfur-rich, oligotrophic zones (low in organic carbon) harbor chemoautotrophic bacteria that synthesize organic carbon using the energy from respiring reduced inorganic sulfur compounds. The bacteria provide the organic compounds to their hosts, allowing the worms to live on carbon from inorganic sources and opening up a new world of habitats for the worms.

Other examples of symbionts that have enabled invertebrates to take advantage of a new environment include nitrogen-fixing, cellulose degrading bacterial symbionts, which have



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allowed their shipworm hosts to live on a diet of wood, and luminescent bacterial symbionts that enable squid to hunt in moonlit waters without casting a shadow that could be detected by predators.

In some cases, obligatory symbioses with microbes can limit the ability of an invertebrate to invade a new habitat. The temperature limits of the bacterial symbiont *Symbiodinium*, for example, appear to curb the number of locations where the host coral species can survive. Studies show *Symbiodinium* may also be more sensitive to stress from anthropogenic (human-made) sources than its host, another factor that could limit the habitats where the coral could establish itself.

Symbionts and the Production of Bioactive Compounds

Many highly bioactive compounds have been isolated from marine invertebrates, including a number of materials with biomedical or industrial significance. It is now known that, in many cases, these substances are produced by symbiotic microbes rather than by the invertebrates themselves. Microbial symbionts synthesize many secondary metabolites, e.g., bryozoan and sponge species.

The exact biological function of the bioactive compounds produced by microbial symbionts is not known, but in the case of cytotoxic compounds found in sponges and tunicates (a type of marine worm), they may be used to prevent fouling of the host. Other materials may be used to deter reef fish from feeding on the invertebrates.

Bioactive compounds produced by microbial symbionts may be useful to humans in any of a variety of ways. If the microbes that produce them can be isolated successfully, it may be possible to achieve large-scale production of these materials by industrial fermentation processes similar to those commonly employed by pharmaceutical companies for the large scale production of antibiotics.

MICROBIAL EFFECTS ON THE ECOLOGY AND LIFE HISTORY OF MARINE INVERTEBRATES

Microbial symbionts can have profound effects on their hosts—effects that have consequences for the ecology and life history of these invertebrates. For example, local microbial diversity has been shown to impact the suite of symbionts harbored by a widespread species of mussel. Studies of the ITS region of the ribosomal DNA of mussel symbionts and their environments have shown that one species of mussel

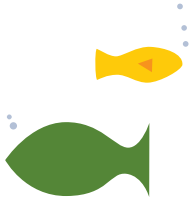
has different populations of bacteria in different geographic areas, a reflection of the bacterial populations available for colonization.

In more rigid, obligatory symbioses, like that between the hydrothermal vent tube worm *Riftia* and its symbionts, the presence of free-living symbionts is necessary for an invertebrate to colonize a given area, posing a limitation on the habitats available to that organism. In another example of a tight relationship (as described previously), the bacterium *Vibrio anguilara* is required for the algae *Ulva* to establish itself on a surface.

There is some evidence that the algae to blame for harmful algal blooms (which have caused as much as \$1 billion damage over the past decade) require not only the appropriate nutrients in order to proliferate into a bloom, but they are also reliant on certain bacterial associates. In other words, if the bacterium is present, an algae bloom is possible and if the bacterium is missing then a bloom may not happen. The mechanism behind this phenomenon is not yet known.

MARINE VIRUSES

At first glance, the numbers of viruses found in marine waters appears to be exceedingly high. Locations studied to date have revealed viral counts on the order of 10,000,000 viruses per milliliter of water. However, these numbers are less surprising when one considers the number of prospective microbial hosts available to those viruses. Approximately 1,000,000 microbes are found in a milliliter of seawater, making the ratio of viruses to hosts roughly 10:1, a reasonable proportion for ensuring that a virus meets up with prospective hosts often enough to propagate itself before is



disintegrates. The half life of viruses averages between two to four days, so a virus population must be sufficiently large to ensure that at least some of its members meet up with an appropriate host during that window of time. Viruses that infect rare hosts may need to live for longer periods of time to survive the interval between infection events.

Marine viruses likely play a number of important roles in the ecology of marine microbes. Obviously, viruses act as predators, causing the mortality of marine microbes. Viruses also help to maintain the high levels of microbial genetic diversity observed in marine ecosystems because hosts are known to manipulate their genomes to evade diseases. By moving DNA between the host cells, viruses act as agents of sex, shuffling genetic information around the community and providing new and surprising combinations of genes in their hosts.

Viruses can also play a role in the ecology of host cells by lysogenic conversion, a phenomenon in which a phage changes the phenotype of a host cell by either introducing genetic material into the host's genome or by other means. It has been found, for example, that marine viruses can carry the genes necessary for photosynthesis, and that these genes are regularly transferred between host cells of *Prochlorococcus*. This temporary storage in viruses and the efficient shuffling of the genes among *Prochlorococcus* species probably has had a profound impact on the evolution of these photosynthesis genes and on the ecology of *Prochlorococcus*.

The diversity of marine viruses is not limited to bacteriophages (viruses that infect and lyse bacteria). Many other forms of viruses, including DNA and RNA viruses with a wide variety of different sizes, host ranges, and biological properties, remain almost entirely uncharacterized. Marine viruses represent a new, unexplored world of diversity.

THE ABUNDANCE AND DISTRIBUTION OF BACTERIAL AND VIRAL PATHOGENS

In the marine environment, as elsewhere, the distribution of a bacterial or viral pathogen is directly determined by the virulence of the pathogen and the number of susceptible hosts available. This balance between hosts and pathogens generates and maintains the diversity of both groups. However, this delicate relationship breaks down in some instances. For example:

>> Lateral gene transfer between bacteria (carried out by viruses) and lysogenic conversion may be important

mediators of change from non-pathogenic to pathogenic states in some bacteria.

