Understanding roles of microbes in marine pelagic food webs: a brief history

Evelyn and Barry Sherr

Introduction

“The sea harbors an extensive population of bacteria, varying greatly in numbers and in the variety of their activities.’ Waksman 1934; “…microorganisms are widely distributed in sea water and on the ocean floor, where they influence chemical, physiochemical, geological, and biological conditions.” ZoBell, 1946. Progress in understanding of microbes in ocean systems has depended both on the research focus of individual scientists, and on the development of novel approaches to study of microbes in the sea. A brief history of the study of ecological roles of microbes in the sea shows that many of the current research thrusts were of interest early on in the development of the field. Often, however, progress on specific facets of marine microbial ecology was impeded by lack of instrumentation and methods to address the problems. A great deal of what we know about marine microbes has only been discovered since the mid-1970’s. New methodology, including molecular genetics approaches and major oceanographic expeditions in the past few decades, have revolutionized our understanding of the vital roles microbes play in marine ecosystems.

Every aquatic microbiologist working during the 1970’s and 1980’s has a unique perspective of the development of the field at that time. Our own perspective is influenced by our years at the University of Georgia Marine Institute on Sapelo Island, where L. R..Pomeroy and colleagues did research that led to novel concepts about the role of microbes in marine ecosystems. We are also grateful for the perspectives of several of our colleagues who have authored highly influential papers.

Pre-1950’s: The early years

In the first half of the 20th century, scientists were aware that a variety of bacteria existed in the sea, and that their activities were likely important in biogeochemical cycling in marine ecosystems. Methods for studying marine bacteria were mainly drawn from clinical microbiology. For example, assessment of the abundance of bacteria in seawater was done by plating diluted water samples onto nutrient agar plates, and then counting the number of bacterial colonies that grew up on the agar per unit volume of original sample. The presumption was that each colony grew from a single cell, thus the number of “culturabe” bacteria per milliliter of seawater could be estimated.

The development of marine microbiology in the first part of the 20th century was greatly influenced by Selman Waksman (1888 – 1973), a soil microbiologist who developed a Department of Marine Bacteriology at the Woods Hole Oceanographic Institution, and by Claude ZoBell (1904-1989) a research scientist at Scripps Institution of Oceanography (Ehrlich 2000, McGraw 2004). Waksman wrote a series of papers on the biogeochemical activities of marine bacteria in the 1930’s, focused mainly on the role of bacteria in decomposing organic matter (Waksman 1934, Waksman et al. 1933). In 1952 Waksman received a Nobel Prize for his work in soil microbiology which led to discovery of a number of new antibiotics. ZoBell (1946) compiled existing knowledge in the field in Marine Microbiology: A Monograph on Hydrobacteriology. Research carried out by ZoBell and his students demonstrated that microbial
life was adapted to living in all parts of the ocean, including abyssal depths and deep in ocean sediments (McGraw 2002). As a PhD student in ZoBell’s laboratory, Richard Morita carried out much of the field work on long ocean cruises, and wondered how bacteria in the deep ocean survived with very little substrate. Morita later focused his long research career at Oregon State University on starvation-survival mechanisms of marine bacteria and, in particular, on how marine microbes persisted at cold temperatures (Morita 1997). ZoBell’s own research was on the roles marine bacteria played in geochemical processes in the biosphere, including the deep subsurface. Deep-rock microbiology in the 1940’s and 1950’s was largely focused on oil deposits. ZoBell published extensively on petroleum microbiology, arguably laying the groundwork for today’s growing field of subsurface microbiology.

It is humbling to realize that many research themes that still occupy microbial oceanographers were either formally investigated or anticipated by Waksman, ZoBell, and other early researchers cited in their publications. These themes included the role of bacteria in elemental cycling in the sea; the problem of decomposition of organic matter, especially dissolved organic matter, by marine bacteria; the idea that there were fractions of bacterial cells that are live/active versus dead/inactive; the potential for allelopathic interactions between microbes; viral lysis as a source of bacterial mortality; the fact that some marine bacteria were adapted to oligotrophic conditions in the open ocean; and the role of bacteria in benthic systems.

