MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, Sept. 2005, p. 440–461 Vol. 69, No. 3
1092-2172/05/$08.00+0 doi:10.1128/MMBR.69.3.440–461.2005

Fate of Heterotrophic Microbes in Pelagic Habitats: Focus on Populations

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INTRODUCTION

Units of Interest: Single Microbial Populations

Molecular biological methods centered on the rRNA gene have developed into powerful tools for the cultivation-independent identification of aquatic microorganisms. Over the last decades, the composition and diversity of microbial assemblages in numerous marine and freshwater environments have been studied by 16S rRNA gene cloning and sequencing (55, 76, 85, 91, 121, 303), community fingerprinting (36, 49, 156, 185, 254), hybridizations with oligo- or polynucleotide probes (2, 89, 136, 188, 218), or by combinations of these approaches (63, 144, 223, 266, 278).

A major goal of aquatic microbial ecology is to understand the specific roles of different microorganisms as mediators of element fluxes, e.g., during the remineralization of nutrients and organic carbon. So far, the novel molecular approaches have focused mainly on the occurrence and evolutionary relatedness of different bacteria, archaea, and picoeukaryotes. Clearly, there is a need to progress beyond a mere descriptive analysis of microbial diversity and community structure, to provide information about which microbes are involved in various biogeochemical processes. An integration of the more diversity-centered and the more biogeochemical perspectives in microbial ecology has been sought in the concept of the "structure and function" of microbial assemblages (73, 256). Such terminology might be fully appropriate for the study of technologically designed environments that serve particular functions, e.g., wastewater treatment plants, of coherent physiological groups, or of habitats like sediments or biofilms that feature a rigid physical structure (37, 257, 308). However, the concept of "structure and function" appears to overemphasize the "bottom-up" aspect of aquatic microbial assemblages, i.e., aspects that are related to microbial substrate and nutrient

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utilization. Pelagic habitats are also zones of high microbial mortality, and a substantial fraction of bacterial biomass may be transferred to higher trophic levels by predation (272). In order to understand the different heterotrophic microbes that inhabit the marine and freshwater plankton, it might thus be more adaeqate to consider both the specific "role" and the "fate" of microbial populations.

In many respects "aquatic microbial communities" are theoretical concepts rather than biologically real entities with (physical) structure, common genome or evolutionary history (66). Although ultimately there might be community assemblage rules also for the aquatic microbes (118), bacterioplankton assemblages could likewise be regarded as more or less loose collections of individual genotypic populations that change over time in features such as growth, mortality, and size. Here we discuss the advantages of adapting such a "Gleasonian" viewpoint (87), i.e., we focus on distinct bacterial populations that can be reliably identified and quantified in aquatic assemblages rather than on microbial community structure, diversity, or function as a whole. Specifically, we review current methodological approaches, and we assemble information about the occurrence, the phenotypic properties, and the possible role and fate of such defined microbial populations in the water column.

Marine Autotrophic Picoplankton as a Model

The advantages of a focus on single microbial populations can be illustrated by looking at autotrophic marine picoplankton. *Prochlorococcus* is an ubiquitous free-living phototrophic cyanobacterial genus that is common in temperate to tropical marine waters (205), where it may account for a high fraction of total primary production. This group was only discovered in 1989 by means of a then-novel technology, flow cytometry (38). The ability to reliably distinguish *Prochlorococcus* populations from other species of autotrophic marine picoplankton (e.g., *Synechococcus*) has resulted in concerted research about the ecology, physiology, and genomic constitution of these organisms that is unparalleled in aquatic microbial ecology. During the past 15 years, numerous strains of *Prochlorococcus* from various locations have been isolated and studied in the laboratory (205). The geographic distribution limits and the contrasting vertical niches of physiologically distinct genotypes have been established (67, 205, 314). Recently, the capacity of *Prochlorococcus* to heterotrophically utilize organic nitrogen sources has been described both in situ (327) and as a feature of its genome (246). The successful unraveling of the functional role of this particular bacterial group based on its reliable in situ identification might also provide a conceptual model for the study of heterotrophic microbes in the water column.

Microbial Phenotypes

Major biogeochemical processes in the water column are related to the activities of heterotrophic microbes, e.g., the mineralization of organic carbon from photosynthesis or its transport to higher trophic levels (13). This is deduced from bulk measurements of microbial growth, respiration, the rates of biomass production, and its loss to predation or to viral lysis (53, 74, 77, 142, 270). Such bulk parameters sum up the potentially different rates of cell replication and mortality of various microbial species. While this may be essential to follow the fate of organic matter as a whole, it has also separated aquatic microbial ecology from other fields of microbiology that insist on well-identified microorganisms as the basic units of research. During the last decade the complexity of microbial phenotypes has moved into the focus of research that aims to explain biogeochemical processes. Our view of microbes as mediators of element flow has matured from the black-box approach of the early food web models to a subtler understanding of the ecophysiology of organisms that thrive in a heterogenous environment of dissolved and particulate organic matter (12).

The diversity of phenotypes within aquatic microbes reflects the complexity of the habitats at a microscale (3, 106, 206, 281). For example, a large fraction of cells in coastal bacterioplankton may be motile during particular seasons or times of the day, and the duration of their actual swimming periods may be affected by substrate availability and patchiness (103). This might be related to chemosensory functions that allow bacteria to actively accumulate in substrate-rich microniches such as the phycosphere of senescent algae (15, 23). The balance between particle colonization efficiencies and detachment dynamics may even reflect species-specific microbial life cycles (100, 140, 141). Culturable genera of aquatic bacteria harbor large numbers of facultative anaerobes, and ten percent of bacteria in oxic coastal marine waters could incorporate glucose both at oxic and at anoxic conditions (4, 243). Some strains isolated from the plankton are capable of degrading complex organic molecules or of utilizing organic sulfur compounds (94, 95). Other aquatic bacteria differ in the duration of the lag phase that precedes growth in batch culture (212), exhibit high tolerance to UV radiation (290), secrete or resist antibiotics (61, 161), or are capable of cell communication by quorum sensing (97). In addition, indications of microbial activity are found in the water column that presently cannot be assigned to particular bacterial groups, e.g., microbial exoenzymes with contrasting kinetic properties (9, 307).

Aquatic microbes have moreover developed a range of specific defense strategies. Some freshwater bacteria form thread-like morphotypes or microcolonies that secrete large amounts of exopolymerous substances, thereby obtaining protection against ingestion by bacterivorous protists (113, 223). In coastal North Sea surface waters >25% of prokaryotes were covered by a polysaccharidic capsule, a feature of many pathogenic species (288) which again might be related to grazing resistance. A high percentage of bacteria in different freshwaters are gram-positive (263), which enhances their protection against digestion by unicellular eukaryotes (114, 125).

However, one must not conceptually merge distinct microbial species into fictional chimeric "aquatic bacteria" (Fig. 1). More appropriately, the various phenotypic features should be regarded as individual elements of unique life strategies that explain the occurrence and distribution patterns of different genotypes in aquatic habitats. Some of these phenotypic features, such as motility or filament formation, may be widespread across very different phylogenetic lineages (115), whereas others, e.g., antibiotic production or resistance, may be highly characteristic for particular species or genera (100, 160). A single genotype may express a variety of phenotypic

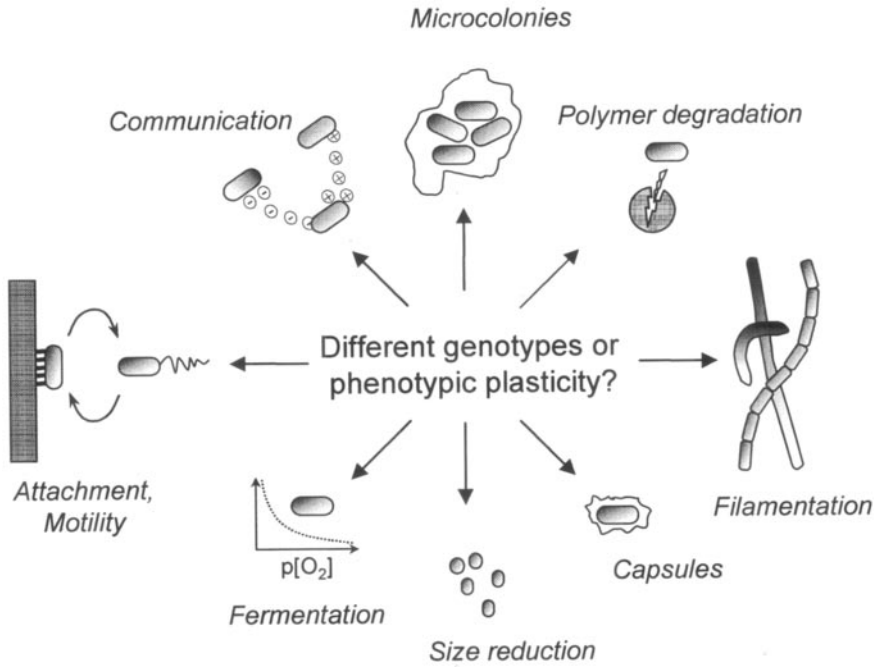

FIG. 1. Schematic example of phenotypic diversification of aquatic bacteria. The relationship between the different genotypes and their specific ecophysiological features, life strategies, and behavior is often poorly understood.

features or even phenotypic plasticity (90, 115), and identical features may be shared by numerous genotypes. Thus, a central task of microbial ecology is to assign the various physiological abilities and phenotypes observed in situ to particular microbial species or genotypes.

