Foundations of Microbial Oceanography

BY DAVID M. KARL AND LITA M. PROCTOR

It's been said, "The farther backwards you look, the farther forward you are likely to see" (Sir Winston Churchill, 1874–1965). This is especially true in science where knowledge is cumulative and contemporary challenges are built on the successes of the past. Microbial oceanography is a relatively new discipline that endeavors to establish a comprehensive understanding of sea microbes, from genomes to biomes, thereby coupling biosystems to ecosystems. Microbial oceanography has its historic roots in the more established fields of microbiology, oceanography, and marine biology. Many current practitioners, however, are unaware of some of the key benchmarks and the contributors who helped to establish their discipline. Our introduction presents a few selected waypoints in microbial oceanography.

DISCOVERY OF MARINE MICROORGANISMS

The earliest study of marine microorganisms can be traced to a Dutch draper and amateur lensmaker, Antony van Leeuwenhoek (1632–1723). Others before him, dating back to Leonardo da Vinci in the fifteenth century, had employed magnifying lenses for the study of small objects, and the compound microscope had already been invented before van Leeuwenhoek's birth. However, van Leeuwenhoek improved the art of polishing lenses of short focal length, and with a single lens instrument ("simple" microscope) he was able to achieve a magnification of approximately 275 diameters (Porter, 1976), enough to observe bacteria in seawater (van Leeuwenhoek, 1677). In a recent essay on the discovery of microorganisms, Gest (2004) suggested that the credit for the discovery of microbes should be shared by Robert Hooke (1635–1703) and van Leeuwenhoek. Hooke had published a monograph, *Micrographia*, in 1665 showing drawings of microbes that he had seen with his compound microscopes, and including a section that described—in some detail—how to construct a microscope from a "very clear piece of Venice glass." The debate over who should get credit for the discovery of microbes is important, but will not be settled here. Rather than create a revisionist view, we will conclude that van Leeuwenhoek probably observed, and certainly published, the first description of a "sea microbe." The authoritative, indeed majestic, biography of van Leeuwenhoek by C. Dobell (1932), published on the 300th anniversary of van Leeuwenhoek's birth, should be required reading for all microbiologists.

Despite his ability to observe "little animalcules," neither van Leeuwenhoek nor his contemporaries had any understanding of the nature of these tiny life forms. Van Leeuwenhoek was not a scientist, and his letters to the Royal Society about his discoveries have been described as "tiresome because of repetition and discontinuity" (Kofoid, 1934); he was even called an "immortal dilettant" (Becking, 1924). It would take more than 150 years until the Prussian microscopist Christian Gottfried Ehrenberg (1795–1876) and others conducted detailed, laboratory-based investigations of microorganisms, including culture studies of isolated marine microbes. In 1828, Ehrenberg coined the term "bacterium" (from the Greek, meaning "little stick"), and in so doing separated them from other microbes.

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Ehrenberg was also one of the first scientists to systematically describe fossil microbes (diatoms, radiolaria, foraminifera, coccolithophorids) in rocks. His influential monograph, *Mikrogeologie*, published in 1854, marked the beginning of micropaleontology as a scientific discipline and was an important precursor for modern studies in geobiology.

During the nineteenth century, there were several other noteworthy advances in our understanding of the microbial world that helped to promote investigations of marine microbes. First was the notion that bacteria were actually a diverse assemblage of related organisms, which lead to various proposals for classification. The Prussian biologist Ferdinand Julius Cohn (1828–1898) identified several unique groups of bacteria based on morphology, cellular structures, pigmentation, and growth characteristics; indeed, he has been credited with founding modern microbiology (Drews, 1999). Cohn was one of the first to conduct detailed laboratory studies of bacterial growth using defined media, and his work helped to debunk the concept of "spontaneous generation" of life by documenting that vegetative cells, but not endospores, were killed by boiling water (Drews, 1999). His achievements became an important foundation for modern microbiology, along with the more well-known studies by Louis Pasteur (1822–1895) and Robert Koch (1843–1910) on infectious disease and pathology, vaccination, and fermentation. A hallmark of this era, sometimes called the "First Golden Age of Microbiology," was the use of pure cultures. This was often considered a requirement, for example, in the application of Koch's postulates[1] (Grimes, 2006).

