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Developments in aquatic microbiology

Summary Major discoveries in marine microbiology over the past 4–5 decades have resulted in the recognition of bacteria as a major biomass component of marine food webs. Such discoveries include chemosynthetic activities in deep-ocean ecosystems, survival processes in oligotrophic waters, and the role of microorganisms in food webs coupled with symbiotic relationships and energy flow. Many discoveries can be attributed to innovative methodologies, including radioisotopes, immunofluorescent-epifluorescent analysis, and flow cytometry. The latter has shown the key role of marine viruses in marine system energetics. Studies of the components of the “microbial loop” have shown the significance of various phagotrophic processes involved in grazing by microinvertebrates. Microbial activities and dissolved organic carbon are closely coupled with the dynamics of fluctuating water masses. New biotechnological approaches and the use of molecular biology techniques still provide new and relevant information on the role of microorganisms in oceanic and estuarine environments. International interdisciplinary studies have explored ecological aspects of marine microorganisms and their significance in biocomplexity. Studies on the origins of both life and ecosystems now focus on microbiological processes in the marine environment. This paper describes earlier and recent discoveries in marine (aquatic) microbiology and the trends for future work, emphasizing improvements in methodology as major catalysts for the progress of this broadly-based field.

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The early years

Marine microbiology is a relatively young field of biology that began in the late 19th century. Table 1 shows major issues dealt with since those early years [57] (Table 1). The era 1855–1890, and oceanic explorations such as the Challenger expedition, laid the foundation for subsequent microbiological studies of the ocean. Initial work studied the survival of water-

Table 1 Sequential development of marine microbiology

- Studies of survival of pathogenic bacteria in the sea
- Are there true marine bacteria?
- Isolation, distribution, abundance
- Identification of major microbial taxa
- Studies of basic cyclic processes
- Enumeration approaches/methodology
- Bacteria as decomposers
- The microbial loop
- Activity/Role of bacteria in diverse marine processes
- Discovery of deep-sea microbiology and thermal-vent communities
- The total ecosystem (microorganisms in toto)
- Molecular methodology

borne pathogens in seawater, and other subjects such as the nitrogen cycle and luminescent bacteria. The latter resulted in numerous reports on the physiology of bioluminescent bacteria, which led to the study of the mechanisms of light production by these organisms, their physiology and nutrition, and their symbiotic association with various marine fishes and invertebrate species [12].

The Galathea expedition [58] was among initial efforts to critically explore microbial aspects of the deep sea and the nature of marine psychrophilic bacteria. Reports on the effects of hydrostatic pressure on marine microorganisms pioneered research in microbial aspects of the deep sea [27].

The importance of obligate psychrophiles was demonstrated, introducing such significant concepts as “starvation–survival” and “feast or famine”, which have served as descriptors of microbial activities under oligotrophic conditions [37]. There have been studies on the effect of pressure and on the mechanisms by which pressure can regulate genetic expression. Other works dealt with nutrient conditions in the deep sea and with the versatility of microbial responses to pressure [36].

As late as the 1960s, researchers focused their attention on descriptive studies of marine microorganisms and on their distribution, using methodology inherent to that era [34]. Currently, marine microbiology is seen as an integral part of global marine science, with vast biological implications unrecognized in earlier years. The decade 1975–1986 saw major review articles on subjects such as biofilms, the role of bacteria in marine food webs, bacterial ecology of the deep sea, psychrophilic bacteria, the concept of starvation survival, and bacterial biomass and marine productivity [19]. In this era, major developments occurred in analyses of estuarine and salt marsh ecosystems, especially anaerobic mineralization of organic matter via sulfate, nitrate and iron reduction processes as well as studies of methanogenesis. Advances in both instrumentation and methodology allowed more accurate detailed sampling of the entire water column. New technology, such as the use of rRNA sequencing [41] to identify marine bacteria, provided significant information on the phylogeny of marine taxa, especially those of the ecologically important Vibrionaceae group. Development of immunofluorescent-epifluorescent techniques has introduced sensitive detection systems, especially to elucidate human pathogens in coastal environments [30].

Research has shifted from studies of microbial distribution and population density to considerations of biogeochemical roles of marine bacteria, including bacterial biomass, energy flow and mineral cycling. Besides, a broad range of techniques employing radioactive precursors have facilitated the determination of microbial activity and biomass production. Significant information obtained on marine bacteria in various planktonic communities has contributed to establish major ecological paradigms such as the “microbial loop” [43].

With the discovery of deep-sea thermal vents, microbial symbiosis has received increasing attention [28]. Systems such as nitrogen-fixing bacteria in boring mollusks, photosynthetic organisms in corals, and sulfur-oxidizing bacteria in hydrothermal-vent organisms, have shown the widespread occurrence of marine symbiosis. Pioneering efforts have contributed to our understanding of deep-sea microbiology, especially the discovery of chemoautotrophic bacterial populations at deep-sea vents in symbiotic associations with the giant hydrothermal-vent tube worm, *Riftia pachyptila* [28]. This, and other significant deep-sea work, has revealed the presence of obligate psychrophiles able to actively metabolize under extreme pressures. It has been possible also to understand the mechanisms by which marine bacteria survive in an oligotrophic ocean; those mechanisms include using high-affinity substrate capture and formation of ultrabacteria [37]. The importance of adsorptive surfaces in microbially-mediated processes has been demonstrated. There is now an appreciation of the role of aquatic microorganisms in biogeochemical cycles along with the recognition that patchiness and physical and chemical gradients are of great

importance in marine microbial ecology. Micro-scale nutrient patches have been reported in mixtures of bacterial isolates consisting of a protozoan, its prey, and chemotrophic bacteria [7].