Operational Phylogeny-Based Definition of Microbial Populations

It is still the subject of debate whether there can be a natural species concept in microbiology (41, 248). Therefore, a definition of "populations" in field investigations of microbial ecology should be more flexible than, e.g., for laboratory studies of ecophysiology or evolution (27, 64, 128). In our view, which is shaped by the available tools and the lack of information about the ecology of 16S rRNA-defined populations in the environment, almost any phylogenetically coherent group of microbes could potentially qualify as a "meaningful" population. The phylogenetic resolution that adequately delineates microbial populations largely depends on the ecological goals of a specific investigation. A meaningful population should represent a unit that (i) is recognizable in subsequent investigations and (ii) exhibits ecologically distinct features from other recognizable units (Fig. 2).

Such genotypically defined populations with a shared set of phenotypic and ecological properties ideally might be single ecotypes sensu Cohan (41) or almost identical clusters of 16S rRNA sequence types (1), e.g., the marine DE2 (144) and NOR5 (63), or the freshwater LD2 (223, 328) clades. However, larger entities may also represent meaningful populations, i.e., 16S rRNA-based clades of sequence types such as the marine *Roseobacter* (63), SAR11 (188), and SAR202 (189) clusters or the freshwater BET1 (278), BET2 (35), or AcI lineages (309). Occasionally it may even be sufficient to differentiate by subphylum (47, 89) or domain (136).

The distinction of genotypic compartments within mixed microbial assemblages helps to compile ecologically relevant information about the abundances and activities of phyloge-

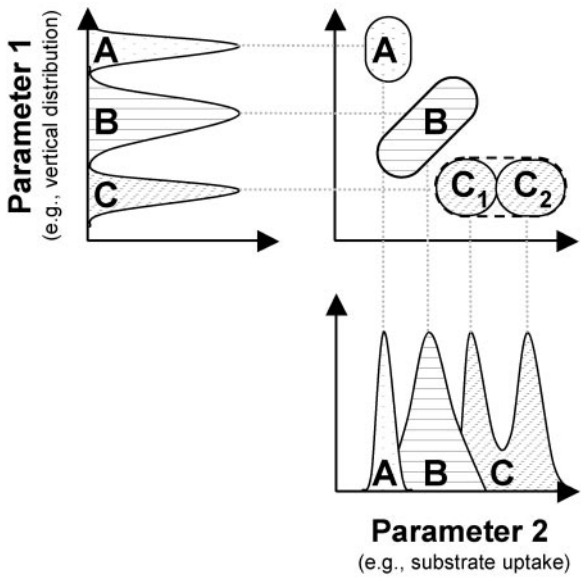

FIG. 2. Conceptual model for an operational definition of microbial populations. In the context of ecological investigations three bacterial taxa, A, B, and C, can be meaningfully defined as different populations if they also differ in an ecologically relevant feature (parameter 1). However, a splitting of population C may be required if it is studied in the context of a second environmental variable (parameter 2).

netically closely related groups of microorganisms, e.g., about their horizontal or vertical distribution patterns (210, 295), seasonality (63, 136), occurrence on particles (261), cell morphology (223), growth response to confinement (61, 72, 254), sensitivity to predation (16), incorporation of particular substrates (47, 169, 306), or growth under anoxic conditions (4). Our conceptual framework thus encompasses investigations of the seasonal occurrence of pathogenic *Vibrio* spp. in coastal waters (83, 117), of the response of a specific clade of freshwater betaproteobacteria to food web manipulations (278), but also of the differences in the vertical distribution of *Bacteria* and *Archaea* in the open ocean (136). On the other hand, it may also refer to the relative contributions of genotypic populations to a single biogeochemical process, e.g., the roles of planktonic members of the *Roseobacter* or SAR11 lineage in sulfur cycling (96, 169, 170, 306, 323).

It should be noted that a microbial population that is defined above the level of the single clone might represent an operational rather than a natural category. Therefore, such an operational taxonomic unit will be limited in its usefulness to a particular study context, and it should be refined or expanded if found inadequate for a specific purpose (Fig. 2). Some phylogenetic clades might harbor organisms with closely related or even identical 16S rRNA gene sequences but rather different ecophysiological properties (128). Sometimes it may be useful to merge such strains into larger categories, e.g., to study the occurrence of genera associated with particular phytoplankton blooms (96, 323). At other instances it might be necessary to distinguish between closely related strains, e.g., to understand the ecological significance of physiological variability (27, 109, 128) (Fig. 2).

Synoptic Discussion of Marine and Freshwater Assemblages

A number of fundamental similarities in the functioning of marine and freshwater microbial assemblages have been described, in particular carbon transfer and food web structure (203, 251, 313) and the role of bacteria attached to aggregates (105, 229). This has traditionally led to a fruitful exchange between limnological and oceanographic research on planktonic microbes, based on shared concepts and on a common set of techniques (79, 81). Recently, the relationship between the two disciplines has somewhat deteriorated, conspicuously paralleled by a shift in research focus from food webs and element cycles to microbial biodiversity. In fact, cultivation-independent approaches have revealed by far more differences (89, 180) than similarities (14) in the composition of the microbial assemblages in the two habitat types.

Can identical ecological concepts be meaningfully applied to communities composed of bacterial groups from completely different phylogenetic lineages? Our review may in parts substantiate such doubts, e.g., with respect to the role of allochthonous (156) or filament-forming (130) microorganisms in the two habitat types. Yet at the same time we illustrate that a focus on individual microbial populations also provides a platform to compare the ecological value of particular phenotypic traits, e.g., of motility and small cell size (103, 221, 238), or of specific growth strategies (65, 68, 212) in very different genotypes and assemblages across aquatic habitats.

The scope of this review is limited in several dimensions. We do not attempt to draw an exhaustive picture of the microbial ecology of heterotrophic aquatic microbes. Instead we try to integrate a rather diverse range of facets, focusing on current methodological and experimental approaches, and on empirical findings related to traditional topics of population ecology. We argue that during the last decade the study of aquatic microbial populations has developed into a rewarding field of research that is based on mature methodology. Since our focus lies on the ecology of different populations rather than on the study of total assemblages, we only marginally touch the rich literature on genetic fingerprinting or on the diversity of aquatic communities. We limit our discussion to the "ordinary saprophytes", i.e., to the predominantly chemoorganoheterotrophic microbes (although admittedly the borders between purely heteroorganotrophic and photoautotrophic energy acquisition by pelagic microbes do become more and more fuzzy [20, 327]). We ignore microbial assemblages in sediments, biofilms, or in engineered systems, and we do not discuss in detail the physicochemical environments of aquatic microbes, but rather point to more specialized reviews, e.g., about macroscopic organic aggregates or exopolymerous substances (206, 281).

TOOLS, TECHNIQUES, AND STUDY CONCEPTS

During the last 15 years aquatic microbial ecology has been shaped by a diversification of methods for the cultivation-independent study of microbial identity, activity, and genomic constitution (11). This trend is reflected in the recent foundation of a scientific journal on methods in limnology and oceanography that is edited by a microbial ecologist (http://aslo.org/lomethods/editor.html). The following section does not give an exhaustive listing of tools for the study of microbial populations in situ. Instead, it covers a suite of complementary approaches which have been applied in various combinations to analyze bacteria in environmental samples. They can provide a coherent framework for ecological investigations about the abundant microbes in the water column, i.e., taxa that contribute approximately 1% or more to the total picoplankton assemblages. Other approaches, e.g., quantitative PCR (17, 283), might be more appropriate to study microbial populations that are substantially smaller.

16S rRNA Gene Clone Libraries: Essential but Prone to Bias

In order to define the important populations in a new environment or at a particular time point, it is often necessary to conduct a prior study about microbial diversity, most commonly of 16S rRNA genes. Full or partial sequences can be amplified from extracts of environmental DNA (75, 301) or directly from cells concentrated on membrane filters (21, 145) by PCR with appropriate primer sets (84, 194). These fragments are then ligated into plasmids, cloned into *Escherichia coli*, and the sequences of vector inserts from an appropriate number of clones are determined. The PCR step is omitted altogether in so-called shotgun clone libraries (305). Since such libraries require a drastically greater screening effort, they are used for obtaining metagenomic information rather than for the mere collection of 16S rRNA genes.

The diversity of sequences in PCR-generated clone libraries may often not quantitatively reflect the diversity of the se-

quence types that are present in the original sample (242, 294). Already the DNA extraction may introduce biases, e.g., against bacteria with gram-positive cell walls (69). Primers that are designed to target the majority of known bacterial 16S rRNA gene sequences may exhibit mismatches to unknown sequence types (45, 287), and the presence of particular sequence types in mixed DNA may influence the PCR amplification efficiencies of other templates (292).

As a consequence some phylogenetic groups of aquatic microorganisms are overrepresented in clone libraries, whereas others are absent. For example, bacteria affiliated to the *Cytophaga/Flexibacter/Flavobacterium* group of the *Bacteroidetes* were rarely found in 16S rRNA gene clone libraries from coastal marine water samples, yet they may represent one third of all bacteria in such habitats (47, 63). In a library of coastal North Sea surface waters 80% of sequence types were related to the marine SAR86 clade, whereas this group formed <10% of microbial cells (63). In addition, PCR may result in chimeric sequences from two templates, and it may thus even produce artificial sequence diversity (124, 155). These biases may affect not only clone libraries, but also PCR-based methods for the genotypic fingerprinting of microbial assemblages (183, 193).

Sometimes it is desirable to limit the analysis of microbial diversity to a defined phylogenetic subset, e.g., to marine archaea or to freshwater actinobacteria (173, 309). This can be achieved by PCR with primers that are specific for the 16S rRNA genes of the group of interest (287). Such primers may cause an underestimation of the potential diversity within a particular phylogenetic group, e.g., some uncultured freshwater actinobacteria show one or more mismatches with the available sets of specific primers (309). Moreover, sequences generated with specific primers often cover only a part of the total 16S rRNA gene, typically less than 1,000 base pairs (55, 287). Partial sequence information negatively affects the accuracy of phylogenetic reconstruction (163), and it limits the range of potential signatures for subsequent in situ population studies by hybridization. Preferably, clone libraries of almost complete 16S rRNA gene sequences should be produced with general bacterial or archaeal primers, and they should then be screened for particular groups of interest (173, 184, 309).