In his writings, Pasteur discussed the importance of the environment in controlling physiology and metabolism, and he expressed great interest in the nascent field of microbial ecology, but time did not permit his full attention to these matters. At the diamond jubilee meeting of the American Society of Microbiology in 1974, René Dubos coined this dichotomy "Pasteur's dilemma —The road not taken," and rhetorically asked where the field of microbial ecology (including marine microbial ecology) would be today if Pasteur had selected it as his primary research thrust. Instead, the discipline lay relatively dormant (at least in comparison to medical/industrial microbiology) for nearly 50 years. Toward the end of the nineteenth century, independent contributions by the Russian microbiologist Sergei Winogradsky (1856–1953) and the Dutch microbiologist Martinus Beijerinck (1851–1931) led to the development of the enrichment culture technique. This single advance in technology facilitated the discovery of chemolithoautotrophic metabolism (ammonium, sulfur, and iron oxidation linked to carbon dioxide fixation), nitrogen fixation, and sulfate reduction, leading to a greater understanding of the role of microorganisms, especially bacteria, in global biogeochemical cycles. Beijerinck also was one of the first to recognize "soluble microbes" and coined the term virus to describe them. He also founded the Delft School of Microbiology, which became an important center for research and training of the next generation of microbial ecologists.

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ACCESS TO THE SEA

The other major root of microbial oceanography, namely developments in oceanography and marine biology, can be traced back to Kiel and Plymouth with the comprehensive studies of

[1] Koch's postulates are four criteria that need to be satisfied to prove that a microorganism causes a disease: the microorganism must found in every case of the disease, the microorganism must be isolated from the diseased animal and grown in pure culture, the same disease must develop when introduced into healthy individuals, and the microorganism must be re-isolated from the new infection.

marine plankton by mostly European scientists, including Victor Hansen, Karl Brandt, Ernst Haeckel, Louis Agassiz, and Anton Dohrn, among others. In the United States, the establishment of the Navy Hydrographic Office following the Civil War and the Fish Commission in 1871 provided new opportunities for at-sea observations and sample collection. In December 1872, HMS *Challenger*, a 2306-ton, steam-assisted British navy corvette, departed from Portsmouth, England, on a four-year, worldwide voyage of ocean discovery. This interdisciplinary expedition provided new information on all aspects of the ocean as an ecosystem, and most historians mark the HMS *Challenger* expedition as the beginning of the discipline of oceanography. Among others, Ernst Haeckel (1834–1919), one of the leading biologists of this era, made pioneering contributions to our understanding of marine microorganisms, especially the distribution, abundance, and taxonomy of foraminifera, radiolaria, and diatoms. Despite the fact that bacteria had already been observed in numerous marine ecosystems and that the First Golden Age of Microbiology was well underway by 1872, there were no systematic attempts to collect or study marine bacteria on the HMS *Challenger* expedition. However, by the end of the nineteenth century, beginning with the French microbiologist A. Certes (1884), scientists from several countries were focusing on the microbial assemblages in the sea. In 1894, German biologist Bernard Fischer published his landmark treatise *Die Bakterien des Meeres* (*Bacteria of the Sea*), which helped to stimulate similar investigations worldwide, leading to modern, integrated studies in the discipline of microbial oceanography.

In addition to numerous global ocean expeditions, several key marine laboratories were established worldwide by the end of the nineteenth century. These facilities provided opportunities for sustained observations and controlled experiments on isolated marine microbes and, more importantly, ready access to the open sea. One of the first and most famous of these marine stations was the Stazione Zoologica founded in Naples, Italy, in 1872 by German zoologist Anton Dohrn. The Sevastopol Biological Station of the Academy of Sciences of the USSR, also founded in 1872, was dedicated primarily to interdisciplinary studies of the Black Sea. The Kristineberg Marine Research Station of the Royal Swedish Academy of Sciences was founded on the west coast of Sweden in 1877 to focus on the study of both coastal and offshore marine habitats. This was followed by establishment of the Plymouth Marine Laboratory in the United Kingdom in 1888, marine laboratories in Canada (Atlantic Biological Station in St. Andrews, New Brunswick, in 1899, and the Pacific Biological Station at Nanaimo, British Columbia, in 1908), Germany (Institut und Museum für Meereskunde in 1900), Bermuda (Bermuda Biological Station for Research in 1903), and Monaco (Musée Océanographique de Monaco, created and endowed by Albert the First, Prince of Monaco, in 1906).

In the United States, several major research and educational facilities emerged near the turn of the twentieth century. The Marine Biological Laboratory at Woods Hole was founded in 1888, the same year as the Plymouth Marine Laboratory. Other facilities included Hopkins Marine Station of Stanford University founded in 1892, Mount Desert Island Biological Laboratory in Maine founded in 1898, Tortugas Marine Biological Laboratory of the Carnegie Institution of Washington founded in 1902, and Coronado Marine Biological Laboratory of the University of California (UC) at Berkeley founded in 1903 (predecessor of the George H. Scripps Memorial Marine Biological Laboratory in 1909,

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Scripps Institution for Biological Research in 1912, and, subsequently, the Scripps Institution of Oceanography in 1925). There were also Marine Biological Station of the University of Southern California founded in 1911, San Juan Islands Biological Station founded in 1904 (predecessor of the Oceanographic Laboratories, University of Washington, 1920); Chesapeake Biological Laboratory of the University of Maryland founded in 1920, and Woods Hole Oceanographic Institution founded in 1930.

