MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, Mar. 2000, p. 69–114 Vol. 64, No. 1
1092-2172/00/$04.00+0

Virioplankton: Viruses in Aquatic Ecosystems†

K. ERIC WOMMACK‡ AND RITA R. COLWELL*

Center of Marine Biotechnology, Baltimore, Maryland 21202, and Department of Cell and Molecular Biology, University of Maryland, College Park, Maryland 20742

INTRODUCTION

From studies of the genetics and biology of viruses has come a more profound understanding of the basic biological processes of life, not the least of which has been the discovery of DNA as the carrier molecule of genetic information (125) and mRNA as an intermediate molecule in the transfer of genetic information to the ribosomes (43). Other breakthroughs in molecular biology attributable to bacteriophage models are the definition and mapping of the first gene (18); discovery of the discontinuous nature of DNA replication (222); discovery of restriction endonucleases (212); and the mechanics of gene regulation (261). Indeed, basic research on the biology of the bacteriophage has been fundamental to the establishment of the field of molecular biology (73). The value of basic research to technological and economic advancement is perhaps best illustrated by the historical link between basic bacteriophage biology and the present-day, multibillion dollar biotechnology industry.

In contrast to extensive information on the biology and genetics of viruses, there is only a limited understanding of the

* Corresponding author. Mailing address: Center of Marine Biotechnology, 701 E. Pratt St., Baltimore, MD 21202. Phone: (703) 306-1000. Fax: (703) 306-0109. E-mail: colwell@umbi.umd.edu.

† Contribution no. 317 from the Center of Marine Biotechnology.

‡ Present address: Department of Marine Sciences, School of Marine Programs, University of Georgia, Athens, GA 30602.

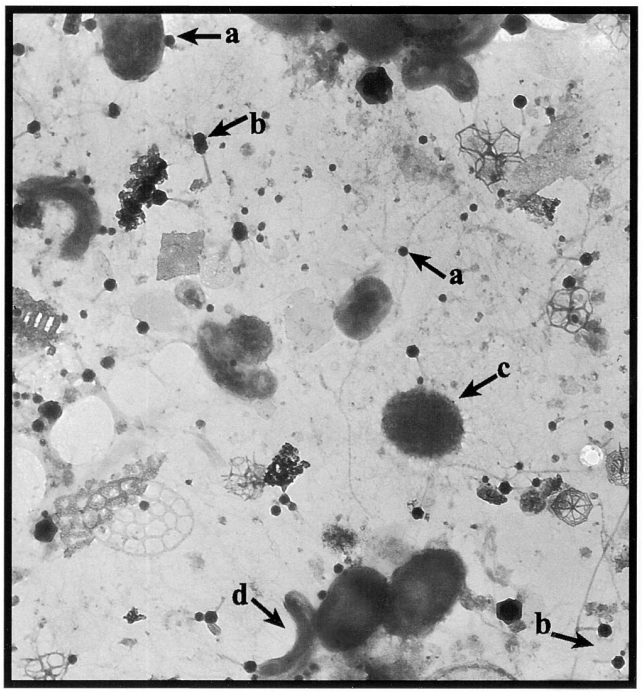

FIG. 1. Transmission electron micrograph of an unfiltered Chesapeake Bay water sample (magnification, ca. ×36,000). a, short-tailed or nontailed virus-like particle; b, tailed virus-like particle; c, bacterium, coccal morphotype; d, bacterium, vibrio morphotype.

occurrence and distribution of viruses in microbial ecosystems and in situ relationships between viral and host communities in the natural environment. The lack of recognition of viruses as naturally occurring organisms was most notably exposed with the discovery that viruses are abundant in a variety of aquatic environments, often exceeding bacterial abundance by an order of magnitude (19, 257). It was a surprise to learn from direct transmission electron microscope examination of marine virio- and bacterioplankton the astounding abundance of virus-like particles in seawater, considering that marine bacteriophages were first described in detail more than 35 years ago (301, 302, 331, 332). As an example, a transmission electron micrograph of unfiltered Chesapeake Bay water is shown in Fig. 1. The realization that in most aquatic environments the virioplankton is the most abundant plankton class has revived scientific investigation into the natural state of viruses in aquatic environments. Important questions raised by discovery of the abundance of viruses in natural ecosystems challenge accepted views of the aquatic microbial food web and the hypothesized singular role of obligate parasites in controlling microorganism population abundance and diversity.

SCOPE OF THE REVIEW

This review is divided into four sections. The first and second sections cover methods for viral direct counting, reports of naturally occurring virioplankton abundance in a variety of aquatic environments, and the correlation between changes in virus abundance and changes in other important ecological parameters. The third examines aspects of aquatic bacteriophage biology which have a significant influence on host infection in aquatic environments. The final section focuses on viral infection and lysis, as both a factor in the mortality of host populations and a mechanism influencing genetic and clonal diversity of host populations. In general, the discussion is focused on in situ measurement of virioplankton populations. Model phage-host systems are included, as appropriate, for background information. Readers interested in aquatic phage-host relationships will find relevant information in reviews by Børsheim (29) and Proctor (253) and an earlier review by Moebus (193). A brief synopsis of current views on marine virus ecology was recently provided by Furhman (91). The distribution and survival of human and animal pathogenic enteroviruses in aquatic environments are not covered herein, since detection and distribution of human disease-causing viruses in natural waters have been extensively reviewed elsewhere (99, 103, 104, 112, 182).

ENUMERATION OF VIRUSES IN WATER SAMPLES

Introduction

Discovery of the abundance of viruses in natural waters reflects the development of direct counting methods for bacterial enumeration. From the use of direct-counting methods to enumerate bacteria in environmental samples (127, 381), it has been found that viable counts, obtained using culture methods, significantly underestimate the number of bacteria in the sample. This finding is not surprising since it was suspected from the early days of bacteriology, when staining methods were used to enumerate bacteria. Newer techniques yielded results that led many investigators to reject conclusions regarding the ecology of bacteria obtained solely by culture. Molecular methods developed for analyzing bacterial population dynamics and diversity have revealed large populations of unculturable bacteria in the environment, further fueling speculation that bacterial diversity may be 100 to 1,000 times greater than that suggested by results of studies involving culture methods (60).

Indirect, Viable Counting of Bacteriophage and Viruses in Water Samples

Indirect titer determination by plaque assay (4), coupled with the most-probable-number method (150, 309), has routinely been used to enumerate viruses in water samples but has only recently been used to elucidate the ecology of viruses. For example, the abundance and distribution of coliphages in natural water samples have been determined by a plaque assay (99, 118, 244). In general, estimates of the abundance of specific viruses by culture methods have been so low that preconcentration of viruses was necessary prior to inoculation and enumeration (titers per liter). The distribution and abundance of phages infecting autochthonous bacterial hosts in a natural body of water, such as the Chesapeake Bay, illustrate the difficulty in interpreting data on the abundance of specific bacteriophages by culture assay (K. E. Wommack, R. T. Hill, J. Ravel, and R. R. Colwell, Abstr. 96th Gen. Meet. Am. Soc. Microbiol. 1996, abstr. N-23, p. 159, 1996). For example, 36 water samples collected at six stations during the year yielded only 10 samples that were positive for bacteriophages infecting one or more of the indicator strains. Of the 10 successful bacteriophage isolations from Chesapeake Bay water samples, only two of the titers exceeded the detection limit of 1 PFU. After taking into account the 10- to 100-fold concentration of virioplankton within the water samples, 7 PFU liter^{-1} was the final abundance estimate. However, direct microscopic examination revealed 100- to 1,000-fold more virus particles in each water sample.

Even though culture-based methods are not efficient in the

enumeration of viruses, they remain indispensable for isolation and purification of virus-host systems. There are exceptions, however, most notably the methods used to study the distribution and abundance of phages of marine *Synechococcus* spp. Cyanophages that infect two *Synechococcus* strains isolated from Woods Hole harbor demonstrated stable annual cycles of abundance, ranging from winter lows of 10 ml^{-1} to late-summer highs of between 10^3 to 10^4 ml^{-1} (341). These abundances were recorded directly from analyses of seawater samples, without preconcentration, using a most-probable-number method and broth dilution cultures. Large numbers of cyanophage, often in excess of 10^5 ml^{-1}, have also been found in hundreds of water samples collected in the western Gulf of Mexico (96, 312, 313).

Obviously, culture-based methods have proven very useful in the study of cyanophage ecology. Cyanobacteria are a relatively well characterized group of aquatic bacteria. *Synechococcus* spp., used as host strains for titer determinations, were similar in genotype to members of the natural host community, a factor helpful in the detection of cyanophages in environmental samples. Waterbury and Valois (341) noted that the *Synechococcus* strains most successfully employed in titer determinations were those which had lost phage resistance, a result of a long time lapse (>2 years) since clonal isolation. The most-probable-number method was used to enumerate cyanophages (308), and broth culture may have enhanced the detection of these bacteriophages in natural waters. In contrast, the most-probable-number technique has been successful in enumerating bacteriophages infecting aquatic heterotrophic bacteria only in the case of *Aeromonas* spp. (85).

The majority of bacterioplankton has been presumed to consist of heterotrophic bacterial species (72, 128). However, very little is known about the community structure of heterotrophic bacteria, not even which species are numerically dominant. In retrospect, reliance on culturing methods can explain why nearly 35 years after Spencer (302) reported the isolation of a marine virus, i.e., *Photobacterium phosphorium* bacteriophage, the dogma persisted that viruses were rare in natural waters and therefore were without significance for their host populations. As we have known for over three decades, ever since the report of MacLeod (169), only a small proportion (1 to 2%) of naturally occurring heterotrophic aquatic bacteria can be grown in culture. Given that hundreds, if not thousands, of bacterial species are believed to comprise the bacterioplankton, any single bacterial species selected for estimation of phage populations will very probably not be a dominant species within the bacterioplankton host community. Therefore, phage titers, obtained with a few host strains, will underestimate virioplankton abundance.

Direct Counting of Viruses in Water Samples

Direct enumeration methods for viruses have facilitated the analysis of viral abundance in natural waters. Transmission electron microscopy is now used for direct enumeration of viruses, with one of the earliest transmission electron microscopy (TEM) observations of viruses in seawater samples having been made by Sieburth (292), who published a compendium on the diversity of marine microbial communities. In 1979, Torella and Morita (326) provided the first direct counts of viruses occurring within the >0.2-μm size fraction of plankton samples collected from Yaquina Bay, Oregon. At $>10^4$ ml^{-1}, the abundance of virus-like particles in filtrates of Yaquina Bay water was the highest recorded at the time. It was postulated that viral numbers were actually much larger, since many viruses pass through a 0.2-μm-pore-size filter. These findings provided the initial supporting evidence for the proposal of Weibe and Liston (354) that viral lysis and infection could both influence the growth and diversity of bacterioplankton and mediate genetic exchange. Despite these early, groundbreaking observations, active research on the ecological importance of viral infection languished for almost 20 years.

In 1988, two important studies showed that viral abundance in the range of 10^6 ml^{-1} occurred in seawater. Using epifluorescence microscopy and a double-stranded DNA (dsDNA) binding fluorochrome, 4′,6-diamidino-2-phenylindole (DAPI), Sieburth et al. (293) reported 5.8×10^6 blue-fluorescing particles per ml in water samples collected during a monospecific bloom of a newly described chrysophyte alga, *Aureococcus anophagefferens*. Prior to enumerating free viruses in water samples collected during the bloom in Narragansett Bay, TEM observations of virus particles within *Aureococcus anophagefferens* cells had been made. In exploratory mesocosm experiments designed to investigate the potential of viral infection for bacterioplankton mortality, Proctor et al. (257) added virioplankton concentrates to water samples collected from the Eastern Caribbean. A significant impact of viral infection was apparent when, after a 24-h incubation period, the bacterioplankton abundance was 25 to 40% lower in mesocosms to which viruses had been added. TEM direct counts of virus-like particles in concentrates indicated a natural virioplankton abundance of between 10^3 and 10^6 ml^{-1}.

Since direct counting can now be considered critical in studies of virioplankton ecology, comparison of the efficiency of methods used to count viruses is useful. Details of methods for direct counting of viruses are provided by Suttle (308, 309) and Bratbak and Heldal (35). The earliest and most commonly used method for viral direct counts (VDC) in water samples is TEM. From the results of studies by Proctor et al. (256, 257), Bergh et al. (19), and Sieburth (293), TEM has evolved into the benchmark for virus enumeration. Virus morphology, including capsid and tail size, can be determined by TEM.

The most commonly used TEM method is direct sedimentation of free virus particles from an unfiltered, glutaraldehyde-fixed water sample onto a fine-mesh, Formvar-coated copper grid. For most water samples, prefiltration prior to centrifugation is not necessary. However, there are technical as well as practical disadvantages of direct virus enumeration. In water samples containing large amounts of particulate matter, viruses are obscured on the grid and therefore cannot be counted. Because of this problem, viruses in water samples collected from the upper reaches of Chesapeake Bay could not be detected (368). Suttle (309) has addressed two additional problems in centrifuge pelleting of viruses onto TEM grids, namely, that viruses do not sediment in parallel paths and therefore are concentrated toward the edges of the bottom of the tube, and that very small viruses are not recovered with 100% efficiency. The result is an underestimation of virioplankton abundance. To minimize centrifugation bias, viruses are counted using replicate grids placed at the bottom of a centrifuge tube. Because of the high magnification required to identify virus-like particles on TEM grids, the practical detection limit of TEM direct counting is 10^5 viruses ml^{-1}, a detection limit high enough to prevent the application of TEM direct counting of samples collected from oligotrophic environments, where the number of viruses in the water is small (138). Finally, there is loss of precision because of uneven staining and nonhomogenous distribution of viruses on the grids. TEM counts of viruses in natural water samples yield an average coefficient of variation of 20 to 25%, versus 7 to 11% for epifluorescence microscopy (122, 349).

To enumerate viruses in samples of oligotrophic water with-

out having to centrifuge the samples, virioplankton can be concentrated by ultrafiltration. In fact, ultrafiltration is essential in virioplankton ecology. Methods for ultrafiltration and concentration of naturally occurring viruses include vacuum filtration (367), centrifugal filtration (97), pressure filtration through either a hollow-fiber filter (254, 256), or a spiral-wound membrane filter (52, 316), and vortex flow filtration, a variant of pressure filtration (139, 240). Viruses in virioplankton concentrates are either pelleted during centrifugation or dried onto a TEM grid and then stained. Ultrafiltration, while improving the detection and enumeration of virioplankton and perhaps even eliminating centrifugation, introduces a selective bias. As a step in obtaining direct counts of viruses, it can affect recovery efficiencies, e.g., 9 to 117% ($\bar{x} = 42 \pm 42$) for vortex flow filtration (138, 240) and 68 to 171% ($\bar{x} = 114 \pm 30$) (mean ± standard error) for vacuum filtration (367), indicating low but variably reduced counts. The strongest evidence of ultrafiltration bias was obtained by analysis of covariance, comparing direct counts of viruses with and without ultrafiltration. Overall, viral abundances estimated using unprocessed samples were nearly three times greater than those estimated using concentrated samples (171). Methodological limitations aside, ultrafiltration has made possible the examination of virioplankton in both oligotrophic water samples and mesocosms in which virus-host population concentrations have been altered. In general, ultrafiltration provides conservative virus counts.