The potential target groups for population studies are defined from sequence data by reconstruction of phylogenetic relationships. This analysis aims at placing the new environmental sequence types into different clades or clusters and to establish stable branching patterns. It forms the base for a phylogenetically meaningful definition of single populations and for the design of the corresponding oligonucleotide probes. A discussion of the reconstruction of microbial phylogenies from 16S rRNA gene sequences would go beyond the range of this review, and we draw the reader's attention to specific publications on this subject (163, 164).

Single-Cell Identification

Since 16S rRNA gene clone libraries do not accurately reflect the abundances of microbes from particular phylogenetic clades in the environment, such analysis needs to be complemented by other strategies for the study of single populations in situ. Hybridization techniques against extracted rRNA or intact cells have developed into an important tool of choice for this purpose. Our review is particularly focused on whole-cell fluorescence in situ hybridization (FISH) with rRNA-targeted oligo- or polynucleotide probes (6, 7, 58, 86, 211, 217). FISH allows the identification and quantification of different microbial taxa in environmental samples. It links information derived from molecular phylogeny with epifluorescence microscopy, a research tool that has considerably formed our understanding of water-column microbes (13, 122, 231).

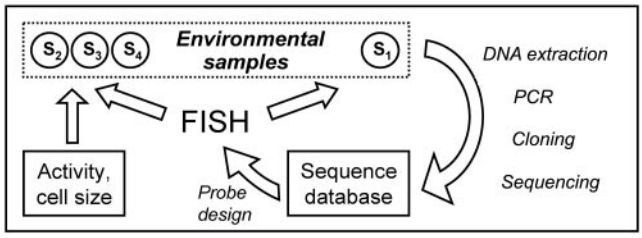

FIG. 3. FISH, a versatile tool for the study of microbial populations in aquatic systems. The FISH approach is embedded in a suite of techniques that are required for the acquisition and processing of rRNA sequence information or that allow simultaneous investigation of microbial geno- and phenotypes at the single-cell level. S_1 to S_4, samples.

The FISH analysis can be carried out at two levels (Fig. 3). For one, investigators may draw upon the rapidly expanding set of available probes (162) (Table 1). Most frequently, probes have been applied that are targeted to higher taxonomic levels, i.e., to *Bacteria*, *Archaea*, *Eucarya*, subgroups of *Proteobacteria*, gram-negative sulfate-reducing bacteria, clades of ammonium-oxidizing bacteria, etc. In an earlier review on FISH, such an analysis was proposed as a first step of a larger "top-to-bottom" strategy (7).

The information obtained by FISH with probes for higher taxonomic levels should guide researchers to choose among probes of increasing phylogenetic specificity for a second and potentially third layer of analysis. This stepwise approach has hardly been realized for the study of pelagic microbial assemblages yet, and currently there are only a few such nested probe sets available. Hierarchic FISH analysis has been applied to distinguish between marine *Gammaproteobacteria* that are favored at different enrichment conditions (16, 61, 175) and to study the seasonal community contribution of a clade of freshwater *Betaproteobacteria* (35). More frequently, probes for large taxonomic groups have been applied as stand-alone tools for explorative investigations of unknown communities. FISH analysis with such probes has revealed that the microbial assemblages in marine and freshwater pelagic habitats differ even at the level of subgroups of *Proteobacteria* (89), that actinobacteria form large populations in lakes (263), that *Crenarchaea* are a major component of the deeper marine water column (136), that protistan and metazoan grazing may influence microbial community structure (132, 147, 275, 279), and that members of the *Cytophaga/Flexibacter/Flavobacterium* group form a substantial fraction of coastal marine communities (45, 63). FISH with probes for large phylogenetic groups has also been used to study community transitions in rivers and estuaries (28, 52, 143)

The second level of FISH analysis is more technically demanding. It includes the design and optimization of new FISH

TABLE 1. Some oligonucleotide probes targeted to clades of marine and freshwater heterotrophic picoplankton

Plankton type	Probe name	Target group	Sequence (5′-3′)	FA[a](%)	*Escherichia coli* 16S rRNA position[b]	Reference or source
Marine	CREN-554	GI *Crenarchaeota*	TTAGGCCCAATAATCMTCCT	20	554–573	176
	EURY-806	GII *Euryarchaeota*	CACAGCGTTTACACCTAG	20	806–823	296
	ROS-537	*Roseobacter* clade (*Alphaproteobacteria*)	CAACGCTAACCCCCTCC	55	537–554	63
	SAR11-441	SAR11 clade (*Alphaproteobacteria*)	TACAGTCATTTTCTTCCCCGAC	45	441–462	188
	SAR116/1-447	Cluster 1 of SAR116 clade (*Alphaproteobacteria*)	GCTACCGTCATCATCTTC	65	447–464	71
	SAR116/2-436	Cluster 2 of SAR116 clade (*Alphaproteobacteria*)	CATCTTCACCAGTGAAAG	45	436–452	71
	OM43-162	OM43 clade (*Betaproteobacteria*)	ATGCGGCATTAGCTAACC	55	162–179	262
	SAR86-1245	SAR86 cluster (*Gammaproteobacteria*)	TTAGCGTCCGTCTGTAT	55	1245–1262	325
	NOR5-730	NOR5 subcluster of OM60 clade (*Gammaproteobacteria*)	TCGAGCCAGGAGGCCGCC	55	730–747	63
	ALT-1413	*Alteromonas* spp., *Colwellia* spp. (*Gammaproteobacteria*)	TTTGCATCCCACTCCCAT	50	1413–1430	62
	GV-841	*Vibrio* spp. (*Gammaproteobacteria*)	AGGCCACAACCTCCAAGTAG	50	841–822	62
	PSA184	*Pseudoalteromonas* spp., *Colwellia* spp. (*Gammaproteobacteria*)	CCCCTTTGGTCCGTAGAC	50	184–210	62
	CF6-1267	DE cluster 2 (*Cytophaga-Flavobacterium*)	GAAGATTCGCTCCTCCTC	40	1267–1284	144
	SAR406-97	SAR406 cluster (*Chlorobium-Fibrobacter*)	CACCCGTTCGCCAGTTTA	65	97–114	71
	SAR202-104	SAR202 clade (*Chloroflexus*)	GTTACTCAGCCGTCTGCC		104–131	189
Freshwater	R-BT065	Beta I lineage of limnic *Betaproteobacteria*	GTTGCCCCCTCTACCGTT	55	65–72	278
	Bet2-870	Beta II lineage of limnic *Betaproteobacteria*	CCCAGGCGGCTGACTTCA	55	870–881	35
	SOL-852	SOL cluster (filamentous *Saprospiraceae*)	ACGCTTTCGCTTGGACAC		852–869	255
	LD2-739	LD2 clade (subgroup of SOL cluster)	GCGTCAATACAGATCCAG	55	739–757	223
	HAL-844	*Haliscomenobacter* cluster (subgroup of SOL cluster)	CGCTTGGACACTCACTCC		844–861	255
	Acl-1214	acl lineage of limnic *Actinobacteria*	CATGCGTGCAGCCCAAGACA	55	1214–1233	310
	Acl-840-1	sub-group 1 of acl lineage	TCGCACAAACCGTGGAAG	30	840–857	310
	Acl-840-2	sub-group 2 of acl lineage	TCGCAGAAACCGTGGAAG	30	840–857	310
	Acl-840-3	sub-group 3 of acl lineage	TCGCAGAGACCGTGGAAG	30	840–857	310
	Acl-840-H1	unlabeled helper for Acl-840 probes[c]	CTAGYGCCCAYCGTTTACGG	30	810–829	91
	Acl-840-H2	unlabeled helper for Acl-840 probes[c]	GTTCSCACAACTAGYGCCCA	30	820–839	91
	Acl-840-H3	unlabeled helper for Acl-840 probes[c]	GGGGCRCTTAATGCGTTAGCTG	30	859–880	91

[a] If available, stringent conditions for hybridization (% of formamide [FA] in the wash buffer) are given for CARD-FISH as described (211).
[b] Probe target position according to reference 33.
[c] Unlabeled helper oligonucleotides are added at a 1:1 concentration to hybridization mixes according to reference 70.

probes for individual phylogenetic clades or single phylotypes based on sequence information from 16S rRNA gene clone libraries of environmental DNA (Fig. 3). This strategy has been termed the "full-cycle rRNA approach" by one of the authors (7). The procedure is comparable to the testing of a scientific hypothesis. Specifically, it is verified if a particular clade of sequence types obtained from environmental DNA after PCR amplification indeed harbors microbes that form a substantial fraction of the assemblage in the studied habitat. The lower limits for a reliable quantification of cell numbers by the FISH approach as set by counting effort and negative controls typically range around 0.1 to 2% of total counts (217). Consequently, researchers applying FISH may need to test a number of newly designed specific probes on field samples to discover microbial populations that are sufficiently abundant for subsequent quantitative population studies. This implicit risk of falsification of a working hypothesis represents a major conceptual difference between the full-cycle rRNA approach and other strategies that analyze microbial assemblages by molecular fingerprinting of PCR-amplified rRNA genes (185, 254).

Problems with FISH and Possible Solutions

In summary, FISH probes represent tools that are rather tedious to construct but easy to apply. The specificity of oligonucleotides for a particular range of phylotypes is deduced from the sequence data that are available at the time of their design. Due to the exponential growth of sequence databases, some probes may lose their originally envisaged specificity or target group coverage with time. For example, one commonly used probe for *Bacteria* (EUB338) has been constructed on a set of <1,000 then-available bacterial 16S rRNA gene sequences (6). At the time of this review, the Ribosomal Database Project (42) has collected >170,000 full and partial bacterial sequences. Thus, it is not surprising that the coverage of probe EUB338 is incomplete, and it has been modified accordingly (51). This points to the need to check the target range of

existing FISH probes on the latest data set before applying them to unknown samples. A useful database about the current specificity and coverage of many published probes is provided by the Molecular Ecology department of the University of Vienna at http://www.microbial-ecology.de/probebase (162).