EMERGENCE OF MICROBIAL OCEANOGRAPHY

By the turn of the twentieth century, oceanographic expeditions routinely collected, enumerated, and analyzed heterotrophic bacteria and other sea microbes. A fundamental scientific question during these early studies was whether there existed truly "marine" bacteria that could be distinguished from their terrestrial and freshwater counterparts. Because most bacteria originally isolated from nonmarine sources were unable to survive for extended periods when inoculated into seawater, a focus of this research on the nature of marine bacteria was on a diagnostic salt requirement or salt tolerance. The existence of marine bacteria remained an open question until the early 1960s when a Na^+ requirement for growth was discovered as a unique characteristic of marine bacteria (MacLeod, 1985).

In 1931, Henry Bryant Bigelow (1879–1967), the inaugural director of the newly established Woods Hole Oceanographic Institution (WHOI), published his benchmark monograph *Oceanography: Its Scope, Problems, and Economic Importance*. This book was based largely on the initial work of the U.S. National Academy of Sciences Committee on Oceanography (1927–1937). Key themes in Bigelow's monograph included: (1) oceanography is impossible unless one goes to sea—observations are fundamental to understanding, (2) technology drives opportunity and facilitates the creation of new knowledge, and (3) the need to shift research from a descriptive approach to an analytical/quantitative one, including mathematical modeling. These are still valid guiding principles for contemporary microbial oceanography. Bigelow also concluded that our knowledge of bacteria is "woefully scant" and emphasized that they must be studied to understand the organic fertility of the sea. He also suggested that the process of nitrogen (N_2) fixation, already reported in many terrestrial habitats since its discovery in the early 1900s, should also occur in the sea. It would take another three decades before Richard Dugdale and his colleagues reported N_2 fixation in the Sargasso Sea (Dugdale et al., 1961).

Perhaps the greatest contribution that Bigelow made to microbial oceanography was to recruit Selman A. Waksman, a well-established soil microbiologist from Rutgers University, to Woods Hole to begin a new program in marine microbiology. Waksman joined the WHOI staff in 1931 and made several fundamental contributions over the next few years, especially on the process of organic matter decomposition and on the marine nitrogen cycle. In 1939, he returned to his "roots," soil microbiology, and within a decade had isolated and characterized 10 antibiotic compounds, including streptomycin. This discovery led to an eventual eradication of tuberculosis and to a Nobel Prize for Physiology/Medicine in 1952. Subsequently, in a special volume of *Deep-Sea Research* dedicated to Henry Bryant Bigelow on the occasion of the twenty-fifth anniversary of the founding of WHOI, Waksman (1955) recounted his early studies in marine bacteriology. Besides his laboratory work, Waksman also attempted some open ocean fieldwork, which he characterized as a complete failure. "Under the threefold movements of the ship, I immediately became seasick and had to leave the work largely to one of the assistants who always accompanied me. After two valiant efforts to carry out studies on the moving boat, I made no further attempts." Bigelow's comment, "Food was wasted on him," was entirely appropriate.

Across the United States, two additional laboratories, one at Stanford University's Hopkins Marine Station in Pacific Grove, California, and the other in San Diego, California, at Scripps Institution of Oceanography (SIO), were beginning to conduct independent studies on sea microbes. At Hopkins, these were organized and led by Cornelis B. van Niel (1897–1985), a distinguished scientist from the Delft School of general microbiology. In addition to many important contributions including his pioneering work on bacterial photosynthesis and the metabolism of sulfur bacteria, van Niel made a lasting contribution by organizing and teaching a summer course at Hopkins. Over the next three decades, this served as an important training ground for many future marine microbiologists (Spath, 2004). At SIO, Haldane Gee was appointed assistant professor of bacteriology in 1928. Others before him, including Charles Kofoid (protozoa) and Winfred Allen (diatoms and dinoflagellates), had studied eukaryotic microorganisms, but Gee was the first to specialize in bacteria.

