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PRINCETON UNIVERSITY PRESS
Princeton & Oxford

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Published by Princeton University Press, 41 William Street,
Princeton, New Jersey 08540
In the United Kingdom: Princeton University Press, 6 Oxford Street,
Woodstock, Oxfordshire OX20 1TW

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LIBRARY OF CONGRESS CATALOGING-IN-PUBLICATION DATA
The Princeton guide to ecology / Simon A. Levin, editor.
p. cm.

Includes bibliographical references and index.

ISBN 978-0-691-12839-9 (hardcover : alk. paper)

1. Ecology. 2. Ecology—Economic aspects.

I. Levin, Simon A.

QH541.p742 2009

577—dc22 2008049649

British Library Cataloging-in-Publication Data are available

This book has been composed in Sabon and Din

Printed on acid-free paper. ∞

press.princeton.edu

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

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Seascape Microbial Ecology: Habitat Structure, Biodiversity, and Ecosystem Function

David M. Karl and Ricardo M. Letelier

OUTLINE

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Seascapes are marine analogs of landscapes in the terrestrial biosphere, namely the physical, chemical, and biological elements that collectively define a particular marine habitat. The field of seascape ecology, also referred to as ecological geography of the sea, seeks fundamental understanding of spatial and temporal variability in habitat structure and its relationships to ecosystem function, including solar energy capture and dissipation, trophic interactions and their effects on nutrient dynamics, and patterns and controls of biodiversity. Implicit in the study of seascape ecology is an interest in the management of global resources through the development of new theory, the establishment of long-term ecological observation programs, and the dissemination of knowledge to society at large.

GLOSSARY

- euphotic zone.** Upper portion of the ocean where there is sufficient light to support net photosynthesis, usually the upper 0–200 m in the clearest ocean water
- genome.** The complete assembly of genes present in a given organism, coded by specific nucleotide se-

quences of DNA, that determines its taxonomic structure, metabolic characteristics, behavior, and ecological function

microorganism. The smallest form of life (<2 μm) on our planet and the most abundant in the open sea, sometimes reaching cell densities of 1 million cells per cubic centimeter

nitrogen fixation. The process whereby relatively inert gaseous nitrogen (N_2) is reduced to ammonia (NH_3) and thus converted into a biologically available form

nutrient. One of several organic or inorganic raw materials that are required for the growth of an organism, for example, nitrogen, phosphorus, iron, and vitamins

oligotrophic. A condition of low nutrient concentration and low standing stock of living organisms, for example, the open ocean

primary production. Metabolic process during which carbon dioxide is incorporated into organic matter by bacteria and eukaryotic algae using any of a variety of energy sources, but usually solar energy

remote sensing. The indirect measurement of habitat characteristics, for example by Earth-orbiting satellites

water mass. A portion of the marine environment that has a characteristic average value of temperature and salinity that is related to its origin and global circulation pattern

1. INTRODUCTION

The global ocean covers 71% of the surface of the Earth to a mean depth of approximately 4 km. In contrast to its terrestrial counterpart, where biomes are associated with characteristic landscapes, differences

in seascapes can be subtle, even when they support unique biological assemblages. Early sailors and naturalists characterized changes in oceanic habitats through differences in water temperature and clarity and the type and abundance of the fisheries they supported. However, hidden within this enormous living space, is a complex mosaic of seascapes, some with well-defined horizontal and vertical limits and others with more cryptic and flexible boundaries (plate 11).

Below the well-illuminated upper layer, known as the euphotic zone, the ocean is well stratified with identifiable stable layers referred to as water masses that can be traced to specific geographic areas of formation and when they were last in contact with the atmosphere. As these water masses move through the interconnected ocean basins, their chemical and biological characteristics change as a result of coupled, integrated effects of particulate organic matter delivery and metabolism. The oldest water masses on the planet (~1500 years old) are in the deep North Pacific Ocean, far removed from their source in Antarctica. However, this water mass is young compared to the very old age of the North Pacific habitat itself: more than 10 million years.

The marine environment supports the growth of a diverse microbial assemblage from all three domains of life: *Bacteria*, *Archaea*, and *Eucarya*. Microbes (especially bacteria) dominate the ocean's genome, and their metabolic activities are responsible for planetary habitability and stability. However, ecologists have not traditionally used microorganisms in the development of ecological models. And although it is likely that existing theory based on the study of macroorganism species and populations is applicable to microbes, there is reason to believe that additional ecological theory may be required to explain microbial genetic and metabolic traits and their relationships to biodiversity, speciation, and evolution.

The relatively new discipline of seascape microbial ecology combines principles, theory, and models of microbiology, ecology, biogeography, genetics, and oceanography to investigate and interpret patterns in the distribution, diversity, and biogeochemistry of microbial assemblages in the sea. A revolution is under way in seascape microbial ecology, ignited in part by the application of novel molecular-based techniques. These approaches have led to the discovery of new organisms, genes, and metabolic processes that define novel marine ecosystem functions. Furthermore, major technological advances in the capability for unattended, remote ocean observation are rapidly changing our view of the structure and the four-dimensional (space and time) variability of marine ecosystems. To illustrate selected advances in seascape microbial

ecology, this chapter focuses primarily on microorganisms that inhabit the sunlit portion (0–200 m) of the open sea where most of the organic matter production occurs.

2. SEASCAPE STRUCTURE, VARIABILITY, AND FUNCTION

Ecological processes in the oceanic realm have been studied for more than a century. However, the open sea is still grossly undersampled. Some of the most basic biological properties of pelagic ecosystems, e.g., rates and controls of oceanic photosynthesis, are still not well understood. Furthermore, many seascapes are currently changing as a consequence of human activities. For this reason, it is vital to establish microbial observatories in selected marine ecosystems to track, characterize, and understand changes in the health of the global ocean.