However, the replacement of some of the first generation of available FISH probes should be seen as a necessary optimization process in a rapidly progressing field rather than as an irresolvable problem of the FISH approach. Analyses of rRNA gene sequences from aquatic habitats indicate that only a limited number of well-defined phylogenetic clades of microorganisms might actually be common in the pelagic zones of marine and freshwater environments (55, 85, 91, 121, 240, 328). A substantial amount of diversity within several of these clades appears to be covered adequately by the presently available sequence data (108). Such knowledge will eventually provide a reliable base for a new generation of more "habitat-specific" FISH probes that discriminate well-established lineages of microbes in a particular environment (Table 1), e.g., the various marine SAR clades (62, 188, 189, 325) or the freshwater actinobacteria (91, 310).

Another drawback of FISH with fluorescently monolabeled oligonucleotide probes is the low fluorescence intensity of hybridized bacteria from natural water samples (214). Bacteria in oligotrophic water are often small, slow growing, or in stationary phase (187), and their ribosome content is typically low (65). Consequently, there are few rRNA target molecules for FISH staining. The fraction of microbial cells that can be visualized microscopically may thus vary with the physiological state of the studied assemblage. For example, a significantly smaller percentage of bacteria could be stained by FISH in coastal North Sea surface waters during the winter months than during the spring and summer seasons (63). In environments such as offshore marine waters, sometimes only a minor fraction of microbes can be visualized by FISH with fluorescently monolabeled oligonucleotide probes (214). Therefore, it is likely that the abundances of some slowly-growing microorganisms with small cell sizes, e.g., of members of the marine SAR86 clade, are underestimated by the standard FISH approach (210).

During the last decade, efforts have been made to increase the sensitivity of FISH, e.g., with peptide nucleic acid probes (320), brighter fluorochromes (88), image intensified video microscopy (78), preincubation with chloramphenicol (200), hybridization with more than one fluorescently labeled oligonucleotide probe (188), and helper probes (70). Two particularly promising alternative strategies to FISH with fluorescently monolabeled oligonucleotides are polynucleotide probes and enzymatic signal amplification (57, 149, 210). Fluorescently multilabeled rRNA-targeted polyribonucleotide probes yield significantly higher signal intensities than oligonucleotide probes (214). They have been successfully applied to discriminate between bacteria and different groups of archaea in coastal and open ocean environments (40, 136, 214). Limitations of polyribonucleotide probes as a routine tool for the identification of aquatic microbes are the relatively high effort and variability of enzymatic probe synthesis, and the rather low phylogenetic resolution. Only three large taxa have been distinguished by this technique in marine waters (57), although potentially a resolution at the genus level is possible (300).

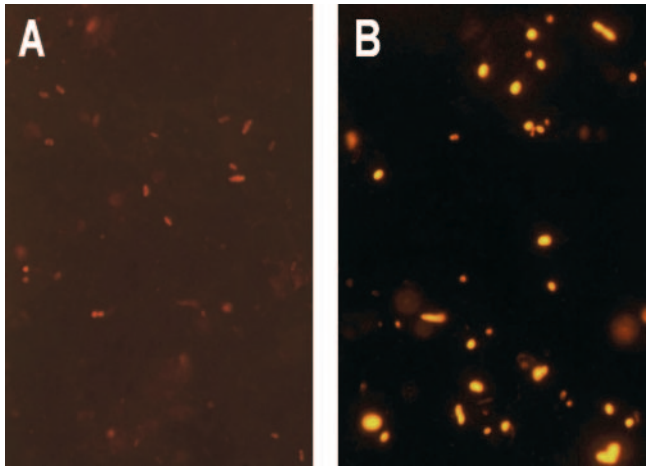

FIG. 4. Comparison of hybridized bacteria from North Sea waters (A) after FISH with fluorescently monolabeled probes and (B) after CARD-FISH. Photographic exposure times were (A) 10 s and (B) 1 s. (Modified from reference 210.)

A working alternative is catalyzed reporter deposition (CARD)-FISH (210, 211, 263), which combines in situ hybridization with horseradish peroxidase labeled oligonucleotide probes and enzymatic signal amplification with fluorescently labeled tyramides (24). This allows the quantitative detection of marine and freshwater pelagic bacteria with low ribosome content that are insufficiently visualized by fluorescently monolabeled probes (210, 263) (Fig. 4).

Quantification of Abundance and Biomass

In order to detect significant net growth or loss of populations, the cell numbers of different microbes in situ have to be determined at a standardized level of precision. This requires manual quantification of the percentage of FISH-stained microbes by epifluorescence microscopy (217). Other potential counting approaches, e.g., flow cytometry (5), so far have failed to provide working alternatives to this tedious evaluation strategy. Eventually, a rapid quantification of bacterial populations by means of automated microscopy (219) might allow us to expand the scale of investigations from a few point measurements to a spatial or temporal resolution that better reflects the true population dynamics of heterotrophic aquatic picoplankton.

A conversion of cell numbers into biomass is required for the reconstruction of carbon fluxes through microbial food webs. Bacterial cell sizes and the relationship between cell size and dry mass can be determined empirically, e.g., by image analyzed microscopy (174, 274) and appropriate conversion factors (159, 197, 233). Some microbial taxa in the plankton form significantly larger cells than others (16), and population changes within such groups may thus contribute disproportionally to changes in total biomass (132, 222). The most extreme example are the filamentous bacteria in the water column of many lakes (130). Such morphotypes rarely form more than a few percent of total cell numbers, but they may temporarily

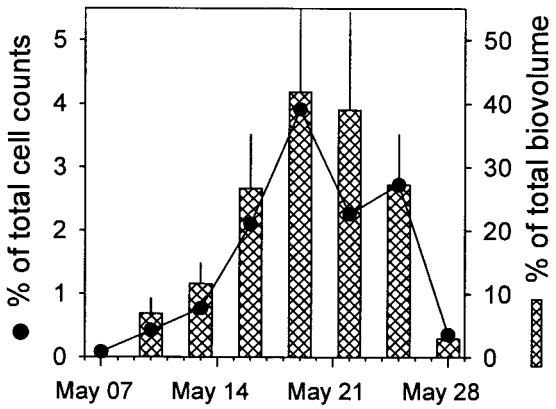

FIG. 5. Contribution of filamentous bacteria from the LD2 clade to total bacterial abundances (line and symbols) and biovolume (bars) in the spring plankton of a lake. (Modified from reference 223.)

constitute half of total microbial biomass or more (130, 286) (Fig. 5). Therefore, the biomasses of different microbial populations may need to be determined separately in order to assess their respective roles in the carbon flux through aquatic systems (46, 132, 216, 237).

Single-Cell Activities of Individual Populations

One important topic of aquatic microbial ecology has been the physiological properties of total microbial assemblages, in particular of the metabolic and respiratory processes that drive biogeochemical transformations (13, 53, 142). In contrast, traditional microbiological research is mainly centered on the physiological properties of bacterial strains in pure culture (109, 127). In between these two levels of analysis there is a conspicuous gap of information. Little is known about the growth-related characteristics of single microbial populations that are realized at environmental conditions in the presence of competitors, predators, viruses, substrate heterogeneity, chemical gradients etc. For example, the genome of the marine planctomycte *Rhodopirellula baltica* strain 1 contains >100 different sulfatases (90), but it is unclear which ecological advantage is associated with this feature (it might, e.g., reflect in situ growth on a complex mix of substrates such as sulfated polysaccharides). In order to understand the stability or fluctuations of a particular biogeochemical process, it would be important to distinguish if it is mediated by a single, physiologically highly versatile microbial population, or if it is carried out by several bacterial groups that may provide a greater functional redundancy (318).

With the exception of stable isotope analysis (236) or pulse-labeling of nucleic acids (302), physiological information is typically lost by methods that use DNA or rRNA extracts for microbial identification. By contrast, physiological properties can be readily related to a particular genotype at the single cell level. FISH by itself may already provide some information about the physiological state of a population, because the signal intensity of hybridization is proportional to cellular ribosome content. The ribosome content of marine isolates tends to increase with growth rate (138). However, rRNA concentration is a parameter that may sometimes be difficult to interpret. Some marine bacteria may maintain high numbers of ribosomes even during periods of prolonged starvation (68). This is probably essential to rapidly respond to changes in growth conditions in a patchy environment (212). Thus, ribosome content may allow a limited assessment of bacterioplankton "activity" at the community level (52, 80), but it needs to be interpreted with caution if single populations are to be compared. In addition, cell identification by FISH can be combined with a number of other methods that visualize particular physiological properties of individual cells, e.g., substrate uptake, DNA synthesis, respiration (47, 196, 213), and even with stable isotope probing (198).