The practical limitation of TEM direct counting is the equipment required, i.e., a transmission electron microscope and ultracentrifuge. Thus, it is not suitable for most field studies and is less likely to be available to investigators at small research institutions. Also, sample preparation and analysis are tediously slow. These limitations can be bypassed by using epifluorescence light microscopy (ELM). In essence, the latter is a variation of direct counting of bacteria. Nucleic acid within the viral capsid is stained with a nucleic acid binding fluorochrome (usually dsDNA). Stained viruses are captured on a small-pore-size filter ($\leq$0.02 μm) and visualized at high magnification ($\geq\times$1,000) by light emission of the bound fluorochrome stain. Successful enumeration of viruses generally requires intensification of the fluorescent signal by means of a charge-coupled device camera (97) or photographic processing designed to increase film speed (116). Several different dsDNA binding fluorochromes, e.g., DAPI, Yo-Pro, and SYBR, have been used as the staining agent. An obvious caveat for those who use ELM to count viruses is that the overlap in size between small bacterioplankton cells and large virus-like particles, in some environments, can be a source of error in direct counts of viruses (299).

With improvements in the specificity and fluorescence yield of dsDNA binding fluorescent stains, ELM methods for direct counting of virioplankton now approach a precision level similar to that of TEM. Initial trials, using DAPI (Table 1), found relatively close agreement between TEM and ELM counts of viruses in Japanese coastal water samples (TEM/ELM ratio $\cong$ 0.8) (115, 116), in phage lysates of laboratory cultures (TEM/ELM ratio $\cong$ 1) (254), and in virioplankton concentrates from Santa Monica Bay (TEM/ELM ratio $\cong$ 1.6 ± 0.37) (97). In only one study, using water samples from the Gulf of Mexico and Tampa Bay, did TEM counts greatly exceed ELM (DAPI) counts (TEM/ELM ratio $\cong$ 10 to 18) (240). Discrepancies among these studies probably can be ascribed to methodological differences, especially for the Gulf of Mexico and Tampa Bay samples, where image intensification was not utilized.

More recently, two other nucleic acid binding fluorochrome stains, Yo-Pro-1 and SYBR Green I, have been used for virioplankton enumeration (144, 173, 214). Specific advantages of these stains are low background staining and stability and brightness of fluorescence greater than that of DAPI. A notable disadvantage of Yo-Pro-1 is the 2-day incubation needed for adequate staining of virioplankton samples trapped on an aluminum oxide filter. Subsequently, it was reported that the cumbersome 48-h incubation time could be reduced to 4 min through microwave pretreatment of virioplankton samples (376). In several trials, Hennes and Suttle (122) found that ELM (Yo-Pro) virus counts exceeded those of TEM by a factor of ca. 3 ± 1.6. Similarly, Weinbauer and Suttle (349) observed that estimates of virioplankton abundance obtained by TEM averaged ca. 66% of those obtained with ELM (Yo-Pro). For the same Gulf of Mexico water samples, ELM virus counts obtained using DAPI staining averaged 86% of the Yo-Pro counts.

Two recent reports indicate that SYBR Green I may emerge as the best solution for epifluorescence counting of virioplankton (173, 214). Unlike Yo-Pro, SYBR Green I stains only viruses and cells in seawater samples with a short (<15-min) incubation and is not affected by the presence of aldehyde fixatives (214). As with Yo-Pro, SYBR Green I virus counts yielded a precision similar to that of TEM counts and generally exceeded TEM direct counts. In a most exciting methodological advancement, SYBR Green I staining has been combined with flow cytometry for rapid enumeration of virioplankton in seawater samples (173). Flow cytometric analysis of virioplankton populations in water samples collected from different depths in the Mediterranean Sea revealed two distinct virus populations, based on size. If the majority of the bright, Yo-Pro-1- or SYBR Green I-stained particles are viruses, most virus counts obtained through TEM (Table 1) are very conservative and probably underestimate the actual population size.

Characteristics of Aquatic Virioplankton

It is likely that virioplankton, besides being most abundant, is the most diverse component of plankton. Hundreds of viruses infecting a wide range of phyto- and bacterioplankton species have been isolated and described. However, as with aquatic bacteria, these represent only a small portion of virioplankton diversity. For example, recently it was discovered that in some marine environments, archaebacteria make up a significant proportion of the bacterioplankton (69, 70). The archaea were previously believed to be restricted to extreme environments characterized by high salinity, high temperature, or anaerobiosis (224). Only a few viruses infecting archaebacteria have been described (380) because of the difficulty of culturing the hosts and the slow growth of archaeal viruses. Archaea make up to 34% of bacterioplankton abundance in Antarctic coastal waters (70) and 40 to 60% of total bacterial abundance in temperate ocean midwaters (200 to 600 m) (94); therefore, it is probable that archaeal viruses constitute a significant proportion of the virioplankton in these environments.

At present, direct TEM examination of aquatic viruses is the most common method of recording virioplankton diversity. While morphological data give only a limited view of virioplankton diversity, these data have been cited as evidence that bacteriophages comprise the majority of viruses within the virioplankton (172, 368). Other observations, such as the lack of significant correlation between algal biomass (chlorophyll *a* concentration) and VDC (Table 1), the ability of changes in bacterial abundance to predict changes in viral abundance (56, 115), the greater abundance of bacteria over that of other planktonic hosts (28, 56), and the predominance of viruses within the virioplankton with bacteriophage-sized genomes (20 to 60 kb), have all been cited as evidence to support this claim

TABLE 1. Virioplankton abundance in aquatic environments

Aquatic environment[a]	Method	Virioplankton data			Depth (m)	Season	Correlation[f] of viral abundance with microbial parameters			Reference(s)
		10^6 VLP/ml[b]	VBR	Capsid (nm)[c]			Chl a[d]	BA[e]	Other	
Marine										
Open ocean										
Northern Pacific (subarctic 45°N 165°W)	ELM (D[g])[x]	0.06–0.38	1.1–4.5		0–5,000	Sp[h]	NC[i]	0.73	Depth (−0.83)	115
N. Pacific (subtropical 24°N 165°W)	ELM(D)[x]	0.4–1.9	1.0–8.7		0–5,000	Sp	NC	0.63		115
Equatorial Pacific	FCM[x] (S[y])	5.3			5			0.6		173
Sargasso Sea [2]	TEM[k]/c[l]	0.003 ± 0.0015			25	S[m]				256
Eastern Caribbean Sea [9]t[t]	TEM/c	1.9 ± 1.3			0–200	Sp				256
Western Caribbean Sea [7]t	TEM/c	4.8 ± 3			0–200	F[n]				256
North Atlantic (49°N, 16°W)	TEM	14.9	50	30–60	10	Sp				19
Barents Sea (71°N 31°E)	TEM	0.06	3		30	W[o]				19
Southern Ocean t	TEM	1.2–5.4			0	S				296
Southern Ocean t	TEM	0.07–0.5			0	W				296
Mediterranean Sea	FCM(S)	2.3–6.5			5–200			0.35		173
Coastal ocean										
Bering (55°N) and Chukchi (73°N) Seas [26]t	TEM	2.5–36	10 ± 5.5		0–406	S		0.81	BP[r] (m[s])	304
Arctic Ocean, Resolute, Canada (sea ice)	TEM	9–130	72–10	50–70	0–4 cm	Sp	M	M	BP (m)	172
(sea water)	TEM	1.1	20–10		0–8	Sp	M	M	BP (m)	172
Pacific Ocean, Japan [4]	ELM(D)[x]	1.4–40	2.3–18		0–200	Sp-F				116
Northern Adriatic Sea [3]	ELM(D)	0.1–95	15 ± 10.4		0–15	Year	0.72	0.8	D-DNA[p] (NC)	343
Northern Adriatic Sea [5]t	TEM	1.2–87		30–60		Sp-S	NC	0.7	S%[q] (−0.51) D-DNA (NC)	342
Gulf of Mexico, Fla. [5]	TEM[x]	0.05–1.6			0	S			D-DNA (m)	138
Gulf of Mexico, Fla. [7]t	TEM/c	0.55–1.3			0–2,500	S	NC	NC	D-DNA (0.74)	28
Gulf of Mexico, Fla. [2]	TEM/c[x]	0.24–0.45			0	S				240
Gulf of Mexico, Tex. [10]t	ELM(Y[r])[x]	10–120			0		NC	NC		122
Gulf of Mexico, Tex.	ELM(Y)* ELM(D)*	0.3–79						0.93		349
English Channel	FCM(S)	18			0–2			0.9		173
Mamala Bay, Hawaii [19]	TEM/c	0.005–31			0–75	Year				244
Bahamas [2]	TEM/c	0.42–2.3			0	S				240
Santa Monica, Calif.	TEM	13–47			0	Sp-S				356
Santa Monica, Calif.	ELM(S)	153			0	Sp				214
Santa Monica, Calif.	TEM	118			0	Sp				214
Santa Monica, Calif.	ELM/c(D)[x]	23 ± 0.04			0	W				97
S. Calif. Bight, Santa Monica, Calif.	ELM(S)	18	14.2		0–800	Sp				214
S. Calif. Bight, Santa Monica, Calif.	TEM	16			0–800	Sp				214
S. Calif. Bight, Santa Monica, Calif. [4]t	TEM	0.3–12.4		<60	0–900	F	NC	S+		56
S. Calif. Bight, San Diego, Calif. [4]t	TEM	12–28		<60	0–2	W	NC			56
Long Island Sound [4]	TEM/c	150 ± 95			1	S				256
Gulf Stream, Fla. [2]	TEM/c	460 ± 28.6			50	F				256
Key Largo, Fla. [8]t	TEM/c	1.7–2			0–35		NC	0.58	S% (−0.97)	243
Raunefjorden, Norway	TEM	11–35			0–25	S		NC	BP (0.6)	120
Raunefjorden, Norway	TEM	0.01–9.9	16 ± 20	30–60	1	Year				19
Raunefjorden, Norway	TEM	0.5–1.8		<60	1.5	Sp-S				35, 27
Paradise Harbor, Antarctica	TEM	0.2–1.3	0.7–6		0–200	Sp				25
Solar salterns (37–372‰)	TEM	50–100		20–50			NC	0.97	BP (NC)	113
Dead Sea, Israel	TEM	9–73	$x = 4.4$		0–70	Sp-F		0.5–10		227
Estuarine										
Lake Saelenvannet, Norway	TEM	20–300	50 ± 30		1 and 2	Sp		0.59		329
Tampa Bay	TEM[x]	6.3–24.3			0	Sp-F			D-DNA (m)	138
Tampa Bay [3]t	TEM/c	27–46			0	S	0.97	0.94	D-DNA (0.74)	28
Tampa Bay [4]	TEM/c[x]	6–34			0–10			0.86	D-DNA (m)	240
Tampa Bay	TEM/c	4.8–20	0.9–9		0	Year	0.725	0.56	Temp.[u] (0.65) S% (−080)	136

Continued on following page

TABLE 1—*Continued*

Aquatic environment[a]	Method	Virioplankton data			Depth (m)	Season	Correlation[f] of viral abundance with microbial parameters			Reference(s)
		10^6 VLP/ml[b]	VBR	Capsid (nm)[c]			Chl a[d]	BA[e]	Other	
Tampa Bay	TEM	5.2–16	0.4–9.3		0	Year				58
Cyanobacterial mat, Tex.	ELM(Y)[x]	960			0	W				122
Chesapeake Bay [6]t	TEM	2.6–140 (x = 25)	3–26	30–60	0–30	Year		M	Depth (NC)	367, 368
Chesapeake Bay	TEM	10.1	3.2	30–60	1	Sp				19
Gulf of Bothnia, Sweden [3]t	TEM	17.5–50	x = 11.6	30–60	25–230	S	NC	S+		56
Freshwater										
Sproat Lake, Canada	TEM	1.5–2		60–200	1	Year				149
22 lakes, Quebec, Canada	TEM[x]	41–250 (x = 110)	76–4.9 (x = 23)	<70	1	S	0.52	NC	DOC[v] (NC), PO_4[w] (0.57), BP (0.5)	171
Pond, Hellebaek, Denmark	ELM(S)	22						4.4		214
European Alpine lakes	TEM	1–21	4–31	40–90	1–9	S				249
Lake Constance, Germany	TEM	10–40		20–50	0–8	Sp-S	M	0.57		121
3 lakes, Tex.	ELM(Y)[x]	2–142			0	Sp				122
Manoa stream, Hawaii	TEM/c	>10			0	Year				244
Lake Superior, surface microlayer	TEM	0.7–2.8	0.2–0.5		20 μm	S-F				320
Lake Superior, water column	TEM	0.15–0.9	0.03–0.7		0–20	S-F				320
4 lakes, Taylor Valley, Antarctica	ELM(Y)	4.2–33.5	3–8.5		0–35	S	M	M	BP(m)	144
Lake Kalandsvannet, Norway	TEM	20–200			0.5–25	S				120
Lake Plußsee, Germany	TEM	254	41	30–60	0.2	Sp				19
Lake Plußsee, Germany	TEM	>100			1–27	Year				71
Lake Plußsee, Germany	ELM(D)	0.3–25.7		40–100		W-S			D-DNA (m)	265
Lake Plußsee, Germany	ELM(D)	13			0–5	F		M		344
Epilimnion										
Metalimnion	ELM(D)	43			5–10	F				344
Hypolimnion	ELM(D)	28			10–25	F				344
Danube River	TEM	12–61	2–17	60–90	0	Year		M	BP (m)	176

[a] Values in brackets are the number of stations sampled.
[b] Viral abundance in 10^6 viruses-like particles (VLP) per milliliter.
[c] Dominant capsid size in nanometers.
[d] Chlorophyll *a* abundance.
[e] Bacterial abundance.
[f] Values in parentheses are r^2 values for correlation of viral abundance and parameter values.
[g] DAPI fluorochrome stain.
[h] Spring.
[i] No significant correlation.
[j] Sampling stations were along a transect.
[k] Transmission electron microscopy.
[l] Preconcentration of virioplankton employed.
[m] Summer.
[n] Fall.
[o] Winter.
[p] Dissolved DNA.
[q] Salinity.
[r] Bacterial productivity.
[s] Parameter was measured but not tested against viral abundance.
[t] Yo-Pro fluorochrome stain.
[u] Temperature.
[v] Dissolved organic carbon.
[w] Phosphate concentration.
[x] Two virioplankton counting methods were used.
[y] Flow cytometry.
[z] SYBR Green I fluorochrome stain.

(116, 372). Further study may fully substantiate the prevalence of bacteriophages within the virioplankton. The morphology of marine bacteriophages has been reviewed extensively by Proctor (257), Børsheim (29), and Frank and Moebus (86).