Oceanographers recognize that there are predictable abiotic and biotic properties that vary systematically with distance from shore and from the equator to the poles (see plate 11). A variety of physical forces, with spatial variability ranging from millimeters to basin scales and temporal variability of minutes to millennia (figure 1) shape seascapes, establishing unique characteristics that promote the exchange and transfer of energy and matter, including genetic information, within the global ocean. Each seascape can be defined by the physical, chemical, and biological variables experienced by an organism during its lifetime; collectively these parameters determine the success or failure of a particular strain, species, or assemblage of microbes. Hence, the size, motility, and lifespan of the organism under consideration define the spatial and temporal scale of that organism's habitat. For this reason, a given seascape is likely to be comprised of numerous microhabitats that collectively support the growth and proliferation of the microbial assemblage as a whole. And here resides one of the potential limitations when we are trying to define marine seascapes: in comparison to landscapes, our senses are unable to perceive changes at the microhabitat level.

Particulate organic matter, ranging in size from small colloids to large aggregates, constitutes the most abundant class of microhabitats in the sea; particles are ecotones or transitional boundaries within a fluid matrix. Furthermore, they are often ephemeral sites of elevated microbial biomass and accelerated metabolism, with a mean life of only a few weeks in the open sea. Life on organic-enriched particles selects for microbes with unique survival adaptations such as motility and attachment mechanisms, specialized chemoreceptors, the ability to produce extracellular enzymes

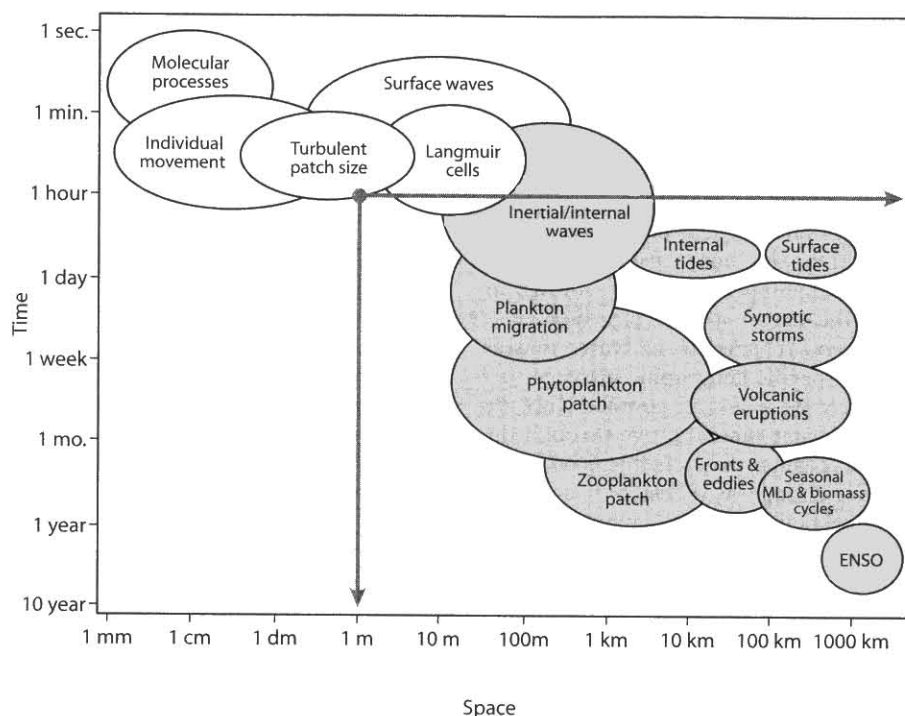


Figure 1. A schematic representation of the relevant time and space scales for key physical and biological seascape processes.

Space

The arrows define the approximate boundaries of at-sea observations using current technologies. (From Dickey, 1991)

(ectozymes), and, possibly, a starvation response for survival after the organic substrates are depleted. Once colonized by microorganisms, the environmental conditions within a given particle (e.g., pH, redox level, nutrients, and dissolved gas contents) change as a result of the metabolic activities of the associated microbial assemblage. During this organic particle aging process, structured multispecies consortia of closely interacting and cooperating microbes can lead to complex biological interactions including cell-to-cell chemical communication using one or more different “languages.”

At any given moment in every seascape, there is a broad spectrum of particle types and ages with both overlapping and unique microbial populations, interacting with the background, low-nutrient populations contained within the seawater matrix. These nutrient-rich patches can provide refuges for extinction-prone, high-nutrient-requiring microbes and may help to explain the high diversity of low-number-abundance microbes that contribute significantly to the resilience of microbial assemblages in the marine environment. Spatial and temporal heterogeneity fragment large-scale biomes into a mosaic of patches and, over time, semi-isolated metapopulations and metacommunities. Therefore, particles can be viewed as ephemeral

microhabitats with source and sink exchange and dynamics with surrounding particle-bound and free-living microbial assemblages.

On a much larger spatial scale, Earth-orbiting satellites equipped with sensors to measure a variety of sea surface parameters have been used to detect and map the dynamics of major biomes globally and in near-real time (plates 11 and 12). For example, the trade wind biomes, which extend from approximately 30°N to 30°S in each ocean basin, dominate our water planet. Key features of these seascapes are a stable vertical temperature/density structure and a downwelling gyre-like circulation pattern that tends to isolate the upper water column from mass exchange with bordering current systems. This stratification and insulation, broken only by an upwelling region close to the equator, lead to a permanent separation of light (above) from nutrients (below) and result in a condition of extreme oligotrophy, including low nutrient concentrations and fluxes, low standing stocks and rates of organic matter production, and, consequently, low rates of organic matter export to the deep sea.

Despite chronic nutrient limitation, oceanic subtropical gyres can support blooms of phytoplankton generally during summer months when the water column is well stratified and most depleted of essential

inorganic nutrients such as nitrate and phosphate. These aperiodic and enigmatic phytoplankton blooms sequester carbon dioxide (CO₂) and recharge the upper water column with dissolved organic matter and oxygen, which support postbloom heterotrophic metabolism. More importantly, blooms contribute to the seascape mosaic that is essential for maintaining genetic diversity in these expansive habitats.

The North Pacific Subtropical Gyre (NPSG) is the largest circulation feature on Earth, but it is not yet fully characterized. Solar energy, both light intensity and spectral quality, sets the upper constraint on ecosystem productivity by determining the energy available to phototrophs. However, the most abundant oxygenic phototroph in the NPSG, the cyanobacterium *Prochlorococcus*, was discovered only two decades ago. Furthermore, recent investigations using molecular-based techniques have revealed a novel proteorhodopsin-based phototrophy as an independent pathway for solar energy capture in "nonphotosynthetic" microorganisms. It now appears that these microbes exist partly on sunlight and partly on dissolved organic matter; they are truly nature's energy hybrids.