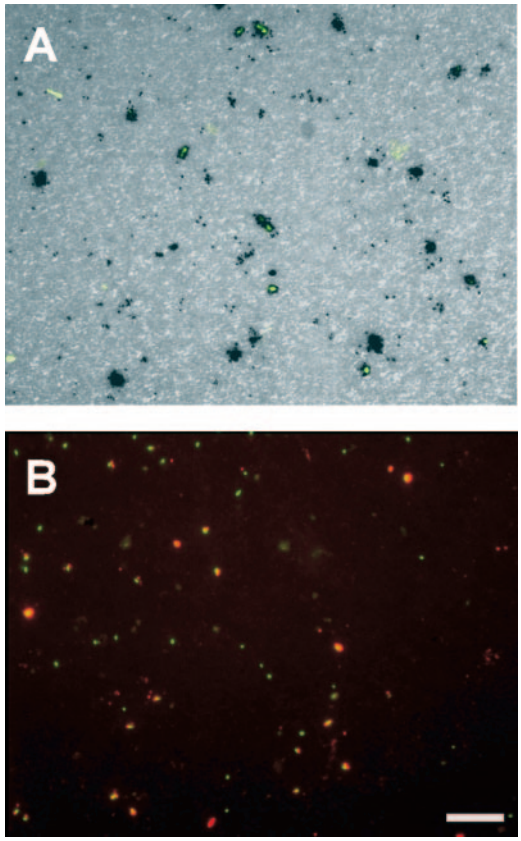

FIG. 6. (A) Glucose uptake into single cells under anoxic conditions as studied by FISH and microautoradiography. Green objects surrounded by black deposits are *Roseobacter* sp. cells from coastal North Sea waters that have incorporated radiolabeled substrate. (B) Immunocytochemical detection of DNA de novo synthesis in freshwater bacteria after incubation with bromodeoxyuridine. Green objects: cells affiliated with actinobacteria; yellow-orange objects: bromodeoxyuridine-positive actinobacteria; red objects: other bromodeoxyuridine-positive bacteria. Bar, 10 μm.

Microautoradiography. One of the most powerful approaches to study physiological activities in mixed microbial assemblages dates back to the 1960s. The uptake of radiolabeled tracer substrates into individual cells can be visualized by a photographic technique termed microautoradiography (30, 31) (Fig. 6a). In combination with cell identification by FISH, this approach allows us to assess the partitioning of substrate consumption between different microbial populations in mixed assemblages. Microautoradiography-FISH was first used to de-

termine organic and inorganic substrate uptake of ammonia-oxidizing bacteria and of other groups in activated sludge (154), but has since then been adapted for microbes in lakes and in the marine water column (4, 47, 98, 169, 199, 296, 306). Recently, protocols have been developed that integrate the superior CARD-FISH staining with microautoradiography for the analysis of bacterial substrate uptake in environments such as the mesopelagic zone (4, 296). It has even been attempted to add a quantitative aspect to microautoradiography by estimating the amount of incorporated radiolabel from the number of grains that are formed around active cells (46, 168, 170).

Without wanting to diminish the potential of microautoradiography, one should be aware of some limitations. Currently, there exist a variety of more or less time-consuming protocols for microautoradiography-FISH of water column bacteria, and some protocols likely cause a high loss of bacterial cells (47, 199). Moreover, it appears rather difficult to standardize some aspects of the procedure (the exposure time and photographic development) to an extent that would meaningfully allow us to quantitatively compare results from different studies. Finally, the choice of adequate tracer substrates may not be trivial. Comparatively little is known about the composition of the dissolved organic matter pool and about the turnover and the concentrations of specific organic carbon compounds in the water column. Sometimes the environmentally relevant substrates are not commercially available and have to be laboriously custom synthesized, e.g., chitin and proteins (47), or the marine algal osmolyte dimethylsulfonopropionate (DMSP) (306). In freshwater systems such as bog lakes much of the dissolved organic matter consists of a complex mix of high molecular weight substances (e.g., humic acids) (299). In these habitats it might be difficult to decide on appropriate model substrates.

Fluorescent activity tracers and flow sorting. In order to study particular aspects of growth in individual microbial populations there are technically less demanding alternatives to microautoradiography. Pelagic bacteria with an active electron transport system of the respiratory chain reduce tetrazolium salts to water-insoluble crystals (322). Such formazan grains are deposited intracellularly, and they can be detected both microscopically (247) and by flow cytometry (54). In combination with FISH cell respiration can thus be visualized in single microbial populations, as was shown for filamentous bacteria from activated sludge (196). However, the tetrazolium reduction method appears to be a rather insensitive means to distinguish between growing and non-growing (or dead) bacteria in the plankton. Accordingly, bacteria with visible formazan deposits are regarded as the most highly active fraction within a larger set of growing cells (268). While there may be good reasons to identify such highly active bacterial populations, no investigation has combined tetrazolium reduction and FISH to study microbes in the water column of natural aquatic systems.

Bromodeoxyuridine is a halogenated nucleotide analogue of thymidine that is incorporated into newly synthesized DNA of bacteria and eukaryotes (213, 245, 302). It provides a nonradioactive alternative to microautoradiography with tritiated thymidine (207) and it allows us to quantify growth rates at the single-cell level (116). Bromodeoxyuridine incorporation has been combined with CARD-FISH to visualize de novo DNA synthesis in different freshwater and marine bacterial populations (209, 213, 310) (Fig. 6b). This offers a sensitive means to detect changes of growth rates in single microbial populations in situ. During bottle incubations of filtered seawater, a rise in the numbers of bromodeoxyuridine incorporating *Alteromonas* sp. cells clearly preceded cell multiplication (213). Significant seasonal differences and short-term variability of growth rates were observed in members of the *Roseobacter* spp. and NOR5 lineages from coastal North Sea picoplankton (209). High bromodeoxyuridine incorporation by actinobacteria in mountain lakes indicated that these bacteria were not passively introduced from soils, but autochthonously growing members of the bacterioplankton community (310).

Flow cytometry may provide an alternative means of detecting activity or substrate uptake in single microbial cells. It allows the physical sorting of particular bacterial populations of interest for further analyses (22, 72). So far, microbial cells from plankton samples have been mainly sorted by phenotypic features, e.g., cell volume or cellular DNA or protein content (72, 150, 267, 326). Sorted bacteria have been analyzed by molecular methods (21, 323) and for radiotracer incorporation (151, 327). In contrast to microautoradiography, the tracer uptake rates of specific cell populations can be readily quantified by this approach.

Direct sorting of microbial cells after FISH staining has first been shown in highly productive wastewater treatment systems (284). Recently this approach has also been adapted for bacterial cells from coastal marine bacterioplankton, taking advantage of the superior signal intensities of CARD-FISH staining (262). Such a combination of cell identification and flow sorting potentially offers the ability to quantitatively investigate substrate uptake of single populations in natural samples (327). Moreover, it might eventually provide a means of obtaining functional genes or larger genome fragments from phylogenetically coherent groups of microbes directly sorted from environmental samples.

Distribution and Dynamics of Different Populations

For unknown reasons, heterotrophic aquatic microbes form large populations in particular habitats or at particular seasons, and are rare at other locations or time points (35, 63, 136, 144, 218, 280). One challenge of population ecology is to explain the observed distribution patterns of different bacterial taxa from their physiological properties and from their interactions with other organisms. Admittedly this may appear a rather far-fetched goal for a discipline that has just started to understand which microbes are frequent in different aquatic environments. However, the ability of macroecology to understand the role of individual plant and animal species is to a large extent based on an understanding of their distribution patterns and population dynamics at various environmental conditions. So far, only a few studies have investigated abundance changes of particular microbial taxa in the water column in a context of physicochemical parameters or food webs (63, 132, 136, 147, 223, 278).

For the purpose of gaining ecological insight from spatial or temporal distribution patterns, binary information about the presence or absence of a particular bacterial group, as provided by cloning or fingerprinting techniques, is probably insufficient. Such approaches allow us to detect fundamental

differences between communities, e.g., between marine and freshwater habitats (180), along rivers (264), or in mesocosm (244, 253), but they can hardly distinguish if a set of environmental variables is more or less favorable for a specific population. A too-coarse division of aquatic microbial assemblages, e.g., into subphyla of *Proteobacteria* by FISH with the respective probes (171), also suffers from drawbacks, since different populations with potentially contrasting dynamics might be put into ecologically meaningless categories (Fig. 2). Such studies may contribute to our understanding of large biogeographic divisions, e.g., between marine and freshwater habitats (89), and of basic discontinuities in the composition of microbial assemblages, e.g., along estuaries (28). However, both the qualitative community analyses by fingerprinting and investigations on large taxonomic units by FISH should be regarded as intermediate steps towards the quantitative study of ecologically coherent and phylogenetically more tightly-defined populations.

Experimental Enrichments in the Field

For decades, aquatic microbial ecologists have complemented descriptive studies on the distribution of microbes in various habitats with a range of simple field experiments. These have been referred to as "bottles, bags and buckets" (203), or more respectfully, as micro- to mesocosms, limnocorrals, enclosures, etc. Typically, various volumes of water are taken directly from the environment studied and this water is incubated at more or less in situ conditions after manipulation of, e.g., substrates and nutrients (61, 152, 177, 202), or of particular functional groups of the food web (16, 133, 293, 317). Depending on the container size, the duration of such experiments ranges from days to weeks. Short-term incubations share a basic logic with tracer uptake experiments: a measurable response to a manipulation should allow a deduction about the original state of the assemblage or some of its members. Sometimes dialysis bags with defined pore sizes may provide a more advanced alternative to bottles (80, 172, 278). Such bags allow readier exchange of dissolved substances if exposed directly in the water column. Even so, some features of the original environment are probably irreversibly destroyed, in particular the assumed continuum between the dissolved and the particulate organic matter (12). Larger containers may provide a useful means to artificially induce blooms of specific phytoplankton groups (244) or to manipulate metazooplankton densities (129, 223).

Such investigations are often plagued by the mysterious "bottle effect," a hard-to-define concept that reflects the worry of whether phenomena observed in confined assemblages are nonspecific consequences of the confinement rather than a result of the planned manipulation. Nevertheless, experimental mesocosms are among the few tools available to microbial ecologists that go beyond a purely descriptive analysis of aquatic microbial assemblages. A number of well-defined hypotheses about microbial populations have been successfully addressed by such approaches (16, 61, 132, 223, 244, 278, 293).