Capsid size provides an index of morphological diversity. Classification according to capsid size has been accomplished, based on data obtained by TEM. In fact, viruses in environmental samples have been classified according to morphotype

by some investigators (353). The most complete of these studies was a survey of bacteriophage diversity in Lake Plußsee, Germany, by Demuth et al. (71), who reported 39 morphologically distinct phages, which were classified into three principal morphotypes: B1 (*Siphoviridae*), A1 (*Myoviridae*), and C1 (*Podoviridae*). These morphotypes made up 50, 18, and 19% of the total diversity, respectively.

Other studies have reported only on the incidence of tailed bacteriophages. Hara et al. (116) and Wommack et al. (368) showed quantitatively that nontailed or short-tailed viruses comprised the majority of virioplankton in Japanese coastal and Chesapeake Bay water samples, respectively. Other investigators have reported (qualitatively) that either long-tailed (contractile and noncontractile) viruses (56, 256, 326) or non- or short-tailed viruses (19, 38) were dominant in water samples. The accuracy of reports on tail morphology, however, has been questioned, since preparation of water samples for viral direct counting may cause separation of phage capsids and tails (253). Since 96% of all known bacteriophages (ca. 4,600) are tailed (1), it is not surprising that careful preparation and exhaustive documentation of virioplankton diversity in aquatic environments (such as that done by Demuth et al. [71]) show that the majority of virioplankters are tailed.

Measuring virioplankton diversity by capsid diameter is appropriate, since this feature varies widely among the bacteriophages described to date (20 to 200 nm) and is, in general, a consistent feature for bacteriophages (3). Data on the frequency distribution of capsid size within virioplankton populations have been reported for a variety of aquatic environments (Table 1), with the dominant virioplankton capsid diameter being in the range of 30 to 70 nm (19, 30, 38, 56, 121, 171, 172, 320, 342, 347, 368). The proportion of virioplankton observed to fall into the 30- to 60-nm size class was ca. 65% or greater. Two investigators have reported the predominance of 30- to 60-nm viruses within bacterioplankton cells (121, 347), suggesting that the free bacteriophages most frequently observed and characterized are indeed produced in situ. The 30- to 60-nm capsid size of aquatic virioplankton is slightly smaller than the 60- to 80-nm range observed for purified marine (29, 86) and other (3) bacteriophages. It has been suggested that the somewhat smaller size of free aquatic bacteriophages indicates that marine phage culture collections do not contain the most abundant bacteriophages that exist in aquatic environments (29). Therefore, conclusions about natural bacteriophage diversity that are based on knowledge gained from experiments with purified bacteriophages must be drawn with caution. For example, Bratbak et al. (34) observed large numbers (10^4 ml^{-1}) of extraordinarily large phage-like particles, with a capsid diameter of 340 to 400 nm and noncontractile tail length of 2.2 to 2.8 μm, in Norwegian and Danish coastal water samples. Abundant large virus particles that overlap the size of small bacteria have also been found in water samples from a eutrophic freshwater reservoir (298), Antarctic lakes (144), European alpine lakes (249), and the food vacuoles of phaeodarian radiolarians (111). Pleiomorphic, spindle- and lemon-shaped viruses have been found in hypersaline environments dominated by halophilic archaea (113, 227).

Changes in the frequency distribution of viral capsid size over time and space suggest that the composition of virioplankton populations and, by inference, cooccurring host populations can vary. Evidence for a correlation between the capsid size of the virioplankton and the composition of host populations comes from the observation of Weinbauer and Peduzzi (347), who showed that bacteria within different morphological groups carry viruses of a particular size class. Analysis of Adriatic Sea water samples revealed that the 30- to <60-nm capsid size class comprised 75 and 100% of intracellular viruses in rods and spirilla, respectively. Cocci, however, more often contained larger viruses (60 to <110 nm) than the smaller 30- to <60-nm viruses (65 and 35%, respectively). Within the bacterioplankton, 84% of the population was rod shaped, supporting the results of earlier work showing a predominance of 30- to <60-nm capsids within virioplankton from Northern Adriatic Sea water samples (342).

Several observations of temporal and spatial changes in the frequency distribution of viral capsid size have been recorded for virioplankton populations. Cochlan et al. (56) found that viruses >60 nm in capsid diameter decreased greatly in abundance, from nearly 50% of the population to almost none, in an onshore-to-offshore transect in the Southern California Bight. The authors observed that differences in virioplankton abundance from water samples collected at different depths or in different environments (Southern California Bight and the Gulf of Bothnia, Sweden) could usually be attributed to the proportion of viruses in the <60-nm size class. A similar observation of the dynamic nature of the <60-nm capsid size class was made by Børsheim and colleagues (30) during a 20-day incubation of a Norwegian coastal seawater sample. In the first 5 days of incubation, the abundance of the <60-nm class increased at a rate of 41% per day. Two studies have documented changes in both virioplankton abundance and composition during Northern high-latitude spring diatom blooms. Bratbak et al. (38) observed two peaks in virioplankton abundance over a 1-month sampling period, during which virioplankton in the <60-nm size class were nearly always dominant and demonstrated the most erratic changes in abundance. Maranger et al. (172) noted a decrease in the abundance of the smallest size class (viruses of <50 nm) and an increase in the 50- to 70-nm size class in samples of Arctic sea ice during a 1-month bloom.

Not every study documenting the capsid diameter of virioplankton has reported changes in the distribution of capsid size with changes in time or location of water sample collection. Mathias et al. (176) noted no change in the frequency of four groups of capsid diameters among the virioplankton in river water samples collected over a 2-year period. It is interesting that viruses larger than 60 nm were most abundant in these water samples (mostly the 60- to <90-nm size class [ca. 40%]). The preponderance of larger viruses in the virioplankton of this riverine environment may be a reflection of differences in host community structure between marine and freshwater environments. In the hypersaline environment of solar salterns, the virioplankton members tend towards smaller capsid diameters, with the dominant size class between 20 and 50 nm (113). In this extreme environment, no correlations were observed between changes in salinity and frequency of capsid diameter; however, the abundance of a pleiomorphic, lemon-shaped virus (believed to be a virus of halophilic archaea) was strongly correlated with increasing salinity (113). Differences in viral size class distribution was noted for water samples collected from 22 Canadian lakes; however, no statistically significant trend connecting these changes to the trophic state of the lacustrine environment was detected (171). Similarly, the frequency distribution of capsid diameters did not change significantly with season or trophic condition for virioplankton populations in water samples from the Northern Adriatic Sea (342).

VIRAL ABUNDANCE AND ENVIRONMENTAL PARAMETERS

In the few years since the presence of large numbers of free virus particles in natural waters was reported, microbial ecologists have used a variety of direct-counting techniques to enumerate viruses in the aquatic environment. As shown in Table 1, the abundance of viruses can range from $<10^4$ ml^{-1} to $>10^8$ ml^{-1}. This extreme variability in numbers is distinctive for this component of the plankton. In similar environments, the number of bacteria ranges between ca. 10^4 and 10^7 ml^{-1}, with ca. 10^6 ml^{-1} reported for coastal marine waters. The data given in Table 1 show that the number of viruses is greater in productive and nutrient-rich environments. The wide variation in numbers suggests that viruses are, indeed, an active component of aquatic microbial communities.

Temporal Variation

Seasonal variation in abundance has been noted since the earliest reports of viruses in seawater by Bergh et al. (19). In Norwegian coastal waters, the concentration of virus particles during the winter fell below 10^4 ml^{-1} from ca. 5×10^6 ml^{-1} in spring through autumn. Counts made at other coastal ocean and estuarine locations have shown similar seasonal trends. In both Tampa Bay and the northern Adriatic Sea, viral abundances changed by an order of magnitude from winter lows of 10^6 ml^{-1} or less to late-summer highs of $>10^7$ ml^{-1} (58, 136, 343). Significant seasonal changes in viral abundance were observed in Chesapeake Bay (368). In the only study done to examine the seasonal dynamics of a freshwater virioplankton population, Mathias et al. (176) recorded viral abundances ranging from 12×10^6 to 61×10^6 ml^{-1} in a backwater system of the Danube River. Bacterial abundances showed similar seasonal variability in these environments but changed only by a factor of 5. An interesting coincidence between these studies is that the greatest viral abundance occurred in late autumn. It is possible that these annual peaks in viral abundance are a result of autumn phytoplankton blooms. Spring phytoplankton blooms also are correlated with increased numbers of viruses; however, the magnitude of the autumn peaks is larger, possibly because of larger initial (summer) virioplankton and bacterioplankton populations at the beginning of the autumn bloom.

The volatile nature of virioplankton abundance is most apparent in shorter-term temporal studies, especially those conducted on or around annual phytoplankton blooms. Initial evidence that viruses were likely to be active within the plankton came from a study examining changes in numbers of free virus particles during a spring diatom bloom in western Norway. Over the course of the March 1989 bloom, employing 2-day sampling intervals, Bratbak et al. (38) documented a rise in numbers of viruses from a prebloom low of 5×10^5 virus-like particles ml^{-1} to a maximum concentration of 1.3×10^7 virus-like particles ml^{-1}. Dramatic peaks in free-virus counts followed peaks in the abundance of diatoms and other photoautotrophs, suggesting a close coupling of viral infection and production to growth of the host population. In a 4-month study of Lake Constance, a large mesotrophic lake, Hennes and Simon (121) documented changes in virioplankton abundance, frequency of infected bacterioplankton, and bacteriophage production rate. Their observations, which commenced just prior to the spring phytoplankton bloom, showed that transient increases in bacterial abundance were closely followed by peaks in the frequency of occurrence of infected bacteria and in the abundance of free bacteriophages. Brief peaks in virioplankton abundance suggested in situ phage production rates of between 0.5×10^6 and 2.5×10^6 phage ml^{-1} day^{-1}. Finally, dramatic changes in virioplankton abundance were observed in Arctic sea ice over the course of the spring phytoplankton bloom. In observations of virioplankton in the lower 4 cm of sea ice, Maranger et al. (172) reported the largest numbers of free virus ever recorded in a marine environment. During the 1-month bloom, viral abundance in sea ice increased by a factor of 100. Surprisingly, viruses were 10 to 100 times more abundant in sea ice than in the underlying water, suggesting that processes controlling virus populations are dramatically different in the two environments.

Short-term changes in virioplankton abundance have been investigated both in large-volume ($\geq$60-liter) mesocosms and in situ. In each case, the number of free viruses in water samples changed significantly over a diel cycle. A mesocosom experiment conducted by Jiang and Paul (136) did not reveal a diel rhythm in changes in virus numbers. During the experiment, a significant increase in VDC was preceded by increases in bacterial abundance and chlorophyll *a* concentration, thereby providing circumstantial evidence for close coupling of host community growth and viral production (136). A diel cycle of viral abundance that was significantly correlated with sunlight levels and bacterial production was detected in 60-liter seawater enclosures (120). In the enclosures, a direct link between host and viral abundance was not observed, since the bacterial direct count (BDC) did not correlate with VDC. However, a correlation of the VDC with the bacterial production rate in the mesocosms supports the assumption that a faster-growing host community is more effective in producing free viruses. Correlation of VDC with global radiation levels led Heldal and Bratbak (120) to suggest that in the microcosms, sunlight stimulated viral production by induction of lysogenic bacteria. In one instance, a significant fluctuation in virioplankton abundance was noted during intervals as short as 10 min. Bratbak et al. (40) recorded two- to fourfold changes in VDC over periods of 10 to 20 min in seawater mesocosms. These short-term changes indicated extremely high viral production (6 to 13 h^{-1}) and loss (5 to 11 h^{-1}) rates. These radical changes in VDC were interpreted as an indication of synchronous lysis of bacterioplankton hosts.

A limitation of mesocosm studies is the experimental error arising from enclosure of the planktonic communities. Many investigators acknowledge that the "bottle effect" can influence conclusions drawn from mesocosm data for natural populations of microorganisms (383). However, observations of virioplankton dynamics in seawater incubations do not indicate effects related to confinement. Rapid changes in virioplankton abundance recorded by Bratbak et al. (40) were not affected by the volume of seawater in mesocosms (ranging from 30 ml to 20 liters), leading the authors to dismiss the bottle effect as an explanation for high short-term virioplankton production rates. Weinbauer et al. (343) monitored diel in situ variability in virioplankton density for 42 h and found changes in VDC and other parameters. Peaks in BDC and chlorophyll *a* concentration preceded significant changes in VDC. These findings, along with those of Jiang and Paul (136) and Hennes and Simon (121), indicate that short-term changes in virioplankton abundance, observed in large-volume mesocosoms, reflect in situ processes.

Depth Variation

Physiochemical changes with depth can have a significant impact on planktonic microorganisms. Within the oceanic euphotic zone, specific distributional patterns of photoautotrophs occur that are related to light intensity and wavelength (225, 226). Water temperature or salinity gradients (clines) can re-

sult in water column stratification into separate physiochemical environments. For instance, in a salt wedge estuary such as the Chesapeake Bay, the water column is stratified by a strong halocline, separating denser seawater from seaward-flowing freshwater. During calm summer weather, the more saline bottom waters of the Chesapeake Bay become anoxic, which, in turn, affects biological processes significantly (218, 330).

In open-ocean waters, virioplankton abundance declines rapidly below the euphotic zone (200 m) to relatively constant, low abundances of $<10^6$ viruses ml^{-1} (25, 28, 115, 240, 304). In one instance, deep-water viral abundance, at a station in the Southern California Bight, was between 1.1×10^6 and 2.5×10^6 ml^{-1} and showed an increase in near-bottom (900 m) water samples (56). Within the upper 200 m, transient subsurface maxima in viral abundance can occur, usually at ca. 15 m (25), 50 m (28, 56, 115) and 75 m (25) to 150 m (28, 56, 115, 304) in the water column. Hara et al. (115) observed subsurface peaks in virioplankton abundance in the north Pacific, above and below the depth of the subsurface chlorophyll maxima. The discontinuity of viral abundance around the subsurface chlorophyll maxima suggests that there are changes in the processes responsible for virioplankton production or loss between 50 and 150 m deep in the open ocean. Cells transported out of the high-productivity zone may be exposed to nutrient limitation or changes in light intensity and therefore may be more susceptible to viral infection or to curing of the lysogenic state, resulting in higher rates of viral production.