Both Waksman (1934) and ZoBell (1946) presented conceptual diagrams which showed bacteria as having central roles in biogeochemical cycles of marine ecosystems. ZoBell’s box diagram of carbon flows in pelagic ecosystems clearly presaged modern versions of such models (Figure 1). Unfortunately, the methodologies and data needed to confirm the inferred importance of bacteria in marine ecosystems and biogeochemical cycles were not available at the time. In fact, it would take another 30 years before the central place of bacteria in marine food webs hypothesized by these early workers was given serious consideration.

1950-1974:

In the years after World War II, oceanography thrived along with other natural sciences, and research in the field of marine microbial ecology expanded in both Europe and in North America. The concept of ecosystems: how they were structured and how they functioned, was a crucial step in the progress of microbial oceanography. The brothers Eugene and Howard Odum collaborated on a book, *Fundamentals of Ecology*, first published in 1953, which became a standard text focused on the structure of ecosystems.

The backstory of one now widely cited paper, Pomeroy (1974), is an example of how interactions between scientific disciplines can result in new concepts. Eugene Odum, a professor University of Georgia in Athens, GA, established an Institute of Ecology on campus, and the University of Georgia Marine Institute on Sapelo Island, a barrier island on the Georgia coast which is still reachable only by boat. In 1954, as a young PhD, Lawrence Pomeroy was recruited as one of the first group of scientists to staff the Marine Institute. Research at the Marine Institute focused on the coastal salt marsh estuaries that surrounded Sapelo Island (Odum and Smalley 1959, Odum and de la Cruz 1963). John Teal (1962) carried out a pioneering study of energy flow through this salt marsh ecosystem. Southeastern salt marshes are composed of vast green swaths of the salt-tolerant cordgrass, *Spartina alterniflora*. The grasses grow quickly in the coastal Georgia heat, producing an amount of biomass per square meter of marsh comparable to that produced in an Iowa corn field. In the fall, the marsh grass turns yellow and dies back. The twice daily tide that floods the marsh carries off much of the grass leaves and stems to the
tidal creeks and rivers. Dead *Spartina* leaves are colonized by marine fungi and bacteria, and then decomposed by the microbes to detrital fragments on the marsh surface and in the estuarine water. In the process, microbial biomass enriches the particles with organic nitrogen and phosphorus, making the leaf fragments a more nutritious food for marsh animals. The consensus from the research carried out by Odum, Teal, and colleagues at the Marine Institute was that the food web of salt marsh estuaries was largely based on this microbially-enriched detritus. The ‘detritus food web’ concept was influential in Pomeroy’s consideration of the quantitative importance of microbial degradation in the cycling of organic matter in pelagic marine systems.

Pomeroy was joined at the Marine Institute by a post-doctoral colleague also interested in elemental cycles in aquatic ecosystems, Robert E. Johannes. Johannes (1965) carried out experiments with bacterivorous flagellates cultured from estuarine water that showed very high biomass-specific rates of phosphorus excretion, much greater than the biomass-specific rates of phosphorus excretion determined for copepods and other zooplankton. This was dramatic evidence that small organisms in the plankton had a much higher ‘rate of living’ than larger sized plankton. This finding in part led Pomeroy and Johannes to collaborate on experiments with plankton to demonstrate that whole water respiration rates routinely measured as disappearance of oxygen in dark bottles was mainly due to activity of microbes rather than to metazoans such as copepods. The colleagues compared rates of respiration in whole seawater and in seawater passed through a No. 2 plankton net with a mesh size of 366 microns (µm, 10^-6 meter). They found that the organisms which passed through the plankton net were responsible for virtually all of the oxygen decrease measured during their experiments (Pomeroy and Johannes 1968). Their results indicated that on a per-volume basis, the smaller organisms in the plankton, mainly microbes, had a 10-fold greater rate of respiration compared to larger plankton.

By the early 1970’s, Pomeroy had concluded that the prevailing concept of the structure and functioning of marine food webs was inadequate. This idea of marine food webs was formalized in John Steele’s 1974 monograph on modeling: ‘The Structure of Marine Ecosystems’. Steele’s simplified diagram of marine food webs relegated heterotrophic microbes to a ‘bacteria’ compartment in the benthos responsible for decomposing fecal material. The model had no formal role at all for bacteria or heterotrophic protists in the plankton. Steele (1974) wrote that:‘The phytoplankton of the open sea is eaten nearly as fast as it is produced, so that effectively all plant production goes through the herbivores.’ From his and Johannes’ remineralization and respiration experiments, Pomeroy knew that heterotrophic microbes played a much bigger role in planktonic food webs than Steele’s model suggested. Pomeroy was able to integrate the ecosystem theory promoted by the Odums with the concept of detritus-based food webs developed at the Marine Institute on Sapelo Island to formulate a new view of the role of microbes in the sea.