- >> Opportunistic pathogens like *Vibrio cholerae* do not comply with the rules of host availability since they can exist outside the host.
- >> Climate-related factors, including temperature, have been shown to trigger a pathogenic state in certain opportunistic pathogens like *Vibrio shiloi* and coral symbiotic algae called zooxanthellae.
- >> Human activities and chemical pollution can also influence the abundance of pathogens by stressing and destabilizing microbial communities.



METABOLIC CAPABILITIES AND ROLES

>> MICROORGANISMS ARE SINGULARLY ADAPTABLE CREATURES, AND THE MICROBES THAT DWELL IN THE WORLD'S OCEANS ARE NO EXCEPTION. MARINE MICROBES HAVE MANAGED TO PUT ALMOST EVERY ENERGETICALLY FAVORABLE CHEMICAL PROCESS TO WORK FOR THEM, MAKING THEM KEY PLAYERS IN THE CYCLES THAT MOVE THE ELEMENTS NECESSARY FOR LIFE AROUND THE BIOSPHERE. THE FLEXIBILITY OF MARINE MICROBES HAS ALSO GRANTED THEM A NUMBER OF UNIQUE METABOLIC CAPABILITIES AND ADAPTATIONS.

SCALE OF MICROBIAL PROCESSES IN THE SEA

Microbial interactions and processes have implications over a very wide range of scales in the oceans, from the nanometer scale (0.000000001 meter) to the kilometer scale (1,000 meters) and greater. The global outcome of microbial metabolic processes is the integration of interactions on very small scales. In designing studies of marine microbial communities, it is important to remember the importance of microscales. They should not be overlooked.

GLOBAL CYCLES OF BIOELEMENTS

The cycles of nitrogen, oxygen, carbon, sulfur, phosphorus, iron, and other bioelements that sustain life on this planet are driven, in part, by the microorganisms in the oceans. Microbes are capable of using every natural compound on the planet and most of the human-made compounds as well. It is this metabolic flexibility that secures microbes' importance in the cycling of the bioelements. Microorganisms control the rate-limiting steps of the cycles that no other organisms can execute. Microbes also strengthen the feedback systems that increase the stability of the cycles of the bioelements. The details of many key processes in the bioelemental cycles remain unknown, and it is largely unknown which microbes are the largest contributors to these cycles, or if it is even possible for one species to be dominant.

Although the exact contributions of marine microbes to the biogeochemical cycles is uncertain, because of their metabolic capabilities and their sheer numbers, marine microorganisms are thought to be major players in every cycle relevant to life. It is estimated that if the oceans were emptied of microbes, the carbon dioxide in earth's atmosphere would increase sevenfold. Moreover, half of the microbially-mediated nitrogen fixation occurs in the oceans, and nitrification and denitrification, two key processes that set the pace of the

nitrogen cycle, are carried out by microbes. Marine microbes may also play a role in cloud formation by cycling compounds such as dimethylsulfide into the atmosphere. A constant efflux of methane from the surface of the oceans has been detected, and although the process is not entirely understood, it is undoubtedly microbially-mediated.

As more and more of the key players in the cycles of bioelements are cultivated and studied in the laboratory, and as metagenomic studies continue to contribute to the mapping of metabolic processes onto ocean depths and provinces, scientists are coming to a better, more sophisticated understanding of how elemental cycling is carried out in the oceans.

UNIQUE METABOLIC CAPABILITIES OF MARINE MICROBES

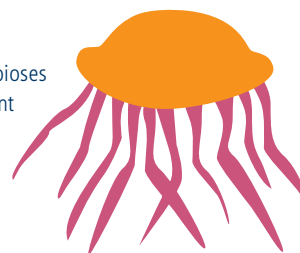
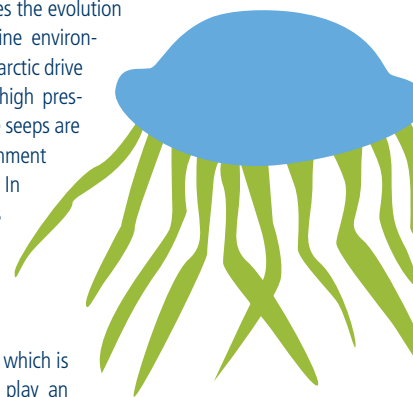
Marine microorganisms possess a number of metabolic capabilities that cannot be found in terrestrial microorganisms. As in other ecosystems, the geochemical habitat drives the evolution of different metabolic capabilities in the marine environment. For example, cold environments near the arctic drive different adaptations than high temperature, high pressure habitats near hydrothermal vents. Methane seeps are another example of a uniquely marine environment that has motivated novel metabolic capabilities. In methane seeps, high sulfate concentrations combine with high methane concentrations to favor the anaerobic oxidation of methane, a metabolism found only in the oceans.

The light-driven proton pump proteorhodopsin, which is found only in marine bacteria, is thought to play an important role in the energy balance of the biosphere because of its ability to efficiently generate energy from light. The use of sodium-dependent transporters is also limited to marine microorganisms.

Marine symbioses, including the symbioses between macroorganisms and bioluminescent bacteria and between shipworms and nitrogen-fixing cellulolytic bacteria, have given rise to many unique metabolic activities.

ADAPTING TO EXTREME ENVIRONMENTS

The oceans are host to many different kinds of extreme environments, and marine microbes have found numerous ways to thrive in those places by either changing their biochemistry to cope with the conditions or by creating barriers to keep the harsh conditions out of their cells.



In cold environments, psychrophilic bacteria cope with the conditions by maintaining flexible membranes and by circulating natural antifreeze compounds throughout the cell. In extremely hot areas of the oceans, hyperthermophilic microbes use subtle changes in their proteins to maximize the number of stabilizing salt bridges and ion pairings that keep enzymes together in the heat. Reverse gyrase, which allows DNA to maintain its structure during replication at high temperatures, is another unique enzyme used by hyperthermophiles.

In areas of high salt concentrations, many halophiles have adapted by altering their proteins, making them acidic, and hence, resistant to the protein-scattering effects of high salt concentrations. Other halophiles circulate osmolytes, small organic molecules that prevent water loss to the highly salty surroundings outside the cell.