Many ecologically significant microbial symbioses, commensalisms, and consortia have been described. Significant diversification in marine microbiology has occurred; the field has moved far beyond its roots in classical bacteriology, and now it comprises the study of many groups of autotrophic and heterotrophic microorganisms, including protozoa and microalgae. The discovery of cyanobacteria and their role in primary production has changed our appreciation of the oceans and food web processes. Cyanobacteria such as *Trichodesmium* are nitrogen fixers and play a major role in the marine nitrogen cycle, especially in nitrogen-limited oligotrophic waters [11]. Contemporary researchers recognize this tremendous diversity of forms and functions, and ecological studies now focus on “systems” approaches [18].

Bacteria in aquatic systems are no longer seen as decomposers and nutrient regenerators; we now recognize their role in the uptake of growth-limiting nutrients, with high conversion efficiencies [3]. This has resulted in re-evaluation of bacteria as biological mechanisms for recycling energy and material lost to the detrital food chain back into the classical food chain, now referred to as a “food web” [44]. The term “strategy” has been used to document microbial activity in oligotrophic waters and stressed environments [37]. The study of a broad range of grazing phagotrophic protozoa has incorporated these concepts into models of energy and nutrient flow in aquatic communities [48]. Size-selective grazing by heterotrophic nanoflagellates is a critical aspect of the microbial loop. Factors such as prey selection, mixotrophy and viral infection of bacteria all play a role in this process involving carbon flow and recycling (regeneration) of essential mineral nutrients. Food chains are now recognized as complex food webs, in which the “microbial loop” plays a major role. A variety of microbial groups, nutritional diversity and different energy sources in a community structure characterize microbial loops, as well as linkages or couplings between organisms and feeding types [29]. Bacteria can use diluted concentrations of energy (dissolved organic matter) and reconvert this into biomass for upper trophic levels, where it serves as food for grazing invertebrates known as bacteriovores.

Food web dynamics

Many diverse biotic and abiotic factors affect the microbial loop. Since 1974 [43], a vast amount of relevant information has been obtained on processes and organisms involved in the microbial loop. Particular attention has been given to molecular approaches to study loop processes, and dissolved organic carbon fluxes and coupling processes, as well as the formation

of high-weight molecular material [25] (Table 2). Considerable research has focused on biotic/abiotic “control” factors with emphasis on phagotrophic (grazers), and more recently on marine viruses. Presence and availability of diverse energy sources has been the focus of numerous investigations [44].

Table 2 Advances in knowledge of the microbial food web

- Establishment of appropriate methodology to measure microbial productivity
- Adaptation of molecular biological methods (ribosomal RNA gene probes, location of specific enzymes)
- Analyses of microbial dynamics within and among different ecosystems
- Studies of predation activities in material/energy transfer
- Recognition of viruses as numerically important components of aquatic ecosystems
- Modeling of microbial/energy fluxes (movement of materials through the food web based on rate/quantity information)

The concept of the microbial loop has developed, in which the role of nanoplanktonic algae (cyanobacteria) in oceanic productivity and that of microflagellates as grazer populations are recognized [55]. The microbial loop is now viewed as an essential link for both oceanic secondary productivity and regeneration of essential rate-limiting nutrients (Fig. 1). Picoplanktonic microorganisms (diameter < 2 μm), primarily bacteria, cyanobacteria, prochlorophytes and some protozoa, are important in energy flow and nutrient cycling in oligotrophic oceanic ecosystems [47]; they contribute significantly to phytoplankton biomass and production. It has been shown that phagotrophy also contributes to dinoflagellate growth by both enhancing photosynthetic performance and by supplying organic

sources of C, N, and P for growth and metabolism [48]. The methodology for measuring microbial productivity has advanced significantly, especially with the use of molecular approaches. This has shown the major role of flagellates in grazing activities, the significance of marine viruses as major components of aquatic ecosystems and has allowed the modeling of microbial and nutrient fluxes through both macro- and micro-food webs [6]. Although microbial cells are small, their high surface-to-volume ratio is significant and their biomass is noteworthy in the sea [2].

The role of biotic and abiotic “decoupling” factors in microbial food webs is evident in flagellate grazing (phagotrophs), substrate (energy) availability and the presence of viruses. Grazing rates can significantly affect the dynamics of microbial associations. Variations in bacterial production and protozoan grazing rates can result in uncoupling, i.e., disruption of normal predator/prey oscillation rates. Selective as well as non-selective grazing, in terms of the particular prey, is prevalent in various trophic systems. The coupling/uncoupling of primary production from growth, and the fate of excess photosynthate, will continue to be a major area of investigation. The intensity of coupling of bacteria to variations in primary production and the variability of dissolved organic carbon, including the occurrence of protein-containing particles, has been addressed by various researchers [33]. The significance of viruses, nutrient limitations and carbon fluxes are critical factors in such processes. Bacteria are now seen as transformers of organic nutrients and dissolved organic matter (DOM) into living biomass and particulate carbon. Furthermore, bacteria play a significant role in structuring the molecular weight