Around this time, John Bardach, the editor of the journal Bioscience, was seeking review papers with wide appeal to promote readership. He asked his friends, one of whom was Robert Johannes, for names of potential authors. Johannes suggested Pomeroy. Pomeroy obliged with a paper summarizing evidence that supported his new view that microbes were central to the functioning of marine ecosystems. According to Pomeroy, his manuscript was sent out to two external referees. One never responded, and the other said the paper was nonsense and should be rejected. However, Bardach liked the review and published it anyway. Although not widely cited at first, by the end of the 1980’s, Pomeroy’s 1974 paper in BioScience: ‘The Ocean’s Food Web: A Changing Paradigm’ was regarded as a pivotal description of new concepts about how
the bulk of elemental flows in planktonic food webs flowed through small planktonic organisms, including small sized phytoplankton, marine bacteria and their protist grazers (Figure 2).

1970’s-1980’s

There was in general great ferment in marine microbiology during the 1970’s and 1980’s, stimulated by application of newly available methodology. In the early 1980’s, a critical mass of work on marine microbes sparked two symposia on marine microbiology sponsored by NATO (Fasham 1984, Hobbie and Williams 1984) that stimulated the publication of influential review papers summarizing the new understanding of the roles of microbes in the sea (Williams 1981, Azam et al. 1983).

Improvement in methods

**Bacterial abundance** The late 1970’s and early 1980’s saw major advancements in methods to quantify the abundance of marine bacteria. Direct count assays based on epifluorescence microscopy were introduced which allowed easy visualization of bacterial cells, which are difficult to detect using regular transmitted light microscopy. The procedures involved staining cells with a fluorochrome such as blue-light excited, green-fluorescing acridine orange, filtering the cells down onto a membrane filter, and then examining the preparation using an epifluorescence microscope. In this type of microscopy, a series of optical filters selects a specific segment of light from a broad spectrum lamp to excite fluorochromes present in cells at the surface (hence epi-) of the stained preparation, and then allows only specific wavelengths of emitted (fluoresced) light to pass up to the observer’s eye at the objective lens. The marine microbiologist E.J. Ferguson Wood (1955) had previously suggested the use of acridine orange and fluorescence microscopy to visualize microbes, based on a paper by a soil microbiologist (Strugger 1948). However, Wood used a transmitted light microscope fitted with special optical filters to look at bacteria suspended in water. It was only after true epifluorescence microscopes became readily available and protocols based on settling cells onto membrane filters were suggested (Zimmerman and Meyer-Reil 1974, Hobbie et al. 1977) that this approach became widely used. Porter and Feig (1980) introduced the UV-excited, blue-fluorescing DNA-stain DAPI as an alternate fluorochrome for bacterial counts, which had the advantage of less background interference from the filter surface compared to acridine orange.

The direct count methods revealed that the abundances of bacterial cells in seawater were orders of magnitude greater than counts made from bacterial colonies on agar plates had indicated. Instead of hundreds to thousands of bacterial cells per milliliter of seawater, in fact there were hundreds of thousands to millions of bacteria per milliliter. This discrepancy was dubbed ‘the great plate count anomaly’ (Staley and Knopka 1985). Once the abundance of bacteria in seawater could be easily quantified, researchers could estimate bacterial growth rates by monitoring the rate of increase over time of bacterial numbers in seawater samples.