Unlike deep-ocean waters, where viral counts at depth are 2 to 10 times lower than surface counts, near-coastal and estuarine water often does not show significantly fewer viruses with depth (56, 244, 343, 368). Consistently large numbers of viruses (usually $\geq 10^7$ viruses ml^{-1}) throughout the water column are observed in near-shore environments. Nevertheless, in a few instances, depth-related variability in VDC has been observed in productive waters. During periods of water column stratification in the northern Adriatic Sea, virioplankton abundance is significantly greater at the thermocline (343). In a permanently stratified Norwegian lake, viruses and bacteria were two- to threefold more abundant at the chemocline (the boundary layer separating anoxic, sufidic bottom water from oxic surface water) than at the surface (329). This observation is not surprising, since bacterioplankton, phytoplankton, and nutrients are known to concentrate within the boundary layer of a stratified water column (189). Similarly, the surface microlayer of natural waters is known to be a zone of high bacterial productivity. In the only report of virus abundance in the surface microlayer, Tapper and Hicks (320) found that the highest abundances of virioplankton in Lake Superior water samples were from the upper 20 μm of the water column. With water column mixing, however, the subsurface thermocline peak in the virus-to-bacterium ratio dissipates and the virioplankton concentration is generally uniform throughout the water column (343, 368). Stratification and deep-water anoxia in Chesapeake Bay were observed to have a significant impact only on bacterioplankton abundance, whereas virioplankton abundance at depth was similar to that at the surface (368). However, the viral abundance at the boundary layer was not measured. Altogether, temporal and depth-related variability in virioplankton abundance provides evidence that viruses play an active and important role in aquatic microbial communities.

Correlation of Virioplankton Abundance with Microbiological Parameters

Chlorophyll *a* concentration and bacterial abundance. The unifying message of the data presented in Table 1 is that the production and distribution of viruses in aquatic environments is, not surprisingly, determined by factors which affect the productivity and density of host populations, especially the bacterioplankton. In most instances, where changes in virioplankton abundance and chlorophyll *a* concentration were recorded, no significant correlation was observed between these parameters. Conversely, in a majority of studies which have examined changes in BDC and VDC, significant correlation between bacterial and viral abundance have been observed (28, 56, 115, 121, 136, 144, 243, 304, 329, 342, 343, 348). Moreover, in nearly every environment where chlorophyll *a* concentration significantly predicted virioplankton density, bacterial abundance did so as well (28, 136, 343).

An exception to a positive correlation between chlorophyll *a* concentration, and BDC and VDC occurred in Canadian lakes. In a study of 22 lakes in Quebec by Maranger and Bird (171), the chlorophyll *a* concentration was significantly correlated with virioplankton abundance whereas bacterioplankton density was not. The authors offered two possible explanations for this observation. First, because freshwater ecosystems contain high concentrations of algae and cyanobacteria, increases in phytoplankton biomass result in more algal viruses. Second, in lacustrine systems, the short-term relationship between viruses and bacteria is negative, which, in effect, obscured the dependence of viral abundance on bacterial abundance in the small data set. The positive correlation between chlorophyll *a* concentration and VDC in the lakes supported the significant correlation found between bacterial production and virioplankton abundance (171). In essence, a high phytoplankton biomass in the lakes was associated with a more productive bacterial community, resulting in increased viral abundance.

There is evidence that factors affecting algal distribution also influence the virioplankton. Most notably, multiple-regression analysis, using combined data from a variety of aquatic environments, indicates that chlorophyll *a* is a slightly better predictor of virioplankton concentration than is BDC (171). Similarly, along an onshore (eutrophic)-to-offshore (oligotrophic) transect in the Gulf of Mexico, VDC was found to be highly correlated with chlorophyll *a* concentration. This association, however, was not observed when data from estuarine and near-coastal stations were excluded (28). The central conclusion to be drawn from these correlative studies is that examination of chlorophyll *a* concentration, BDC, and VDC data from water samples taken over small temporal and spatial scales generally reveals only a correlation between BDC and VDC, whereas studies examining larger data sets, such as an onshore-to-offshore transect (28) or regression analysis of reported literature values (171), reveal the ultimate correlation of all biological parameters with levels of primary production.

Bacterial production. It is possible that the confusion in determining factors that are significantly related to virioplankton distribution will be cleared up by examining the correlation of bacterial activity (production) with viral abundance. On the few occasions where viral abundance and bacterioplankton production have been measured simultaneously, significant correlations have been observed (Table 1) (120, 171). Other supporting evidence that bacterial activity can be correlated with virioplankton abundance is derived from data obtained from Arctic sea ice, where the region of maximal bacterial production corresponds to the region of highest viral count (172). A more direct indication that increased viral production coincides with the level of bacterial secondary production was noted by Steward et al. (304). A significant correlation ($r^2 = 0.64$) was observed between the frequency of visibly infected cells (FVIC) (measured by TEM) and bacterial production rates (304). While it would appear obvious that a link between

viral abundance and rates of bacterial production should exist, a clear demonstration of such a link provides strong evidence that maintenance of abundant virioplankton populations is dependent on an active bacterioplankton host community.

The dissolved-DNA dilemma. In addition to chlorophyll *a* levels, BDC, and bacterial production measurements, the connection between virioplankton abundance and dissolved-DNA (D-DNA) concentration has been investigated. Interest in the abundance and distribution of DNA in aquatic environments has been fueled both by research on the biological cycling of carbon through aquatic microbial communities and concerns about the consequences of the release of genetically engineered microorganisms into the environment. In the first instance, D-DNA (the DNA fraction which passes through a 0.2-μm-pore-size filter) is a readily measurable portion of dissolved organic matter (DOM) which is useful in studies of DOM dynamics. Because of its ubiquity in living cells and its specialized role in cell function and reproduction, DNA also serves as a useful tracer molecule for analyzing planktonic rate processes (142). An excellent model of the distribution of total DNA (particulate and dissolved) in the oceanic environment has been provided by Jiang and Paul (138). Secondly, the possibility has been offered that D-DNA serves as a reservoir through which exotic or engineered gene sequences could be introduced via transformation into autochthonous, aquatic bacteria (68, 238).

Our current understanding of the dynamics and distribution of D-DNA in aquatic environments is largely attributable to the work of Paul et al. (233–239, 241) and DeFlaun et al. (66–68). Through their efforts, measurement of D-DNA has become an important addition to the list of parameters used to characterize aquatic microbial communities. Paul and DeFlaun carried out their early studies prior to 1989, before the recognition of viruses as an abundant component of aquatic environments. Methods for quantifying dissolved DNA in water samples did not distinguish soluble DNA (sDNA) from encapsulated, viral DNA (vDNA) (67, 142). Thus, the discovery of abundant virus populations raised skepticism among microbial ecologists with respect to the existence of a large pool of free, soluble DNA. Therefore, prior to a discussion of the correlation between D-DNA concentration and VDC, it is necessary to review the studies examining the preponderance of vDNA in the D-DNA pool.

In recent years, several authors, using a variety of approaches, have produced conflicting evidence about the contribution of vDNA to the aquatic D-DNA pool. Beebee (16), using ultracentrifugation and liquid chromatography, separated D-DNA into pellet (vDNA) and supernatant (sDNA) fractions. Pellet DNA comprised between 76 and 95% of total D-DNA and was of viral genomic size, i.e., >20 kb. Soluble DNA was generally 500 bp in size. Maruyama et al. (175) used DNase to quantify the proportion of free and coated (non-DNase digestible) DNA in Tokyo Bay estuarine water. They found that nearly 90% of the DNA contained within the <0.2-μm size fraction was non-DNase digestible. Furthermore, most coated DNA (assumed to be vDNA) was 20 to 30 kb in size, leading the authors to conclude that coated DNA originated from viruses within the <0.2-μm size fraction. The concentration of vDNA in Tokyo Bay samples was between 9 and 19 ng ml^{-1} which, using the standard conversion factor of 0.09 fg of dsDNA $virus^{-1}$ (2), yields an estimate of ca. 10^8 viruses ml^{-1} (175).

As pointed out by Paul et al. (241), neither of the studies cited above enumerated viruses in water samples, which is necessary for a direct test of their conclusions concerning the predominance of encapsulated vDNA in the total D-DNA pool. Using the simple approach of converting viral abundance to vDNA concentration, several authors have demonstrated that vDNA is actually a minor contributor, generally less than 20%, to D-DNA pools (28, 138, 240, 342, 343). It is possible that the criteria of nuclease insensitivity extend to other forms of D-DNA besides vDNA. Paul et al. (240) reported anecdotal evidence that the proportion of D-DNA which is DNase insensitive is greater than that of viral origin (estimated by conversion of VDC). Jiang and Paul (138) presented further evidence refuting the hypothesis that D-DNA is nuclease insensitive. After careful development of a centrifugation method, they were able to separate D-DNA into putative sDNA (supernatant) and vDNA (pellet) fractions. In the two water samples tested, approximately half of the DNA within the pellet was DNase insensitive, yet only 34 and 66% of that pellet DNA was vDNA. Using these and earlier results, Jiang and Paul (138), postulated that D-DNA exists in two forms, sDNA and stable or bound DNA. These fractions each make up 50% of the total D-DNA in seawater. The stable form of DNA is composed of vDNA (17 to 30%) and an as yet uncharacterized bound form of DNA (70 to 83%) (138). The nature of nonviral, nonsoluble D-DNA can only be speculated. It is possible that this D-DNA fraction is associated with small colloids, known to be abundant in ocean water (153, 352), or polymeric molecules such as histone proteins (240) and exopolysaccharides. Such associations could render D-DNA inaccessible to DNase and fluorochomogenic dyes, such as Hoechst 33258, which have been utilized for quantification of D-DNA.

Finally, two observations lend additional evidence to the notion that vDNA is not the principal source of D-DNA. Paul et al. (240) observed that the abundance of D-DNA, estimated using the DNA-specific fluorochrome Hoechst 33258 (242), increases after ethanol precipitation (240). They demonstrated that ethanol precipitation of D-DNA in water samples released both bound and vDNA and increased the fluorescence signal of the Hoechst 33258 stain. Thus, the difference between vDNA abundance and the post-ethanol fluorescence readings can be concluded to represent a bound form of D-DNA. This form of D-DNA would be wrongly judged to be vDNA, based on the methods of Maruyama et al. (175) and Beebee (16). If the source of D-DNA is primarily viral, D-DNA would not be expected to contain a detectable amount of bacterium- or eukaryote-specific 16S and 18S ribosomal gene sequences. Jiang and Paul (138) tested this supposition by hybridization analysis. In two of three trials, significant hybridization (over background levels) was observed between D-DNA and oligonucleotide probes specific for small-subunit rDNA genes, suggesting a cell and not virus origin of D-DNA.

In three cases, D-DNA abundance was measured as part of a larger study of virioplankton dynamics. In two reports from studies of virioplankton dynamics in the northern Adriatic Sea, Weinbauer et al. (342, 343) found no significant correlation between changes in virioplankton abundance and D-DNA concentration (Table 1). Furthermore, the D-DNA concentration, unlike the virioplankton abundance, showed no significant change over a transect which ranged from mesotrophic to eutrophic nutrient conditions (342). This suggests that factors controlling D-DNA and virioplankton abundance are probably different. In the third study, results of multiple-regression analysis showed that viral abundance in seawater collected at Gulf of Mexico stations could be explained by bacterial and cyanobacterial abundance and chlorophyll *a* and D-DNA concentration. However, when analyzed separately, VDC and D-DNA abundance showed the poorest correlation of the four independent variables (28). While the data are limited, the lack of correlation between VDC and D-DNA concentration lends

support to the view that vDNA is likely to be only a minor contributor to D-DNA pools in seawater.

Altogether, the findings of Jiang, Paul, and others provide a convincing argument against viruses being the principal source of D-DNA. Nevertheless, it is intuitive that viral lysis, by mediating a portion of the flux of cellular DNA into D-DNA, should contribute to the dynamics of the D-DNA pool. Through a series of conversions, using measurements of viral abundance and the FVIC, Weinbauer et al. (342, 343) estimated that release of DNA from bacteriophage-induced lysis contributed between 2 and 74% of total D-DNA per day. A direct demonstration of lytic release of D-DNA was presented by Reisser et al. (265, 266). From in vitro experiments with a phycovirus (host, *Chlorella* sp.) and a cyanophage (host, *Synechococcus cedrorum*), they demonstrated that D-DNA released during lysis was of host and viral origin and that the sDNA fraction (0.5% of the total D-DNA released) consisted of DNA less than 500 bp long (266). In situ observations of an algal population decline supported their in vitro observations. Immediately after the significant decline, virioplankton abundance increased over 20-fold and the D-DNA concentration doubled (265). These estimates and observations provide some insight into the role of viral infection in the dynamics of DNA in aquatic ecosystems. However, many interesting questions concerning the dynamics and character of aquatic D-DNA remain unanswered.

Virus-to-Bacterium Ratio

In addition to correlations between VDC and environmental parameters, the virus-to-bacterium ratio (VBR) has been used to study the relationship between virioplankton and bacterioplankton populations. From VBR data presented in Table 1, it is easy to appreciate that even in oligotrophic environments, viral abundance exceeds bacterial abundance. With the sole exception of Lake Superior waters, where VBR values were consistently low (i.e., <1) (320), generally the ratio of virus to bacterial abundance falls between 3 and 10. VBR values are higher for more nutrient-rich, productive environments. This simple observation suggests that bacterioplankton host populations produce greater numbers of viruses under environmental conditions favoring fast growth and high productivity. These increases in virioplankton production under nutrient-replete conditions are also due, in part, to higher infection rates and larger burst sizes, two biological parameters which can strongly influence VBR. VBR can be useful in constructing theories on the effect of viral infection on aquatic bacterial host communities; however, it is important to also consider that this ratio can be influenced by a multitude of factors which control the production and loss of viruses and bacteria.

Studies of changes in VBR have revealed several associations between VBR and bacterial density. Results of field studies off the coast of Japan (116) and in a Norwegian meromictic lake (329) revealed that while BDC and VDC change dramatically with depth and over short temporal scales, VBR values remained consistent, suggesting a tight coupling between bacterial and viral concentrations and relatively constant levels of virus production and loss. An unchanging phage-to-bacterium ratio was also noted by Ogunseitan et al. (220) in freshwater mesocosm studies of *Pseudomonas aeruginosa* and bacteriophages UT1 and M1.

A positive relationship between VBR and bacterial numbers was noted in both oligotrophic water samples collected in the North Pacific (115) and eutrophic water samples collected from the northern Adriatic Sea (343). Hara et al. (115) suggested that the positive relationship between these parameters in Pacific Ocean water samples arises from a direct dependence of virus production on bacterial host cell density. They also suggested that, based on the observation that higher VBR values coincided with larger numbers of small bacteria, increased numbers of viruses may act as a positive selective pressure for reduced cell volume. This suggestion is also supported by the colloidal aggregation theory, which predicts that a smaller bacterium would encounter fewer phages through Brownian motion (115).

While these studies offer intriguing theories, they represent special cases of viral and bacterial community interaction. The most commonly observed relationship between VBR and bacterial abundance is either negative or inverse. Field studies of Tampa Bay (136), Arctic sea ice (172), Canadian lakes (171), and seawater mesocosms (328) have all provided results showing that the VBR decreases as bacterial abundance increases. In our analysis of Chesapeake Bay, we noted that the VBR was inversely related to bacterial abundance (368). The highest VBR values were recorded at times of relatively low bacterial abundance and vice versa, suggesting seasonality in virioplankton production in the nutrient-rich ecosystem of Chesapeake Bay. Seasons where a high VBR occurs would show more virioplankton production and hence more bacterioplankton lysis. Maranger et al. (172) reported an extremely high VBR of 72 in sea ice at the onset of the spring phytoplankton bloom, which declined precipitously as bacterial numbers increased. They speculated that as the bloom proceeded, the pressure of viral infection selected for phage-resistant mutant bacteria among the bacterioplankton. Toward the end of the bloom, resistant bacteria were dominant among the sea ice bacterioplankton, resulting in reduced active production of phage. The dominance of resistant bacteria could also explain the lower bacterial production rates recorded at the end of the bloom. Phage resistance often results in lower physiological efficiency (160).