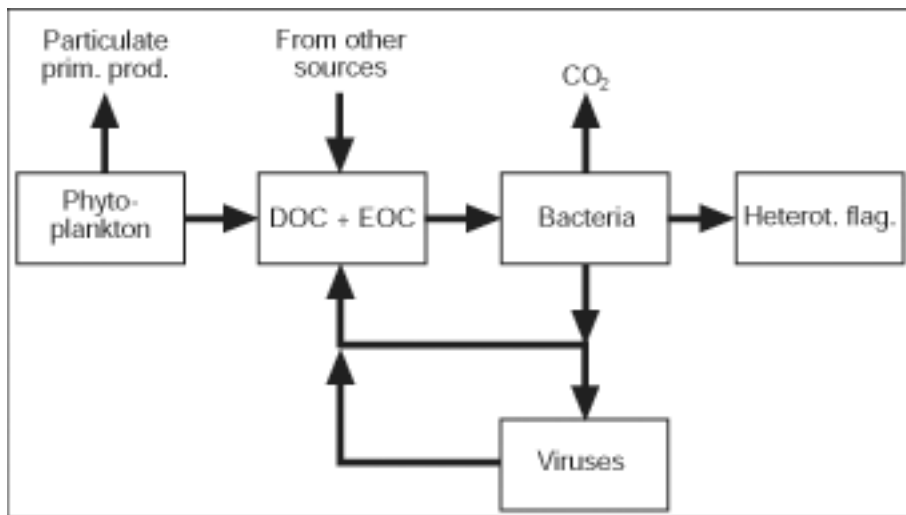


Fig. 1 Diagram of flows of organic material assumed to be associated with heterotrophic bacteria. The pool of dissolved organic carbon (DOC) and extracellular organic carbon (EOC) is fed by excretion from phytoplankton (and other sources not indicated here); bacteria growing on this material are removed, either by predation from heterotrophic flagellates, or by viral lysis. Viral lysis releases the organic material of the bacterial cells, in part as new viral particles. Assuming that only a minor fraction of the viruses are successful in new infections, or adsorbing to larger particles, the virus production is also returned to the pool of DOC (From *Mar Ecol Prog Ser* 83:273–280, 1982)

distribution of organic matter in the sea. The DOM pool is a major energy source and play a major role in nutrient regeneration (Fig. 2).

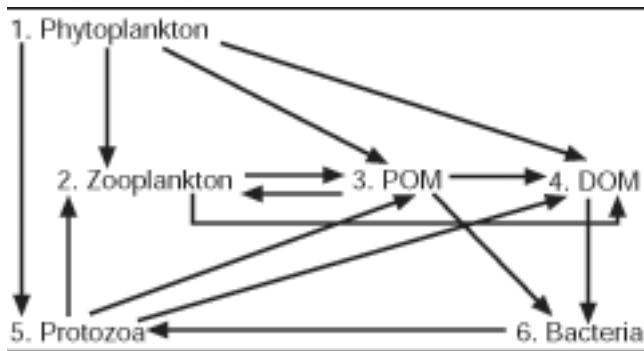


Fig. 2 Conceptual model of carbon flux through the ocean's food web and some of the major pathways of carbon from phytoplankton to bacteria. POM: Particulate organic matter. DOM: Dissolved organic matter.

1-2: Grazing on phytoplankton by zooplankton, with release of feces, murchates (2-3) and soluble products (2-4)

1-3: Death of phytoplankton from virus infection or nutrient depletion, followed by solubilization of bacteria (3-6)

1-4: Release of DOM by phytoplankton

1-5: Grazing on picophytoplankton by protozoans, with release of feces (5-3) and soluble products (5-4)

Transparent exopolymer particles (TEP) found in marine food webs, especially during algal blooms, may represent a significant pathway for dissolved organic carbon in the sea [42]. Marine colloids are the most abundant particles in the ocean, accounting for 30–50% of the dissolved organic carbon (DOC) in seawater. Such colloids, at the boundary between chemical species and sinking particles, may support the bulk of heterotrophic microbial production in seawater. Also involved is “marine snow” (macroaggregates) and non-specific surface-related interactions in colloid cycling [13]. Marine snow, known as particulate organic carbon (POC), serves as a vehicle for vertical flux of organic matter and enriched “hotspots” of microbial respiration and sites of rapid turnover of particulate carbon in the sea [1, 31]. The stability of a broad range of biologically-active compounds within the DOM component of seawater may depend largely on their interfacial characteristics [49].

Early studies on the physiology and biochemistry of marine bacteria have provided a better understanding of such organisms compared to those of terrestrial origin [34]. Investigations have elucidated specific requirements and the role of various ions, i.e., Na^+ and Mg^{2+} , in marine bacterial metabolism and specific cellular processes. Marine bacteria have been shown to require Na^+ for their growth, as well as to have a specific requirement for Na^+ for membrane transport activities. Magnesium interacts with the cell walls of marine bacteria and is required to maintain them intact. There has been a growing effort to determine mechanisms, processes, and control of microbial productivity in the ocean, including the effect of diel variations [23].

Increased emphasis has been on basin-wide and global scale approaches, leading to automation in sampling and analytical capabilities. Besides, there has been a trend toward interdisciplinary research utilizing the combined knowledge of scientists over a broad range of oceanic subjects. Mixotrophy, combination of phagotrophic and phototrophic nutrition in a single species, is of noteworthy significance in microbial food webs [46]. Work in the Mediterranean has shown that abundant mixotrophic algae also function as potential bacterivores [23]. Feeding, pigmentation and growth in a species of the mixotrophic dinoflagellate *Gyrodinium* is reported in which both photosynthesis and phagotrophy support higher growth rates associated with mixotrophy than with strictly autotrophic growth alone.

Sediment processes

The discovery of the metabolic diversity of sulfate and sulfur reducers in marine sediment has been a major contribution to marine microbiology. The benthic sediment-water interface, the benthic boundary layer (BBL), is the focus of considerable contemporary research. Modification of organic matter close to the seafloor may be as rapid as that determined in the euphotic zone. The BBL could be considered a distinct environment with small-scale variations in the vertical distribution of particle composition along with enhanced microbial activity, affecting energy budgets for benthic communities. A 1997 review on sediment bacteria [38] stressed the need for further studies of the mechanisms whereby sedimentary microbes interact with each other and with their environment, forming critical linkages. New microscopic techniques such as confocal laser scanning microscope (CLSM) and environmental scanning electron microscope (ESEM), along with fluorescent probes, will have a major impact to understand the activities of sediment bacteria. Major areas for further study are the structural and functional processes within sediments and their loss due to a variety of factors.