**Bacterial activity:** More sophisticated approaches to quantifying bacterial activity were soon developed. Hobbie et al. (1968) were among the first to demonstrate, using radiolabeled amino acids, that marine bacteria were able to rapidly assimilate the small molecular weight organic substrates present in seawater. Assaying the rate at which bacterial cells incorporated radiolabeled substrates into biomolecules was an obvious approach to measuring the rate of bacterial biomass production. Organic substrates such as sugars and amino acids, while readily assimilated by bacteria, were also respired. Fuhrman and Azam (1980) suggested using tritium-
 labeled thymidine (TdR), a nucleotide which was incorporated into DNA with little respiratory loss. Empirical conversion factors of amount of radiolabeled thymidine incorporated per number of bacterial cells produced allowed estimation of the rate of bacterial biomass production. Kirchman et al. (1985) subsequently developed a method based on incorporation of radiolabeled leucine, a common amino acid in protein, to quantify the rate of protein production by marine bacteria. Since protein is a large and relatively constant proportion of bacterial cell biomass, the rate of leucine incorporation into bacterial protein could be directly converted into rate of production of bacterial biomass, without need for empirically determined conversion factors. These approaches were quickly adopted by the community of marine microbiologists, and soon there was a growing data set of bacterial productivity in various parts of the world ocean (Ducklow and Carlson 1992, Ducklow 2000).

Reviews by Peter Williams (1981, 1984) focused on the importance of microbes in a variety of marine systems based on respiration rates in various size fractions of seawater. Williams (1981) reported results from his own work in an experiment in a British Columbia, Canada, fjord, in which a very large plastic bag was filled and suspended in the water column. Enclosure, or ‘mesocosm’ experiments such as this allowed researchers to track changes in a planktonic ecosystem over time within a single, isolated water mass. Comparison of rates of respiration in water collected from the mesocosm bag that was passed through sequentially finer filters demonstrated that organisms smaller than 30 µm accounted for almost all of the respiratory activity (Fig. 3). Williams also noted that although the biomass of bacteria was smaller than that of phytoplankton or zooplankton, when the surface areas of the various categories of planktonic organisms were compared, bacteria were overwhelmingly dominate (Table 1). This interesting idea, which Williams credited to Scripps microbiologist Farooq Azam (Williams 1984), highlights a major reason why marine bacteria are so important in material and energy fluxes in marine ecosystems: because of their large surface area, these small but abundant organisms have the greatest probability of encountering, and interacting with, chemical substances in seawater. Williams presented a simple compartment diagram in his 1981 review which showed bacteria consuming dissolved organic matter produced from phytoplankton exudates (and also from zooplankton feeding processes, which he charmingly termed ‘munchates’) and presumably regenerating inorganic nitrogen and phosphorus nutrients to further fuel phytoplankton growth.

During the same period, Holgar Jannasch and A. Aristides Yayanos developed methods of studying bacterial abundance and activity in the deep ocean, which involved use of special sampling and incubation chambers which allowed microbes to be kept at the high pressure of the depths at which water samples were collected during measurements of activity rates (Jannasch and Wirsen 1977, Jannasch 1984, Yayanos 1986).

**Marine heterotrophic protists**

Russian scientists have had a long history of study of marine microbes. One of the most influential Russian microbial ecologists, Yuri Sorokin, was broadly interested in the roles of heterotrophic microbes in the sea, including bacteria and heterotrophic protists. He was one of the first marine microbial ecologists who attempted to quantify the abundance and biomass of colorless, heterotrophic flagellates as well as ciliates in the open ocean (Sorokin 1981).

In the west, John Sieburth, a professor at the University of Rhode Island, was also interested in the whole spectrum of marine microbes, from viruses to bacteria to protists. Sieburth authored two books that served to greatly spark interest in both marine bacteria and in
the wide diversity of unicellular eukaryotes in the sea: *Microbial Seascapes* (1975), a compendium of scanning electron micrographs of marine microbes, and *Sea Microbes* (1979), an encyclopedic narrative of what was then known about marine microbes, with a strong emphasis on protists. He and colleagues (Sieburth et al. 1978) formalized terms that were used to describe various size categories of marine organisms (Figure 4). Their terms pico- (0.2-20 µm), nano- (2-20 µm), and micro- (20 – 200 µm) are now routinely used by aquatic microbial ecologists in referring to size classes of microorganisms. Davis and Sieburth (1982) advanced the study of protists in marine ecosystems by adapting the acridine orange staining and epifluorescence microscopy method used to count bacteria to enumerate non-pigmented, presumably bacterivorous, flagellates in seawater. A number of methods based on epifluorescence microscopy to determine in situ abundance, growth rates, and rates of prey ingestion of these small, colorless protists soon followed (Caron 1983, Sherr et al. 1983, McManus and Fuhrman 1986, Sherr et al. 1987).