Defined Laboratory Communities and Pure Culture Studies

Experimental systems such as the Winogradsky column (316) have fundamentally shaped our understanding of environmental microbiology. However, concomitant with the rise of cultivation-independent methods, laboratory investigations on experimental communities have somewhat suffered from a lack of popularity among microbial ecologists. This may have been in parts a consequence of Thomas Brock's harsh words about studies on "mixed cultures of unknown provenance . . . at some ill-defined state" (32). It may also be related to the realization that many laboratory investigations have been performed on microorganisms that are readily culturable (112, 212) but that are rare in the water column (62, 312). These drawbacks may no longer apply. For one, an increasing number of aquatic microbes have been isolated during the past years that also form large populations in situ (63, 110, 238) and that thus represent adequate model organisms for laboratory investigations (27, 109). Second, 16S rRNA-based molecular tools now provide new means to precisely follow the population dynamics of different bacteria in mixed experimental assemblages (16, 221). An increasing wealth of genomic information from isolated environmental bacteria may eventually even allow us to link the performances of individual members of such model assemblages to the expression of particular genes in the context of a well-defined experimental setup.

Thus, laboratory investigations on bacterial isolates or model communities add another important dimension to the understanding of microbial population ecology. For example, particular physical structures within aquatic habitats cannot be preserved in experimental approaches in the field. Laboratory systems can artificially produce aggregated organic matter in natural water samples (101) and trap single flocs in a three-dimensional flux field (230). Such designs provide the adequate physical and chemical environment to study microbial activities and successions on organic aggregates and particles (100–102).

Laboratory studies may also provide a better control over parameters that might mask the hypothesized relationship between the studied population and the variable of interest. Continuous-cultivation systems, not necessarily chemostats (126), stabilize the composition of mixed assemblages by enforcing a minimal growth rate and by providing constant temperature, illumination, and input of substrates and nutrients. This allows us to experimentally sustain transient ecological phenomena over prolonged periods of time, e.g., the rise of particular predator populations (112, 221, 232, 234, 250, 279), bacterial-viral interactions (181), or the breakdown of cyanobacterial blooms (304).

Investigations on pure cultures offer the possibility to distinguish between phenotypic and physiological properties of different aquatic bacteria, e.g., in their interactions with algae (29, 99, 146), their chemotactic responses (15, 23), motility patterns (140), grazing sensitivity (27, 114), or cell filamentation (115, 273). This provides the opportunity to test hypotheses about the ecological relevance of such features.

OCCURRENCE OF MICROBIAL POPULATIONS IN PLANKTON

A Few Words on Diversity

Our knowledge of aquatic microbial diversity is largely derived from the analysis of 16S rRNA gene sequences directly PCR amplified from environmental DNA (239) and from cul-

ture collections predominantly established on rich solid media (107). Both approaches have revealed complementary and often nonoverlapping facets of diversity (but see reference 228). Nevertheless, our perception of the total diversity of aquatic microbes is most probably incomplete. A recent investigation by shotgun cloning of large genome fragments from Sargasso Sea picoplankton concentrates and high-throughput genome analysis has produced >100 novel bacterial 16S rRNA sequence types in a single sample (305).

Evidence has accumulated that there is relatively little overlap between the phylogenetic groups that are present in marine and freshwater environments (89, 180, 241), e.g., specific groups of archaea appear to be entirely limited to the marine water column (59, 76, 173). Lineages of 16 rRNA gene sequence types that occur in both habitat types include the SAR11 clade of *Alphaproteobacteria* (14, 85, 329), and the ammonia-oxidizing *Betaproteobacteria* (123, 297). Even within these clades, distinct clusters of marine and freshwater sequence types can be distinguished (329). Some clades of actinobacteria typically found in freshwater habitats also contain sequences from estuaries and marine waters (48, 309), whereas other clades within this phylum are exclusively of either marine or freshwater origin (91, 240, 309, 328). Members of the very diverse *Cytophaga/Flexibacter/Flavobacterium* group are common in some coastal and offshore marine habitats (45, 63, 144, 280), but they also occur in rivers and lakes (28, 143, 218, 223). Bacteria from this group appear to have radiated across a range of aquatic habitats, including biofilms and sediments (34, 158).

The phylogenetic ties between freshwater and soil microbes are still unclear. Sequence types from both environments have, e.g., been found within the freshwater acIV lineage of the actinobacteria (309). There are furthermore indications that microbial assemblages in some lakes may be similar to those in the influx from the catchment (156).

In the context of this review we distinguish between the multitude of microbial phylogenetic lineages that may occur in various aquatic systems and the few groups of microbes that have been shown to form substantial populations in such environments. Clearly, archival listings of microbial diversity from different habitats are a crucial first step to investigate the role and fate of aquatic microbes, since they provide the essential fundament for subsequent studies about the ecology of particular populations. However, it is equally important to progress from a purely qualitative appreciation of microbial diversity to the quantification of the abundances, biomasses, and activities of different phylogenetic groups. For example, it is presently still unclear if members of the *Verrucomicrobia* are an important component of freshwater assemblages. Such bacteria are frequently detected in lakes by PCR-based methods (157, 330, 331). From all we know, their densities might be one in a million but potentially also >10% of all cells. The following sections thus specifically discuss investigations that have established the cell concentrations, spatial distributions, temporal successions, or physiological features of specific microbial taxa in the plankton of marine and freshwater systems.

Bacterial Populations in the Marine Water Column

Some groups of marine bacteria had been known for years from their 16S rRNA gene sequences before their abundances in the water column were determined. This is the case for bacteria related to the marine SAR11 (188), SAR86 (210), SAR116 (71), SAR202 (189), and SAR406 (71) clades, whereas for the various lineages of marine actinobacteria (240) such evidence is still lacking. Members of the SAR11 clade are believed to be among the most common prokaryotes in the marine plankton. It has been reported that these bacteria may seasonally represent >50% of total bacterial abundances in surface waters of the northwestern Sargasso Sea and 25% of subeuphotic microbial assemblages (188). Bacteria related to *Roseobacter* sp. (also referred to as the SAR83 cluster, (241) are another common component of coastal and offshore microbial assemblages, and they may constitute up to 25% of the marine picoplankton (63, 95, 295). The seasonal abundance of *Roseobacter* spp. in coastal North Sea picoplankton closely followed the development of phytoplankton biomass (Fig. 7) (63, 95, 295). The geographic distribution of one particular subcluster from this lineage appears to be limited to temperate and polar oceans (266). In transects across the German Bight *Gammaproteobacteria* related to the SAR86 lineage on average formed 7% of total cell numbers (210), and 3 to 6% of all bacterial 16S rRNA genes in Monterey Bay surface waters were affiliated with this group during an upwelling event (295). Genes encoding proteorhodopsin were first described in members of the SAR86 clade (19), but recent findings indicate that such light-driven proton pumps might be a widespread feature of marine bacterioplankton (305).

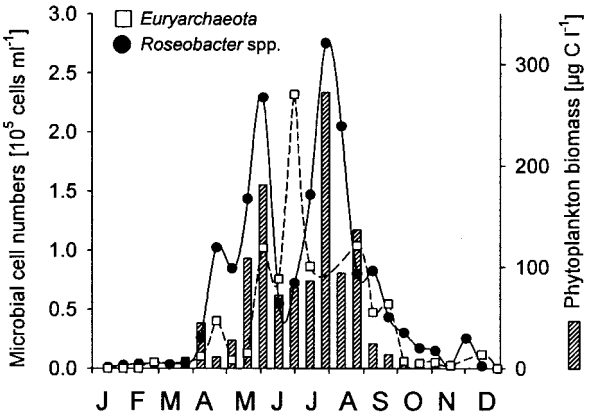

FIG. 7. Seasonal population dynamics of marine *Euryarchaeota* and of bacteria from the *Roseobacter* lineage in the German Bight of the North Sea. Bars: phytoplankton biomass. (Combined from references 63 and 214.)

In addition to the well-established clades of marine bacteria, new groups have been described that may reach high densities in the water column. The NOR5 lineage of the OM60 clade of *Gammaproteobacteria* (39) seasonally represented between 5 and 10% of coastal picoplankton in the North Sea (63) and 2–3% in the western Mediterranean Sea (L. Alonso and J. Pernthaler, unpublished data). Uncultured members of the novel DE2 cluster (*Cytophaga/Flexibacter/Flavobacterium* group) accounted for 10% of total cells in samples from the Delaware Estuary and from the Chukchi Sea (Arctic Ocean) (144). In brackish waters of the Baltic Sea and in samples from the Skagerrak-Kattegat front, substantial populations of species related to *Alphaproteobacteria* and to the *Cytophaga/Flexi-*

bacter/Flavobacterium group were detected using radioactively labeled whole-genome probes (226, 228). Interestingly, these bacteria also readily formed colonies on solid media, which clearly contrasts with findings from other marine sites (62).

The composition of microbial communities in more extreme habitats might sometimes be very simple. Hydrothermal circulation activities in deep sea environments produce buoyant plumes with substantially elevated levels of reduced chemicals. The bacterial assemblages within such a plume inside the caldera of the Suiyo Seamount volcano consisted almost entirely of two distinct phylogenetic populations that were related to sulfur-oxidizing symbionts of hydrothermal vent fauna (291). The caldera might thus represent a giant natural continuous-cultivation system for these two groups.

Sometimes, it may be necessary to define microbial populations at the level of single strains, e.g., for the study of pathogenic *Vibrio* spp. in marine waters. In coastal environments *Vibrio cholerae* can be found both attached on particles and free-living in the water column (44), and pronounced seasonal and horizontal differences in population sizes have been reported (83, 117). In microcosms spiked with *V. cholerae*, rapid growth of these bacteria was observed after addition of organic carbon (190). It is thus conceivable that anthropogenic eutrophication might indirectly favor the growth and dispersal of pathogenic *Vibrio* strains.