Finally, Bratbak and Heldal (36) and Tuomi et al. (328), in explaining the negative or inverse relationships they observed between VBR and bacterial abundance, formulated the intriguing hypothesis that the VBR may be an indicator of host community diversity. In a bacterioplankton community dominated by only a few bacterial species, specific adsorption of viruses to their cooccurring host would be favored. Once adsorbed to their specific receptor, free viruses are effectively lost from the virioplankton. In environments with high host diversity, virioplankton would presumably spend more time as free viruses, since specific adsorption would be slower. This argument hinges on two plausible assumptions: (i) that specific adsorption is an important factor in viral loss, and (ii) that bacterial numbers increase as the overall community diversity decreases. The latter assumption can easily be accepted for mono- or polyspecific blooms, when populations can dramatically increase in number, with simultaneous loss in overall species diversity.

IMPACT OF VIRUSES AND VIRAL INFECTION ON THE AQUATIC FOOD WEB

Significant Biological Factors for Modeling In Situ Phage-Host Interaction

In situ burst size. Burst size, i.e., the number of virus particles released upon host cell lysis, is an essential component in calculations that relate estimates of in situ viral production to the level of virus-mediated mortality of bacterioplankton. Intuitively, there is an inverse relationship of burst size and the level of host mortality due to viral infection. The greater the

TABLE 2. Burst size based on in situ observations

Aquatic environment	No. of viruses/burst (range)	Method	Comments	Reference(s)
Marine				
Raunefjorden, Norway	50 (10–300)[e]	LFWO[a]		120
Raunefjorden, Norway	ca. 500[e]	TEM[b]	TEM observation of virus-like particles surrounding *E. huxleyi* cells	33
Bay of Århus, Denmark	100[e]	LFWO		39
North Sea	ca. 400[e]	VIC[c]	*E. huxleyi*	44
Gulf of Mexico, Tex.	30 (21–45)	VIC	Avg of maximum estimates of burst size from lysogens	350
Gulf of Mexico, Tex.	21 (11–29)	VIC	Avg of minimum estimates of burst size from lysogens	350
Aransas Pass, Tex.	92–324	Calc.[d]	Calculation of cyanophage burst sizes from viral decay rate and contact rate	312
Gulf of Mexico, Tex.	10–23	VIC	Oligotrophic conditions	357
	29–64	VIC	Mesotrophic conditions	357
Northern Adriatic Sea	16–20	VIC	Mesotrophic conditions	342
Northern Adriatic Sea	ca. 30	VIC	Eutrophic conditions	342
Northern Adriatic Sea	48 (28–51)	VIC	Estimated burst sizes for different bacterial morphological groups	347
Freshwater				
Lake Constance, Germany	21–121[e]	VIC		121
Danube River, Austria	17–36	VIC	Estimated burst sizes for different bacterial morphological groups	176
Lake Plußsee				
Epilimnion	34 (19–35)	VIC		344, 345
Metalimnion	44 (32–55)	VIC		344, 345
Hypolimnion	63 (44–87)	VIC		344, 345

[a] Lysis from without using streptomycin.
[b] TEM observations of lysed cells.
[c] Burst size estimated from the number of virus-like particles within visibly infected cells.
[d] Burst size necessary to support viral production or decay rate based on theoretical contact rates.
[e] Estimates are ranges of burst sizes from individual cells rather than an average burst size calculated from observations of several cells.

burst size, the smaller the number of host cells lysed to support a given level of virus production. Noting the dramatic effect of burst size estimates on modeling the impact of viral infection on host populations, many authors have chosen to use a range of burst sizes for these calculations (see Tables 2 through 6). However, it is likely that improvements in the precision of estimates of virus-mediated mortality can most easily be achieved through more accurate estimates of burst size under in situ conditions. To this end, several studies have used burst size estimates, based on in situ observations of bacterio- and phytoplankton, to calculate virus-mediated mortality. Burst size estimates are summarized in Table 2.

In a comprehensive review, Børsheim (29) compiled a table of reported burst sizes for 26 marine bacteriophages. These estimates, based on one-step growth curve experiments, averaged 185 phages/lysed bacterium. This value is significantly higher than the estimates for in situ burst size reported in Table 2. It is not surprising that estimates of burst sizes, based on growth conditions in culture media, are larger than those in situ. In general, bacterial cells grown in the laboratory on bacteriological media are larger and support the production of greater numbers of phage particles (29, 152, 200, 275, 347). Weinbauer et al. (344, 347) found a significant correlation between in situ burst size and host cell volume. Increases in cell volume were paralleled by significant increases in burst size, especially for smaller phages (30 to <60 nm in capsid size). Stepwise multiple-regression analysis showed for Lake Plußsee water samples that cell size was the only parameter capable of explaining changes in burst size (344). Furthermore, in situ observations of infected bacteria in the northern Adriatic Sea found that burst size estimates for bacterioplankton in eutrophic environments are significantly larger than those in mesotrophic environments (342).

Nearly all of the estimates of in situ burst size (Table 2) are based on TEM observations of virus particles within intact (176, 342) or thin-sectioned (121) bacterioplankton cells. In most studies, in situ burst size estimates have been reported as averages from a number of single-cell observations; however, in a few instances, burst size has been reported simply as a range of individual observations (33, 39, 44, 120, 121). While direct TEM estimates of in situ burst size are limited, there is surprisingly good agreement among the data. With the exception of the latter group of studies, the average burst size of bacterioplankton is 24 phage per cell across the range of environments examined (Table 2). This average estimate is lower than the burst size conversion factor of 50 used in other studies (97, 120, 304, 318). For methods which utilize burst size estimates to calculate virus-mediated mortality of bacterioplankton, the use of the lower burst size estimate results in an increase in mortality estimates. Finally, TEM observations of intracellular viruses of the bloom-forming coccolithophorid *Emiliania huxleyi* indicate that the in situ burst size for viruses of this important oceanic phytoplankter is ca. 400 to 500 viruses (33, 44). With this burst size estimate, Bratbak et al. (33) estimated that viral infection accounted for 25 to 100% of *E. huxleyi* mortality during a bloom.

The most innovative method of estimating in situ burst size introduced thus far was reported by Heldal and Bratbak (120). In their approach, bacteriophage within bacterioplankton cells are released by lysis of the cells from without through addition of streptomycin. Bratbak et al. (39) show examples of this method in a series of TEM micrographs of streptomycin-lysed

bacterioplankton cells. The authors regard estimates obtained by this method to be minimum, since all bacterioplankters may not respond to streptomycin treatment and since streptomycin, in some cases, prematurely terminates phage production; thus, the actual, full-term, burst size would be underestimated (39, 120).

Another interesting approach to estimating in situ burst size is to calculate the burst size necessary to balance viral production with estimates of viral decay. Suttle and Chan (312) used this approach for estimating in situ cyanophage burst sizes for near-coastal *Synechococcus* populations. From estimations of *Synechococcus* and cyanophage abundance, it was possible to calculate theoretical in situ contact rates between cyanophage and host. Assuming that all contacts result in infection, the burst size is simply the number of cyanophage produced per day, i.e., the cyanophage decay rate, divided by the abundance of cyanophage-infected *Synechococcus*. Cyanophage burst sizes of between 92 and 324 were necessary to balance the range of cyanophage decay rates in Texas Gulf coastal water (Table 2) (312). These in situ burst size estimates are the highest reported for any bacteriophage. It would be interesting to estimate in situ cyanophage burst size, both theoretically and directly, thereby allowing an assessment of the rigor of in situ burst size estimates and of the underlying assumptions required for the calculation.

The principal advantage of in situ burst size estimates is that these estimates account for the effect of environmental and biological variables on phage development. It has been demonstrated, using the in situ observation techniques discussed above, that burst size estimates can change significantly with temperature (176), as well as with the trophic state of the aquatic environment (342). It has also been observed that bacterioplankton morphological groups (rods, cocci, and spirilla) demonstrate significantly different burst sizes (176, 342, 347), affecting the relative contributions of these groups to virioplankton production. Seasonal changes in burst size have also been noted (121); however, these changes were only trends and were not statistically significant.

Viral inactivation and particle destruction. Since the earliest studies of bacteriophage physiology (79), researchers have routinely reported data on the stability of purified viral isolates. Initially, data on viral inactivation were of practical use only in research; however, public health concerns about the safety of recreational, drinking, and shellfish-producing waters soon added significance to investigations on the survival of viruses in aquatic environments. Thus, most studies on the survival and fate of viruses in natural waters has focused directly on enteroviruses or on coliphages used as indicators of enteroviral pollution. The latest stimulus for investigating virus decay and destruction in natural waters has come from interest in the impact of viral infection on bacterial productivity. If, as enumeration studies seem to indicate, virioplankton abundance is relatively stable over seasonal scales, rates of viral production and loss should be equal. Therefore, estimations of viral loss and inactivation in aquatic environments should indicate the virus production rate needed for steady-state conditions. In turn, estimates of viral loss rates can lead to estimates of the level of virus-mediated mortality of bacterioplankton hosts. For excellent, comprehensive reviews of the early literature on viral inactivation, see references 5 and 141.

The difficulty in detecting viral inactivation factors in natural waters arises from the complex interplay of physical, chemical, and biological variables which influence the survival of an infective virus. Nevertheless, from the wealth of data on this topic, it is possible to draw conclusions about the viricidal qualities of various physical, chemical, and biological factors. Among studies of environmental virus inactivation, the most prevalent finding is that the presence of a natural bacterial community and particulate organic matter (POM) greatly increases the rate of viral inactivation. As shown in Table 3, in nearly every case the removal of bacterioplankton and POM (>0.22 μm) from water by filtration results in a lower decay rate than in untreated natural water. The rare exception to this general conclusion was the observation that water filtration slightly increased the decay of coliphage ϕX174 (190, 191) or did not change the decay of poliovirus (6).

Further support for the involvement of bacteria, as well as heat-labile substances, in viral inactivation comes from the general observation that either heating or autoclaving natural water samples prior to viral inoculation improves virus survival. The positive effect of heating on virus survival has been observed in viral inactivation experiments with enteroviruses (168, 178, 217, 291) coliphage (21, 46, 190), and marine bacteriophage (215). It is likely that observations of increased viral inactivation at higher incubation temperatures (4 to 25°C) are related to temperature-mediated enhancement of bacterial and enzymatic activity in natural waters (101, 107, 166, 176, 213, 377). The exact nature of the virucidal heat-labile substances within natural waters is not known; however, it is suspected that these substances exhibit both protease and nuclease activity. Attempts to re-create the virus-inactivating capability of activated sludge, with RNase and trypsin (340), or of seawater, with a bacterial cell lysate (178), were unsuccessful. However, Noble and Fuhrman (215) were able to reconstitute 20 to 25% of the virucidal activity of natural seawater by addition of high-molecular-weight DOM (colloids and dissolved particles in seawater, 0.2 μm and 30,000 Da size range) to heat-treated or filtered seawater. Finally, the implication of nonliving biological or colloidal material in viral decay is supported by the observation that a low level of viral inactivation occurs in cyanide-treated seawater (318).

While some biological and chemical components of natural waters have aggressive virucidal properties, others serve to promote virus survival. In particular, the inactivating effects of bacterioplankton and heat-labile dissolved compounds can be offset by the protective effect of viral adsorption to sediment and particulates suspended in the water column. Indeed, the general sorptive properties of viruses to suspended particulates in water (see the review by Bitton [26]) have been exploited in several methods for concentrating viruses from natural water samples (7, 81, 82, 252, 287, 288, 336). In controlled experiments, the addition of clay or sediment to water, prior to the addition of virus, lowered the decay rate of both enteroviruses (158, 297) and coliphages (27, 105). In addition to inorganic particulates, heat- or UV-killed bacterial cells can protect viruses through adsorption (191). The degree to which sorption onto particulates counteracts viral inactivation is affected by the ionic environment and whether adsorption is reversible. It is possible that the differential and sometimes contradictory effects of salinity on viral inactivation in natural waters (106, 166, 178, 193, 378) are related to the influence of ionic environment on the adsorption of a particular virus strain to water column particulates.

From the wealth of scientific literature gathered prior to 1990, concerning viral inactivation in natural waters, it appeared that most of the important environmental factors involved in decay had been identified. However, the role of sunlight as a dominant factor in the decay of marine bacteriophages brought a new perspective to the issue (318). The most recent review, to 1992, by Kapuscinski and Mitchell (141) on the topic of viral inactivation in seawater, concluded that the levels of solar radiation penetrating surface waters is not im-

TABLE 3. Decay rates of various bacteriophages in natural waters

PHS	Decay rate (% h^{-1})	Expt treatment	Assay	Reference
Coliphages				
MS2	5.2–4.4	FW[b]	Viable[a]	107
F-RNAΦ	3.5	FW		
MS-2	1.2–32.5	GW[c]	Viable	377
Coliphage[n]	0.4	SW[d]	Viable	46
	0.18	Filt.[e] SW		
	0	AcSW[f]		
ϕX174[n]	15	SW	Viable	190
	20	Filt. SW		
T7[n]	4–1.4	EW[g]	Viable	213
T7[n]	3–1	SW	Viable	27
	0.8–0.5	SW+clay		
T2[n]	6.7–4.8	ASW,[h] SW	Viable	105
	1.6–1.2	ASW, SW+clay		
Aquatic bacteriophages				
9 marine PHS	1	SW	Viable	194
9 marine PHS	0.7	SW	Viable	197
	0.2	Filt. SW		
UT1	5.4	FW	Viable	220
	2.7	Filt. FW		
LB1VL-P1b	2.5	SW	Viable	318
PWH3a-P1	1.4	SW		
Both phages	40–80	SW (sl)[i]		
PWH3a-P1	0.7–0.85	SW (sl), Filt. SW (sl)	Viable	357
*Mp*V[o]	0.4–0.25	SW (d)[j]	Viable	62
	30–28	SW (sl)		
	6	ASW(sl)		
CB 7Φ	3.7	ASW(d)	Viable	369
CB 38Φ	5.2	ASW(d)		
	11	ASW(sl)		
Both phage	2.8	ASW(sl+d)	VDC[j]	
H series (4 marine PHS)	6.6–11	SW(sl), Filt. SW(sl), AcSW (sl) SW(d), FSW(d), AcSW (d)	Viable	215
	ca. 3.9			
PR series (4 marine PHS)	4.1–7.2	SW(sl), Filt. SW(sl), AcSW (sl)		
	ca. 3.85	SW(d), Filt. SW(d), AcSW (d)		
S-PWM 1 and 3	25–65	Filt. SW(sl)	Viable	101
Viroplankton loss				
Viroplankton (Danube River)	6–8	SW+KCN[k], initial 4 h (5–15°C)	VDC	176
	8–15	SW+KCN, initial 4 h (18–25°C)		
	2–5	SW+KCN >5 h (all T°C)		
Virioplankton (Santa Monica, Calif.)	5.5	SW+KCN (d)	VDC	97
	6.9	SW+KCN (sl)		
Virioplankton (Ranuefjorden, Norway)	54	SW+KCN(sl)	VDC	120
	30	SW+KCN(dph)[m]		
Virioplankton (Gulf of Mexico, Tex.)	2.2–3.0	SW(sl), Filt. SW(sl)	Viable	101
	0.14–0.5	SW (d), Filt. SW (d)		
Virioplankton (Gulf of Mexico, Tex.)	1–2.5	SW+KCN (sl)	VDC	357

[a] Decay assessed through loss of viable phage titer.
[b] Freshwater.
[c] Groundwater.
[d] Natural seawater.
[e] 0.22-μm-pore-size filtered.
[f] Autoclaved or heated seawater.
[g] Estuarine water.
[h] Artificial seawater.
[i] Sunlight.
[j] Dark.
[k] Natural seawater with added potassium cyanide.
[l] Surface water.
[m] At depth.
[n] Decay rate calculated from viral titers given in reference.
[o] Virus of *Micromonas pusilla*.

portant in the degradation of enteric viruses discharged into natural waters. In studies using coliphages that were conducted prior to 1992, sunlight was reported to have only a mildly enhancing effect on viral inactivation (21, 213). More recent studies, utilizing various marine bacteriophages (215, 312, 318, 357, 369), cyanophage (101, 312), and a virus of *Micromonas pusilla*, a cosmopolitan marine phytoplankter (62), have shown that unattenuated sunlight is a dominant factor in controlling the decay of viral infectivity in surface waters. In most cases, exposure of experimental treatments to sunlight results in a doubling of viral inactivation rates (Table 3).