A leading light of marine protozoology from the mid 1960’s to the present has been the Danish scientist, Tom Fenchel. A benthic ecologist, Fenchel focused his research on how organisms lived in their natural habitats (Fenchel 1967, 1977), and was particularly interested in bioenergetics of protists. A four-paper series on the ecology of marine heterotrophic flagellates (Fenchel 1982 a,b,c&d) was extraordinarily important in focusing attention on the role of marine bacterivores in the sea.

The “microbial loop”

At the NATO Advanced Research Institute Flows of Energy and Material in Marine Ecosystems in Caiscais, Portugal, in 1981, a group of marine microbial ecologists drew together emerging information about microbial abundance and activity in the sea, which resulted in the paper of Azam et al (1983). These authors suggested that the microbial components of pelagic food webs formed a separate entity they termed the ‘microbial loop’. They segregated the ‘classic food web’ of larger sized phytoplankton to zooplankton to fish and the ‘microbial loop’ which began with heterotrophic bacteria consuming dissolved organic matter (Figure 5). The size spectrum of the various components of their food web was based on the terminology of Sieburth et al. (1978). Some of the phytoplankton in their diagram were too small to be consumed by copepods, thus herbivorous protists were suggested to be an important pathway from small sized phytoplankton to multicellular zooplankton such as copepods. [We now know that phagotrophic protists are significant grazers of all size classes of phytoplankton.]

The idea of a ‘microbial loop’ in marine food webs was reinforced by another paper the same year by Hugh Ducklow (1983). Ducklow reviewed the basis for the importance of heterotrophic microbes, bacteria and protists, in marine ecosystems. He also presented a simple block diagram (Figure 6) which clearly identified the microbial side of the food web outlined in Pomeroy (1974) as a three step pathway from heterotrophic bacteria to bacterivorous protists (mainly flagellates), to larger protists (mainly ciliates) which consumed the bacterivores. Ducklow’s figure stressed that a multi-step pathway between heterotrophic microbes, and not simply degradation of organic matter by bacteria, was necessary for regeneration of inorganic nutrients for phytoplankton production. He also demonstrated that the ‘microbial loop’ was mainly a sink for organic carbon, with virtually all of the organic matter that flowed through the loop being lost to the food web as respiratory carbon dioxide. Ducklow’s diagram neatly captured all of the previous concepts and data concerning the roles of bacteria and protists in marine food webs up to that time. This model emphasized the role of the microbial loop in
regeneration of macro-nutrients, particularly nitrogen and phosphorus, which allowed further phytoplankton growth. When oceanographers considered the ‘microbial loop’ concept, they often cited Azam et al. (1983), but visualized the diagram of Ducklow (1983).

However, Ducklow’s simplified ‘microbial loop’ diagram (Figure 6) did leave out the smaller size class phytoplankton as components of the microbial food web depicted in the diagrams of Pomeroy (Figure 2) and Azam et al. (Fig. 5). During the 1980’s, there was a growing body of data on the large fraction of phytoplankton production consumed by planktonic grazers smaller than 200 µm (the microzooplankton, mainly ciliates and phagotrophic dinoflagellates), based on the dilution assay protocol of Landry and Hassett (1982). At the same time, pico- and nano- sized phytoplankton including coccoid cyanobacteria (Waterbury et al. 1979) and small eukaryotes (Murphy and Haugen 1985, Shapiro and Guillard 1986) were found to comprise a large proportion of phytoplankton biomass in the open ocean; these cells are too small to be effectively grazed by copepods. Sherr and Sherr (1988) reintroduced the small phytoplankton and herbivorous protist components of the Azam et al. (1983) paper in an expanded conceptual diagram in which the ‘microbial loop’ as depicted by Ducklow (1984) was embedded in an overall microbial food web (Fig. 7). In this conceptual model, the multicellular components of pelagic food webs were supported both by ‘classic’ large phytoplankton and by the heterotrophic protist component of the ‘microbial’ side. The model shown in Figure 7 has been updated by including the potential for viral lysis of bacteria and phytoplankton to affect carbon flows in marine food webs (Fuhrman and Suttle 1993).