Marine *Archaea*

The two major pelagic lineages of *Crenarchaeota* and *Euryarchaeota* are among the most well studied phylogenetic group of uncultured microbes in marine picoplankton. Both oligo- and polynucleotide probes have been developed for the direct visualization of such microbes in water samples (57, 78, 192, 296). Recently, a protocol for CARD-FISH staining of *Archaea* in samples from the deep sea has been described (296). Originally it was believed that creanarchaeota only form large populations in the meso- and bathypelagic layers below the euphotic zone (59, 76). In a seasonal study in the North Pacific subtropical gyre, the mean annual abundances of *Crenarchaeota* below 200 m water depth ranged between 20 and 40% of total picoplankton cells, which corresponded to 3×10^3 to 1×10^5 cells ml^{-1} (136). Comparable abundances of this archaeal group were also reported from the deeper waters of the Antarctic circumpolar deep water (40). However, the same study also detected large crenarchaeal populations by FISH in the surface waters of the Southern Ocean during the winter months. Such contrasting vertical distribution patterns are currently difficult to interpret.

The metabolic capacities of planktonic crenarchaea are unclear, but there are indications that members of this lineage might be auto- or mixotrophic (120, 321). The planktonic marine *Euryarchaeota* on the other hand appear to be a common element of coastal assemblages and surface waters. Members of this lineage seasonally formed >30% of all cells in the surface picoplankton of the North Sea (214) (Fig. 7). Seasonal blooms of *Euryarchaeota* were also observed during a long-term study in surface waters of the upper Santa Barbara Channel (191).

Numerically Important Bacteria in Freshwater

Seasonal dynamics of different freshwater bacterioplankton populations have first been reported from an ultraoligotrophic mountain lake (218). So far, bacteria from two of the four typical freshwater lineages of *Betaproteobacteria* as defined by Glöckner et al. (91) have been detected in high abundance in the environment. Bacteria affiliated with the beta I clade (also termed the "*Rhodoferax* sp. BAL47 lineage") (328) formed populations of >10% in the summer plankton of a eutrophic reservoir (278). A second lineage of *Betaproteobacteria* related to *Polynucleobacter necessarius* (beta II) seasonally constituted up to 50% of all pelagic microbes in the aerobic waters of a meromictic humic lake (35). Filamentous bacteria from the LD2 subclade (328) that is closely related to *Haliscomenobacter hydrossis* (255) (*Cytophaga/Flexibacter/Flavobacterium*) transiently formed >40% of total bacterial biomass in a mesotrophic lake (223). Actinobacteria from the uncultured acI clade (91, 309, 328) are another ubiquitous group of freshwater prokaryotes (91, 309, 328) that seasonally occur in high densities in habitats of very different limnological characteristics, e.g., humic (35) or high mountain lakes (91, 263, 310).

Bacterial Populations Attached to Organic Particles

Microbial assemblages on suspended organic aggregates differ from those of the water column (56). So far the particle-attached communities in marine habitats have only been investigated qualitatively by fingerprinting and comparative sequence analysis (48, 56). More information is available about limnic and riverine aggregates. Microbial populations on aggregates in fresh waters change with the ageing of such particles (101) or during their transport from rivers into estuaries (265). Three populations related to the genera *Duganella*, *Hydrogenophaga*, and *Acidovorax* formed almost half of the *Betaproteobacteria* on organic aggregates obtained from Lake Constance at a depth of 50 m (261). These bacteria effectively colonized artificially produced microaggregates within 24 h. Interestingly, members of the three genera were rarely detected in the planktonic microbial assemblage, and they were not affiliated with the presently defined clades of typical freshwater *Betaproteobacteria* (91, 329). Instead, these bacteria are known from highly eutrophic environments such as activated sludge (249, 258).

In contrast to the lake snow assemblages, riverine organic particles have been described to mainly harbor *Betaproteobacteria* related to the drinking water biofilm bacterium *Aquabacterium commune*. These bacteria formed up to 30% of all cells on lotic organic aggregates in the river Elbe (135). The composition of the microbial assemblages on such aggregates showed pronounced seasonal changes, and bacteria related to the *Planctomycetales* were absent in winter.

FACTORS CONTROLLING MICROBIAL POPULATION SIZES

Competition for Different Substrates

The shortness of the following section does not reflect the importance we assign to the topic. Our current understanding of the relationship between the growth of individual bacterial

populations and the availability of particular substrates or nutrients is still rudimentary. For example, it is established that pelagic bacteria and archaea may incorporate amino acids (201), but with a few exceptions (see below) it is unclear if different genotypes show preferences for individual components of such mixes. Studies on the interactions between bacteria and specific algae indirectly suggest that substrate quality may play a role in the competition between microbial populations. Pure cultures of various algal species may harbor distinct microbial assemblages (252). Different microbial communities could be established from identical inocula in seawater micrososms if blooms of specific algal groups were induced (225). Cottrell and Kirchman reported (47) that various cooccurring microbial populations in pelagic habitats may be specialized on the degradation and uptake of particular substrate classes. Using microautoradiography, these authors showed that different bacterial genotypes also differed in their uptake of ^{3}H from radiolabeled chitin, proteins, amino acids, and *N*-acetylglucosamine. A preference for simple monomers over proteins in bacteria from the SAR11 clade has recently been reported by the same laboratory (168).

A set of recent investigations on the transfer and processing of phytoplankton-derived organic sulfur compounds has provided a first model for future studies of the relationship between particular bacterial populations and a specific biogeochemical process. Dissolved dimethylsulfonopropionate (dDMSP) is an algal osmolyte that is released during viral lysis or sloppy zooplankton feeding (50, 167). dDMSP is a growth substrate for heterotrophic bacteria (153), and it represents an important precursor of protein-sulfur in marine bacterioplankton (139). An increasing body of evidence is pointing to a few groups of marine bacteria as key elements in dDMSP biogeochemistry. Laboratory studies on isolates of the diverse *Roseobacter* clade have indicated a widespread ability of members of this lineage to degrade dDMSP and to incorporate the sulfur derived from this organic compound into cellular protein (93). In addition, large populations of bacteria affiliated with the *Roseobacter* clade were observed during dDMSP-producing algal blooms in the North Atlantic. In two independent studies the horizontal or vertical distribution of microbes from this group was positively correlated to dDMSP concentrations or fluxes (96, 323). Direct evidence of DMSP uptake was recently obtained by microautoradiography (170, 306). This approach also revealed that DMSP uptake may be a feature of different bacterial populations, and bacteria from the SAR11 clade rather than *Roseobacter* mediated the bulk of DMSP turnover in an offshore planktonic assemblage (169).

Patchiness and Gradients

Selective preference for particular substrates may not be the only factor affecting the competition between aquatic microbes. Specialization on a single resource might in fact be disadvantageous in an energy-deficient environment (60), and the majority of bacteria and archaea in pelagic marine environments are capable of incorporating mixes of radiolabeled amino acids (137).

A considerable proportion of the substrates and of bacterial productivity in freshwater and coastal habitats are distributed in organic particles and microscale patches (12). In contrast, the bathypelagic zone of the open oceans represents a rather desert-like environment poor in particulate organic matter. Individual microbial species or phylogenetic lineages within the bacterioplankton thus likely differ in their ability to succeed in habitats with steeper or flatter substrate gradients. An "opportunistic" growth strategy might be widespread among those bacterial groups that successfully colonize organic aggregates or other nutrient-rich microniches. A marine *Pseudoalteromonas* sp. strain exhibited a significantly shorter growth delay than an *Oceanospirillum* sp. under "feast-and-famine" batch culture conditions, but the former strain experienced a growth disadvantage if substrate changes were gradual (212). The importance of such bacteria may vary between habitats, and different species of "opportunistically" growing microbes are found in marine and freshwater environments (224).

Representatives of "opportunistic" genera of *Gammaproteobacteria* that are readily culturable on solid media were generally rare in the bacterioplankton of shallow North Sea waters (62). In contrast, colony-forming bacteria represented a prominent fraction of the microbial assemblages in brackish waters of the coastal Baltic Sea (227, 228). Readily enrichable bacteria were also found in high concentrations in the plankton of a lake with high irregular input of organic carbon from the catchment (35). The identification and quantification of such "r-strategists" within heterotrophic picoplankton assemblages might thus eventually allow deductions about short-term fluctuations in the availability of organic matter in aquatic habitats, e.g., for a biological monitoring of pollution effects. In contrast, many if not most of the typical free-living marine and freshwater bacteria appear to lack the ability to form colonies on solid media (282), and their growth is negatively affected by enhanced substrate levels (289).

It is likely that many bacterial species in the water column combine elements of the oligotrophic and the "opportunistic" growth strategies in their life cycles, e.g., by alternating between free-living and attached growth forms (141). Isolates from freshwater plankton that were initially oligocarbophilic could gradually be adapted to richer conditions (114). Bacteria from the marine NOR5 lineage, which are apparently free-living in the water column, also formed colonies on low-nutrient agar plates, albeit significantly later than other "opportunistic" strains (63). A dual-niche existence may also explain why facultatively anaerobic bacteria appear to be such a common component of coastal bacterioplankton assemblages (4, 243). In shallow waters of the North Sea >80% of free-living *Roseobacter* spp. cells were able to incorporate glucose at both oxic and anoxic conditions (4). Since the average depth of the German Bight is only 20 m, bacteria from the *Roseobacter* spp. lineage that colonize aggregated senescent algae (97) might experience temporary anoxia while settled on the sediment surface. These bacteria could be reintroduced into the water column by the periodic resuspension of particulate organic matter (179).

Mortality

So far there is little evidence that a lack of resources is a major cause of bacterioplankton mortality. Cultured strains that are thought to be representative for the pelagic environment are often oligocarbophilic (238, 259), or they survive

extended periods of starvation (68). Predation and viral lysis are believed to be the key factors that counterbalance microbial growth in the water column (13, 77, 271). Growth inhibition or cell damage induced by UV radiation may be another important ecological factor in some aquatic habitats (10, 119, 285). *Nevskia ramosa*, a species inhabiting the neuston layer of fresh waters, exhibited elevated resistance to UV (290). Some alpine lakes exposed to intense levels of UV-B radiation (148) feature conspicuously high abundances of actinobacteria, a phylogenetic lineage of mainly gram-positive bacteria with a high genomic G+C content (91, 263, 310). Gram-positive bacterial isolates are often less affected by UV radiation than gram-negative bacteria (8), and a high genomic G+C content has been suggested to mediate higher resistance to radiation damage (166).