Methodology Innovative methodological approaches have revealed new ecologically-significant information. This can be seen in studies of grazing-loss rates of bacteria [52], wherein fluorescently-labeled tracers are combined with flow cytometry to estimate in situ loss rates of natural planktonic bacteria to grazers. Flow cytometry has significant application in analysis of bacterial distribution, especially in analysis of grazing rates of nanoflagellates. It also allows the recognition of more than a single virus population in natural samples and can be applied to analyze the heterotrophic bacterial community [10]. Increased use of the cytometric method in analyses of flagellate grazing rates can be expected, especially to discern the complex distribution of such rates under a variety of natural conditions, and the linkage of the key nanoflagellate genera to specific grazing rates. Molecular approaches are being used to study the

microbiology of sediments, and to characterize total and specific microbial biomass and the microenvironment itself [16]. Traditional colony-counting techniques have been supplemented, and in many instances they have been replaced by cloning of bacterial 16 rRNA genes from the environment. Such approaches have demonstrated the biogeographical diversity among marine bacterioplankton and their varied physiological states.

There has been notable development of quantitative aquatic microbiology, including a variety of methods to estimate microbial biomass, metabolic activity, growth rate and production [50]. The use of radioisotopes has provided the sensitivity necessary to assess microbial rate processes in natural systems. The trend has been toward in situ experimentation for obtaining "real world" information [18]. Methodology has uncovered significant members of the phytoplankton, including the nanoplankton, the picoplankton (the cyanobacteria) and the prochlorophytes. The discovery and recognition of the magnitude of the cyanobacteria alone has completely changed the concept of marine productivity. The growth rates and biomass of phototrophic nanoplankton exceed those of large photosynthetic net plankton. Furthermore, the importance of microzooplankton (the grazers or phagotrophs) in the transfer of biomass to higher trophic levels has been recognized [51]. Use of submersibles to collect samples and for deep-sea in situ experiments has provided new information. Similarly, the use of microelectrodes to study microenvironments, both estuarine and deep sea, comprise major advances at the critical microscale site level in different ecosystems [17]. Molecular approaches, begun several decades ago, are now being applied as marine biotechnology becomes the "wave of the future" [30]. In this regard, rRNA sequencing research has allowed to establish a phylogeny of the marine bacteria [41]. Such biotechnological work will allow the exploitation of unique ecosystems, especially the recovery of biologically-active compounds.

Biotechnology Biotechnological approaches, including the use of immunochemical methodology, will be increasingly applied to all phases of research in marine biology in general [50]. Indeed, molecular biology is changing our understanding of ocean microbiology in terms of what is there and what it does. Flow-cytometry methods [10], combined with the nucleic acid specific dye SYBR Green-1, have proven to be very effective to enumerate marine viruses. This and other methodologies will clarify the structure and dynamics of virus populations in natural water bodies. The application of molecular biology to oceanic studies has allowed both a more definitive analysis of the structure of the microbial community and the determination of the metabolic status of different components of microbial populations [16]. Equally important has been the use of innovative methodology, including techniques in microbial genetics and computer-driven analytical instrumentation. Marine biotechnology has given increasing attention to the discovery of novel microbial biocatalysts along with the improvement of methods to isolate microorganisms from the deep sea. The 1998 International Conference on Toxic

Cyanobacteria focused attention on the production of secondary metabolites by this group of phytoplankton. Related research concerns toxic algae as well as production of toxic compounds by bacteria comparable to those found in algal species.

Research is now documenting the significance of ectoenzymes [15] in the aquatic environment, which will reveal worthwhile information on the kinetics of material turnover, especially that of dissolved organic matter. Bacteria inhabiting marine snow have cell-specific ectoenzymatic activities up to three orders of magnitude higher than bacteria in the surrounding water [31, 35]. The variability of ectoenzymatic profiles among different bacterial isolates indicates the importance of species composition of the bacterial communities as well as grazing activities [45]. Researchers have shown the release of dissolved amino acids in the form of primary amines by flagellates and ciliates grazing on bacteria. A Japanese team has reported that specific proteins survive in seawater as dissolved protein, the origin of these proteins being bacterial porin [50]. Studies in biological oceanography have revealed the presence of ecologically important peptides and their function as chemical signal molecules in aquatic ecosystems. These various findings provide new and significant information on carbon and nitrogen cycling within overall aquatic food web processes (Fig. 3).

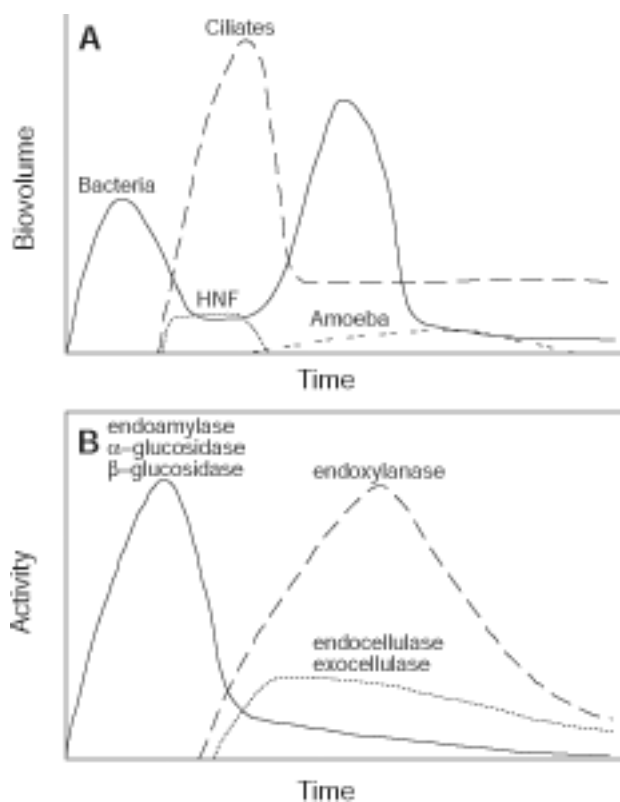


Fig. 3 Scheme of the succession of microbial populations (A) and ectoenzymatic activities (B) involved in the degradation of polysaccharides. After an initial peak of bacteria, heterotrophic nanoflagellates (HNF) appeared and, through grazing controlled bacterial numbers. Ectoenzymatic activities reflect shifts in the microbial populations (from *Aquat Microb Ecol* 20:75–82, 1999)