The marine environment presents an unparalleled opportunity for studying life in extreme environments. Nowhere else on earth do so many different environmental conditions exist in such compact areas. The oceans are home to extremely high pressures and to the highest temperatures of any aquatic system. Deep sea brines, which have high concentrations of both salts and heavy metals, are singular habitats where life thrives despite the difficulties. Because of these diverse habitats, the oceans are unique with respect to the challenges posed to life and with respect to the wealth of biological diversity that has arisen to cope with those challenges.

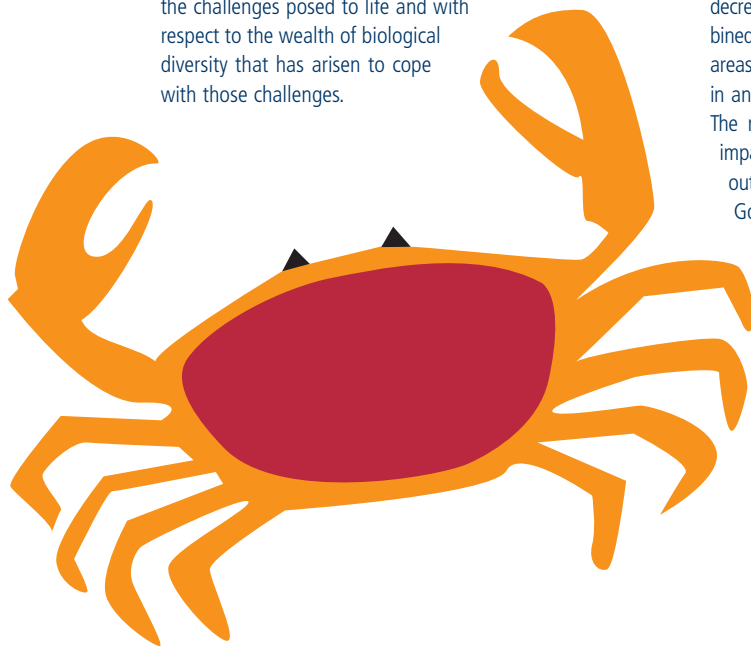
IMPACTS OF HUMAN-INDUCED CHANGE ON THE CYCLING OF ELEMENTS IN THE OCEANS

Since the industrial revolution, vast quantities of many important bioelements have been taken from relatively inert or inaccessible sources and released into the planet's air, soil, and water. Inputs to the environment on this scale directly and indirectly impact the oceans, and atmospheric inputs of greenhouse gases have induced rapid climate change. As world populations climb and resource exploitation soars, environmental inputs and climate change are accelerating at an alarming rate. Some significant alterations in the microbial communities of the oceans, and, therefore, in the global cycling of the bioelements, are anticipated in the wake of these escalating changes.

The impacts of global temperature changes resulting from climate changes will be felt by microbial enzyme systems. Different enzyme systems have different temperature optima, so microecological processes in a given area may be depressed or stimulated depending on the nature of the local temperature change (up or down) and the optima of the enzyme systems involved. These changes are not predictable, since the optimum temperature for enzymes are not easy to decipher from an organism's habitat.

In some areas of the world, climate change has resulted in a decrease in rainfall. In Saharan Africa, a decline in rainfall combined with human activities has led to desertification of many areas and encouraged an increase in wind-born dust, resulting in an increase in the deposition of African dust in the oceans. The metals carried in this dust, including iron, are likely to impact marine microbial communities and the cycles they carry out that sustain the biosphere. (As a side note, in China's Gobi Desert, irrigation and other land use practices have actually decreased the size of selected deserts. This has led to a decrease in dust deposition, and could cause iron limitation in the marine waters where dust and the iron it carries is otherwise deposited.)

Human inputs of nitrogen to the oceans, which comprise roughly half of the total nitrogen inputs, are impacting the global cycling of nitrogen in both known and unknown ways. For example, nitrogen pollution has had an impact on nitrous oxide cycling in coastal systems, appar-



ently resulting in a net increase in nitrous oxide production. Nitrogen-burdened, oxygen-depleted coastal regions account for only 2% of the ocean surface area, but these regions contribute about 20% of the nitrous oxide the oceans release to the atmosphere. The concentration of nitrous oxide in the atmosphere is increasing at an accelerating rate, and it is not clear how this trajectory will change with global climate change. It is

acid (H_2CO_3) which subsequently dissociates into H^+ and HCO_3^- . These materials lower the pH of the water. The concentration of carbon dioxide from human sources is also on the rise. Hence, a decrease in the pH of seawater is expected to echo the global warming trend and ongoing increases in carbon emissions. Ocean acidification will have a direct impact on corals. They are expected to grow more slowly and become

THE CONSEQUENCES OF GLOBAL CLIMATE CHANGE AND HUMAN INPUTS OF BIOELEMENTS TO THE OCEANS ARE POTENTIALLY DISASTROUS.