Viral lysis. The current concepts of specific viral-bacterial interaction in aquatic systems are largely shaped by theoretical models (298), and presently there are almost no investigations about the influence of viral lysis on the coexistence of individual microbial populations in the plankton (260, 317). Viral influence on the growth of different bacteria might act both directly by "killing the winner" (298), but also indirectly via the release of dissolved organic matter and nutrients from lysed pro- and eukaryotic cells (92, 182). Rapid development of resistance to viral infection has been observed in some bacterial strains during continuous-culture experiments (181), but it is unclear if such a process will also occur in natural aquatic assemblages. Recently, changes of the species richness of marine archaea have been reported as a likely consequence of experimentally manipulating viral densities (317). For a detailed discussion of the influence of viruses on aquatic microbial assemblages, readers should refer to specific reviews on the subject (311, 319).

Selective predation. Hetero- and mixotrophic protists, in particular nanoflagellates and ciliates, are the main consumers of picoplankton in the marine and freshwater pelagic zone. Their role in controlling the abundance, biomass, and productivity of microbial assemblages has been amply documented (269, 298). Many of these predators are omnivorous, i.e., they can feed on a large range of bacterial species. However, they are not unselective feeders. In the following we will focus on predation selectivity that is related to prey cell morphology, in order to illustrate the influence of a phenotypic feature on the success of different genotypic microbial populations. Other aspects of microbial predator-prey interactions are discussed elsewhere (25, 82, 131, 215, 277).

Due to their specific particle uptake mechanisms and handling procedures, protistan predators cannot feed on all bacterial shapes or cell size classes with equal efficiency (26, 178). As a consequence, microbial cells within a length range of 1 to 3 μm are preferably ingested by heterotrophic flagellates and ciliates, whereas smaller or larger cells profit from reduced loss rates (130, 186, 276). Even small differences in cell sizes between strains of freshwater ultramicrobacteria may have large effects on their survival rates (27). Microbial assemblages that are exposed to high grazing pressure thus typically harbor high abundances of extremely small cells (222). In contrast, large filamentous bacterial morphotypes and bacteria that form microcolonies are substantially more protected from protistan grazing (115, 273). Filamentous morphotypes accumulate during periods of high predation in fresh waters (134), but they are rarely observed in marine systems. Some strains of freshwater *Betaproteobacteria* produce microcolonies within a sponge-like matrix of extrapolymerous substances that renders them resistant to flagellate predation (113).

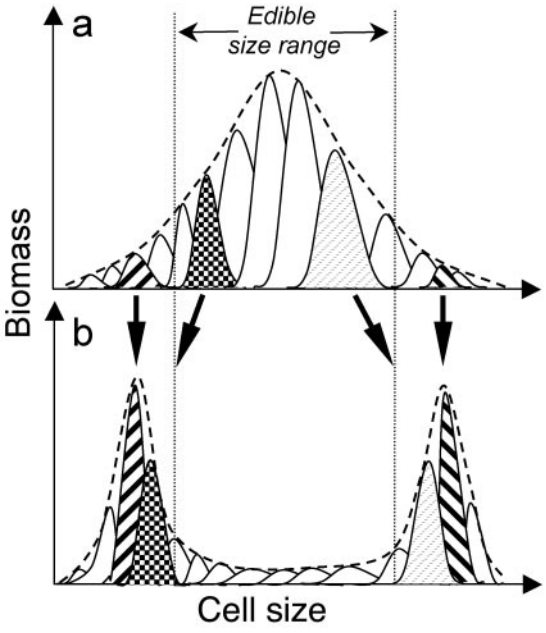

FIG. 8. Effect of size-selective protistan grazing on bacterioplankton biomass size distribution. Several microbial populations with different mean cell sizes are highlighted. (a) Low or no grazing: unimodal cell size distribution. Most biomass is distributed in populations close to the mean size, whereas very small or large populations are rare. (b) Heavy grazing: populations within the edible cell size range are eliminated unless they can escape predation by a change in their mean cell sizes. Under these conditions the growth of very small or very large phenotypes is favored. (Reproduced with permission from *Nature Reviews Microbiology* [215] copyright Macmillan Magazines Ltd.)

Size-selective predation induces shifts in the genotypic composition of mixed assemblages by imposing different mortality rates on bacterial species with different mean cell sizes (Fig. 8) (221). First evidence for such community changes originated from continuous-cultivation experiments on microbial assemblages that formed stable associations with freshwater algae (221, 234). Selective grazing mortality is moreover believed to set an upper limit to the standing stocks of some microbial populations in the plankton. For example, bacteria from a cosmopolitan lineage of freshwater *Betaproteobacteria* (beta I) constituted approximately 10% of the summer assemblage in a eutrophic drinking-water reservoir (278). After removal of protists and in situ incubations in dialysis bags, members of the beta I clade increased to almost 30% of total cells within 24 h. It is thus likely that these bacteria contributed disproportionally to the flux of organic carbon from the picoplankton to the higher trophic levels. Some genera of "opportunistically" growing *Gammaproteobacteria* (*Alteromonas*, *Vibrio*, and *Pseudoalteromonas*) are probably rare in coastal surface picoplankton because they are almost completely suppressed by size-selective grazing (16). This mechanism might also play a role in eliminating pathogenic *Vibrio* spp. from the water column

(165).

On the other hand, grazing-resistant genotypes rapidly accumulate in assemblages exposed to high protistan predation. In nonaxenic continuous cultures of the phytoflagellate *Cryptomonas* sp., filamentous *Betaproteobacteria* were only observed if protistan predators were added (279). A filament-forming *Comamonas acidovorans* strain outcompeted a *Vibrio* sp. strain after the addition of bacterivorous flagellates to chemostat cocultures (112). Grazing-resistant bacterial morphotypes from different phylogenetic lineages increased rapidly after food web manipulation in water from fishless ponds (132, 147). A substantial enrichment of filamentous *Flectobacillus* sp. was observed during artificially induced blooms of nanoflagellates in samples from a eutrophic freshwater reservoir (278). Threadlike bacteria of >99% 16S rRNA sequence similarity formed >40% of total bacterial biomass in a mesotrophic lake during high protistan grazing (223). Such a natural enrichment of grazing-resistant genotypes in fresh waters appears to be an ephemeral phenomenon, as these bacteria are typically sensitive to predation by larger, filter-feeding metazooplankton (129).

The interplay between resource availability and mortality in determining the population sizes of different bacterial taxa is poorly understood. Changes in bacterial community composition might be related to changes in the ratio of bacterial mortality rate to growth rate (277). This would imply that communities with apparently stable taxonomic composition may be encountered at very different levels of total microbial productivity or mortality and that profound community changes are induced by shifts from top down to bottom up or vice versa (235). One type of natural model system to study such interactions might be freshwater reservoirs that feature pronounced longitudinal substrate and nutrient gradients between the river influx and the dam areas. Experiments that combine the transplant of water from different positions of the gradient with food web manipulations (80) might eventually shed more light on the complex control of different microbial populations by top-down and bottom-up forces.

OUTLOOK

The future of environmental microbiology will be shaped by the current advances in genome sequence analysis of cultured and uncultured microbes. This appears to create a favorable conceptual environment for a population-based perspective in aquatic microbial ecology. Already, the analysis of genomic information has strengthened the interest in the cultivation of abundant heterotrophic microbes from the water column (39, 63, 110, 204, 238). Such activities will probably draw increased attention to the unique physiological features of different genotypes and thus to the potential ecological differences between cooccurring microbial populations in mixed assemblages.

Some genomic information will moreover doubtlessly be of direct interest for the study of aquatic microbial populations in situ. Emerging techniques such as a combined FISH staining of mRNA and rRNA (208) will allow us to detect the expression of metabolic genes within bacterioplankton populations defined at the level of single ecotypes (41). Ideally, such gene-specific probing could be applied in combination with other ecologically relevant features of bacterial cells, e.g., morphology (223), proliferation (213), substrate uptake and turnover (324), motility (104), exoenzyme activity (195), or polysaccharide sheath formation (288). Moreover, a more complete knowledge of microbial genomes will allow us to detect far more subtle phylogenetic differences between bacteria than what can presently be derived from the analysis of 16S rRNA genes (315). Questions addressing the biogeography (43, 315) and physiological or evolutionary adaptation of microbial ecotypes (27, 109, 128) might therefore form another area of future research in environmental microbiology.

However, the ties between environmental genomics and microbial population ecology should not be overstated. The population sizes of different microbes in the environment cannot be predicted from their genomic constitutions only, and the success of different bacterial taxa is codetermined by their interactions with competitors and predators in the context of the physicochemical environment. Moreover, while the existence of microbial biogeography arguably is debatable (66), this controversy cannot be a central concern of population ecology. Even if in principle "everything is everywhere" (18), it is known that different groups of heterotrophic aquatic microbes exhibit distinct temporal, spatial, and geographic distribution optima. Thus, a major challenge of the coming decade will be to understand the underlying reasons for the heterogenous distribution patterns of different microbial populations in marine and freshwater plankton.

ACKNOWLEDGMENTS

We thank our students and colleagues for all the fruitful discussions and controversies that helped to shape our ideas and David Kirchman and two anonymous reviewers for their helpful comments on the manuscript.

We acknowledge the institutions that have supported our research on the topics discussed here, in particular the European Union (EVK3-2001-00194 BASICS), the German Ministry of Education and Research (BMBF01 LC0021/TP4), the German Research Foundation (PE-918/2-1), and the Max Planck Society.

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