Ecological trends Such trends include recognition of microhabitat events, increased focus on composite group (consortia) of microorganisms, and a variety of new techniques for analyses of biomass, growth rates, and metabolic activities on a surface/volume basis. The microbial component is now viewed as a critical biomass portion of the total marine food web. There is growing evidence that microbial heterotrophs may play a major role in ocean iron cycling, affecting critical nutrient-limited availability. Laboratory and pure culture studies have been replaced in part with analyses of actual rates of activity in the field under ambient "real world" conditions. A new field of research has involved the chemical and microbial ecology of biologically-active compounds and specific ecosystems such as sponges and corals. A diversity of chemical compounds are found in marine sponges with a variety of postulated ecosystem functions. How such compounds enter into microbial ecology warrants investigation in terms of chemical ecology [39]. Increased attention is being given to micro-scale nutrient patches, consisting of protozoa, their preys, and chemotrophic-heterotrophic bacteria. Events leading to such patches are associated with bacterial cell lysis and predation events, and can have significant implications for the flow of energy and nutrients in the oceans. Focus in microbial ecology is needed to study bacterial foraging strategies and constraints imposed by the physical microenvironment.

Microbial aspects of food web dynamics include reports on marine bacteria that kill noxious red-tide flagellates. Surface-active polysaccharides have been shown to participate in the formation of transparent exopolymer particles (TEP) by bubble adsorption of seawater [42]. This may be a major pathway for the formation of such particles in surface waters, especially during algal blooms. TEP and their precursors appear to be a chemically distinct group of polysaccharides whose production and standing crop are uncoupled from bulk carbohydrates [56]. Recent studies [42] have noted the formation of TEP and their origin from dissolved precursor material. It is stressed that mechanisms and rates of transformation between the different carbon pools (dissolved organic carbon [DOC], particulate organic carbon [POC] and colloidal organic carbon [COC]) must be studied to understand oceanic processes. Lastly, it is postulated that close correlations may exist between outbreaks of cholera and sea-surface warming brought about by such critical climatic factors as El Niño.

Marine viruses The presence of large concentrations of viruses in marine ecosystems has been established [5]. Flow-cytometry has facilitated virus detection and discrimination between a wide range of different viruses [9]. Numerous studies indicate that indigenous marine bacteriophages are dynamic components of microbial food webs and are now included in models of carbon transfer within the microbial loop [8] (see Fig. 2). Virus-like particles are extremely abundant in oligotrophic waters with viral infection being

higher in nutrient-rich environments [24]. Factors involve viral lysis of bacterial cells with consequences for nutrient and energy cycling, control of species diversity and exchange of genetic material among bacteria in marine environments. Potential impact on dissemination of the cholera toxin has been noted [53]. Research suggests that the effects of phages on mortality, nutrient flow and species diversity in microbial food webs depend largely on the lytic pathway of viral infection [40]. There is a need to study more environments, such as the open ocean and deep-sea, and more phage-host systems to find general trends and to determine the variability of viral activity. Mortality of microorganisms due to viral infection has significant implications in both nutrient and energy cycling. Reports of virus concentrations in aquatic environments of 2.5×10^8 virus particles per milliliter suggest that viral infection could be significant in the ecological control of planktonic microorganisms [40]. It is estimated that as much as 10–20% of marine bacteria is lysed daily with 2–3% of primary production lost through viral activity [54]. Further implications are seen in the exchange of genetic materials among bacteria in marine ecosystems following phage attack.

Researchers recently have reported on viruses in the bacterioplankton of oligotrophic waters [40]. Although bacterial mortality due to viral infection seemed low, finer detection techniques should be developed. Viral impact will vary depending on the nutrient characteristics of the particular water mass. In this regard, further studies are needed in the vast oligotrophic environment of the open-sea. Elsewhere, there are reports on marine viruses in microbial food webs and the rapid turnover of viruses and lysis products [14]. Such viral lysis products are likely to be major sources of organic N and P, especially in oligotrophic systems. Further microbial/virus studies are needed to assess the importance of virus-mediated processes to biogeochemical cycling in the main microbial food web.

Deep-sea microbiology The discovery of deep-sea hydrothermal vents in 1977 ranks among the major events in the history of oceanography and marine microbiology [28]. In unique life systems, H_2S is used as a prime energy source for chemoautotrophic bacterial production. The discovery of symbiotic or mutualistic associations between such bacteria and giant tube worms (*Riftia*) have led to a better understanding of microbial diversity and the unique physiological breadth of organisms referred to as "extremophiles." Complex interactions between marine bacteria and closely-associated animal populations and specific ecosystems have been shown in other parts of the ocean ecosystem. Such associations are widespread and can be found in shallow-water settlements. Deep-sea microbiological studies will accelerate as increasing focus is placed on the isolation and study of extremophiles and their production of biologically-active compounds.