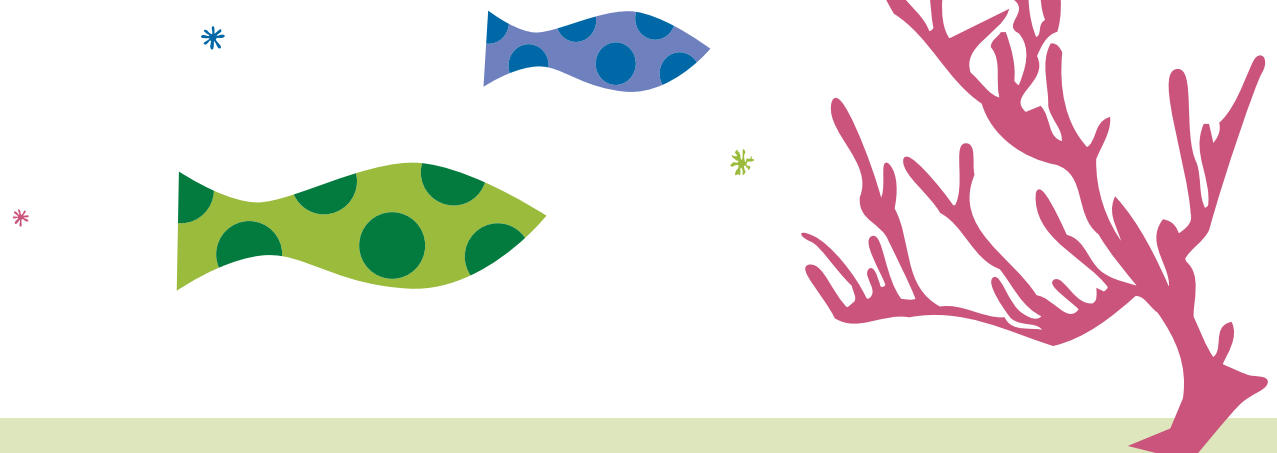
likely that escalating nitrogen inputs will continue to perturb and transform the nitrogen cycle and that nitrous oxide emissions from the oceans will continue to climb.

Because of their rapid growth rates and metabolic flexibility, marine microbes could possibly serve as a buffer that helps dampen large scale changes resulting from human-induced modifications of the oceans. There is a great deal of uncertainty about how well this dampening effect works in practice, however. It may be that marine microbes have a certain buffering capacity, but when it is exceeded conditions will decline precipitously.

The consequences of global climate change and human inputs of bioelements to the oceans are potentially disastrous. Temperature increases can have an indirect effect on the acidity of ocean water, and thus, on the marine habitat. The solubility of carbon dioxide increases with temperature, and dissolved carbon dioxide combines with water to form carbonic

more fragile under these circumstances, decreasing the ability of coral reefs to act as protective island barriers even as storm intensity increases because of climate change.

Climate change may also bring about big changes in the stratification of the oceans, reducing mixing and reducing the output of marine fisheries.





>> MARINE MICROORGANISMS ARE INEXTRICABLY LINKED TO HUMAN LIFE THROUGH BIOGEOCHEMICAL CYCLES, PATHOGENIC RELATIONSHIPS, AND MICROBIAL INFLUENCES ON OCEAN FOOD WEBS. HOWEVER, MANY HUMAN ACTIVITIES CAN IMPACT MICROBIAL DIVERSITY, WHICH, IN TURN, IMPACTS THE SERVICES MARINE MICROBES OFFER US.

Aside from their natural roles in human affairs, microbes from the oceans can also be put to use in biotechnology applications or in helping to alleviate environmental deterioration.

HUMAN IMPACTS ON MARINE MICROBIAL DIVERSITY

Over six billion people now populate the world, and the impacts of our activities are felt in every corner of the globe, including the oceans. The biodiversity of marine microbes is likely to be affected by human activities on both global and local scales.



On the global scale, all organisms in the oceans, including microbes, will respond to the chief feature of global climate change: temperature changes. Elevated temperatures have been found to bring about increases in the numbers of certain waterborne pathogens.



Higher temperatures can also trigger the activity of certain microbial genes, including virulence genes that can lead environmental pathogens to cause disease in humans, corals, and other macroorganisms.

Temperature changes in the oceans may also disturb the delicate balance between the numbers of bacteria and phages. The checks and balances system between viruses and their bacterial hosts has recently been recognized as a determinant of bacterial abundance and diversity and of the efficiency of carbon cycling in a given microbial community. It is possible that climate change may not have drastic outward effects on the balance between bacteria and phage, however, given the flexibility of the system and the adaptability of microorganisms.

On a local level, the ozone hole over the Antarctic, a consequence of human releases of ozone-depleting chemicals, is probably having effects on microbial populations there. The gap in the ozone layer allows high levels of ultraviolet rays to reach the surface of the ocean, and is likely to increase microbial mutation rates and change microbial community composition in Antarctic waters.

Local impacts also include the release of exotic ballast water. The practice of ballast water dumping is known to release both invasive macroorganisms and potentially harmful microorganisms in coastal waters.

Nutrient loading, whether from fish farming activities or from runoff, has had massive impacts on coastal environments. Fish farms have proven responsible for eutrophication (additions of high concentrations of nutrients) of surrounding waters, resulting in large changes in the local microbial communities. Fish farming activities and runoff together have resulted in an increase in the frequency of coastal phytoplankton blooms, including harmful algal blooms that cost fishermen and governments millions of dollars every year.

Large-scale harvesting of the marine macrofauna by humans has changed the food webs of the oceans and is likely to be affecting marine equilibria and microbial diversity, driving the numbers of some species up and others down. In Peru in the 1970s, for example, over-fishing led to large phytoplankton blooms and a succession of bacteria that created large zones of depleted oxygen, called “dead zones,” in coastal waters. In this case, human activities led directly to a fundamental and devastating change in the microbial ecosystem. The exact nature of the effects of food web changes resulting from over-fishing is little understood and requires further study.

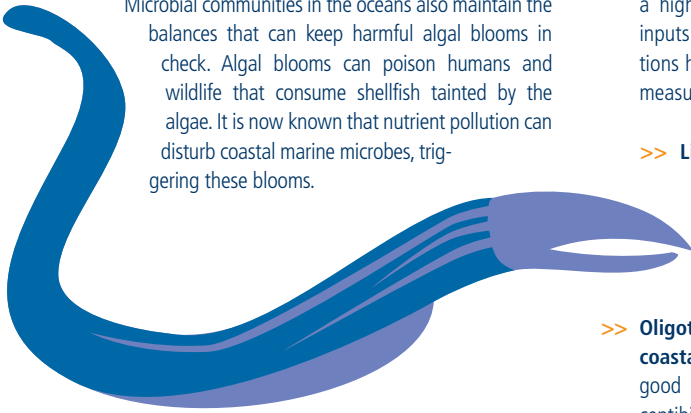
CRITICAL MICROBIALLY-MEDIATED EQUILIBRIA THAT IMPACT ENVIRONMENTAL AND HUMAN HEALTH

While it is true that marine microbial systems drive the biogeochemical cycles that make life possible on this planet, marine microbes also have more direct, immediate effects on human health and the well-being of the ocean ecosystem. Many of these direct effects are the result of fragile microbial equilibria, balancing points between opposing trends that could lead to serious repercussions for humans and our environment.