Although ecological processes in terrestrial biomes are controlled primarily by temperature and the flow of water, marine ecological processes are controlled primarily by temperature and turbulence; the latter impacts nutrient delivery and constrains rates of primary production in marine habitats where nutrients are limiting (figure 2). Near-surface mixing also determines the mean light field of planktonic microorganisms by defining their position in the water column. Consequently, there are complex relationships among turbulence, nutrients, light, and photosynthesis that tend to select for, or against, certain traits at the individual and community levels.

In addition to a physically favorable environment, the metabolism and proliferation of microorganisms also require a renewable supply of energy, electrons for energy generation, carbon and other bioelements, and, occasionally, organic growth factors such as vitamins. Depending on how these requirements are met, all living organisms can be classified into one of several metabolic categories (table 1). Only obligate photolithoautotrophs are self-sufficient, even if they must tie their growth and survival to other microbes that are vital in sustaining nutrient availability over longer time scales. All other microbes ultimately rely on photosynthesis for a supply of energy, dissolved oxygen, or both.

Among the sea microbes there are a variety of metabolic strategies for nutrient capture, transport, and assimilation, all under genetic control. Some microbes are specialists, able to grow on only a single form of a required nutrient; others have less stringent growth

requirements. For those microbes that compete for the same substrate, some specialize in the ability to capture substrate at very low ambient concentrations, whereas others have high-capacity uptake and intracellular storage capabilities, being adapted to a feast-and-famine type of existence. Maximum potential growth rates are also variable and probably under genetic control. In low-nutrient-supply habitats such as the NPSG, rapid growth and reproduction may not be the best survival strategy; in this environment the defense against protozoan grazing and viral lysis may also be of great selective advantage.

3. ASSESSMENTS OF MICROBIAL "SPECIES" DIVERSITY AND FUNCTION

Two major challenges in seascape microbial ecology are to identify the proper time and space scales to assess diversity and to define the exact nature of the diversity that should be studied. Beyond taxonomic or phylogenetic diversity, one needs to consider diversity of metabolic or physiological potential within a given species, population, or assemblage as well as niche diversity and the temporal dynamics of the seascape. The former is essential for establishing the possible flux pathways for energy and matter, and the latter is crucial for understanding interactions including competition, resource partitioning, natural selection, and speciation in marine ecosystems.

Microbes assemble in nonrandom fashion similar to patterns that are observed for macroorganisms (plate 12). If multiple environmental parameters and processes are to be compared, sample size and sampling frequency need to be matched. For example, if flow cytometric characterization of bacteria in a deep sea habitat requires a sample size of 0.1 cm³, whereas microbial DNA to prepare a clone library needs 0.1 m³ of seawater, diversity measured by these two procedures may be mismatched by sample size. If more than one microhabitat is combined, then the diversity may be overestimated relative to the scale that is relevant for microbial interactions to occur.

Sea microbes are ubiquitous and abundant; at typical concentrations of more than 100 million cells per liter, marine bacteria are by far the largest contributors to living organic matter in the sea. Their genetic diversity is believed to be large but at present is poorly known. Microorganisms have a very long evolutionary history (3–4 billion years) and appear to have low extinction rates, which may help to explain their enormous extant diversity. Furthermore, habitats like the open sea that are chronically nutrient stressed or energy limited can trigger a starvation–survival response in microorganisms, promoting mutations and, over

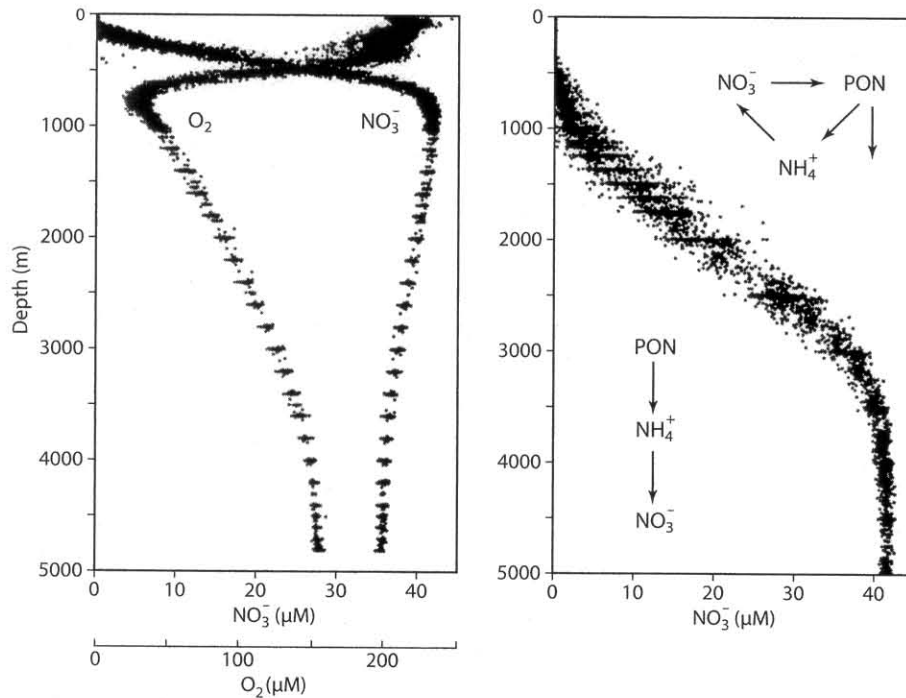
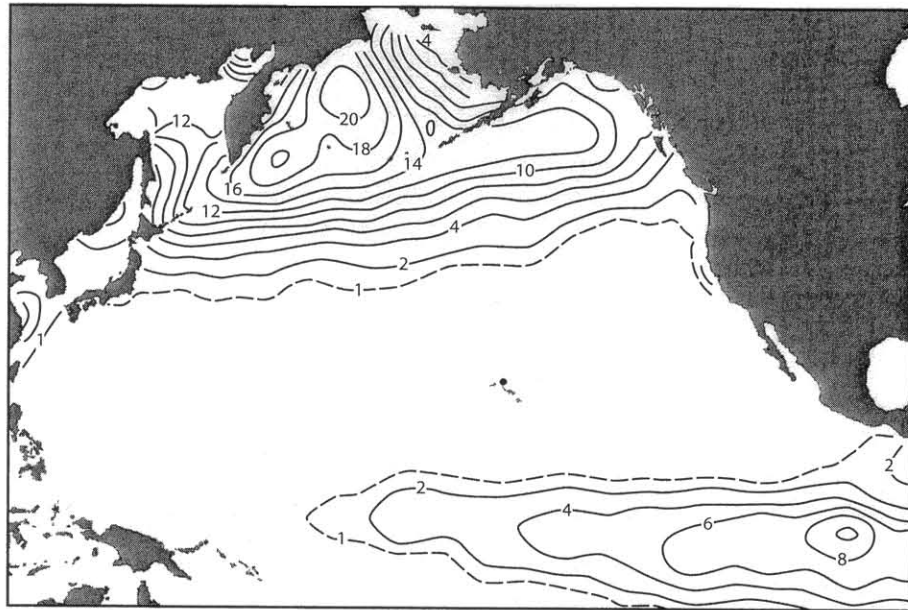