At the thirty-first meeting of the Society of American Bacteriologists (predecessor of the American Society for Microbiology), December 30, 1929, to January 1, 1930, Gee described a new bacteriological sampler for use in deep water. He also reported preliminary data for total bacterial counts from several surveys off southern California. He concluded that "significant counts in the water are restricted to the topmost 25 m, below which there is apparent sterility until the bottom zone is reached" (Gee, 1930a). That same year, Gee (1930b) reported on the status of marine bacteriology at the fourth Pacific Science Congress in Java. Based on a compilation of 136 scientific papers on the subject, he concluded that, "Much of this work must be regarded as incomplete and some of it can be discarded entirely in view of developments in bacteriology in the past 10 years." He went on to state, "The first prerequisite for a general survey is the development of special methods." Unfortunately, Gee's career was cut short due to illness and he left SIO in 1932 (Yayanos, 2003). Gee was replaced by Claude E. ZoBell, who worked tirelessly for the next five decades to help establish and promote the discipline. The publication of his monograph, *Marine Microbiology*, is cited by many as the beginning of a new era (see below).

By the early 1960s, many new methods had been introduced for microbial-cell enumeration and estimation of community respiration, metabolism, or growth. In 1963, the Institute of Marine Resources at SIO established the Food Chain Research Group (FCRG). This group was founded and led by John D.H. Strickland, an analytical chemist by training, born and educated in the United Kingdom. In 1952, he emigrated to Canada and worked at the University of British Columbia and at the Fisheries Research Board of Canada at Nanaimo, British Columbia, before moving to Scripps. At Nanaimo he began a long-term collaboration with Tim Parsons, and in 1968 they published the first edition of their classic monograph, *Handbook of Seawater Analysis* (Strickland and Parsons, 1968). As leader of the FCRG, Strickland assembled an outstanding, interdisciplinary scientific staff, including Angelo Carlucci (bacteria), Richard Eppley (phytoplankton), John Beers (protozoa), Osmund Holm-Hansen (productivity and biochemistry), Peter Williams (organic biogeochemistry), and Michael Mullin (mesozooplankton). Collectively, this team developed new methods, promoted systematic studies of trophic structure and interactions with an emphasis on pelagic marine ecosystems, and trained a large number of students and postdoctoral scholars that helped to continue the tradition. After Strickland's death in 1970, the FCRG sustained interdisciplinary research in microbial biogeochemistry under shared leadership. Farooq Azam and George Jackson were added to the staff to further enhance leadership in microbial food webs and food-web modeling/physical-biological interactions, respectively. Unfortunately, when their core funding from the U.S. Atomic Energy Commission (predecessor of the Energy Research and Development Agency and, subsequently, the Department of Energy) dried up, the FCRG dissolved, although the individual scientists continued to make pioneering contributions, especially in the area of marine microbial ecology. During this same period, circa 1960–1980, other scientists worldwide began making fundamental contributions regarding the biology and ecology of sea microbes. By the early 1970s, most marine science/oceanography departments, and many departments of microbiology/bacteriology/environmental science had at least one "marine microbiologist" on staff. This led to rapid growth in training and recruitment of new scientists into the collective workforce of this challenging and expanding discipline. Ironically, very few universities offer advanced degrees in marine microbiology (or marine microbial ecology or microbial oceanography). The International Max Planck Research School of Marine Microbiology (MPI-MM), a joint program between the MPI-MM in Bremen, the University of Bremen, The Alfred Wegener Institute for Polar and Marine Research (AWI), and the International University of Bremen (IUB) is the best and, perhaps only, contemporary example.

ZOBELL'S *MARINE MICROBIOLOGY* AND RELATED WORKS

Claude E. ZoBell (1904–1989) began his training in medical microbiology at the Hooper Foundation for Medical Research at the University of California, Berkeley. His Ph.D. research, completed in 1931, was on the growth and metabolism of *Brucella*. In 1932, he moved to the "temporary" position of instructor in marine microbiology at Scripps Institution of Oceanography, where he remained active until approximately 18 months before his death in 1989. According to published records, ZoBell's initial responsibility was "to determine the extent to which bacteria are active in the open ocean and to assess their possible importance as geochemical agents" (Yayanos, 2003). While significant progress has been made in the intervening 75 years, these themes remain as the contemporary challenges of microbial oceanography, collectively comprising the "Second Golden Age of Microbiology."