The equilibrium between bacteria and viruses in the oceans is an example of the kind of critical balancing act microbes perform every day. Changes in water temperature and ultraviolet radiation (UV), two factors known to be impacted by human activities, are known to disturb the relative numbers of bacteria and viruses in the oceans, with possibly disastrous results for human health. The virulence of a virus that preys on the bacterium responsible for cholera (*Vibrio cholerae*), for example, is affected by subtle changes in temperature. Hence, coastal temperature is a key determinant of the burden of cholera in coastal waters—an important matter to humans who live near those areas. Studies indicate UV can convert

viruses between active and dormant forms, so atmospheric changes that increase the amount of light in these wavelengths that reaches the oceans can upset the balance between viruses and their hosts, possibly leading to uncontrolled epidemics in fish, invertebrates, or humans.

Microbial communities in the oceans also maintain the balances that can keep harmful algal blooms in check. Algal blooms can poison humans and wildlife that consume shellfish tainted by the algae. It is now known that nutrient pollution can disturb coastal marine microbes, triggering these blooms.



Symbiotic equilibria between marine microbes and their hosts could also be upset by human activities. Increases in water temperature, for example, compel corals to drive out their bacterial symbionts. Marine microbes mediate the nutrient ratios in seawater, and releases of nutrients from runoff, wastewater treatment facilities, or other sources that upset those ratios can seriously unbalance ecosystem health.

Marine systems are highly connected with one another—more so than terrestrial systems. As a result, altering microbially-mediated equilibria in one part of the ocean will often have impacts on adjacent areas and far-flung regions. The coupling between the sediment (called the benthic zone) and water (called the pelagic zone) in coastal areas is particularly tight and changing one of those components will inevitably affect the other. Similarly, the deep ocean collects material from the upper ocean—the two are somewhat separate but inextricably linked.

Models for Studying the Microbially-Mediated Equilibria

Model systems are needed for studying the microbially-mediated equilibria that relate to human and environmental health. Some candidate models include:

>> **Cyanobacteria and cyanophage** would serve as a good model for investigating the balances between hosts and viruses.

>> The **corals** that have been driven north by warm waters that stimulate *Vibrio* toxicity to the coral's symbiotic zooxanthellae would be an excellent system for studying the impacts of human activities on symbioses.



>> The **Chesapeake Bay** could be a good model system of a highly-impacted coastal marine ecosystem. Nutrient inputs to the bay have been curtailed, but these restrictions have not yet resulted in a marked improvement on measures of ecological health.

>> **Lightly stratified marine systems**, like the Red Sea, and **highly stratified pristine coastal systems** could serve as good models for comparison against one another to learn about the conditions that can lead to anoxia.

>> **Oligotrophic systems** (nutrient-poor) and **eutrophic coastal systems** (nutrient-contaminated) could make good comparative systems for understanding the susceptibility and resilience of equilibria to nutrient loading.

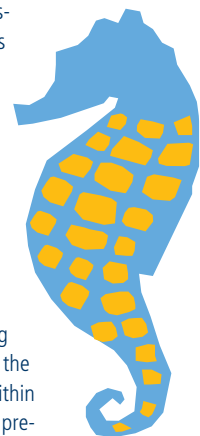


A model of harmful algal blooms is critically needed. The mechanisms and triggering factors behind these phenomena are not known, and a suitable system for study has yet to be found. Model ballast water systems are also needed. A great deal of water and many billions of microbes are being moved around the world as ballast, but the effects of these activities are little understood.

Reversibility of Changes in Microbially-Mediated Equilibria

Clearly, the critical equilibria that marine microbes maintain can be easily perturbed by human actions, and many of these conditions, like the balances that keep harmful algal blooms in check, are already in a disturbed state. However, some of these impacts may be reversible. Nutrient pollution, for example, which affects the equilibria that control algal blooms and nutrient concentrations in coastal waters, could be controlled by preventing sewage dumping into the ocean and by restoring the wetlands and salt marshes that filter nutrients in runoff before it reaches the ocean.

Preventing the emission of ozone-depleting chlorofluorocarbons would allow the hole in the ozone layer over the Antarctic to recover within decades. A more intact ozone layer would pre-



vent a great deal of harmful UV from reaching the oceans and would relieve bacterial-viral equilibria of the adverse effects of UV.

In an effort to limit pathogen releases, water used in fish farming operations could be treated and recycled within the system. Limiting releases to the oceans would lower the numbers of farm-associated pathogens to which wild populations are exposed and would restore local balances between pathogens and hosts. Recycling would also eliminate (or at least curtail) the release of nutrients to local waters. Nitrogen could be removed from farm water using an anaerobic ammonia process carried out by marine bacteria. Aquaculture without biological and chemical pollution is a worthy goal for the industry.

Humans have impacted marine equilibria in many ways, and the time required for these systems to recover is not known. Education on these topics is necessary to convey the severity of these impacts to the global community so that aggressive steps can be taken to reverse them.

APPLYING MARINE MICROBES USING BIOTECHNOLOGY

The great metabolic diversity of marine microbes makes the oceans a rich source of biological material for biotechnology applications. A variety of secondary metabolites, enzymes, polymers, and metabolic processes can be found only in the microbes that inhabit the oceans, and it is likely that many more useful products will be found as research continues. The biotechnology potential of these organisms is a major driving force behind the push to characterize marine microbial diversity.

Different marine habitats will yield microbes suited to different applications. Exploring hot environments, for example, will unearth thermophilic bacteria that produce thermostable polymerases that could be used in industrial applications.

