ROLE OF MARINE MICROBES IN CARBON AND NUTRIENT CYCLES

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Contents

Summary

Microbes are important components of the planktonic food web. In this chapter, we review their ecological properties that make them globally relevant. We also revise the evolution of the main concepts on their role in the nutrient and carbon circulation in the

ocean. Many discoveries have occurred during the last 30 years in the microbial part of the ocean's food web that have forced important changes of paradigms. Most of these discoveries have come after a methodological development shedding light to a new area, to a new group of organisms or to a new metabolism. A particular emphasis is made in the current attemps of opening the microbial black box with the use of phylogenetic and physiological probes. We conclude that these efforts will help to answer the currently most relevant questions now still pending: how each microorganism participate in the global carbon and nutrient oceanic fluxes.

1. Microbes in the sea. Defining the subject of study in marine microbiology

Marine biologists have classically divided the object of their study following either taxonomical or physiological rules. Probably because of the old traditions by which the living world had been divided ever since the Greeks, marine biologists were either botanists, and studied the classical primary producers, or zoologists, and studied zooplankton and fishes. However, we have learned in the past years that most of the biomass in the illuminated depths of the sea is composed of microbial primary producers, while heterotrophic activity in the dark deep sea is performed almost exclusively by chemoheterotrophic prokaryotes. As it will become evident throughout this article, the old botany/zoology dichotomy is no longer a pertinent way of studying sealife even if some textbooks still maintain the tradition alive. Life in the sea is dominated by microbes, both in terms of numbers, biomass and activity.

Microbes are organisms so small that have to be watched with a microscope. They are either prokaryotic (Bacteria and Archaea) or eukaryotic (protists: "algae" and "protozoa") single-celled organisms, which at times can grow in clumps, filaments or colonies that can be seen with the naked eye. They are placed in all sides of the traditional metabolic and physiological barriers, as we find primary producers (both photo- and chemoautotrophs) in all the groups, we find consumers of the particulate primary production ("secondary consumers") also in all the groups and "decomposers" (consumers or respirers of dissolved primary production) again in all of these taxonomic groups. Parasitism, in its general acceptation of absolute dependence on a live organism, can also be found in all groups, and particularly in the viruses (see chapter "Virus and Heterotrophic Microplankton").

Group	Nuclear structure	Sizes	Main Metabolisms	Source of carbon	*Other terms*
Viruses	-	0.01 – 1	-	Living cells (parasitism)	
Archaea	Prokaryotes	0.2 – 2	Chemotrophs ?	DOM, POM ?	*"bacteria"*
Bacteria	Prokaryotes	0.2 – 2	Chemotrophs Phototrophs	DOM, POM, Inorganic	*"bacteria" "algae", "cyanobacteria" "cyanophyceae"*
Protists	*Eukaryotes*	*~1 – >20*	*Chemotrophs Phototrophs Mixotrophs*	*POM, DOM Inorganic*	*Protozoa, Yeasts Algae, "Microscopic plants"*

Table 1: Characteristics of the different groups of sea microbes

All these microorganisms (viruses, archaea, bacteria, protists) tend to be studied in connection because of several reasons: (i) they share a rather narrow size range, from 0.02 to 0.2 µm ("femtoplancton", including most viruses and some bacteria), from 0.2 to 2 µm ("picoplankton", including most bacteria and archaea and some protists), and from 2 to 20 µm ("nanoplankton", mostly including protists); (ii) the methods needed for their study are similar, based on electron microscopy, epifluorescence or optical microscopy, and flow cytometry. (iii) Because of the well-known general relationship between cell size and concentration, they are usually very abundant: in a single milliliter of water we can find 10^7 viruses, 10^5 bacteria and between 10^3 down to 10^1 protists. And (iv) their diversity is mostly physiological and phylogenetic (specially in the case of bacteria and archaea) and not morphological, as is in the case of metazoans and plants. Because of that reason, and as we will see below, molecular and phylogenetic probes are needed for their study.

Other than the relationship with abundance, size is also relevant in terms of nutrient uptake, which is proportional to cell surface. The smaller an organism is, a larger surface-to-volume will have. This is one of the explanations for the dominance of very small primary producers in the most oligotrophic areas of the ocean, and a probable explanation for the relative better performance of heterotrophic bacteria than nanoplanktonic algae in the uptake of nutrients in these oligotrophic environments. Viruses and small bacteria have also a size that prevents, or at least diminishes, their sedimentation. In fact, and for many years, geochemists and geologists have considered "dissolved organic matter" everything that was below 0.45 µm (thus including most bacteria and archaea). Small microbes also have a property that has ecosystem-level implications: they tend to have less water for a given cellular volume, and a higher nutrient content (relative to carbon content) than algae, protozoans and other marine biota. The lower water content might have to do with the lack of need of buoyancy mechanisms (there are exceptions, however) and the lack of biochemical tools to accumulate unused nutrients; and the relatively low C:N and C:P contents can be related to the lack of need of external "space-filling" structures (analogous to the trees' wood) in non-sedimenting microbes. Whatever the reason, the high nutrient contents (low C:N and C:P), and the large surface-to-area values of the smaller bacteria make them the best competitors for nutrients when these are scarce. Some authors have called bacteria "nutrient traps": Fuhrman et al. (1989) calculated that heterotrophic bacteria were 70% of the C of the photic zone in the Sargasso Sea, but > 80% of the photic zone nitrogen. Only biotic mechanisms, such as viral death or protozoan grazing, would free up the nutrients accumulated by bacteria and allow their recycling. These mechanisms will control nutrient supply to planktonic biota in those cases.