In 1946, ZoBell published his now classic book *Marine Microbiology: A Monograph on Hydrobacteriology*. Many science historians consider this to be the modern beginning of the discipline; Donald McGraw recently characterized ZoBell's magnum opus as a pioneering "manifesto" in the founding of marine microbiology (McGraw, 2006). Although others before him had written scholarly monographs on sea microbes, ZoBell's was the first that was written by a "twentieth century scientist" (he was born in Utah just after the turn of the century), and his monograph reviews the many key discoveries that had taken place in the decades since the monographs by Fischer (1894) and Benecke (1933) had been published. However, not everyone was uniformly pleased with the scope and quality of ZoBell's monograph. A book review that appeared in *Ecology* (Burrows, 1946) was critical of the fact that the volume was much less inclusive than the title, *Marine Microbiology*, implied because it focused almost exclusively on marine bacteria and not "microbes" in a more general sense of the term. In fact, the main title *Marine Microbiology* and the subtitle *A Monograph on Hydrobacteriology* are somewhat contradictory, because the former implies all marine microbes (including bacteria, yeasts and fungi, algae, and viruses), while the latter covers only bacteria but includes all aquatic (marine, freshwater, estuarine, groundwater, wastewater) environments. This important point on the relatively narrow scope of the monograph relative to the potential scope of the discipline itself, despite the broad title, was echoed by van Niel, a recognized leader in general microbiology at that time. In a book review that appeared in *The Quarterly Review of Biology*, van Niel (1946) stated, "This preference on the part of ZoBell is hardly an adequate reason for the use of a title which suggests an account of the nature and activities of marine microorganisms. Instead, the contents are almost exclusively limited to a rather elementary treatise of the bacterial population of the ocean, with a few brief sections on bacteria in marine air, in brines, and in salt- and freshwater lakes. The yeasts and molds are dealt with in seven pages; beyond the re-iterated statement that the phytoplankton is largely responsible for the production of organic matter in the ocean, the algae are not mentioned; nor are the protozoa." Van Niel ended his review by stating, "as a monograph it does not reach a very high standard, and seems, on the whole, rather trifling."

> ...the foundation of our discipline continues to expand with new fundamental knowledge about life in the sea.

Despite those contemporary criticisms, ZoBell's collective contributions, including his monograph *Marine Microbiology*, form a significant benchmark in the history of microbial oceanography. ZoBell was only 42 years old when his monograph was published; he went on to publish nearly 300 scientific papers on various aspects of marine microbiology, geomicrobiology, and petroleum microbiology, and to participate in many important research expeditions during his career. For example, during the Danish *Galathea* deep-sea expedition (1950–1952) led by Anton Bruun, ZoBell was able to document bacterial life at the bottom of the 10,462-m-deep Philippine Trench. He also demonstrated a growth preference in these populations for low temperature (2.5°C) and high pressure (1000 atm), conditions that were uniquely characteristic of their native habitats (ZoBell, 1952). Donald McGraw has written extensively about ZoBell and his impact on marine microbiology (McGraw 2001, 2004, 2006), and four mini-biographies on various aspects of ZoBell's scientific career appeared in the *Proceedings of the 8th International Symposium on Microbial Ecology* (e.g., see Lappin-Scott, 1999). In 1985, a *festschrift* of the *Geomicrobiology Journal* was dedicated to ZoBell, who was the journal's founder and editor-in-chief (Morita, 1985).

It was quite a while before another major synthesis of research in marine microbiology was undertaken in the form of a monograph. In 1955, Jean Brisou's *La Microbiologie du Milieu Marin* was published, but to our knowledge this work was never translated into English and, therefore, it had comparatively minor impact on the field as a whole. A.E. Kriss completed the Russian version of *Marine Microbiology (Deep Sea)* that the Academy of Sciences, USSR, published in 1959; a year later it was awarded the Lenin Prize. J.M. Shewan and Z. Kabata subsequently translated this book into English, and Oliver & Boyd published it under the same title (Kriss, 1963). According to ZoBell's review, the English translation has some new material not found in either the original Russian or previous German version (ZoBell, 1963). The monograph also includes a comprehensive summary of the Russian research effort in marine microbiology, and a bibliography of more than 400 Russian papers. The bulk of the 536-page volume summarized the "quantitative distribution of heterotrophic microorganisms throughout the seas and oceans," including bacteria and yeasts, using a variety of different methods of enumeration, complete with numerous tables and figures, and an exhaustive description of many of the species that were isolated. Typically, fewer than 100 bacterial cells per milliliter (and often, zero) were reported for growth on various media, compared to orders of magnitude greater cell abundances derived from direct microscopic examination of seawater samples concentrated on membrane filters. Jannasch and Jones (1959) also discussed this methodological mismatch, and even the direct microscopic methods using membrane filters systematically underestimated the true abundance of marine bacteria. One of the more bizarre aspects of Kriss's work is his report of strange and morphologically complex "microbial forms" in many samples that were observed under the microscope. These unusual "microbes" (called *Krassilnikoviae* [ZoBell, 1963]) were never cultured, and it now seems highly unlikely they were actually microorganisms at all, but rather body parts of larger marine organisms. A second monograph by A.E. Kriss and others, *Microbial Population of Oceans and Seas*, also written in Russian, was later translated into English by K. Syers, edited by G.E. Fogg, and published in 1967.

It is remarkable how rapidly our paradigms are shifting...