Collections of libraries and culture collections of marine microorganisms would be very useful for harvesting biotechnological products from marine microbes. Basic research

needs to provide the fundamental libraries, tools, and meta-data that can be linked and passed on to biotechnological and biomedical interests for devising applications for marine microbes and their products. Innovative ways of linking research to real-world applications are needed.

Industrial Applications

Potential industrial applications of marine microorganisms are many and varied, including:

- >> The interface between microbiology, nanotechnology, and engineering may yield new nanostructures or electronic components produced with the help of marine microbes.
- >> Magnetic beads from magnetotactic marine bacteria could have applications in optics and electronics.
- >> Certain marine organisms, including *Prochlorococcus* and *Pelagibacter*, have streamlined genomes that may be useful as metabolic engines for manufacturing industrial products.
- >> Alkylphiles may produce useful products for use in laundry detergents.

Energy Production

Humankind faces vast energy needs, but with the help of marine microorganisms, biofuels, cleaner energy production, and alternative fuel technologies may all be within reach. New processes that could build sustainable sources of energy may arise from a better understanding the diversity of processes available in marine microorganisms. Understanding the diversity of microbial processes will provide the evolutionary, structural, and functional information that are needed for process organization and optimization. Microbial hydrogen production from light and water, nitrogen fixation, and carbon sequestration processes each need to be studied at a basic level. The redox gradients created by microorganisms in marine sediment could possibly be used to generate electricity.

Medical Applications

Enzymes and bioactive compounds from marine microbes can be very useful in medical applications. Today, polysaccharides are being used in many new products, and several promising marine compounds are in the drug development pipeline. Promising bioactive compounds initially discovered in marine invertebrates may be produced by symbionts rather than by the invertebrates themselves. Successful cul-

ture of these symbionts can ensure an economic, sustainable supply of these compounds, improving the odds of their commercial development as drugs.

Marine microbes may also be used to manufacture compounds called “nutriceuticals,” natural products used as diet supplements to promote health. Docosahexaenoic acid, a fatty acid necessary for proper brain function, for example, could be harvested from cultures of marine dinoflagellates and processed for sale as a nutraceutical.

Taxonomy has some predictive value in linking physiological potentials to specific taxa. For example, some groups, like actinomycetes and cyanobacteria, are reliable producers of bioactive compounds. Similarly, thermostable enzymes are likely to be found in taxa that thrive in hot environments, like *Thermocales*. However, researchers should take caution in following these types of generalities so as to avoid the self-fulfilling prophesy: finding bioactive compounds only where they were pursued. Currently, the allocation of resources for taxonomic research is disproportionately allocated towards macroorganisms; more resources are needed in taxonomic research on microbial species.

USING MARINE MICROBES TO AMELIORATE ENVIRONMENTAL DETERIORATION

The metabolic diversity of marine microorganisms not only makes them useful in biotechnology applications, it makes them versatile tools for addressing environmental problems. One prime environmental application for these organisms is bioremediation—the treatment of chemical contamination using microorganisms. The cleanup of hydrocarbons, specifically petroleum products, is an especially pressing matter in the oceans, where accidents aboard oil tankers can release thousands of gallons of oil in a single incident. In some cases, fertilizing the indigenous microbial communities on the affected beaches with nitrogen and phosphorus can speed the degradation of the spilled oil, but bioremediation options in open water are limited because of difficulties in delivering sufficient nutrients to sustain biodegradation.

It is now known that the actions of an entire microbial community are necessary to break down complex organic matter, including petroleum. A super-organism that could

carry out the entire process was sought, but was never isolated or designed.

A cluster of genes that carry out the degradation of chlorinated biphenyls, called the BPH cluster, have been isolated and are now being applied in treating dredged contaminants from the Hudson River estuary.

Today, most research in bioremediation in the marine environment is focused on the organisms already present in the affected areas. For a number of reasons, few efforts are underway to develop engineered microorganisms to address problems of chemical contamination. There are particular problems with respect to releasing microbes for bioremediation into aquatic environments; confining the organisms to the site of concern, for example, would be difficult.

Finally, there is a possibility that marine viruses can be used to control harmful algal blooms in a sort of “viral therapy.” Viruses may be isolated from the algal species responsible for blooms, then engineered or otherwise enhanced in the laboratory, and released in a bloom. This approach holds great potential for controlling diseases in aquaculture settings.



>> THE FUTURE OF MARINE MICROBIOLOGY LIES IN INNOVATIVE RESEARCH AND IN EFFECTIVE EDUCATION AND TRAINING OF THE NEXT GENERATION OF SCIENTISTS. AN INTEGRATED APPROACH WILL BE NEEDED TO MENTOR STUDENTS WHO WILL TAKE ON THE CHALLENGE OF ADDRESSING MUCH OF WHAT WE STILL DO NOT UNDERSTAND IN THE REALM OF MARINE MICROBIOLOGY.

GENOMICS AND MARINE MICROBES

Genomics, the study of all or part of the genome of an organism to answer questions about that organism, describes a relatively new and powerful set of research techniques that can be applied in marine microbiology. Genomics will allow researchers to uncover previously unknown abilities and metabolisms and define the interrelationships that hold marine microbial communities together, revolutionizing our understanding of the field in the coming years.

Despite their power, genomics approaches cannot be the only tools used to address marine microbial biology. Traditional laboratory approaches in genetics, biochemistry, physiology, and ecology are still necessary to validate the biology. Functional studies will often be necessary to follow up on genomics studies that uncover unknown genes, for example. Conserved hypothetical genes pose another problem; it is not often possible to discern protein function from the sequence of a hypothetical gene alone. Better motif recognition tools could help improve the ability to discern function from sequence. Microarray studies could also help by identifying where and under what conditions the gene of interest is expressed. The integration of genomics, transcriptomics, proteomics, with ecological, biophysical, and chemical techniques will be necessary to delineate fundamental physiological processes, understand their regulation, and determine how they relate to biogeochemical cycles.