Symbiosis Microbial diversity in the marine environment is seen in a variety of symbiotic relationships [12]. Endosymbiotic bacteria have also been found associated with marine nematodes [26] (Fig. 4). Work beginning in the 1970s has provided a wealth of information on bacterial bioluminescence and the relationship of luminous bacteria and luciferase with various marine mammals. This is evident today in our understanding of the function of diverse light-inducing mechanisms with the so-called flashlight fish. Members of at least two families of squids and several families of marine fishes harbor monospecific cultures of luminous bacteria in specialized light organs. The presence of a phylogenetically diverse assemblage of endosymbiotic bacteria is being reported from other marine invertebrates [26]. Recent pioneering work has provided additional significant information on the role of *Vibrio* symbionts with various species of squid [21], reporting two different species of luminous *Vibrio* bacteria co-occurring within a single light organ.



Fig. 4 Transmission electron micrograph of bacterial symbionts in the gutless marine oligochaete *Olarius loisae* showing a symbiont-containing region just below the cuticle of the worm. Note three distinct bacterial morphotypes: large bacteria (**la**) with cytoplasmic globules and small bacteria (arrows) interspersed among the large bacteria which both occur in all six specimens examined, whereas intermediate-sized bacteria (**i**) (also referred to as the third morphotype) occurred in two out of six worms. Bar = 1 μ m. (From *Mar Ecol Prog Ser* 178:271–280, 1999)

This symbiotic relationship of species of *Vibrio* with several genera of squid has introduced exciting observations on luminous bacteria associated with light organs of squid, as well as with species of flashlight fishes. Earlier work studied the species *V. fisheri* as a light organ symbiont of Pacific squids, whereas recent reports focus on *V. logei*, the predominant light-organ symbiont of Mediterranean squids (*Sepioloa* sp.). The effect of ambient water temperature on the nature of the

predominant symbiont in the light organ at any particular time illustrates the importance of abiotic factors in such associations. The presence of intracellular bacteria (endosymbionts) in *Noctiluca scintillans* [32], a cause of red tides in European waters, is of special interest in view of increasing speculations on the role of bacteria in toxic algal blooms, particularly those of dinoflagellates. Changes in the composition of free-living microbial communities associated with algal blooms have been reported, and these changes have been suggested to have toxicological implications [22]. As an example, the ecological significance of such relationships and phylogenetic diversity in a gutless marine oligochaete, *Olavius loisae*, has been reported. Recent work on bacterial endosymbionts suggests that this could be a phylogenetically diverse assemblage of bacteria unknown from other marine invertebrates.

Coral reef studies Coral-related research has shown the importance of bacteria in carbon and nutrient transfer in such critical environments [20]. We can expect to see methodology focusing on ectoenzymatic activities related to the microbial turnover of both dissolved and particulate organic matter. Micro-sensors have allowed to study photosynthesis and calcium uptake simultaneously [17]. With accelerated global warming, there are more and more reports of diseases and stress-induced mortalities of corals. In coral communities, worldwide occurrence of bleaching and disruption of the microalgal endosymbionts (zooxanthellae) and reports of related bacterial activities warrants serious attention. Recent studies carried out in Israel [4] have shown the role of *Vibrio shiloi* as a pathogen in both the inhibition of photosynthesis and the bleaching of the coral zooxanthella *Oculina patagonica* in the Mediterranean Sea.

Future developments

Both microbial diversity and function of the ecosystem will be significantly affected by critical phenomena such as ocean warming and El Niño oscillation. Future research will focus on micro-scale nutrient patches [7] and invertebrate grazing activities. The study of such patches may identify additional significant pathways to recycle growth-related nutrients in the microbial food web. Studies of bacterial biomass in the ocean have already revealed the release of copious amounts of capsular material as semi-labile to refractory dissolved organic carbon. The viroplankton is now recognized as a major component of the traditional microbial loop affecting both autotrophs and heterotrophs. Perhaps of even greater significance in aquatic ecosystems is gene transfer through phages and lysogenic conversion, which involves potentially biological hazards such as the dissemination of the cholera toxin.

The increasing use of molecular techniques will allow a better insight into microbial processes that regulate aquatic systems, including the role of viruses. The ability to predict

the detailed physiological traits of an organism from its rRNA sequences is of great importance in microbial-ecology studies. Comparison of genetic sequences of prokaryotes, using rRNA as a phylogenetic marker molecule, has changed concepts of bacterial evolution. Genotypic analyses provide invaluable information on the extremophile group, especially on archaeobacteria from deep-sea thermal vents [27]. As sequences from a greater variety of organisms with diverse physiologies are obtained, this will provide a more complete picture of the biochemical diversity of marine microorganisms. Recent investigations show that small amounts of coral exudates, rich in dissolved and particulate organic matter, greatly stimulate microbial growth, with bacterial biomass 6-fold high in coral [20, 39].

Advancements in technology and instrumentation in microbial ecology, including an increasing level of automation, data processing capabilities and computerization, can be anticipated. Other suggested developments include remote sensing approaches (both satellite and unmanned submersibles) and continuous monitoring of microbial biomass, metabolic activity, and growth. Emerging areas in marine microbiology include the increasing identification of assemblages, or consortia, of a variety of microorganisms ranging from autotrophs to diverse grazers. Research will continue to stress the importance of recycling processes and phytoplankton fluxes in the food web structure. Equally noteworthy is the prevalence of mixotrophy involving heterotrophic nanoflagellates grazing on bacteria as well as on picoplankton.

Future research will focus on the quality of carbon fluxes under diverse oceanographic regimes, including diel changes in bacterial activity correlated with photosynthetically-dissolved organic carbon. Developments in oceanography including monitored measurement systems and mechanisms for retrieval of synoptic data will continue to have significant application in marine microbiology. For example, researchers at the Marine Science and Technology Center (JAMSTEC) in Japan have been using automated monitoring systems in deep-sea studies to provide valid data of biological events at in situ habitats where pressure and temperature play a critical role. The use of nanotechnology, i.e., miniaturization of methods for a broad range of applications, will play a major role in the development of methods to discern events at the molecular level.