In 1965, P. Gray and J.D. McCarthy initiated a new publication series, *Modern Biological Studies*. The idea was to commission scholarly monographs on various topics that were identified by them to be timely and important. For Volume 1 of this series, they turned to E.J. Ferguson Wood (1904–1972), then professor of marine microbiology at the Institute of Marine Sciences at the University of Miami (predecessor of the Rosensteil School of Marine and Atmospheric Sciences) to prepare a synthesis entitled *Marine Microbial Ecology*. Wood, an Australian-born and -educated bacteriologist began his career as a marine scientist studying fish spoilage and the biology of sea grasses, later moving into studies of marine phytoplankton. After a successful, 30-year career at the Commonwealth Scientific and Industrial Research Organization (CSIRO), he moved to the University of Miami in 1963 where he worked until his return to Australia in 1970. His contributions, especially as a prolific scholar and author in the last decade of his life, established him as one of the leaders of his generation. In the preface to his monograph, Wood stated, "In marine microbiology our facts are so discrete and disconnected that they do not form a story, and the most important truths of our discipline are still unknown." He went on to conclude that "we now need breadth and vision rather than specialists … (people) who can integrate rather than dissect." Although he was one of ZoBell's close colleagues, Wood pointed out that ZoBell's book *Marine Microbiology* was rather narrowly focused on bacteria and also felt that the terms "microbiology" and "bacteriology" were often used synonymously and therefore incorrectly. He called for an integrated study of the ocean as an ecosystem. Wood published a second monograph, *Microbiology of Oceans and Estuaries*, just two years later, and he served as founding co-editor of the series *Advances in Microbiology of the Sea* in 1968 (see next section). His contributions were indeed significant.

In 1972, Rita Colwell and Michael Zambruski prepared a translated, edited, and revised version of A.G. Rodina's monograph on *Methods in Aquatic Microbiology,* which had last appeared in Russian in 1965. Because the expanding discipline of marine microbiology was still very much methods limited at that time, the introduction of novel methods and instrumentation—some known only to those working in Russia—was invaluable.

During the next three decades (1976–present), several additional monographs on aquatic microbiology/marine microbiology have appeared, including Sieburth (1979), Rheinheimer (1980), Austin (1988), Sorokin (1999), and Munn (2004). All serious practitioners and apprentices should read these works, cover to cover, to gain an appreciation for the origin and evolution of ideas in the discipline. John Sieburth also prepared a wonderful pictorial essay on marine microorganisms and their habitats, *Microbial Seascapes*, which helped to illuminate the microbial world (Sieburth, 1975).

SYMPOSIA, EDITED VOLUMES, AND THE PROLIFERATION OF TRADE JOURNALS

As scientific disciplines develop and mature, they leave an archival legacy of new information. Oftentimes, monographs do not adequately capture the details and, sometimes, subtle implications of all advances in methodology and concept. Special symposia, key review articles, and original refereed publications are the primary vehicles for advancing the state of knowledge. In 1957, a special symposium on "Marine Microbiology" was held in conjunction with the thirty-second meeting of the Australian and New Zealand Association for the Advancement of Science at Dunedin, New Zealand. T. M. Skerman compiled the proceedings of this international symposium and published them in 1959 as *Contributions to Marine Microbiology* (Skerman, 1959). This publication included a broad range of papers by C.E. ZoBell, D.J. Rochford, V.B.D. Skerman, E.J. Ferguson Wood/P.S. Davis, and L.G.M. Bass Becking.

A few years later, C.H. Oppenheimer compiled and edited a series of papers based on a special "Symposium on Marine Microbiology" that was held in conjunction with the sixty-first annual meeting of the American Society of Microbiology (ASM) in April 1961. In the preface to the volume, Oppenheimer states that this symposium was the "first attempt to bring together the leading investigators in this field from all parts of the world to exchange ideas, present research results and discuss problems of mutual concern" (Oppenheimer, 1963). Apparently, he was either unaware of the 1957 symposium in Dunedin, or didn't think much of it! In any case, the 1961 ASM symposium was a much larger, more comprehensive event attended by a veritable who-was-who in the discipline at that time. The published volume contains 66 chapters divided into seven sections: (1) introduction—5 chapters; (2) the producers and their relation to the chemical and biological environment—9 chapters; (3) geomicrobiological activities of marine microorganisms—7 chapters, (4) ecology of algae, protozoa, fungi, and viruses—14 chapters; (5) heterotrophy in marine microbiology—10 chapters; (6) distribution and function of marine bacteria—14 chapters; and (7) marine bacteriology and the problem of mineralization—7 chapters. If a symposium were to be organized today, many of these sections from the 1961 event would align with contemporary issues and challenges, which is somewhat sobering to contemplate given the enormous progress that we have achieved during the past four decades. In chapter 48 of the symposium volume, A.E. Kriss discusses "The immediate tasks of marine microbiology." He lists four challenges: (1) extension of at-sea observations and integration of marine microbiology into "mainstream" oceanography, especially marine geochemistry; (2) initiation of year-round, repeat observations to resolve daily, seasonal, and interannual variations in microbial population structure and processes; (3) estimation of primary productivity and determination of production: biomass ratios; and (4) initiation of a systematic study of bacterial taxonomy, especially in the deep sea. These "tasks" are all still relevant today; we apparently have some work to do.