METAGENOMICS

Genomic sequencing of single species has made great contributions to our understanding of marine microbial systems, and metagenomics (in which the genomes of a group of organisms are lumped and studied together) approaches will extend that knowledge and add to our understanding of marine microbial processes.

Metagenomics confers the potential to map the metabolisms of microbes in space and time. It may even be possible to identify differences in metabolic potential between different ocean regions using metagenomics approaches. Archiving of genomic and metagenomic samples will be necessary to provide baseline data against which microbial community structures can be assessed over time. Moreover, genomic and metagenomic information provide the fundamental for microarray and proteomics-based technologies that have shown the promise for unveiling complex microbial functions. Community-based proteomics have been applied to investigate the major expressed proteins from estuarine bacterioplankton.

Protein expression of Chesapeake Bay picoplankton community shown by 2-D gel analysis (Courtesy of Feng Chen). The gel was stained with SYPRO Ruby. The IP ranged from 5 to 8, and the molecular mass from 10-100Kda.

To make the best progress in the field, it will be critical to tie metagenomics studies to the existing knowledgebase in biological oceanography. One way to accomplish this is to locate large scale studies at existing study sites, like the ocean time series stations Hawaiian Ocean Time-series (HOT) and the Bermuda Atlantic Time-series Study (BATS), in order to have a rich context of existing data available for understanding new metagenomics data.

It will be important to make metagenomic data and other genomics datasets accessible to other researchers and as easy to use as possible.

16S DEPENDENT TECHNIQUES

The level of resolution of techniques that rely on the 16S ribosomal RNA gene is another pressing issue in research on marine microbes. Ribosomal RNA gene sequences are often used to identify microorganisms and 16S group probes are used to assess the diversity of groups of microbes. New information has brought up questions about whether 16S genes offer sufficient resolution to inform researchers about the distribution of different microbes. It may be that 16S genes are simply too highly conserved across related strains of bacteria and archaea to be useful in many analyses and that studies that rely on denaturing gradient gel electrophoresis (DGGE) or other 16S-dependent methods are seriously underestimating microbial diversity.

Greater resolution of microbial species can now be achieved using other tools, including the internal transcribed spacer (ITS) region between the 16S and 23S genes, and even meas-

ures such as genome size. Studies using these methods have shown that 16S genes can miss out on a lot of genetic diversity, confirming that the definition of a microbial species is indeed a complicated issue.

RESEARCH FUNDING

There are a number of urgent questions in marine microbial ecology, like how climate change will affect marine microbes and biogeochemical cycling that can only be addressed by large national initiatives. More interagency programs are needed to target these types of projects. There is also a need for centers of excellence in marine microbiology to focus the efforts of the community on the big questions of the day.

NEW COLLABORATIONS

Penetrating questions in marine microbiology rarely stay within the confines of one person's expertise—they require the input of many varied perspectives. Collaboration across international boundaries and across disciplines needs to be encouraged.

International Collaboration

International cooperation in marine microbiology research can serve to foster new ideas and fertilize research with technology exchange. Research vessels and submersibles are in limited supply, and sharing them with scientists from other nations can only help the field. Also, the oceans of the world are a shared resource, and research on marine life and marine processes that sustain the planet is in the interest of all people, regardless of national origin.

Differences exist in the strengths of scientists in the U.S. and abroad, and training in some of the fields that pertain to marine microbiology is admittedly better in other countries. Close collaborations can draw on the strengths of the participating countries and foster improvements where they are needed.

There are many fewer possibilities for international collaboration today than 20 years ago. European Union projects, for example, do not usually support work by Americans, and National Science Foundation grants do not fund European scientists. Modern political issues, including visas and national security measures, are also a hindrance.

Federal agencies can stimulate international work by establishing joint funding projects with their equivalent organizations in the European Union, Asia, and elsewhere. International post-doctoral appointments could also facilitate cooperation across borders. A federally-funded exchange pro-

gram that would involve 10 postdoctoral associates in marine microbiology in an exchange program every year would go a long way toward encouraging international projects. The International Society for Microbial Ecology (ISME) could be another effective facilitator of international collaborations.

Interdisciplinary Collaboration

Much of the most innovative work in marine microbiology takes place on interdisciplinary boundaries. For example, physical geobiology, the intersection of geochemistry, microbiology, and geology, has made great strides in recent years. Other fields need to be brought together to encourage this kind of innovation. Physical oceanographers, chemical oceanographers, and microbiologists should be able to interact fruitfully in water column studies.

Microbiologists should also build interfaces with engineers to develop instrumentation for the field. Secondary metabolite discovery and study could be pursued in collaborations between marine microbiologists, pharmacologists, organic and bioinorganic chemists. Other potential collaborative partners for marine microbiologists include mathematicians, ecologists, computer scientists, informaticists, physicists, mathematicians, and marine biologists.

Biology would benefit from better integration at the university level. Currently, administrative divides separate ecology and evolution departments from cell and molecular biology departments and microbiology departments, but all of



these fields bear direct relevance to marine microbiology. Marine microbiology research would benefit from better-integrated universities that promote interactions between the specialties. One way of achieving integration is to establish cross-departmental research initiatives within universities in order to bring together the people concerned with “microbial science,” for example.

TEACHING AND EDUCATIONAL OPPORTUNITIES

There are a number of innovative ways to convey marine microbiology to the undergraduate students, graduate students, and postdoctoral associates who will be the next generation of scientists.

Training in marine microbiology should be multidisciplinary, exposing students to such diverse disciplines as molecular biology, laboratory microbiology, ecological theory, oceanography, and bioinformatics. The challenge here lies in expressing all the basic subjects in these fields to the students in a coherent manner.

A semester at sea is a valuable part of training in marine microbiology. It may be advisable to coordinate with the University-National Oceanographic Laboratory System (UNOLS) to get more students involved in projects onboard research vessels.

Summer internships or “lab swap” arrangements in which a student spends a semester in a new lab would foster interdisciplinary interactions among graduate students, undergraduates, and postdoctoral associates. Arrangements like this would inevitably lead to productive interactions between the student’s mentors, as well. “Research experience for undergraduates” programs would also help to improve the quality of undergraduate training in marine microbiology.