Joel Goldman (1984) further elaborated the microbial loop concept by proposing that an important source of regenerated ammonium and phosphate was the ‘spinning wheel’ of microbial activity associated with suspended organic aggregates. Goldman and his student/colleague David Caron subsequently evaluated the potential for nitrogen and phosphorous regeneration by protists fed prey with differing C:N and C:P ratios (Caron and Goldman 1988), and wrote a key review of the major contribution of phagotrophic protists to nutrient regeneration in the sea (Caron and Goldman 1990).

The revised ideas about the role of heterotrophic microbes: bacteria and phagotrophic protists, and the importance of smaller sized phytoplankton in marine food webs had a great influence in the sampling and experimental design of large oceanographic field projects carried out in the world ocean during the 1980’s and 1990’s. The Subarctic Pacific Ecosystem Research (or SUPER) program (1984-1987), led by Charles B. Miller at Oregon State University, investigated the cause of uniformly low phytoplankton stocks year-round, despite high nutrient concentrations, in the subarctic Pacific Ocean. Experiments that evaluated rates of recycling of nitrogen and grazing by phagotrophic protists on phytoplankton lead to a combined iron-limitation, micrograzer/recycled ammonium hypothesis to explain the observation of constantly low phytoplankton biomass in this region (Miller et al. 1991). Subsequently, during the decade long Joint Ocean Flux Study (JGOFS) starting in 1989, multinational efforts to understand time-varying fluxes of carbon, nitrogen, and other biogenic elements in major regions of the world ocean included measurement of the biomass and activity of heterotrophic bacteria and of phagotrophic protists (Landry 2003, Ducklow et al. 2004).

The data gained from these large programs, as well as from other studies carried out in marine systems during the same period, was incorporated into mathematical models of marine ecosystems, which demonstrated that the relative magnitude of material flow through small sized phytoplankton and heterotrophic microbes is fundamental to the fates of organic matter in the sea, i.e. how much is respired/regenerated, stored, or exported. The first influential model that
incorporated microbial processes into elemental flows in pelagic ecosystems was that of Pace et al. (1984). Subsequent nitrogen- or carbon-based models in which microbes were of central importance were developed by Michael Fasham, Hugh Ducklow, Louis Legendre and their colleagues (Fasham et al. 1990, Legendre and Levefre 1995, Ducklow et al. 2004).

While this history of the development of marine microbial ecology has focused on understanding the role of heterotrophic microbes in pelagic food webs, study of microbes in benthic systems has proceeded as well. Much of this effort has involved the study of mediation of specific biogeochemical processes by microbes (Fenchel et al. 1998). However, work on chemosynthetic processes and symbiotic relationships in hydrothermal vent and methane seep environments has led to spectacular new discoveries about marine microbes in benthic systems (Jannasch and Wirsen 1979, Karl 1995, Boetius et al. 2000, Michaelis et al. 2002, Van Dover et al. 2003).

1990-present: The molecular revolution

Up until the 1990’s, heterotrophic bacteria in the sea were lumped together in what microbial ecologists acknowledged was a ‘black box’ in terms of species diversity. The ‘plate count anomaly’, i.e. the difference in abundance between the number of bacterial cells that could be grown up as colonies on agar plates and the number of bacterial cells actually enumerated in seawater by epifluorescence microscopy, suggested that a very large portion of bacterial diversity was composed of strains that had not yet been brought into culture, and was therefore unknown. The bacterial isolates that were obtained for use in laboratory experimentation were typically microbes that grew well on surfaces or at high substrate concentration.

The groundwork for the current transformation of our understanding of microbial diversity in the sea was laid by Carl Woese and his colleague George Fox with a 1977 publication in the Proceedings of the National Academy of Sciences. Woese and Fox used differences in gene sequences of evolutionarily conserved regions of the bacterial genome which coded for the 16S portion of the ribosome to show that bacteria, or prokaryotes, actually were split into two quite separate domains of life: the true bacteria, or Bacteria, and the Archaea. Their completely unexpected finding was stiffly resisted by traditional microbiologists for years (Morell 1997). However, a powerful new approach to getting inside the ‘black box’ of microbial diversity was launched. Molecular biologist Norman Pace and colleagues developed methods for cloning specific gene sequences from DNA extracted from environmental samples (Pace et al 1986, Olson et al 1986). Stephen Giovannoni, who worked in Norman Pace’s lab, led a research team in the first description of prokaryotic diversity in seawater based on 16S ribosomal DNA gene sequences (Giovannoni et al. 1990). This research team discovered novel prokaryotic gene sequences that were unrelated to those of any previously cultured marine bacteria. Among these sequences were those of an uncultured bacterial ‘phylotype’ that appeared to comprise a large fraction of all the gene sequences isolated from samples collected in the Sargasso Sea. This gene clone was labeled SAR11. The phylogenetic subgroup, or clade, of Bacteria with close affiliation to the initial SAR11 16S rRNA gene sequence has subsequently been found to be widely distributed and abundant in all regions of the world ocean (Giovannoni and Rappe 2000) (see also Chapter 3)