Figure 2. Map of the North Pacific Ocean basin showing the spatial variability in nitrate. Shown on top is the mean annual surface nitrate concentration ($mmol\ NO_3^-\ m^{-3}$) based on the World Ocean Atlas (2001) Ocean Climate Laboratory/NODC. Areas of high NO_3^- (and presumably high NO_3^- flux) correspond to high chlorophyll ($>1\ mg\ m^{-3}$) as a result of net plant growth. The NPSG is the central region characterized by low ambient NO_3^- concentrations ($<1\ mmol\ m^{-3}$) and low standing stocks of chlorophyll ($<0.1\ mg\ m^{-3}$). The circle in the central portion of the basin is the approximate location of Station ALOHA ($22^\circ 45' N$, $158^\circ W$). The data in the

bottom panels, from Station ALOHA, show (left) the vertical distributions of nitrate and dissolved oxygen for the full water column (0–4800 m) and (right) for the upper 0–1000 m to emphasize the absence of nitrate in the upper euphotic zone (0–100 m) and the steep gradient in nitrate concentration versus water depth beneath the euphotic zone. Also shown are schematics of the key ecological processes of nitrate (NO_3^-) and ammonium (NH_4^+): nutrient uptake into particulate organic nitrogen (PON) and gravitational flux of PON from the upper layers coupled to remineralization back to nitrate in the deep sea.

Table 1. Variations in microbial metabolism based on sources of energy, electrons, and carbon

Source of energy ^a	Source of electrons	Source of carbon
Sunlight <i>photo-</i>	Inorganic <i>-litho-</i>	CO ₂ <i>-autotroph</i>
	Organic <i>-organo-</i>	Organic <i>-heterotroph</i>
Chemical <i>chemo-</i>	Inorganic <i>-litho-</i>	CO ₂ <i>-autotroph</i>
	Organic <i>-organo-</i>	Organic <i>-heterotroph</i>
Radioactive decay <i>radio-</i>	Inorganic <i>-litho-</i>	CO ₂ <i>-autotroph</i>
	Organic <i>-organo-</i>	Organic <i>-heterotroph</i>

Note: A "mixotroph" is an organism that uses more than one source of energy, electrons, or carbon.

^aAccording to Karl (2007).

time, enhancing biodiversity. Some scientists estimate that there may be 1–10 million different "species" of microbes on Earth; others suggest the number of species may be 1 billion.

In a recent study of bacterial diversity in the North Atlantic Ocean as part of the International Census of Marine Microbes (ICoMM), tens of thousands of low-number-abundance microbes per liter were found in association with a relatively small number of very abundant microbes. This type of rank-order abundance curve, with a very long tail, is also characteristic of most macroorganism distributions. It reveals a "rare biosphere" of nearly inexhaustible genetic variability, a gene bank that can be used as necessary to maintain ecosystem stability and function if the seascape is perturbed or permanently altered.

The Linnaean paradigm of hierarchical organization of all living organisms, now 300 years old, is a basic building block of biology. However, mechanisms such as mate preference, spawning synchrony, gamete recognition, and reproductive isolation that are important for speciation in sexually reproducing populations are not applicable to most microorganisms (especially bacteria) because they reproduce by binary fission and pass genetic material vertically from parent to two identical offspring. Additionally, genes can also be passed horizontally between otherwise unrelated species, providing a vehicle for adaptation and evolution that is much more common for microorganisms than invertebrate and vertebrate taxa. Horizontal gene transfer has profound biological, ecological, and bio-

geochemical consequences and may promote microbial diversity in nature. Consequently, an ever-changing weblike topology rather than the traditional tree of life may be a more accurate framework to represent the evolutionary history of microbes. Indeed, we may need to consider a continuum of ecological functions within marine microbial assemblages. If so, any attempt to group or classify sea microbes may be a static representation of a dynamic system.

There is currently no widely accepted criterion for the designation of a microbial species. This has tended to isolate environmental microbiology from mainstream ecology, where species are the fundamental units of theory and models. Taxonomic assignments of microbes are usually made on genetic relatedness based on similarity of DNA or one or more marker genes (e.g., small subunit ribosomal RNA, so-called 16S rRNA). Phylogenetic surveys using 16S rRNA sequence analyses have revealed two important facts about seascape. First, most marine diversity is microbial. Second, most of the 16S rRNA sequences recovered from natural habitats are distinct from those of the model marine microbes held captive in our laboratories. In selected seascape, the "species" list retrieved by culture versus culture-independent approaches are distinct, suggesting that one, or both, surveys may be in error. The 16S rRNA method requires DNA amplification, cloning, and sequencing but not cell growth, whereas the pure culture isolation method requires cell growth and division. It is conceivable, even probable, that many sea microbes are not actively growing (or have growth requirements that are difficult to reproduce in the laboratory) even though their 16S rRNA genes can be isolated and identified. Furthermore, microbial diversity analysis using the 16S rRNA criterion will likely underestimate the true physiological diversity because most 16S "ribotypes" contain genetically distinct ecotypes with similar, but not identical, niches (see section 5). It is equally plausible that rare microorganisms not well represented in the gene surveys because of their low abundances in nature might be crucial for ecosystem function. Other species, although rare most of the time, might be responsible for microbial blooms following the addition of nutrients or other environmental perturbations. This disparity between phylogeny and physiology, and between contemporaneous and future potential metabolism, remains a major analytical and conceptual challenge for the discipline as a whole.