Postdoctoral programs that train individuals in cross-disciplinary fields, like bioinformatics, should be targeted with a slant on marine microbiology.

There is a need for a good textbook in marine microbiology. A book compiled with the input of many experts that inte-

grates information across the disciplines of microbiology, ecology, evolution, and oceanography, would probably be most welcome by instructors.

It is important to train marine biologists and marine ecologists in the basics of microbiology and molecular techniques during undergraduate and graduate training. In order to imprint upon these students the importance of microbial systems, it is critical to present microbiology in a way that makes a big impact, conveying the importance of microbiology to our understanding of the oceans, rather than detailed concepts. One way to accomplish this is by appointing effective guest lecturers.

COMMUNICATING THE ISSUES

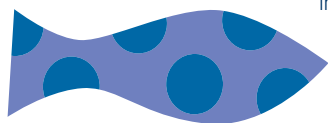
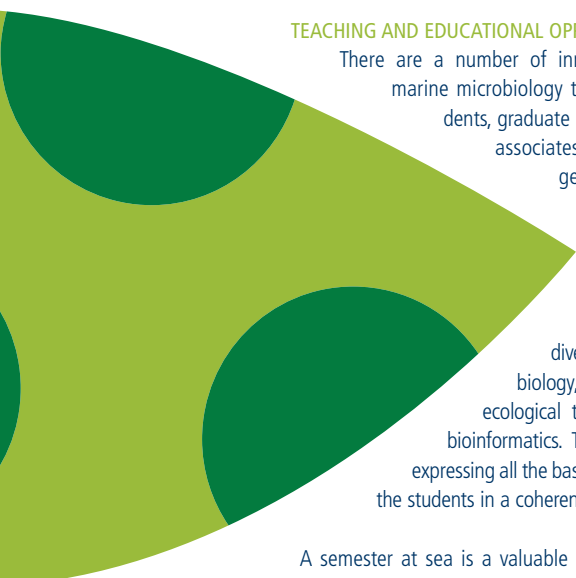
The scientific community needs to take a more active role in conveying the issues surrounding marine microbes to the public and to policy makers. A number of different approaches can be used in educating nonscientists on the importance of microscopic creatures (including the microbes that live in the oceans) to our everyday lives.

Video is a very appealing way to reach the public, particularly younger audiences. A film festival for graduate students, to be held annually at the meeting of an invested scientific society, would be an excellent way to foster projects of this kind and to highlight the research of graduate students. The film festival sponsored by the European Life Scientist Organization would be a good model on which to base a new film festival endeavor.

Video games and children’s books with microbiology themes are other ways to reach young audiences.

Television shows with forensic science themes have boosted the interest of students and the public in forensics education. Perhaps television shows with microbiology themes could be used to stimulate interest in this field.

To reach policymakers, it may be a good idea for marine microbiologists to participate in the existing program of short courses for congressional interns. Topics specific to marine science could be presented to these fast-track nascent politicians.



RECOMMENDATIONS

1. There are a number of outstanding questions about marine microbial distributions that need to be addressed, including:

- >> What is the ecological relevance of functional gene diversity observed in marine microbial communities?
- >> Does diversity have an impact on ecosystem function or long-term stability?
- >> Are changes in microbial communities predictive of changes in environmental function, like the cycling of bioelements?
- >> The long-term variability of marine microbial communities is poorly understood. Can intermittent sampling, like taking community snapshots, address this question or is continuous sampling necessary to evaluate community composition shifts?
- >> Are there keystone species in marine microbial communities that are critical to a given function?
- >> How can scientists identify which organisms they are?

2. Climate change will undoubtedly alter the diversity of marine microbial communities in unforeseen ways. Climate change should be considered a major top-down controller of microbial communities.

3. Microbial interactions and processes have implications over a very wide range of scales in the oceans. In designing research targeted toward marine microbial communities, the importance of microscales should not be overlooked.

4. Large-scale harvesting of ocean fish by humans has changed the food webs of the oceans and is probably affecting marine equilibria and microbial diversity. The exact nature of the effects of food web changes resulting from over fishing is little understood and requires further study.

5. Much of the most innovative work in marine microbiology is carried out by interdisciplinary teams of scientists. Collaborations among microbiologists and geochemists, geologists, physical oceanographers, chemical oceanographers, natural products chemists, engineers, physicists, mathematicians, bioinformaticists, and marine biologists should be fostered and encouraged by federal agencies and professional societies.

6. Training in marine microbiology should be multidisciplinary, exposing students to such diverse disciplines as molecular biology, laboratory microbiology, ecological theory, oceanography, and bioinformatics.

7. Humans adversely affect marine equilibria in many ways, and the time required for these systems to recover is not known. Education on human impacts on the oceans is necessary to convey the severity of environmental degradation to the global community so that aggressive steps can be taken to reverse them.

8. The interactions between the microbial communities of marine sediments and communities in the water column are little understood. This presents a clear gap in the current knowledge that should be addressed in future research.



9. Marine microbial diversity needs to be more fully explored, including the remarkable diversity found in microbial symbionts of marine invertebrates. Marine viruses represent a new, unexplored world of diversity.

11. It will be important to make metagenomic data and other genomics data-sets accessible to other researchers and as easy to use as possible. Genomic characterization provides only potential activities, application of community based proteomics (metaproteomics) could be a useful way to investigate the actual microbial processes and link them with important microgeochemical functions.

10. A model of harmful algal blooms is critically needed. The mechanisms and triggering factors behind harmful algal blooms are not known, and a suitable system for study has yet to be found. Model ballast water systems are also needed. A great deal of water and many billions of microbes are being moved around the world as ballast, but the effects of these activities are little understood.

12. There is a need for a good textbook in marine microbiology. A book compiled with the input of many experts that integrates information across the disciplines of microbiology, ecology, evolution, and oceanography, would probably be most welcome by instructors.