The molecular biology revolution has swept through the various fields of aquatic microbial ecology. Studies on diversity and distribution of phylotypes of both marine prokaryotes and eukaryotes, and on the distribution of specific functional genes, now are a large component of research in this field. Spectacular discoveries have been made, which will be
highlighted in subsequent chapters. This new wave of research is only at the beginning, though, with exciting prospects for the future, including study of how microbial genes function and how gene products (specific proteins produced) affect biogeochemical processes in the sea.

**SUMMARY**

1. Roles of heterotrophic microbes in marine plankton were mainly speculative before the 1950’s, because methods for accurately assessing bacterial abundance and activity in situ were not yet available, and only a few scientists, notably Selman Waksman and Claude ZoBell, had research programs focused on marine microbes.

2. The paper of L.R Pomeroy in BioScience in 1974 marked a renewed interest in elucidating the importance of heterotrophic microbes, including heterotrophic bacteria and phagotrophic protists, in pelagic food webs.

3. In the 1970’s, new methods of enumerating heterotrophic microbes, and quantifying rates of microbial activity, led to discoveries that bacteria and heterotrophic protists were much more abundant in the sea than had previously been recognized, and were major consumers of phytoplankton production.

4. International workshops on marine microbes in the early 1980’s compiled growing data on marine bacteria and protists, and resulted in a number of influential review papers, including the ‘microbial loop’ paper of Azam et al. (1983).

5. Beginning in the 1990’s, molecular genetic approaches to understanding phylogenetic diversity and gene function of bacteria and protists have yielded exciting new information about microbes in the sea. Molecular biology approaches continue to be a major theme in the field of marine microbiology.
References


Caron, D.A. 1983. Technique for enumeration of heterotrophic and phototrophic nanoplanckton, using epifluorescence microscopy, and comparison with other procedures. Appl. Environ. Microbiol. 46: 491-


Table 1. Comparison of biomass and living surface area of various groups of plankton in CEPEX experimental enclosure CEE-2 (from Williams 1981, Table 5)

<table>
<thead>
<tr>
<th>Planktonic group</th>
<th>Biomass µg dry wt / L (% total)</th>
<th>Surface area cm² L⁻¹ (% total)</th>
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<tbody>
<tr>
<td>Bacterioplankton</td>
<td>26 (4.6%)</td>
<td>24.6 (69%)</td>
</tr>
<tr>
<td>Protozoa</td>
<td>9.2 (1.7%)</td>
<td>0.3 (0.7%)</td>
</tr>
<tr>
<td>Phytoplankton</td>
<td>310 (56%)</td>
<td>10.7 (30%)</td>
</tr>
<tr>
<td>Zooplankton</td>
<td>206 (37%)</td>
<td>0.3 (0.9%)</td>
</tr>
<tr>
<td>Total:</td>
<td>551</td>
<td>35.9</td>
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Figure legends

1. Diagram redrawn from Fig. 10, Chapter X, ZoBell 1946, depicting the carbon cycle in the sea. In ZoBell’s diagram the solid lines represented processes in which bacteria were thought to participate, and the dashed lines processes in which bacteria did not participate. The number codes represented specific biological processes: (1) respiration, (2) nutrition, (3) decomposition, and (4) carbon dioxide fixation.

2. Diagram redrawn from Pomeroy’s 1974 Bioscience paper. Classic pelagic food web components are inside the circle, black arrows show transfer of materials and energy between these components of the food web. Microbial components added by Pomeroy to the classic food web are shown outside the circle.

3. Distribution of plankton respiratory activity with size (oxygen consumption per unit time, measured for sequentially smaller size fractions in a mesocosm (from data of Williams, 1981).