4. THE OCEAN GENOME

The genomics revolution has redirected the marine microbial research prospectus, perhaps at the expense

of ecological investigation and field experimentation. However, application of these cutting-edge technologies, including whole microbial genome sequencing and marine metagenomics, has enabled major conceptual advances, helped to recruit new intellectual and funding partners, and invigorated the discipline of microbial oceanography as a whole.

A *metagenome* is the term used to describe the total inventory of genes contained within a given sample. The theoretical summation of all marine metagenomes is equal to the panocenic genome. In relatively simple environments with limited microbial diversity, the most dominant microbial genomes can be assembled from the short fragments of environmental DNA that are isolated, cloned, and sequenced. If successful, this genome assembly provides a direct estimate of taxonomic diversity of the organisms present in that sample, regardless of whether the microbes can be cultured or not. It also provides the entire genomic parts list for the assemblage as a whole, helping to define and constrain ecosystem function. However, the marine metagenomes constructed to date have proven to be much too complex for whole genome assembly. Furthermore, novel genes and proteins (i.e., no homologs in the extant database) and even new protein families have been recovered from open-ocean samples, confirming the presence of an extremely diverse and poorly characterized microbial assemblage. Because it is impossible to know which genes are associated with which microbes from a metagenome, let alone which genes are being actively expressed and what functions they might code for, the oceanic genome provides only limited ecological information at the present time. Although metagenomics is an important first step, neither the activities of sea microbes nor their ecological function can be predicted from the parts list alone; assembly and operation manuals are also required.

5. ECOTYPE VARIABILITY AND RESOURCE COMPETITION

Competition theory predicts that, at equilibrium, the number of species cannot exceed the number of limiting resources; G. E. Hutchinson termed the observed coexistence of many species of marine phototrophic algae with similar, if not identical, growth requirements the "paradox of the plankton." Temporal and spatial heterogeneity of the habitat (contemporaneous disequilibrium), niche diversification, selective predation or viral infection (kill the winner), and allopatric (microhabitat) speciation, among other factors, have been proposed to explain this paradox.

It is now known that many closely related, even "identical" (at 16S rRNA level), microbes have fun-

damentally distinct genomes, physiological potentials, and ecological niches. Genetic differentiation leads to the diversification of these related strains (clones) into clusters that are referred to as ecotypes. F. Cohan's formal definition of an ecotype is: "a set of strains using the same ecological resources such that an adaptive mutant from within the ecotype leads to the extinction of all other strains of the same ecotype but does not impact the success of strains from other ecotypes." In other words, resource competition is more acute within a given ecotype than between related ecotypes, allowing these related groups to coexist within a given habitat.

Ecotypes retain many characteristics of the parent strain but are diversified genetically and ecologically because of acquisition of new genes. Much of the genetic variation that is observed between ecotypes is present as genomic islands that are inherited by virus-mediated horizontal gene transfer. These coherent blocks of functional genes confer selective advantage on the recipient strain so they are retained in the population and readily exchanged. Consequently, ecotypes should be viewed as plastic, even ephemeral, and able to respond rapidly to changing environmental conditions.

Some of the most extensive research on marine microbial ecotypes has been conducted using *Prochlorococcus* as a model system. Variation in pigmentation and photophysiology allows selected ecotypes to grow optimally under either high light intensity ($\geq 100 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$) or low light intensity ($\leq 20 \mu\text{mol quanta m}^{-2} \text{sec}^{-1}$). Additionally, these specific ecotypes have nutrient uptake mechanisms that match their habitat. For example, the high-light-adapted ecotype cannot use nitrate or nitrite as a N source and grows only on reduced forms of N including ammonium and selected dissolved organic N compounds (e.g., urea). However, because the main supply of new N from below the euphotic zone is almost exclusively as nitrate, the high-light ecotype must rely on other organisms for its supply of chemically reduced N and, possibly, other growth substrates. Field studies have shown that *Prochlorococcus* ecotypes are stratified in both horizontal and vertical space along environmental gradients in light, temperature, and nutrients. This resource partitioning enables *Prochlorococcus* to exist, indeed, dominate, photosynthetic biomass over the entire euphotic zone (0–200 m) in many temperate and tropical seascapes.

Of the 12 *Prochlorococcus* genomes currently available, the number of protein-coding genes ranges from approximately 1900 to 3000 in high-light- and low-light-adapted ecotypes, respectively. A "core" genome, shared by all ecotypes, of 1250 genes has been identified, with an additional approximately 6000 unique genes (many with unknown function) collec-

tively present in the *Prochlorococcus* "pangenome." The shapes of the accumulation curves for both the core and pangenomes suggest that the latter, but not the former, will continue to increase as the genomes of additional isolates are sequenced. The remarkable genotypic diversity, only partly charted at the present time, is undoubtedly responsible for its successful invasion into warm water marine ecosystems worldwide.