Other key symposia were organized to bring international researchers with a common interest in sea microbes together to exchange data, knowledge, and their plans for future research. Rita Colwell, Richard Morita, and others were instrumental in the organization of two U.S.-Japan conferences on marine microbiology. The first was held in Tokyo in 1966, and the second was held at the University of Maryland in 1972 (Colwell and Morita, 1974). Edited symposia proceedings also included Stevenson and Colwell (1973) and Colwell and Foster (1979). In 1962, Samuel Meyers of Louisiana State University initiated the *Aquatic Microbiology Newsletter*, which he edited, printed, and distributed for more than four decades. The newsletter covered a broad range of topics, including reports of meetings and related activities of mutual interest. When Meyers retired, so too did the *Newsletter*, unfortunately.

With great expressed need, a new contributed series, *Advances in Microbiology of the Sea*, edited by M.R. Droop and E.J. Ferguson Wood appeared in 1968 (Droop and Wood, 1968). The scope was summarized in the preface by M.R. Droop: "In so far as it can be considered a corporate science, marine microbiology may be defined as the study of marine processes effected by microorganisms. This, of course, includes the study of the agents themselves." In Chapter 1, "Perspectives in Marine Microbiology," E.J. Ferguson Wood provided a status report of the discipline. Among several other provocative statements, he concluded that "so many recent discoveries cast doubt on theories which seemed to be solidly founded that the reviewer finds it hard to give a factual evaluation," but that "valuable information on all subjects is coming to hand." Therefore, "it seems high time for marine microbiologists to sum up the work that has been done, consider how they can fill the gaps in existing studies and proceed to solve what may be of global importance, the true role of microorganisms in the oceans, seas and estuaries of the world." Amen! This volume also contained eight scholarly review articles on subjects ranging from the kinetics of phytoplankton growth (R. Eppley and J.D.H. Strickland), to numerical taxonomy of marine bacteria (W. Hodgkiss and J.M. Shewan), to the distribution and ecology of marine yeasts (N. van Uden and J.W. Fell). By the end of this 239-page work, most readers were already looking forward to volume 2; unfortunately, it never appeared.

The series was reincarnated nearly a decade later as volume 1 of *Advances in Aquatic Microbiology* edited by M.R. Droop and H.W. Jannasch (1977). The scope was broadened to include studies of both marine and freshwater ecosystems, from microbial physiology to ecology, with a stated goal of identifying common ground across aquatic habitats. Unlike some other "Advances in…" series, this one was not intended to be an annual publication, and volume 2 did not appear until 1980 (also edited by M.R. Droop and H.W. Jannasch). Despite the growing importance of aquatic microbiology, especially marine microbiology, the publication series ended in 1985 with volume 3, edited by H.W. Jannasch and P.J. leB. Williams. In his review of *Advances in Aquatic Microbiology* volume 1, Tom Brock expressed doubt about the long-term success of "one more review series in microbial ecology." Unfortunately, Brock was right on with this assertion, and the series was eventually terminated. When asked about the demise of this important publication, volume 3 editor Williams noted that the series was perceived by some to be in competition with others in the growing field of microbial ecology, and that it required the full-time attention of one or more editors to sustain it (personal communication to DMK, February 2007).

Other edited volumes have also appeared since the publication of ZoBell's monograph. In 1976, Carol D. Litchfield, then a professor at Rutgers University, edited a volume entitled *Marine Microbiology*, as the eleventh book in the Benchmark Papers in Microbiology series. This volume was comprehensive in scope and included annotations by Litchfield on the significance of each benchmark paper that she had selected for presentation. Other works edited by Sleigh (1987), Ford (1993), Cooksey (1998), and Kirchman (2000), and two comprehensive aquatic/marine microbiology methods manuals edited by Kemp et al. (1993) and Paul (2001), have also appeared during the past two decades. Ironically, Kirchman's *Microbial Ecology of the Oceans* (2000, second edition due in 2007) had an acknowledged and deliberate focus on heterotrophic bacteria in the upper euphotic zone, so it does not provide a comprehensive coverage of the discipline. In this regard, it is a flashback to the criticism that van Niel and others voiced when ZoBell's book was first published in 1946. Probably no book, edited series, or monograph will ever be complete, given the breadth and scope of the modern discipline of microbial oceanography, so compromises must be made.