Dynamic interactions of biology and physical factors of diverse ecological systems are seen in the key role of biocomplexity in both pelagic and benthic ecosystems. For example, changes in microbial loop components during algal blooms [32] indicate the need to critically examine such trophic interactions in highly-productive marine environments. Monitoring of grazing loss rates of bacteria, using fluorescently-labeled compounds and flow cytometry will continue to reveal significant information on ratios of natural planktonic bacteria to grazers. There is a need for more field observations combined with experiments designed carefully to test key hypothesis. Future discoveries in marine microbiology may well be based

on instrumentation yet to be built.

The Millennium issue (Vol. 38 No. 1) of the *Aquatic Microbiology Newsletter* notes the following quotation from O. Kinne, showing the significance of contemporary research in marine microbiology: "It has taken marine researchers a long time to fully recognize the greatness of smallness—to appreciate that the ecological dynamics of life processes are first of all linked to and directed by the activities of microorganisms. It is in the microbial realm that the scene for the unfolding of more complex expressions of life is set, where the basic driving forces of ecology are at home."

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References

- Allredge AL, Silver M (1988) Characteristics, dynamics, and significance of marine snow. *Prog Oceanogr* 20:41–82
- Azam F, Hodson RE (1977) Size distribution and activity of marine microheterotrophs. *Limnol Oceanogr* 22:492–501
- Azam F, Fenchel T, Field JK, Gray JS, Meyer-Reil LA, Thingstad F (1983) The biological role of water-column microbes in the sea. *Mar Ecol Prog Ser* 10:257–263
- Ben-Haim Y, Banim E, Kushmaro A, Loya Y, Rosenberg E (1999) Inhibition of photosynthesis and bleaching of zooxanthellae by the coral pathogen *Vibrio shiloi*. *Environ Microbiol* 1:223–229
- Bergh O, Bersheim KY, Bratbak G, Haldal M (1989) High abundance of viruses found in aquatic environments. *Nature* 340:467–468
- Berman TM, Nawrocki M, Taylor GT, Karl DM (1987) Nutrient flux between bacteria, bacterivorous nanoplanktonic protists, and algae. *Mar Microb Food Webs* 2:69–82
- Blackburn N, Fenchel T (1999) Influence of bacteria, diffusion and shear on micro-scale nutrient patches, and implications for bacterial chemotaxis. *Mar Ecol Prog Ser* 189:1–7
- Bratbak G, Haldal M, Thingstad TF, Riemann B, Haslund OH (1992) Incorporation of viruses into the budget of microbial C-transfer. A first approach. *Mar Ecol Prog Ser* 83:273–280
- Bratvold D, Srien F, Taub SR (2000) Analysis of the distribution of ingested bacteria in nanoflagellates and estimation of grazing rates with flow cytometry. *Aquat Microb Ecol* 21:1–12
- Brussard, Corina PD, Dominique M, Bratbak G (2000) Flow cytometric detection of viruses. *J Virol Methods* 85:175–182
- Capone DG, Zehr JP, Paerl HW, Bergman B, Carpenter EJ (1997) *Trichodesmium*, a globally significant marine cyanobacteria. *Science* 276:1221–1229
- Cavanaugh CM (1994) Microbial symbiosis: Patterns of diversity in the marine environment. *Am Zool* 34:79–89
- Chin W-C, Orellana MV, Verdugo P (1998) Spontaneous assembly of marine dissolved organic matter into polymer gels. *Nature* 391:568–572
- Chiura HX (1997) Generalized gene transfer by virus-like particles from marine bacteria. *Aquat Microb Ecol* 13:75–83
- Chróst RJ (1990) Microbial enzymes in aquatic environments. In: Overbeck J, Chróst RJ (eds) *Aquatic microbial ecology: Biochemical and molecular approaches*. Springer-Verlag, New York, pp 47–48
- Cooksey KE (ed) (1998) *Molecular approaches to the study of the ocean*. Chapman & Hall, London, 549 pp
- de Beer D, Glud A, Epping E, Kühi M (1997) A fast-responding CO₂ microelectrode for profiling sediments, microbial mats, and biofilms. *Limnol Oceanogr* 42:1590–1600