6. THE STREAMLINED GENOME OF SAR 11

The size of a microbial genome, usually expressed as total number of nucleotide base pairs (bp), is highly correlated with cell size and number of protein-coding genes. Obligate symbiotic or parasitic microorganisms have minimal genomes that reflect a reduction in the size of the genome relative to their free-living relatives; this process has been termed *genome streamlining*. Genome reduction can also occur in free-living marine microorganisms, but this evolutionary strategy usually results in an organism that is a metabolic ward of the seascape, dependent on other microbes for continued survival. Selective pressures that favor genome streamlining include growth in complete nutritional medium that contains biosynthetic organic precursors (amino acids, nucleic acid bases) and vitamins, or life in an energy-limited habitat where resource competition is keen. The most abundant microorganism in the sea, *Pelagibacter ubique* strain HTCC 1062 (SAR 11 clade), is an evolutionary product of genome streamlining. The genome of *P. ubique* is one of the smallest of any known free-living microbes, 1,308,759 bp and 1354 protein-coding genes. The genome contains no pseudogenes, introns, transposons, or extrachromosomal elements and has the shortest intergenic spacers yet reported. *P. ubique* cannot synthesize vitamins (B₆, B₁₂, thiamine, biotin), so it is dependent on the metabolic activities and biosynthetic processes of other sea microbes with more complete genomes.

The strategy of genome streamlining involves survival risk, but it appears to have been very successful for microbes in the open sea. It is possible, even likely, that the coexistence of similar but genotypically distinct microbes may be a consequence of a complex series of codependencies in such a way that no one isolate would be able to survive by itself. In this regard, community-level interactions may be much more important than population interactions.

7. STATION ALOHA: A MICROBIAL OBSERVATORY IN THE OPEN SEA

Long-term ecological studies are predicated on the assertion that certain processes, such as climate-driven

changes in microbial community structure and productivity, are time dependent and must be studied as such. In October 1988, Station ALOHA (A Long-term Oligotrophic Habitat Assessment) was established in the North Pacific Ocean, approximately 100 km north of Oahu, Hawaii, for long-term observations of coupled physical, chemical, and microbiological processes as the deep water benchmark of the Hawaii Ocean Time-series (HOT) program.

Open-ocean tropical seascapes such as Station ALOHA are the aquatic analogs of terrestrial deserts because the standing stocks of living organisms and rates of photosynthesis are very low. Rather than being limited by water availability, oceanic deserts are nutrient starved, in particular by a chronic shortage of bioavailable nitrogen (N) in the surface layers where photosynthesis takes place (figure 2). This situation is a consequence of the density-induced vertical stratification between warm surface waters and the cold abyss that leads to a spatial separation of solar energy from the nutrients required to support net photosynthesis. Typically, 90–95% of the nutrients that are consumed daily in the euphotic zone at Station ALOHA are derived from local remineralization; the remaining 5–10% of the quota, termed "new" nutrients, is delivered from external sources. In the open sea, new nutrients (e.g., nitrate) are supplied mostly via the relatively slow process of vertical eddy diffusion from the deep water nutrient reservoir (figure 3); these supply rates ultimately control microbial biomass and productivity and, hence, the ecosystem's function.

Despite the chronic limitation of nitrate in the surface waters of most open ocean habitats, dissolved gaseous N₂ is present in unlimited supply (>500 μM). However, the relative stability of the triple bond of N₂ renders this form inert to all but a few specialized N₂-fixing microbes, dubbed diazotrophs. N₂ fixation in most open-ocean ecosystems is solar powered, and most diazotrophs are cyanobacteria. Diazotrophs require an ample supply of iron (Fe), which is an obligate cofactor for the enzyme nitrogenase. The Fe supply to the surface waters of most open-ocean habitats is via atmospheric dust delivery, and thus Fe flux varies considerably with geographic location and distance from dust sources (e.g., deserts). Furthermore, in order for N₂-dependent net growth to occur, diazotrophs require a suite of macro- and micronutrients, especially phosphate. If light, Fe, and phosphate are present in excess, phototrophic diazotrophs would have a competitive advantage in N-limited seascapes, and N₂ fixation may be a significant pathway for the introduction of new N into the ecosystem.

When the HOT program began, N₂ fixation was not considered to be a significant process in the NPSG.

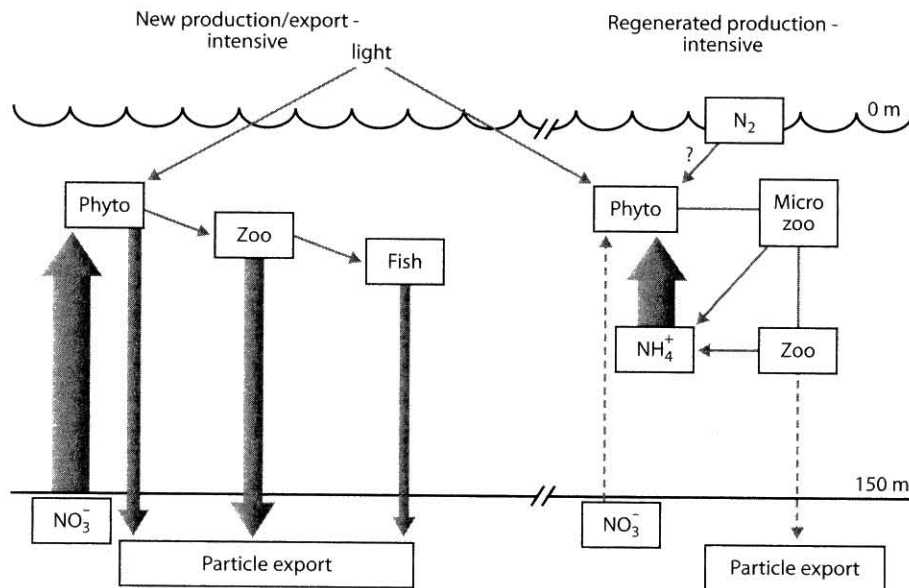


Figure 3. Conceptual view of the new versus regenerated N model for primary production in the sea. Shown are two contrasting marine ecosystems: (left) an upwelling habitat where allochthonous NO_3^- -supported new production dominates total primary productivity, and (right) an open-ocean habitat where locally produced NH_4^+ -supported

regenerated production dominates total primary productivity. N_2 fixation represents a potential enhancement of new production in N-limited seascapes. New production-intensive biomes also support much greater export, usually in the form of sinking particulate matter, than remineralization-intensive systems such as the NPSG.

Indeed, the climax community paradigm for this seascape circa 1970 was one of a time-independent, nitrate-controlled, eukaryote-dominated, low-productivity biome. Whereas nitrate resupply from deep waters was considered the ultimate source of new N in the historical view, we now recognize N_2 fixation as an approximately equal new N flux pathway (table 2).