The effect of sunlight on bacteriophage viability in seawater is directly proportional to the amount of sunlight (101, 318). From data correlating decay rates of five marine bacteriophages with irradiance, Suttle and Chan (312) developed a predictive model for estimating the phage inactivation rate from measurements of incident quantum irradiance. By combining surface irradiance measurements with sunlight attenuation coefficients for water samples of different turbidities, they found that sunlight-induced inactivation was significant to a depth of 200 m in clear oceanic waters (318). Even in turbid estuarine and near-coastal waters, a modest effect of sunlight on viral decay can occur at a depth of 2.5 m (62). Predictably, light wavelengths of <320 nm (UV-B) had the greatest virucidal effect on marine bacteriophages (207, 318, 357, 369), accounting for up to two-thirds of the total decay (215). Exclusion of UV-B or reduction of incident sunlight to 20% of surface levels still resulted in significant decay, compared to dark controls (318). Recent experiments with natural cyanophage populations in the Gulf of Mexico found UV-A (320 to 400 nm) to have the greatest impact on the inactivation of this virioplankton group (101). However, involvement of wavelengths longer than 400 nm in marine phage inactivation has not been supported (215).

Conclusions presented to date on virucidal factors in natural water come from experiments assessing the loss of viral infectivity under various environmental conditions. A connection between a loss of viral infectivity (inactivation) and destruction of virus particles has a special relevance for our understanding of in situ virioplankton production. If the mechanism of viral inactivation is directly related to, or coincident with, destruction of the virus particle, then VDC should be an accurate estimate of the abundance of infective viruses. In a few studies (Table 3), the loss of viral particles has been examined using natural seawater (97, 101, 120, 176, 357). The rate of viral particle loss found in these studies ranged from 1% h^{-1}, which compares well with viral inactivation rates, up to an astounding 54% h^{-1} (120). The broad range of these estimates does not support or nullify a connection between particle destruction (loss) and inactivation. Similarly, experiments examining the destruction of enterovirus in natural water have not supported a direct connection between particle loss and inactivation. Studies of the destruction of poliovirus and coxsackievirus in experimental microcosms concluded that inactivation of enteroviruses coincides with cleavage or damage of viral RNA (217, 340). However, loss of infectivity precedes destruction of viral capsids (124, 325).

Wommack et al. (369) determined rates of inactivation and loss of VDC in artificial seawater microcosms seeded with bacteriophage strains CB 7Φ and CB 38Φ isolated from Chesapeake Bay (K. E. Wommack, R. T. Hill, and R. R. Colwell, Abstr. 93rd Gen. Meet. Am. Soc. Microbiol. 1993, abstr. Q-288, p. 399, 1993). The results are shown in Fig. 2 and 3. As expected, for both phages the infectivity declined rapidly under surface sunlight conditions, at a rate twice that of low-light, control, and dark treatments. CB 7Φ, however, was more resistant to the inactivating effects of sunlight than was CB 38Φ. Others have also noted marked differences in the UV tolerance of bacteriophage strains (133). Under surface sunlight conditions, the abundance of virus particles significantly exceeded the number of infectious viruses, whereas, under low-light, control, and dark treatments, the titers were in close agreement. Unlike inactivation rates, the rate of loss for CB 7Φ and CB 38Φ phage particles was identical, regardless of experimental treatment.

Altogether, the findings of Wommack et al. (369) indicate that destruction of phage particles is a process separate from loss of infectivity, especially for sunlit waters. The marked difference between inactivation and particle destruction was also noted for phages LB1VL (317) and PWH3a-P1 (357) in Texas coastal waters. In the absence of sunlight, similar processes may be responsible for the loss of infectivity and the destruction of virus particles. Finally, while CB 7Φ and CB 38Φ had substantially different tolerances to the inactivating effects of sunlight, they showed similar rates of particle loss, regardless of treatment, indicating that in seawater, all viral capsids may have a characteristic degradation rate. The notion of an "expiration date" for viral capsids in aquatic environments is supported by the observation that in lake water, capsid destruction rates were similar for coxsackievirus and poliovirus but inactivation rates were not (124). Conversely, however, data from studies documenting total virioplankton loss rates indicate that there is a range of capsid degradation rates. Two studies in which virioplankton loss was calculated from changes in VDC reported that the <60-nm capsid size class exhibited the most rapid decrease whereas larger viruses comprised the more refractory portion of the virioplankton (120, 176). Overall, the important implication of these findings for understanding viral abundance is that VDC most probably overestimate the actual number of infectious virioplankton in sunlit surface waters.

The extreme sunlight sensitivity of viruses leads to a prediction of very low or nonexistent titers of infective viruses in surface waters. However, as noted previously, titers of infective cyanophages can be as high as 10^4 to 10^5 ml^{-1} in surface waters (312, 313, 341). As pointed out by Suttle et al. (317), this suggests a paradox, especially for viruses such as cyanophages and algal viruses, which infect photoautotrophic hosts. A plausible explanation of this paradox is that the infectivity of sunlight-inactivated bacteriophages is restored by either host- or phage-mediated DNA repair. The phenomenon of photoreactivation of UV-inactivated bacteriophage is well known. Indeed, this phenomenon alerted researchers to the existence of specific DNA repair machinery in bacteria (74, 75, 231). Subsequently, a great deal of the basic enzymatic and genetic mechanisms involved in DNA repair have been elucidated using coliphages T4 and λ (20). However, the importance of photoreactivation or other repair mechanisms for the survival of viruses in natural waters has only recently been investigated.

Weinbauer et al. (351) demonstrated that infectivity could be restored and virus production could be increased by incubation of sunlight-damaged viruses with a host population treated with photoreactivating wavelengths of light. Generalized light-dependent repair of the virioplankton was demonstrated by the observation that incubation of sunlight-damaged virioplankton with bacterioplankton under photoreactivating conditions always resulted in a greater production of viruses than did incubation of sunlight-damaged virioplankton nonactivated bacterioplankton (351). The degree to which host cell light-dependent repair was capable of restoring viral infectivity varied according to the aquatic environment. In oligotrophic ocean samples, VDC of sunlight-damaged virioplankton incu-

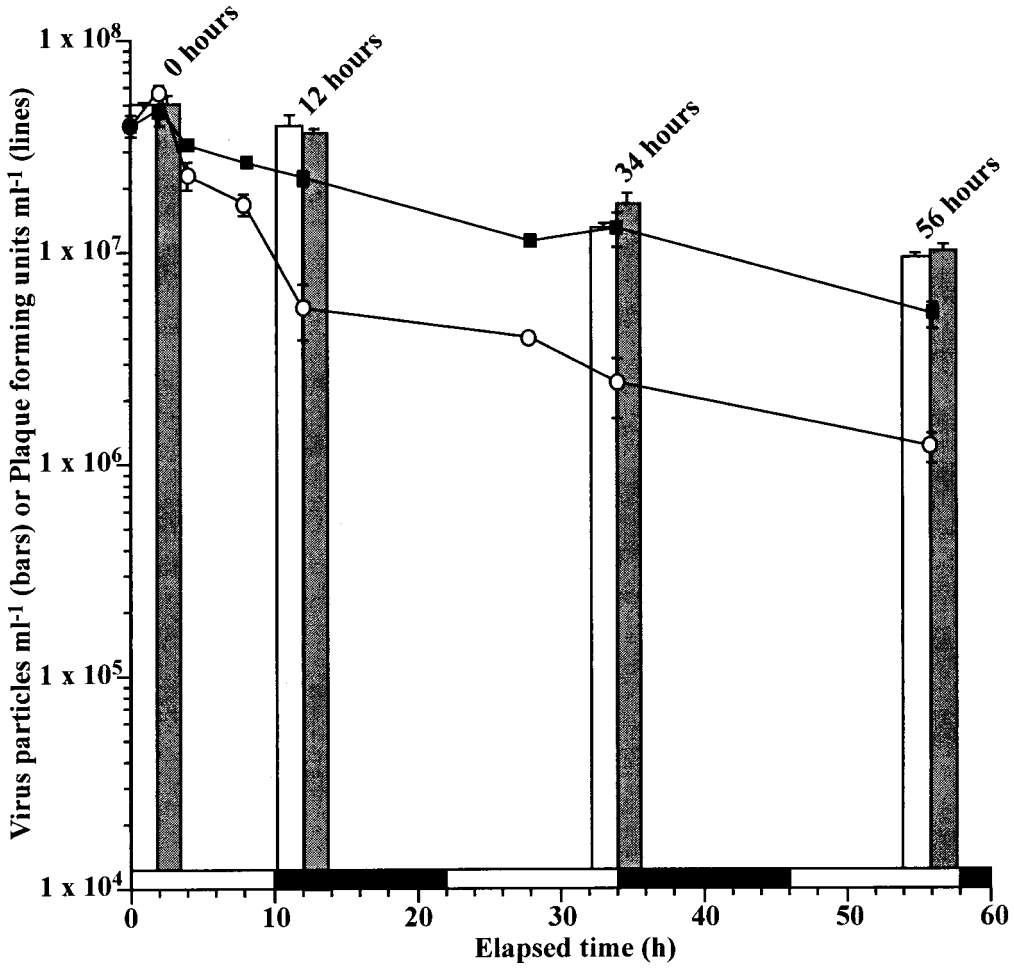

FIG. 2. CB 7Φ inactivation (lines) and viral capsid loss (bars). Symbols: ○, PFU per milliliter for microcosms under surface sunlight conditions; ■, PFU per milliliter for microcosms under low light and dark conditions; open bars, VDC per milliliter under surface light conditions; shaded bars, VDC per milliliter under low light and dark conditions. Alternating light and dark boxes represent day and night periods, respectively. Error bars are standard errors of duplicate determinations. Adapted from reference 369.

bated with activated bacterioplankton recovered to only 25% of control titers, whereas for coastal-water samples, VDC recovery was nearly 50% of control titers. The authors proposed that due to water clarity, oceanic virioplankton suffered a greater degree of nonrepairable damage (351). The repair of virioplankton infectivity has also been estimated from the difference between loss rates of infectivity and virioplankton particles in mesocosm incubations. Wilhelm et al. (357) argued that to maintain the steady state (i.e., a stable virioplankton population size) . . . "infectivity cannot be lost faster than virus particles," assuming that the pool of infectious viruses is derived from the production of new viruses. Based on these assumptions, it was calculated that for Gulf of Mexico waters, between 54 and 78% and between 39 and 67% of viral infectivity was restored daily at oligotrophic and mesotrophic stations, respectively.

The principal mechanism of UV-mediated viral inactivation is the formation of DNA photoproducts such as cyclobutane pyrimidine dimers (CPDs). Immunochemical detection and quantification of CPDs within viral DNA was recently utilized to determine the relationship between DNA damage and loss of infectivity in marine virus strains (358). As with experiments in sunlight, viral strains showed marked differences in susceptibility to UV inactivation; however, in each of the four phage-host systems examined, host-mediated photorepair systems were responsible for significant recovery of inactivated viruses. Subsequently, the relationship of CPD abundance to loss of infectivity for one virus strain, PWH3a-P1, was used to estimate inactivation in natural viral communities. From measurements of CPD abundance within sunlight-exposed virioplankton communities, it was determined that under photoreactivating conditions, more than 50% of the viruses remained active, even at the height of daily CPD formation (358).