4. Distribution of different taxonomic-trophic compartments of plankton in a spectrum of size fractions, with a comparison of size range of nekton. Diagram redrawn from Figure 1, page 1259, Sieburth et al. (Limnology and Oceanography, Vol. 23, No. 6, Nov. 1978).

5. Conceptual diagram of the ‘microbial loop’ idea of Azam et al. (1983), based on the presentation of the relation of the microbial and classical components of pelagic food webs in Figure 8.1 in Fenchel (1987). DOC = dissolved organic carbon.

6. Simplified box model diagram of the microbial loop concept redrawn from Figure 1 of Ducklow (1986).

7. Trophic interactions within the microbial food web, which is separated here into phytoplankton and ‘microbial loop’ (i.e. bacteria and heterotrophic protist compartments). In this model, production of < 5 µm sized algae is accessible to metazoan zooplankton only after being ‘repackaged’ into larger protist cells. Diagram redrawn from Figure 2, page 1226, Sherr and Sherr (Limnology and Oceanography, Vol. 33, No. 5, Sep., 1988).
Figure 1

Diagram showing the carbon dioxide cycle involving plants, animals, bacteria, dissolved colloidal & particulate organic matter.
Figure 2

- **FECES AND EXCRETA**
- **NET ZOOPLANKTON**
- **NET PHYTOPLANKTON**
- **FISHES**
- **TOP CARNIVORES**
- **MUCUS NET MAKERS**
- **NANNOPLANKTON**
- **PARTICULATE ORGANIC MATTER**
- **DISSOLVED ORGANIC MATTER**
- **BACTERIA**
- **PROTOZOA**
- **Dissolved Organic Matter**
- **Net Phytoplankton**
- **Net Zooplankton**
- **Feces and Excreta**
- **Top Carnivores**
- **Mucus Net Makers**
- **Nannoplankton**
- **Particulate Organic Matter**
- **Bacteria**
- **Protozoa**
Figure 3

Cumulative respiration rate as % of whole water rate

Nominal size limit

< 1 μm < 3 μm < 10 μm < 30 μm < 100 μm < 300 μm

100 %
Figure 4

<table>
<thead>
<tr>
<th>PLANKTON</th>
<th>FEMTO-0.02 – 0.2 µm</th>
<th>PICO-0.2 – 2 µm</th>
<th>NANO-2 – 20 µm</th>
<th>MICRO-20 – 200 µm</th>
<th>MESO-0.2 – 20 mm</th>
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<th>MEGA-20 – 200 cm</th>
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<td>Virio-plankton</td>
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<td>Bacterio-plankton</td>
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<td>Phyto-plankton</td>
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<td>Protozooplankton</td>
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<td>Metazoo-plankton</td>
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<td>Nekton</td>
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Primary producers
Microbial consumers
Multicellular Consumers

Centimeter nekton
Decimeter nekton
Meter nekton

Primary producers:
- Phyto-plankton
- Protozooplankton
- Metazoo-plankton

Microbial consumers:
- Virio-plankton

Multicellular Consumers:
- Nekton
Figure 5

Microbial side  pico-  nano-  micro-  size, µm  Classical side

0.2  2  20  200  > 200

DOC

Heterotrophs feeding on dissolved substrates

Heterotrophic picoplankton

Autotrophic microplankton

Autotrophic nanoplankton

Autotrophic picoplankton

Phagotrophic nano-flagellates

Microzooplankton, ciliates and heterotrophic dinoflagellates

Mesozooplankton, copepods, other metazoan grazers

Nutrient recycling

Fish
Figure 6

CLASSIC FOOD CHAIN

Phytoplankton → Herbivorous zooplankton → Fish, etc

Dissolved organic matter
Heterotrophic bacteria
Bacterivorous flagellates
Ciliates

Regeneration of N & P

Respiratory loss of organic carbon from the ‘classic food chain’

CO₂
Figure 7

CARBON FIXATION PATHWAY

< 5 µm phytoplankton

Heterotrophic bacterioplankton

> 5 µm phytoplankton

Bacterivorous protists

DOM & POM

Herbivorous protists

mixotrophy

viral lysis

FIXED CARBON REPACKAGING AND RECOVERY PATHWAYS

ZOOPLANKTON