N_2 fixation can be viewed as a keystone ecological process in the N-stressed NPSG, supplying new nitrogen via a delivery pathway that is independent of turbulence. The impact of diazotrophs is disproportional to their relatively low abundance. With the possible exception of stochastic bloom events, diazotroph biomass rarely exceeds a few percent, at most, of phytoplankton carbon in these habitats. However, the removal of all N_2 fixers from the NPSG would likely lead to a significant decrease in phytoplankton biomass, net primary production, fish production, CO_2 sequestration, and a corresponding reduction of the export of carbon and energy to the mesopelagic and deep sea, with attendant ecological consequences—the hallmark of an ecological keystone.

We currently recognize at least three fundamentally different groups of diazotrophs at Station ALOHA (see table 3): (1) small, free-living unicellular cyanobacteria (*Crocospaera*-like), (2) large filamentous and colonial morphologies of the cyanobacterium *Trichodesmium*,

and (3) *Richelia*-like cyanobacteria living as ecto- and endosymbionts with several species of large aggregate-forming diatoms (e.g., *Rhizosolenia*, *Hemiaulus*). The N_2 fixed by each of these groups has a different impact on the ecology and biogeochemistry of the NPSG, despite the fact that all belong to the same diazotroph guild (table 3). In addition to alleviating N stress, N_2 fixation-based organic matter production enhances the sequestration of CO_2 because the import of N_2 is decoupled from the delivery of deep water nitrate that also contains a high concentration of CO_2 . Gravitational settling of N_2 -based particulate organic matter pumps excess carbon into the deep sea. This diazotroph-based sequestration is further enhanced because the C:P molar ratios of most diazotrophs growing under P control are higher than that of the total C:P ratio of upwelled nutrients.

It has been hypothesized that the environmental conditions necessary to promote the selection for N_2 -fixing microorganisms (e.g., water column stratification, nutrient resupply rates, and N:P ratios) have systematically changed since the later 1970s, resulting in an epoch of N_2 fixation that continues today. If the biomass of N_2 -fixing microbes and the rates of N_2 fixation in the NPSG are increasing over time because of climate-driven changes in the environment, then the biome is being forced into severe P limitation.

Table 2. Biological and biogeochemical indicators of N₂ fixation at Station ALOHA

<i>Observation</i>	<i>Method</i>
N ₂ -fixing microbes	Direct microscopy, <i>nif</i> H gene abundances by quantitative polymerase chain reaction (QPCR)
N ₂ fixation rates	In situ measurements using acetylene reduction and ¹⁵ N-N ₂ isotopic methods, <i>nif</i> H gene expression by reverse-transcribed QPCR
Long-term changes in the inventories of soluble and particulate phosphorus and in C:P/N:P ratios	Time-series collections and direct chemical measurements
Changes in the rate of particulate-P export and in the ¹⁵ N isotopic abundance and elemental ratios	Field collections using sediment traps and direct chemical and N isotopic measurements
Summertime drawdown of dissolved inorganic carbon in absence of nitrate	Direct measurements from repeat sampling of surface waters

Table 3. Diversity of form and ecological function of three major groups of diazotrophic microbes in the North Pacific Subtropical Gyre

<i>Nanoplankton</i>	<i>Trichodesmium</i>	<i>Diatoms/Richelia</i>
Small (<10 μm), high growth rate	Large (>20 μm), low growth rate	Large (>20 μm), high growth rate
“Background” population	Bloom forming	Bloom forming
Dispersed	Floater/migrators	Sinkers/migrators
Consumed by protozoans	Not readily consumed	Consumed by zooplankton
High turnover/low export	Low turnover/low export	Variable turnover/high export

Ecological consequences might include changes in the standing stocks and turnover rates of dissolved and particulate P and altered C-N-P composition of new biomass production, which could in turn select for microorganisms that do not require as much P for growth, or for slower-growing microbes with lower P requirements because of reduced ribosomal RNA content.

Although the phosphate inventory at Station ALOHA has decreased by more than 50% over the past two decades (figure 4), most likely as a consequence of N₂ fixation, there still seems to be a surplus. Further reduction of phosphate to subnanomolar concentrations can be expected along with a selection for alternative P capture and a further shift in the cell size and activity spectra to smaller, slower-growing microorganisms. This prediction has numerous potential impacts on the trophic structure, selecting for smaller predators and thereby altering top-down grazing control of microbial populations and nutrient cycling rates. Without an adequate resupply of phosphate and other nutrients, these P-stressed ecosystems could lose biomass, biodiversity, and possibly their ability to respond to habitat fluctuations and climate change.

A key negative feedback to enhanced N₂ fixation—decoupling of N and P cycles and the export of high-N:P-ratio organic matter—is the eventual buildup of a subeuphotic zone nutrient reservoir that has an elevated nitrate:phosphate ratio relative to cellular needs (e.g., N:P > 16:1). As these regenerated nutrients slowly feed back into the euphotic zone, they will select against N₂ fixers because the excess phosphate is assimilated by competing nitrate-utilizing microorganisms. This would lead to another shift in community structure, ecological stoichiometry, grazing control, and organic matter export. An alternation of ecosystem states between N limitation and P (or P/Fe) limitation in the NPSG is expected to occur on an approximately 20- to 50-year cycle based on the estimated residence time of nutrients in the upper mesopelagic zone reservoir. However, the extent to which greenhouse gas-induced warming and other changes to the surface ocean will impact the dynamics of these hypothesized alternate ecosystem states is currently unknown. In global climate model simulations, higher dust deposition to the open ocean enhances global primary productivity, N₂ fixation, and CO₂ sequestration on time scales of a decade or less. Furthermore, it is almost certain that the global dimensions of subtropical gyres