Finally, the study of marine microbes ecology is an extremely dynamic area, with new knowledge being added, old paradigms being dismissed and new paradigms being created every few years. As an example, just consider the fact that the most abundant primary producer in the whole Atlantic and Pacific Oceans, probably the most abundant primary producer on Earth, was unknown to science until 1988. The discovery of *Prochlorococcus*, the evaluation of its large abundance and large primary production share (~50% in the equatorial oceans) is as relevant and surprising as it would be to find out that a single unknown species of tree was dominating in all the world's tropical forests. The rhythm of discoveries is high, and today's knowledge will probably be outdated in a

few years time: textbooks have no time to create a benefit for the publisher before being outdated so no textbooks are published. As prestigious ecologist E.O. Wilson has put it, "If I could do it all over again, I would be a microbial ecologist. Ten billion bacteria live in a gram of soil... They represent thousands of species, almost none of each are known to science". It is in the study of microbes where the excitement of the unknown is now.

2. The role of microbes in the cycles of nutrients and carbon. A historical view.

2.1 The abundance of pelagic microbes. How many are there ?

In spite of the obvious nature of such a question, researchers had a hard time in finding an answer, specially for the smallest protists, viruses and bacteria. In part this was due to the fact that traditional microbiological techniques relied on cultivation, and the culture media did not seem to replicate nature very well. And because of the low concentration of bacteria detected growing on plates (four to five orders of magnitude lower than the direct bacterial counts), researchers believed that the role of bacteria in the cycling of carbon and nutrients in the ocean was very limited. A linear food web consisting of phytoplankton, zooplankton and fish (the "classical" food web) dominated carbon cycling in the ocean, and the few bacteria there simply had a role in decomposing the dying organisms (Figure 1). However, this view was at odds with the fact that most of oceanic plankton respiration occurred in size classes below 1 µm, and the fact that most of the pelagic particles had sizes below 10 µm. Could it be possible that few bacteria existed in the plankton but were extremely active, with extremely large cell-specific respiration rates ?

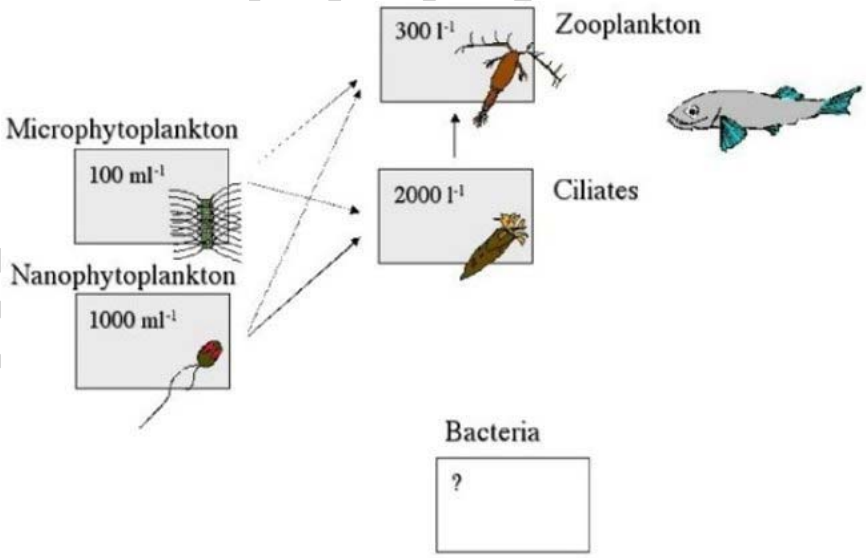

Figure 1: An example of the classical food web, which ignores microbes.