The findings reviewed above indicate that host-dependent, light-mediated repair may be the primary mechanism of phage recovery; however, phage-encoded light-independent repair mechanisms exist, the best studied of which is mediated by T4 endonuclease V, encoded by *denV* (20). Recently, a genetic and phenotypic homolog of *denV* (A50L) was discovered in *Chlorella* virus PBCV-1 (100). DNA sequences homologous to the PBCV-1 A50L gene were found in several other *Chlorella* virus strains. The possibility that other, unique, phage-encoded repair systems remain to be discovered was recently demonstrated with a *P. aeruginosa* phage, UNL-1, which is capable of *recA*-independent repair in UV-A-irradiated hosts (290). The existence of both host-mediated photoreactivation repair and

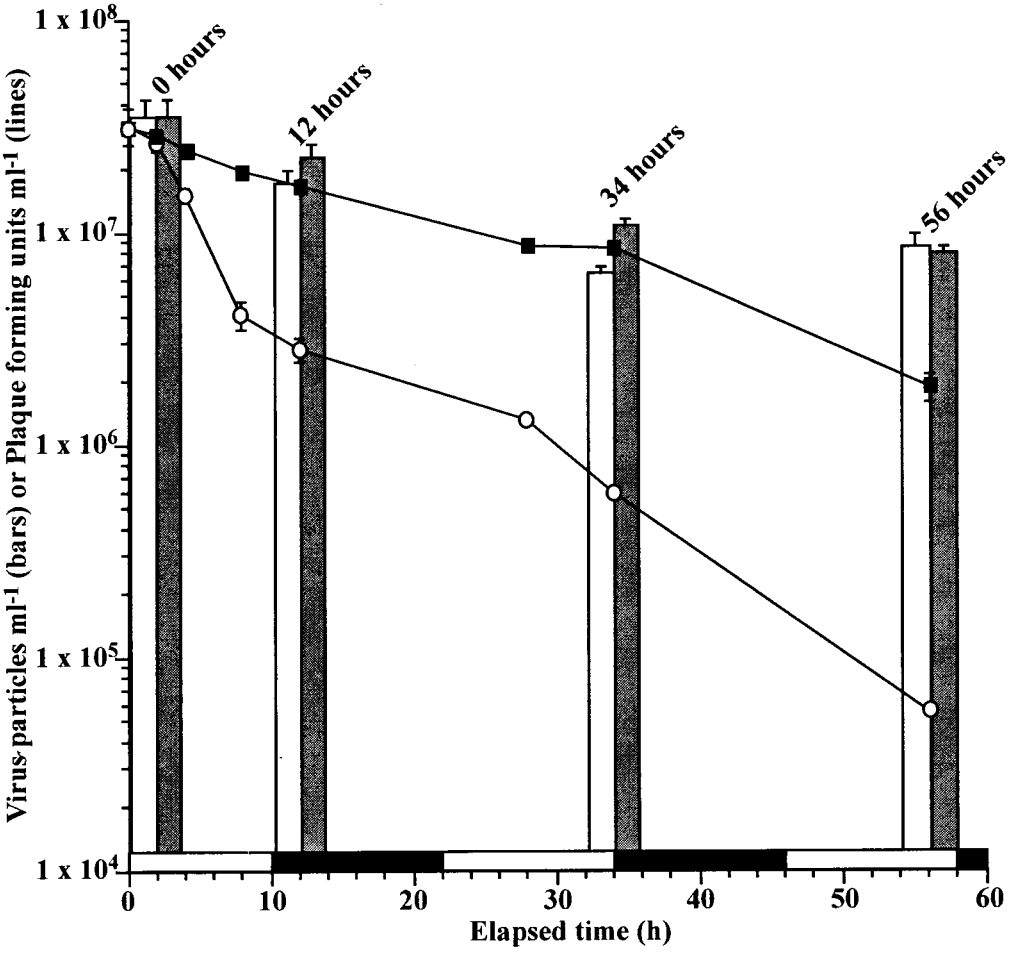

FIG. 3. CB 38Φ inactivation (lines) and viral capsid loss (bars). Symbols: ○, PFU per milliliter for microcosms under surface sunlight conditions; ■, PFU per milliliter for microcosms under low light and dark conditions; open bars: VDC per milliliter under surface light conditions; shaded bars, VDC per milliliter under low light and dark conditions. Alternating light and dark boxes represent day and night periods, respectively. Error bars are standard errors of duplicate determinations. Adapted from reference 369.

phage-encoded endonuclease repair may explain how high titers of viruses infecting photoautotrophic hosts are maintained in nature (333, 379). Furthermore, the discovery of similar dark repair enzymes in unrelated viruses is exciting, since it suggests that such mechanisms may be widespread among viruses (100).

Differences in sunlight inactivation rates of CB 7Φ and CB 38Φ (Figs. 2 and 3) demonstrate that environmental persistence is a trait particular to each phage strain. As is evident from the data on decay rates presented in Table 3, viruses exhibit a range of susceptibilities to the virucidal effects of the aquatic environment, related in part to phenotypic traits, such as phage-encoded DNA repair. Under in situ conditions, autochthonous bacteriophages appear to survive better than their allochthonous counterparts (107, 215, 220). The difference between sunlight tolerance of native (Southern California Bight) and nonnative (North Sea) bacteriophages was especially pronounced when both were exposed to southern California sunlight conditions (215), suggesting that virucidal factors provide a selective pressure influencing the composition of virioplankton communities. Support for this hypothesis includes the recent observation that there is a strong seasonal component to the susceptibility of natural cyanophage populations to light-dependent decay (101). In environments where the amounts of particulates and UV-adsorbing compounds are small and the level of incident sunlight is high, a large proportion of the virioplankton may be nonviable or inactivated. However, it is probable that in more turbid waters, a large proportion of viruses remain infective. Through mathematical modeling, it has been demonstrated that the degree to which virioplankton populations are affected by sunlight is also strongly influenced by the degree of stratification and mixing that occurs within the water column (207).

Host cell concentration and viral replication. To maintain a steady-state concentration of a virulent bacteriophage population requires that a single progeny phage from each burst event survive to infect and replicate (89, 208). Intuitively, as the host cell concentration declines, the theoretical time to successful host-virus contact increases. At a critical threshold of host cell density, viral inactivation and destruction rates exceed the contact rate and therefore the number of virulent phage declines. Viewed in these terms, virus-host contact has been described mathematically as diffusive transport (208).

Considering only the physical properties of the water column, (e.g., size, abundance, and motility of a virus and its single cell host) Murray and Jackson (208) devised a predictive model for contact rates between viruses and planktonic hosts of various sizes. From simulations, close agreement was ob-

served between theoretical and empirically derived attachment rates for a number of virus-host systems. Important conclusions derived from the diffusive-transport model were that the enhancing effect of host motility on viral adsorption is inversely related to host size and that the process of contact and adsorption to nonhost particles may be an important factor in virus mortality. Despite a significantly greater contact rate for viruses and large host cells, it is more probable that a virus and bacterium will connect because of the sheer abundance of bacterioplankton within aquatic ecosystems.

The diffusive-transport model, while useful for estimating an ideal host-virus adsorption rate, has not been widely applied to predicting the concentration of host cells required for the maintenance of a population of virulent viruses. This is principally because important model parameters, e.g., in situ concentration, burst size, and production rate of a specific virus, are not known. An elegant example of the application of diffusive transport theory to predicting the concentration of hosts necessary for viral propagation was a study of the dynamics of a lytic virus, *Mp*V, infecting the ubiquitous marine picoflagellate *Micromonas pusilla* (62). Because in situ titers of *Mp*V could be easily obtained, Cottrell and Suttle (62) were able to estimate the concentration of 1.1×10^3 to 5.4×10^3 *M. pusilla* ml^{-1} required to maintain the *Mp*V production rate in Texas Gulf Coast water samples. The fivefold difference in threshold density of *M. pusilla* arose from differences between theoretical and measured adsorption rates. The lower adsorption rate measured for the *Mp*V clone from the Texas water samples illustrated that the rate can vary among virus clones and that theoretical rates are likely to be maximal estimates (62).

Other studies carried out to examine changes in viral production relative to host cell concentration have relied on in vitro approaches. In experiments using three bacteriophage-host systems, 80α (*Staphylococcus aureus*), T4 (*Escherichia coli*), and SPβ *c*I (*Bacillus subtilus*), it was found that bacteriophage production did not occur until the host cell concentrations reached approximately 10^4 cells ml^{-1} (355). Because similar threshold concentrations were found in phylogenetically dissimilar hosts, the authors suggested that this trait may be universal for virulent phage-host systems. This idea was challenged, however, when a series of one-step growth curve experiments, employing three *Pseudomonas aeruginosa* bacteriophages, demonstrated both successful phage attachment and production at low host concentrations (10^2 cell ml^{-1}), with little or no change in burst size or latent period (156).

The discrepancy between these two studies most probably arises from differences in experimental design. Kokjohn et al. (156) separated the effect of host cell density on the two independent processes of infection and replication by using the classical one-step growth curve experimental design (79), whereas Wiggins and Alexander (355) examined coincident phage production in a batch culture of growing host cells. Of the two studies, the former, employing *Pseudomonas aeruginosa* phage-host system (PHS) models, lends stronger support for survival of a virulent phage population in an aquatic environment. Even if a higher concentration, such as that suggested by Wiggins and Alexander (355), is necessary, virulent-phage survival would theoretically still be possible. Near-coastal and estuarine waters, where bacterioplankton concentrations are typically 10^6 cells ml^{-1}, may contain up to 100 different bacterial hosts, each at a concentration of 10^4 ml^{-1}.

In addition to pure-culture studies, observations of natural virioplankton dynamics indicate the existence of a minimum host cell concentration necessary for viral replication. Through linear regression analysis, it was found that a minimum bacterioplankton concentration of between 4.7×10^5 and 7.2×10^5 bacteria ml^{-1} is necessary for measurable levels of virioplankton production in Southern California Bight water samples (306). A similar statistical analysis of bacterioplankton and virioplankton abundance data for Southern California Bight and Gulf of Bothnia water samples did not yield a minimum concentration of bacterioplankton before a measurable number of viruses could be obtained (56). However, in the latter study it is possible that the actual threshold concentration of host cells was below the detection limit for both viral and bacterial direct counts (10^4 ml^{-1}). The former approach of comparing the viral production rate with bacterial abundance is more likely to detect an in situ threshold host cell concentration, because, as shown in Table 1, it appears that no natural aquatic environment is devoid of viruses as free virus particles. Finally, FVIC data from northern Adriatic Sea water samples indicated that when the numbers of rod-shaped bacteria was below 2×10^5 ml^{-1}, no intracellular viruses were observed in this dominant morphological group (347).

Observations of the in situ dynamics of marine *Synechococcus* phage indicate that a minimum number of host cells is necessary for replication of these phage. In an analysis of changes in the natural abundance of cyanophages infecting specific strains of marine *Synechococcus* and total *Synechococcus* abundance, it was demonstrated that synechophage numbers increased dramatically when the total *Synechococcus* abundance reached ca. 10^3 ml^{-1} (312, 313). Long-term, seasonal observations of marine *Synechococcus* and cyanophages in Woods Hole Harbor indicated that if a minimum number of *Synechococcus* cells, i.e., a threshold density, exists, it is likely to be very small, since titers of specific synechophage were detected when the number of *Synechococcus* cells was as small as 10^2 ml^{-1} (341).

Perhaps the strongest evidence that maintenance of a stable virus population requires a specific number of host cells was provided by the observations of Wilcox and Fuhrman (356). In mesocosm experiments, natural virioplankton and bacterioplankton densities were altered and changes in VDC and BDC were monitored for 6 days. For all experimental treatments, an initial titer of $>10^6$ viruses ml^{-1} was necessary for VDC to rise to in situ levels. Interpreting these results in light of the diffusional transport theory (208), the authors concluded that the product of bacterioplankton and virioplankton concentrations had to be $>10^{12}$ in order for lytic virus production to occur (356). Therefore, in aquatic environments, such as estuarine and near-coastal waters, where the product of the number of bacteria and virus is typically on the order of 10^{13} (10^6 bacteria $ml^{-1} \times 10^7$ viruses ml^{-1}), lytic viral production would be high.

While these in situ observations indicate the population densities of bacterioplankton and virioplankton at which virus production seems to occur, they cannot predict the threshold density of a single bacterial host species necessary for replication of a particular virulent phage strain. Estimates of threshold density under in situ conditions require data on the temporal dynamics of the several naturally occurring PHS. With current technology, direct observation of PHS dynamics is difficult. However, based on theoretical calculations of phage-host encounters (303), production of a lytic virus appears to occur under natural conditions. In an examination of bacterial populations in a meromictic lake, Tuomi et al. (329) postulated that lytic-virus production could explain in situ fluctuations in the densities of individual bacterial strains. In this system, VBR was a relatively constant high value of ca. 50; therefore, if individual bacterial populations were on the order of 10^4 ml^{-1} and each virus strain was, on average, 50-fold more abundant than its host, then ca. 750 virus contacts h^{-1} occurred.

The authors concluded that in this system, lytic-virus infection could account for dramatic changes in the abundance of bacterioplankton populations.

Host metabolic state. It is widely understood that the physiological state of bacteria under laboratory culture conditions is dramatically different from that under natural conditions. Even in the most productive aquatic environments, bacteria are hypothesized to exist in a condition of "feast-or-famine" (157), in which periods of nongrowth are punctuated by short periods of rapid growth. Therefore, the normal state of aquatic bacteria is generally believed to be approximated by the stationary phase of a typical bacterial growth curve (157). Besides obvious metabolic slowdown, the transition from exponential growth to the stationary state results in dramatic changes in cell morphology, rates of macromolecular synthesis and degradation, and constitution and surface characteristics of the cell wall (146, 294). The genetic mechanisms behind these adaptations to starvation survival in nonsporulating bacteria are only beginning to be revealed (see the review by Matin et al. [177]). After some time in a nongrowth state, cells may enter the viable-but-nonculturable state characterized by loss of culturability on standard media but with retention of substrate responsiveness (viability) (61, 277).

The bias toward the study of exponentially growing bacteria is based on the requirement for balanced growth in physiological experiments. Similarly, this bias has extended to the study of the biology of phage-host interactions, since nearly all of the thousands of described bacteriophages infect and replicate apparently only in rapidly growing hosts (272). The belief that most aquatic bacteria exist in a slow-growth or starvation state is at odds with our current understanding of the nature of phage-host interactions and calls into question whether bacteriophage infection and replication occur in aquatic environments.

Detailed studies of bacteriophage infection of senescent host cells are few. The one detailed examination of phage infection and growth in stationary-phase cells utilized *Achromobacter* bacteriophage α3a (375). Compared with growth on logarithmic-phase, wild-type *Achromobacter*, the growth of α3a on strain 14 (an *Achromobacter* strain supporting α3a replication only during stationary-phase growth) showed dramatic increases during the latent period (100 min versus 6 to 9 h) and in burst size (153 versus 700). Significant production of α3a by stationary-phase strain 14 cells required microaerophilic conditions, was observed in stationary (nonshaken) cultures for up to 19 days, and was not due to infection of a small subset of growing cells (273, 375). Increases in the latent period and changes in the burst size of bacteriophages infecting starved or stationary-phase host cells were recently documented for several bacteriophages specific for either *P. aeruginosa* or *E. coli* hosts. Astoundingly, two of the *P. aeruginosa* phages, ACQ and UT1, were able to infect and replicate in host cells which had existed under starvation conditions for 5 years (286). Delayed plaque formation or continuous growth in plaque size on an established lawn of host cells have been speculated to be characteristic of a stationary-phase bacteriophage (272). When isolating several PHS from Chesapeake Bay and Bahamian waters, we have observed two instances of bacteriophages demonstrating growing plaques. We concluded these bacteriophages are likely to infect host cells both during rapid growth and during host cell senescence (Wommack et al., Abstr. 93rd Gen. Meet. Am. Soc. Microbiol. 1993).

Mechanisms responsible for stationary-phase-specific infection and replication of α3a on strain 14 were suggested to be related to template specificity of the host RNA polymerase (274) and not to selective attachment of α3a only to stationary-phase cells (273). Similarly, the RNA coliphage MS2 demonstrated the curious ability to attach to, infect, and replicate in both stationary- and logarithmic-phase host cells; however, MS2 production in stationary-phase hosts was not followed by immediate lysis. Delay of cell lysis appeared to be linked to cell division, since infected stationary-phase cells released progeny virus only after resumption of cell growth (259, 260). In an ecological sense, the behavior of coliphage MS2 is a possible explanation for the persistence of a virulent phage within a population of nongrowing hosts. By delaying lysis but not production until cell division, progeny phage are released during periods of rapid growth when conditions are more favorable for further rounds of phage infection and replication.