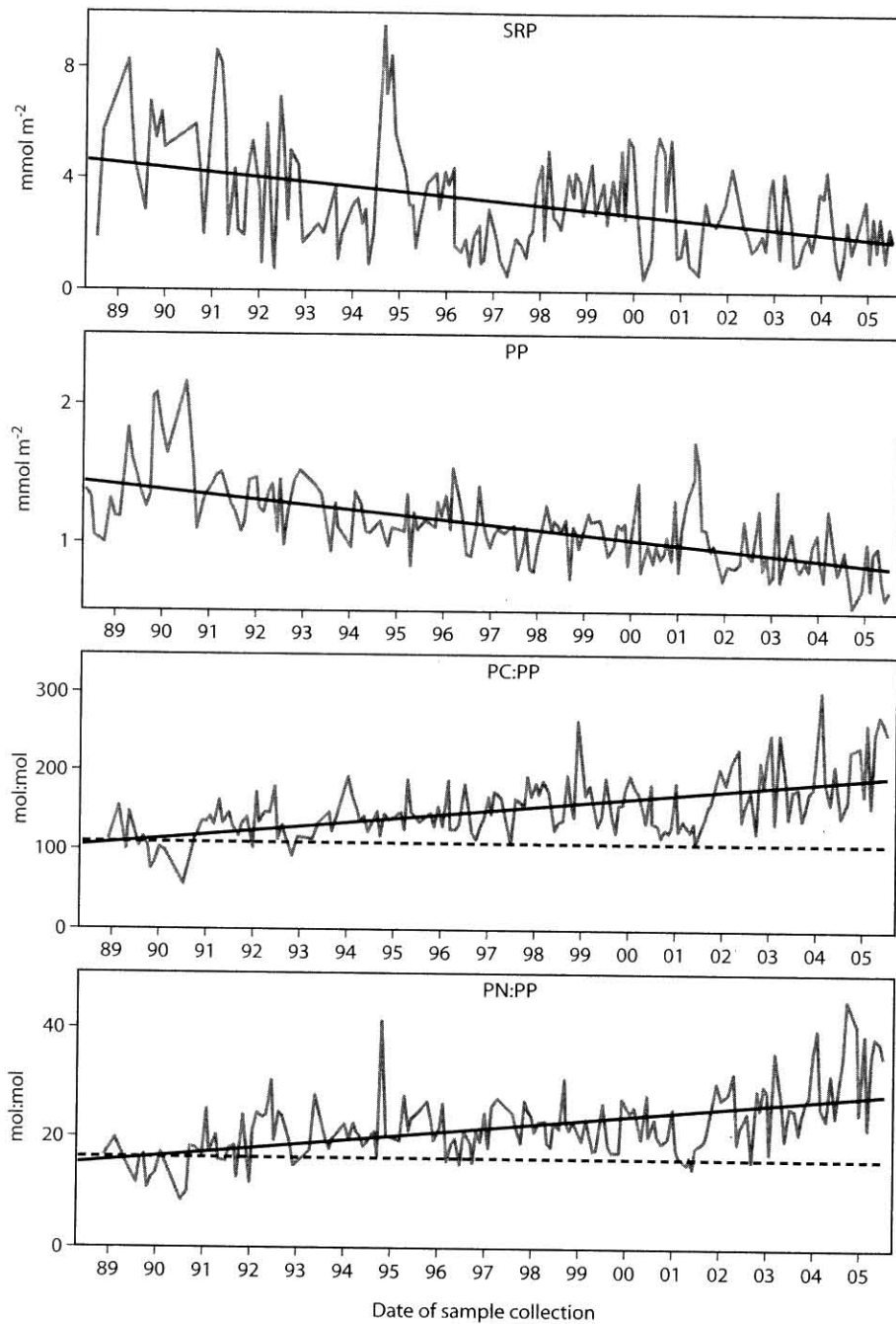


Figure 4. Station ALOHA phosphorus pool dynamics. Shown are the 15-year time-series records of soluble reactive phosphorus (e.g., phosphate, SRP) integrated over the upper 0–60m of the water column, particulate phosphorus (PP) integrated over the upper 0–75m of the water column, and the average (0–75m) carbon-to-phosphorus and nitrogen-to-phosphorus ratios of the particulate matter pools (PC:PP and PN:PP, respectively). The solid

lines represent the best-fit linear regression analyses for each data set, and the dashed lines in the PC:PP and PN:PP plots represent the global average stoichiometry [106C:16N:1P] for marine particulate matter. The decreasing concentrations of SRP and PP and increasing PC:PP and PN:PP ratios are all predicted from the hypothesized enhancement of N₂ fixation at Station ALOHA over the past two decades.

will expand as the Earth warms, and the open ocean as a whole will become more stratified and nutrient depleted, setting the stage for the selection of N_2 -fixing microorganisms with attendant ecological consequences.

8. CONCLUSION

Seascape ecologists are at a distinct disadvantage relative to their land-based colleagues. Whereas early terrestrial naturalists could readily observe environmental changes in space and time, open-ocean ecologists had to develop tools to observe biological changes in the vast pelagic environment; consequently, the discipline of seascape ecology is young. Furthermore, it is difficult to conduct replicated, long-term manipulation studies in the sea as has been done in lakes and on land. Finally, the ephemeral nature of many seascapes, including, but not limited to, complex, nonlinear physical-biological interactions and unpredicted climate variability, precludes the development of predictive models at present.

The field of landscape ecology has an explicit connection to humans as components that actively alter natural habitats. Likewise, seascape microbial ecology seeks fundamental information on how humans are beginning to alter some of the most remote ocean habitats. The vehicle for seascape change is global climate variability and especially the impacts of gases that affect the transmission of long-wavelength radiations through the atmosphere, such as CO_2 , methane, and nitrous oxide. As these greenhouse gases accumulate in the atmosphere, the planet will get warmer. This heating, in turn, will impact the atmospheric circulation and global hydrology, thereby altering ocean circulation patterns, stratification, and dust fluxes to ocean. As the surface ocean absorbs more and more CO_2 , it will become more acidic, further altering the physical/chemical characteristics and microbial biodiversity.

Seascape microbial ecology requires a sound theoretical framework for analyzing spatial and temporal patterns in the distribution, abundance, and biodiversity of microorganisms and the ecological services they perform. Continued progress in this area must be considered a priority and will require an integration of laboratory, field, and modeling efforts and new collaborations among scientists who traditionally have not interacted. The next decade should be both challenging and exciting.

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