The problem was solved with the introduction of a new technique, the microscopy of cells collected in the surface of a filter and then stained with a fluorescent dye (epifluorescent microscopy) at the end of the seventies, first by Russian researchers. This new group of methods, that have been used until recently when they start to be substituted by flow cytometry, allowed the quantification of pelagic bacteria, which we now know exist in

concentrations of 10^5 to 10^6 cells ml^{-1} in the ocean. We could then realize that there are many bacteria in the ocean, to the point that a very important fraction of the total planktonic biomass (up to 40% in oligotrophic systems) is made up by bacteria. We also know that many pelagic ecosystems support more bacterial biomass than biomass of primary producers. A further general observation was made: while primary producers abundance can change over a large range of values (from almost 0 up to very large abundances – i.e. 10^5 cells ml^{-1}), bacterial abundances seem to be relatively stable over time, ranging from a minimum of ca. 10^5 up to a maximum (in marine plankton) of ca. 8 x 10^6 cells ml^{-1}.

As in any breakthrough, not all oceanographers accepted that microbes had such an important role in the cycling of carbon in the ocean. The hypothesis that most of the detected microbial biomass was dead or inactive and thus, not a dynamic part of the pelagic community, was soon posted.

2.2 The growth and production of bacteria

Answer of this question required again strong efforts in method development. The first methods that were used, like the dark incorporation of CO_2, generated little uncontested data. Microbial ecologists were searching for a universal substrate that could simply and reliably provide microbe growth rate estimates with minimal experimental disturbances to natural populations. While some still think that the feasibility of achieving this goal is remote, the incorporation of nucleotide precursors of DNA (mainly thymidine) and aminoacid protein precursors (mainly leucine) are reliable methods that are currently used, not without problems, to estimate bacterial growth rates and bacterial production. The first results with these techniques, at the beginning of the 80s allowed the measurement of rates of growth of the same order than those of phytoplankton (biomass duplication times of 2-15 days for bacteria are common as are biomass duplication times of 1-6 days for phytoplankton). See Table 2.

	Growth rate (d^{-1})	Dt range	Dt Average (d)
Coastal Seas			
Great Sippewisett Marsh	5	0.1 – 0.2	0.14
Hudson river plume	1.4		0.5
Antarctic coast	0.22	1.2 – 4.6	3.2
St. Lawrence Gulf	0.07	2.9 – 29	10.4
Adriatic coast	0.03	1 – 8	
Open Oceans			
Ross Sea, Antarctica	0.25		
Equatorial Pacific	0.19	4 – 6	
Arabian Sea	0.18		
Equatorial Pacific	0.12		
NW Mediterranean	0.11	0.6 – 46	
North Atlantic Bloom	0.08	2 – 64	
Sargasso Sea	0.08	5 – 15	

Indian Ocean	0.07	2.5 – 16	
Subarctic Pacific	0.05		
Bermuda	0.05		
Equatorial Atlantic	0.04	0.6 – 119	
Drake Passage	0.03	1.4 – 298	
Great Sippewisett marsh	5	0.1 – 0.2	0.14
Hudson river plume	1.4		0.5
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St. Lawrence Gulf	0.07	2.9 – 29	10.4
Adriatic coast	0.03	1 – 8	25.1
Open Oceans			
Ross Sea, Antarctica	0.25		2.8
Equatorial Pacific	0.19	4 – 6	3.5
Arabian Sea	0.18		3.9
Equatorial Pacific	0.12		5.6
NW Mediterranean	0.11	0.6 – 46	6.0
North Atlantic Bloom	0.08	2 – 64	8.4
Sargasso Sea	0.08	5 – 15	8.5
Indian Ocean	0.07	2.5 – 16	10.5
Subarctic Pacific	0.05		13.9
Bermuda	0.05		13.9
Equatorial Atlantic	0.04	0.6 – 119	15.2
Drake Passage	0.03	1.4 – 298	25.1

Table 2: Some values of bacterial growth in the ocean

Two of the most relevant studies published in the 80s were the cross-system analyses of Bird and Kalff (1984) and Cole et al. (1988). The first study presented a good relationship between bacterial abundance and chlorophyll concentration in a set of planktonic systems. The second presented a good correlation between primary production and bacterial production, again, in various aquatic ecosystems, also showing a relative constancy in the proportion of primary production carbon circulated through bacteria. Moreover, the later study suggested that most methods used to that date for the estimation of bacterial production offered relatively close estimates. For the first time it was shown that the measures of bacterial abundance and production had ecological sense, and varied following common wisdom.

However, soon a question was posed: if bacteria are very abundant and their concentrations are relatively constant with time, but we are measuring rates of turnover of such high speeds (2-10 days needed to generate 10^6 cells ml^{-1}), what is the mechanism that removes bacterial production ? Are zooplankton removing bacterial production in the same way that they are removing algal primary production ? Can copepods, nauplis, large ciliates and other zooplankters remove particles of < 1 μm in size ?