When ZoBell published his monograph in 1946, the journals *Applied and Environmental Microbiology*, *Aquatic Microbial Ecology*, *Biological Oceanography*, *Deep-Sea Research*, *Ecosystems*, *Environmental Microbiology*, *Geomicrobiology, Journal of Plankton Research*, *Limnology and Oceanography*, *Marine Biology*, *Marine Ecology Progress Series*, *Microbial Ecology,* and others that currently publish most of the original papers on sea microbes did not exist. There were fewer scientists overall, and the accumulation rate of new knowledge was manageable. Today, we have more scientists, more journals, and more information that needs to be carefully sorted and assimilated. In the "good old days," all scientists routinely spent significant amounts of their time, usually between laboratory experiments or field expeditions, reading books and journals in the library. This was both necessary and enjoyable. There was an element of excitement being among the first to scan the current issue of your favorite periodical, to read about the latest discovery or contribution. One of us (DMK) remembers attending a seminar, circa 1975, by marine ecologist Joel Hedgpeth who argued that the invention of the photocopy machine was one of the worst things that had happened to the dissemination of scientific information, because students started photocopying, rather than carefully and critically reading, the scientific literature. Today we have even more rapid Internet-based information exchange, and scientific papers are downloaded in digital format and stored electronically, but oftentimes still not carefully or critically read. Carlos Duarte (professor of marine sciences and president-elect of the American Society of Limnology and Oceanography) estimated in a personal communication that approximately 3000 papers are published each year in the field of marine microbial ecology, and that number will only increase in the future. How do we deal with this avalanche of new knowledge? And how can the field as a whole maintain a high standard if there is an exponential increase in the burden for peer review? These are new, twenty-first century challenges for our discipline and for science as a whole.

...we may not yet be in a position to provide an ecumenical account of even the most basic processes of carbon and energy flow through marine systems.

TECHNOLOGY, DISCOVERY, AND SHIFTING PARADIGMS

The field of microbial oceanography is currently methods, data, and knowledge limited. As new methods, instruments, or technologies are introduced, novel data sets are obtained about microbial life in the sea, which leads to an improvement in understanding and the development of improved paradigms and models (Doney et al., 2004; Rothstein et al., 2006). This interplay of theory, engineering, and scientific application continues to define progress in our discipline.

Space limitations preclude a detailed accounting of the many significant technology-driven advances in the field of microbial oceanography in the past few decades, but we itemize a few here to serve as examples: (1) the discovery of a long-lived radioactive isotope of carbon, carbon-14 (Ruben and Kamen, 1940), that led to novel methodologies for measurements of oceanic primary production (Steemann Nielsen, 1951) and heterotrophic bacterial production (Parsons and Strickland, 1962); (2) the invention of the "plastics-irradiated-etched" (PIE, later known by the trade name Nuclepore®) membrane filters (Fleischer et al., 1964) that enabled routine, accurate estimation of marine bacterial abundance by epifluorescence microscopy (Hobbie et al., 1977) and to the discovery of the "widespread occurrence of a unicellular, marine, planktonic cyanobacterium" belonging to the genus *Synechococcus* (Waterbury et al., 1979), the second most abundant phototroph in the sea; (3) the application of satellite-based remote sensing of ocean color (Smith and Baker, 1982), which led to synoptic global maps of near-surface chlorophyll and estimates of total oceanic primary production (Behrenfeld and Falkowski, 1997); and (4) the transition of laser-based flow cytometry and cell-sorting techniques from the medical laboratory to the ocean research vessel (Yentsch et al., 1983) that led to the discovery of *Prochlorococcus*, the most abundant phototroph in the sea (Chisholm et al., 1988). These are just a few of the many examples of how technology drives discovery in the field of microbial oceanography. Without an accurate inventory and basic ecological understanding of the dominant groups of sea microbes, we cannot yet claim to have achieved success, though we have made great progress.

EPILOGUE

The rest, as they say, is "history." The more recent application of molecular and metagenomic methodologies to marine sciences has facilitated the unexpected discovery of new organisms, new genes and proteins, and even new metabolic pathways (DeLong and Karl, 2005; Rusch et al., 2007). Many of these more recent contributions are covered in subsequent articles in this issue. Suffice it to say that the foundation of our discipline continues to expand with new fundamental knowledge about life in the sea. It is remarkable how rapidly our paradigms are shifting, and sobering to think that we may not yet be in a position to provide an ecumenical account of even the most basic processes of carbon and energy flow through marine systems. There is hard work ahead in this exciting discipline.

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