18. Duarte CM, Gasol JM, Vaqué D (1997) Role of experimental approaches in marine microbial ecology. *Aquat Microb Ecol* 13:101–111
19. Ducklow HW, Carlson CA (1992) Oceanic bacterial production. *Adv Microbiol Ecol* 12:113–181
20. Ferrier-Pages C, Jaubert J, Leclercq N, Pelegri SP (2000) Enhancement of pico- and nanoplankton growth by coral exudates. *Aquat Microb Ecol* 21:203–209
21. Fidopiastis PM, Von Boletzky S, Ruby EG (1998) A new niche for *Vibrio logei*, the predominant light organ symbiont of squids in the genus *Sepiola*. *J Bacteriol* 180:59–64
22. Gallacher S (1997) Evidence for production of paralytic shellfish toxins by bacteria associated with *Alexandrium* spp. (Dinophyta) in culture. *Appl Environ Microbiol* 63:239–245
23. Gasol JM, Doval MD, Pinhassi J, Calderón-Paz JI, Guixa-Boixareu N, Vaqué D, Pedrós-Alió C (1998) Diel variations in bacterial heterotrophic activity and growth in the northwestern Mediterranean Sea. *Mar Ecol Prog Ser* 164:107–118
24. Guixa-Boixareu N, Vaqué D, Gasol JM, Pedrós-Alió C (1999) Distributions of viruses and their potential effect on bacterioplankton in an oligotrophic marine system. *Aquat Microb Ecol* 19:205–213
25. Heissenberger A, Herndl GJ (1994) Formation of high molecular weight material by free-living marine bacteria. *Mar Ecol Prog Ser* 111:129–135
26. Hentschel U, Berger EC, Bright M, Felbeck H, Ott JA (2000) Metabolism of nitrogen and sulfur in ectosymbiotic bacteria of marine nematodes (Nematoda, Stibonematinae). *Mar Ecol Prog Ser* 183:149–158
27. Jannasch H, Taylor CD (1984) Deep-sea microbiology. *Annu Rev Microbiol* 38:487–514
28. Karl DM (1995). Ecology of free living, hydrothermal vent microbial communities. In: Karl DM (ed) *The microbiology of deep-sea hydrothermal vents*. CRC Press, Boca Raton, FL, pp 35–124
29. Karl DM (1986) Determination of *in situ* microbial biomass, viability, metabolism, and growth. In: Poindexter JS, Leadbetter ER (eds) *Bacteria in nature*, Vol 2, Plenum Press, New York, pp 85–176
30. Karl DM, Dobbs FC (1998) Molecular approaches to microbial biomass estimation in the sea. In: Cooksey KE (ed) *Molecular approaches to the study of the ocean*. Chapman & Hall, London
31. Karner M, Herndl G (1992) Extracellular enzymatic activity and secondary production in free-living and marine snow-associated bacteria. *Mar Biol* 113:341–347
32. Kirchner M, Sawling G, Schott C, Döpka H (1999) Intracellular bacteria in the red tide-forming heterotrophic dinoflagellate *Noctiluca scintillans*. *Arch Hydrobiol* 54:297–310
33. Long RA, Azam F (1996) Abundant protein-containing particles in the sea. *Aquat Microb Ecol* 10:213–221
34. MacLeod RA (1965) The question of the existence of specific marine bacteria. *Bacteriol Rev* 39:9–23
35. Martinez J, Smith DC, Steward GF, Azam F (1996) Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. *Aquat Microb Ecol* 10:223–230
36. Morita RY (1968) The basic nature of marine psychrophilic bacteria. *Bull Masaki Mar Biol Inst Kyoto Univ* 12:163–177
37. Morita RY (1997) Bacteria in oligotrophic environments: Starvation-survival lifestyles. Chapman & Hall, London, 529 pp
38. Neelson KH (1997) Sediment bacteria: Who's there, what are they doing, and what's new? *Annu Rev Planet Sci* 25:403–434
39. Newbold RW, Jensen PR, Fenical W, Pawlik JR (1999). Antimicrobial activity of Caribbean sponge extracts. *Aquat Microb Ecol* 19:279–284
40. Noble RT, Fuhrman JA (1999) Breakdown and microbial uptake of marine viruses and other lysis products. *Aquat Microb Ecol* 20:1–11
41. Pace NR, Stall DA, Lane DJ, Olsen GY (1985) Analyzing natural microbial populations by rRNA sequence. *American Society for Microbiology News* 51:4–11
42. Passow U (2000) Formation of transparent exopolymer particles, TEP, from dissolved precursor material. *Mar Ecol Prog Ser* 192:1–11
43. Pomeroy LR (1974) The ocean's food web, a changing paradigm. *BioScience* 24:499–504
44. Pomeroy LR, Wiebe WJ (1993) Energy sources for microbial food webs. *Mar Microb Food Webs* 7:101–118
45. Sala MM, Güde H (1999) Role of protozoans on the microbial ectoenzymatic activity during the degradation of macrophytes. *Aquat Microb Ecol* 20:75–82
46. Sherr BF, Sherr EB, Pedrós-Alió C (1989) Simultaneous measurement of bacterioplankton production and protozoan bacterivory in estuarine waters. *Mar Ecol Prog Ser* 54:209–219
47. Sherr EB, Sherr BF (1991) Planktonic microbes: Tiny cells at the base of the oceanic food webs. *Trends Ecol Evol* 6:50–54
48. Sherr EB, Sherr BF (1994) Bacterivory and herbivory: key roles of phagotrophic protists in pelagic food webs. *Microb Ecol* 28:223–235
49. Simone M, Alldredge AL, Azam F (1990) Bacterial carbon dynamics on marine snow. *Mar Ecol Prog Ser* 65:205–211
50. Suzuki S, Kogure K, Tanoue E (1997) Immunochemical detection of dissolved proteins and their source bacteria in marine environments. *Mar Ecol Prog Ser* 158:1–9
51. Vaqué D, Gasol JM, Marrasé C (1994) Protist grazing rates: The significance of methodology and ecological factors. *Mar Ecol Prog Ser* 109:263–274
52. Vazquez-Dominguez E, Peters F, Gasol JM, Vaqué D (1999) Measuring the grazing losses of picoplankton: Methodological improvements in the use of fluorescently-labeled tracers combined with flow cytometry. *Aquat Microb Ecol* 20:119–128
53. Waldor MK, Mekalamus JJ (1996) Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science* 272:1910–1914
54. Weinbauer MC, Suttle CA (1996) Potential significance of lysogeny to bacteriophage production and bacterial mortality in coastal waters of the Gulf of Mexico. *Appl Environ Microbiol* 62:4374–4380
55. Williams PJ, Le B (1981) Incorporation of microheterotrophic processes into the classical paradigm of the planktonic food web. *Kieler Meeresforsch Sanderh* 5:1–28
56. Zhou J, Mopper K (1998) The role of surface-active carbohydrates in the formation of transparent exopolymer particles by bubble adsorption of seawater. *Limnol Oceanogr* 43:1860–1871
57. ZoBell CE (1946) *Marine microbiology*. Chronica Botanica Co., Waltham, MA
58. ZoBell CE, Morita RY (1959) Deep-sea bacteria. *Galathea Reports* 1:139–154