2.3 The control of bacterial abundance

While in freshwater some cladocerans (*Daphnia*) were soon found to be able to feed on bacteria, the main marine zooplankters, copepods and nauplia, can not feed efficiently on particles as small as bacteria. In 1982, however, Tom Fenchel presented the results of his research on the ecology of heterotrophic "microflagellates" (we now call them nanoflagellates) which he identified as the organisms responsible for most of the removal of bacterial production in aquatic systems. These organisms, initially described as being of sizes between 2 and 5 µm –but we now know that they can be as small as 1 µm– , are very diverse and abundant (roughly 10^3 flagellates ml^{-1}) and while they had escaped detection in the past, were most probable the "missing link" between bacteria and zooplankton.

Further work showed how common were predator-prey-like oscillations when bacteria and heterotrophic nanoflagellates (HNF) were let to grow, and the use of bacterial "surrogates" in the form of fluorescent latex beads and bacterial and eukaryote chemical inhibitors, demonstrated that protozoans were effectively ingesting bacteria. It is currently known that nanociliates can also efficiently feed on bacteria and that there are probably several steps in the transfer of carbon between the smallest bacteria and zooplankton involving several organisms feeding on each other. We also know that active benthic filter-feeding organisms like mussels or passive suspension feeders like gorgonian corals can use bacterial production and thus become an effective carbon linkage between planktonic and benthic aquatic subsystems.

While bacterial production use by protozoans indicates transfer of carbon between trophic levels, predation on bacteria fulfills another important ecological role, because grazing frees up nutrients previously accumulated in the bacterial biomass. This being most important in those ecosystems where there is a strong nutrient limitation of total production.

Heterotrophic nanoflagellates were found to be phylogenetically close to some of the previously known flagellated algae (phototrophic nanoflagellates), and it was encountered that the presence of chloroplasts in a given species was no predictor as to whether the flagellate was hetero- or autotrophic. Mixotrophy, the use of both chemoorganoheterotrophic and photolitoautotrophic nutrition modes, was found to be widespread in most flagellate groups in an strategy particularly adequate for guaranteeing nutrient supply to the flagellates in nutrient-poor environments. Mixotrophy is also present in ciliates, where it appears to be a phenomenon more related to the maintenance of the plasts of ingested algae that still keep their photosynthetic potential. The mixotrophic behavior of these protists, flagellates and ciliates, demonstrates how outdated is the traditional botany/zoology division based on trophic mode when it has to be applied to microbes.

2.4 The rediscovery of planktonic viruses

Flagellates and ciliates seemed to compensate all bacterial growth in most but not all cases. Furthermore, the presence of a relatively large bacterial concentration in the ocean and the knowledge that bacteriophags existed –and were commonly used and isolated in

microbiological laboratories– , conspired to reduce the possible surprise originated in the discovery, at the beginning of the 90s of large abundances of free viruses in the ocean. Viruses were found to be in concentrations of up to 10^8 viruses ml^{-1}, to form virions inside phototrophic and heterotrophic bacteria, to be able to significantly reduce primary production and to account for a variable portion of bacterial production ranging from 20 to 70%. Viruses were soon found to be also dynamic members of the plankton community, with high production and loss rates.

While the impossibility of differentiating algal from bacterial phages has limited our knowledge of their ecological role, we now understand the role of bacteriophages primarily as generators of the so-called "viral loop", a reduction of bacterial carbon conversion efficiency because of an increase in bacterial carbon "lost" as respiration. This process also increases the recycling of inorganic nutrients. Viral activity is an efficient way of converting living biomass into dissolved and particulate organic carbon available to bacteria.

Taking into account the high specificity of the host-parasite viral systems, the other role hypothesized for viruses is the maintenance of microbial diversity by the mechanism known as "killing the winner". When one of various competing organisms wins and produces a "bloom", the high concentrations achieved facilitates viral infection, spreading of the infection, and termination of the bloom.

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Biographical Sketch

Born in 1962. Ph.D. in Biology in 1989, under the supervision of **Prof. Dr. C. Pedrós-Alió**.
Postdoctoral work in McGill University, Canada and in the Departament de Genètica i Microbiologia of the Universitat Autònoma de Barcelona.

I have participated in more than 12 international and national research projects, in 10 scientific cruises and I have published more than 50 papers in peer-reviewed international journals. Reviewer in more than 10 international journals.

I'm interested in planktonic microbe abundance and activity, and the ecosystem effects of their abundance and activity. This implies the study of the factors that regulate the abundance of planktonic microorganisms and those that regulate microorganism community structure (size, functional and taxonomical structure), and how the physical changes in the marine environment (macro-, meso – and microscale structures) affect these parameters. As an example, how predation and resource availability regulate bacteria abundances, bacterial use of DOC, and how they regulate the composition of the microbial "black box" in terms of size structure and metabolic characteristics of the community. This is approached by empirical analysis of data bases of organism abundance, growth and loss rates, generated mainly in cruises; by mesocosm and microcosm experiments; and by the combined use of image analysis and flow cytometry and metabolic fluorescent probes.