The aforementioned studies demonstrate that phage attachment and production can occur in physiologically stationary cells under ideal culture conditions; however, the stationary state of bacterial host cells under natural conditions has not been examined. The effect of the natural starvation state on phage infection was examined in experiments with two bacteriophages, UT1 and Φ116L, and starved *P. aeruginosa* cells placed in autoclaved river water (156). Both the virulent UT1 and the temperate Φ116L phages were capable of infecting and replicating in starved (156) cells under riverine conditions, albeit with a lengthened latent period and greatly reduced burst size, compared to logarithmic-phase infection. Significant attachment (≥44%) of UT1 and Φ116L to starved *P. aeruginosa* cells was observed as long as 40 days after the onset of starvation conditions.

At present, the number of bacteriophage species capable of infecting nongrowing hosts appears to be limited; however, due to the wealth of evidence for active viral infection in aquatic microbial communities, it is intuitive that stationary-phase infection should be common within the virioplankton. Recent studies by Moebus (199, 200) offer a first step toward wider demonstration of this phenomenon. Four of six marine PHS that were assayed showed some degree of stationary-phase infection after host propagation on low-nutrient media. Dramatic differences were observed in the ability of senescent cells to propagate phage. For example, PHS H40-H40/1 showed no decrease in the ability to produce phage throughout the 30-day assay period, whereas, bacteriophage H2/1 could not infect and grow on stationary-phase H2 host cells that were more than 3 days old. All PHS showed decreases in the rate and extent of phage production during the logarithmic-phase-to-stationary-phase transition (199). This observation may indicate that cellular changes occurring during prolonged nongrowth are necessary for phage infection. It is interesting that, like phage α3a, production of phage H3/1 on stationary-phase H3 cells was significantly faster under microaerophilic conditions. It is exciting to speculate that physiological changes occurring with the adjustment to microaerophilic conditions may coincide with those necessary for phage infection and production in nongrowing cells.

From the few studies presented, it is not possible to estimate the fraction of marine bacteriophage within the virioplankton which are capable of infection and replication on nongrowing hosts. The interaction between phage and bacterial host cells in a natural state of physiological stasis, such as the viable-but-nonculturable state, also remains an open question. Finally, it has been suggested that study of stationary-phase bacteriophage infection may provide insight into this enigmatic physiological state (273).

Host range. It is possible to view the bacterioplankton host community as comprising nongrowing cells within a diverse assemblage of species, each present at small numbers. Under these conditions, propagation of highly strain-specific viruses

seems unlikely; however, a bacteriophage capable of infecting a broad range of bacterial hosts would, conceivably, have better potential for replication under in situ conditions. In general, phage do not "trespass generic boundaries" (2). Therefore, among the ca. 4,000 known bacteriophages, host specificity is the rule. Similarly, most aquatic viruses (102, 264) and bacteriophages (13, 22, 64, 117, 151, 321) examined to date have demonstrated either species or strain specificity. Nonetheless, broad-host-range (polyvalent) bacteriophage have been demonstrated on several occasions.

Most notable of these are the lipid-containing phages of the virus family *Tectiviridae* (see reference 10 for a review of the *Tectiviridae* type phage PRD-1). Several phages within the *Tectiviridae* have demonstrated the ability to infect multiple genera within either gram-positive or gram-negative domains (10). This unusually broad host range is explained by the fact that the phage receptor is encoded on conjugative plasmids (32, 374). Marine bacteriophages described to date have generally shown species specificity with commonly observed resistant strains (see reference 29 for a review). One exception is the vibriophage KVP40, which was shown to infect eight *Vibrio* species and the closely related bacterium *Photobacterium leiognathi* (180). The basis of the broad host range of KVP40 is the OmpK receptor, which, from sequence information and immunoblotting, is unique to *Vibrio* and *Photobacterium* strains (130, 131). The central role of the receptor in determining host specificity is also evident from the observation that the phage host range is often extended by mutations in the phage genome, which allow the use of an additional receptor molecule for attachment (203). Investigation of bacterial strains showing a reduced efficiency of plating for KVP40 revealed a restriction-modification system which may be common to several species of the *Vibrionaceae* (179). The existence of a common restriction-modification system is noteworthy, since R-M systems are generally species or strain specific (31). It is interesting to speculate that the widespread R-M phenotype found in the *Vibrio* strains is a result of the selective pressure of the broad host range of KVP40.

Perhaps the most careful analysis of host specificity among a group of aquatic bacteriophages has been done for cyanophages. In a collection of 14 cyanophage isolates infecting marine *Synechococcus* strains, only 2 phage were specific to a single host strain. All other phage infected at least three different strains of *Synechococcus* marine cluster A (341). Among seven cyanophages isolated from Texas Gulf coast waters, most were specific to a single host strain (five out of seven), while two, S-PWM3 and S-PWM4, were polyvalent (313). In both studies, host range assays identified marine *Synechococcus* strains which were particularly susceptible to cyanophage attack and thus were useful as indicator strains. Among freshwater cyanophages, a similar variability in host range has been reported, ranging from a virus strain which infects several filamentous species across three genera (279) to more host-specific strains infecting only two or three strains of heterocystous cyanobacteria (295) or unicellular forms (278, 280) (see reference 229 for a review).

It appears that polyvalence may be more common among cyanophages. However, none of the studies gave results that correlated host phylogeny with cyanophage susceptibility. Both studies involving marine *Synechococcus* assayed strains containing either phycocyanin or phycoerythrin. Based on this criterion, only three cyanophages were capable of infecting representatives of both pigment groups (313, 341). As pointed out by Ackerman and DuBow (3), coliphages often appear to be polyvalent because the family *Enterobacteriaceae* is such a closely related bacterial group. At present, there is not enough information on the host range of aquatic bacteriophages to determine the degree of polyvalence among virioplankters. A study of activated-sludge PHS showed that polyvalence could be as high as 10% in that natural bacteriophage consortia (114). A method, recently developed by Hennes et al. (123), which involves labeling of bacteriophage particles with fluorescent dyes, could be a promising means of rapidly determining whether a new phage isolate is potentially polyvalent. With this approach, they demonstrated that phage PWH3a specifically attached only to its host and not to other cells within a natural bacterioplankton consortia. Conversely, the known polyvalent cyanophage S-PWM3 attached to ca. 3% of *Synechococcus* cells in water samples.

The varied susceptibility of bacteria to phage infection and the generally restricted host range of bacteriophage have been exploited to construct highly selective schemes for typing bacterial strains (250). In an application of phage typing, Moebus and coworkers (201, 202) utilized a large collection of marine PHS to examine the geographic distribution of bacterial populations. From the large set of phage-typing data, it was evident that bacterioplankton populations west of the Azores were phenotypically distinct from those east of the Azores. Furthermore, eastern North Atlantic bacteria were often susceptible to infection by "western" phages, whereas the converse was only rarely seen (202). These susceptibility patterns, the authors argued, reflect the general circulation of surface waters in the North Atlantic from west to east. Interestingly, out of the 217 Atlantic series phages positive for infection, only 41 (18.9%) were specific solely to the original host strain. Because the taxonomy of the bacterial hosts tested was unknown, the authors made no claim that phages infecting several strains were actually polyvalent.

More recently, the phage-mapping approach was applied to German coastal waters. Unlike the Atlantic series, the Helgoland series of bacteriophages appeared to have a higher incidence of host specificity. Among the 194 phages of this typing set, 88 were propagated solely on their original host (194). However, the Helgoland collection also contained a few polyvalent members, since a recent study extensively characterizing 85 of these phages showed that 27% had moderate to broad host ranges (353). In general, these phage-typing experiments found a greater degree of polyvalence among phages collected from low-nutrient, pelagic ocean waters than among those collected from productive coastal waters. This observation raises the intriguing possibility that bacteriophages occurring in oligotrophic oceanic waters with a low bacterioplankton abundance have a tendency toward polyvalence whereas phage found in nutrient- and host-rich coastal waters are generally host specific. This hypothesis is tenable because, in a low-host-density environment, broad-host-range phages would intuitively have a greater chance for successful host encounter. Conversely, high-host-density environments, where host-phage encounter is more common, would presumably select for hosts with broad-range resistance to abundant cooccurring viruses.

Finally, recent work suggests that the dogma of extreme host specificity among bacteriophages may be a methodological artifact (132). Tests of two bacteriophage collections revealed that 90% of the phages were polyvalent. The first group, phages infecting *Sphaerotilus natans*, were capable of infecting *Pseudomonas aeruginosa* with the same efficiency as the original host due to a general resistance of these phages to type I and II restriction endonucleases. The second group was more typical of polyvalent phages, since these phages plated much less efficiently on the alternate host. The latter group was isolated using a novel two-host enrichment protocol instead of the traditional single-host enrichment. The authors argued that

the two-host enrichment prevented selection for narrow-host-range phages and thus demonstrates that polyvalent phages can be readily isolated from environmental samples (132). The implications of these findings are best illustrated in the observation that two of the nine polyvalent phages mediated generalized transduction. Therefore, if polyvalent viruses are common in the virioplankton, it is possible that indiscriminate transfer of genetic material between bacterioplankton species occurs frequently.

There are two open questions concerning the relationship of phage-host range and virioplankton ecology. First, in most aquatic environments, it seems that a broad-host-range phenotype would be favored; however, most aquatic phages are thought to have highly specific host ranges. Therefore, what is the selective advantage of a narrow host range for a bacteriophage? The second question relates to the hypothesis discussed above. What is the relationship between the frequency of polyvalent phenotypes and environmental conditions?

Phage life cycle. (i) Temperance versus virulence. The biological components of the phage-host relationship discussed thus far have focused principally on factors which impact the production of virulent phage. For example, a virulent phage which has limited host range and a rapid decay rate would be quickly eliminated in an environment of slow-growing hosts present in small numbers. The fact that many aquatic environments are characterized by a low concentration ($<10^5$ ml^{-1}) of slow-growing bacterioplankton and that free phage are quickly inactivated under in situ conditions has led to speculation that temperate bacteriophages may be most numerous in aquatic environments (37–39, 323). This speculation has been supported by observations, from short-interval samplings (10 min to 2 h), of rapid changes in virioplankton abundance. These bursts in virioplankton production are presumably a result of synchronous lysis caused either by mass infection and subsequent lysis of a susceptible host population or mass induction of a population of lysogens (36, 40, 42). The supposition that temperate phages predominate within the virioplankton has also been supported by the often cited claim, made by Freifelder (87), that more than 90% of known bacteriophages are temperate. While these simple points are alluring for building a hypothesis of widespread lysogeny among aquatic bacterioplankton, there is little hard evidence to support Freifelder's claim for aquatic bacteriophages (195, 364). Certainly, a better understanding of the mechanisms of in situ phage production will assist in determining the impact of phage infection in aquatic environments.

Production and survival of a virulent phage requires the rate of successful host-phage encounter to exceed the rate of virus destruction and inactivation (350). In contrast, the production of a temperate phage is independent of host cell density and requires only a suitable number of lysogenic carrier cells and the occasional presence of an inducing agent to stimulate the lysis and release of free viruses (19, 37, 350). Virioplankton communities composed principally of virulent phage would be predicted to control the number of susceptible hosts and exert significant selection pressure toward phage resistance. Conversely, communities of temperate phage may have little influence over the population size of lysogenic hosts and instead could be instrumental in the distribution of genotypic and phenotypic characteristics.

Before considering the prevalence of either temperate or virulent phage in aquatic ecosystems, it is important to discuss the relative selective advantage of each to the persistence of a bacteriophage and its host. The advantages of virulence essentially address the disadvantages of temperance, namely, that given an ample supply of susceptible hosts, a virulent phage population can grow rapidly and utilize limited available resources quickly. Presumably, in a closed system, a population of virulent phage would reduce host abundance to a level below that capable of sustaining phage production. However, numerous chemostat experiments have demonstrated that the concentrations of hosts and virulent phage inevitably reach an equilibrium in which phage abundance is two to three orders of magnitude lower than host abundance (160). For the temperate bacteriophage, lysogeny provides a means of persistence through ". . . 'hard times' when host bacterial density falls below that necessary to maintain the viruses by lytic infection alone" . . . (307). However, when contained in the cryptic, prophage state, a temperate phage is restricted to a level of multiplication dictated by the growth rate of the host (174). Lysogenic host populations are burdened with the maintenance of extra prophage DNA and the possibility of cell lysis from prophage induction (174). Noting the stability and prevalence of lysogeny among bacteria, it is teleologically obvious that these disadvantages must be outweighed by the beneficial effects of the lysogenic state. At a minimum, the host cell, via lysogeny, aquires immunity to further infection from other homoimmune phages (2).

Besides superinfection immunity, lysogenic hosts commonly gain new phenotypic characteristics. Known as phage conversions, these phenotypes include antibiotic resistance, antigenic changes, enterotoxin production, and virulence factors (see references 12 and 48 for reviews). A noteworthy example of phage conversion was the recent discovery that cholera toxin is encoded on a filamentous bacteriophage (337). This finding has helped to explain the enigmatic nature of cholera epidemics and suggests that the lysogenic relationship between *Vibrio cholerae* and the cholera toxin phage could be a key determinant of the incidence of cholera (361). This discovery also prompted speculation that another important virulence determinant, the type III secretion system, present in a variety of pathogens (e.g., *Yersinia*, *Shigella*, and *Salmonella*), may also be transmitted via a bacteriophage vector (11). While many phage conversions are associated with pathogenic phenotypes, it is possible that a range of genetic properties important to host survival, fitness, and resource utilization are phage encoded.

Evidence of physiological changes which enhance reproductive fitness have been documented for several *E. coli* lysogens. When grown in glucose-limited chemostats, lysogens of phages P1, P2, lambda, and Mu all demonstrated higher metabolic activity, faster growth, and extended growth phase than did nonlysogens (77, 78, 165). In the case of lambda lysogens, increased reproductive fitness was also noted for growth substrates, such as glycerol, lactate, and acetate (78). While specific details concerning the mechanism of these lysogenic phenomena are not known, it is possible that outer membrane proteins are involved, since greater expression of certain outer membrane proteins was noted in lambda lysogens under experimental conditions (76). Finally, an unsubstantiated advantage of lysogeny may be the elimination of competing, phage-susceptible nonlysogens (307). Under conditions of high-density growth, induction and release of phage from a small subset of a lysogenic population could selectively destroy nonlysogens, thereby effectively reducing competition for a limiting resource.

To estimate the potential contribution of lysogeny to the maintenance of abundant virioplankton populations, it is necessary to determine either the percentage of bacterioplankton which are lysogens or the frequency of temperate bacteriophages. There have been numerous reports of temperate bacteriophages isolated from marine (14, 134, 192, 205, 221, 230, 362) and freshwater (220, 271) environments. Until the recent

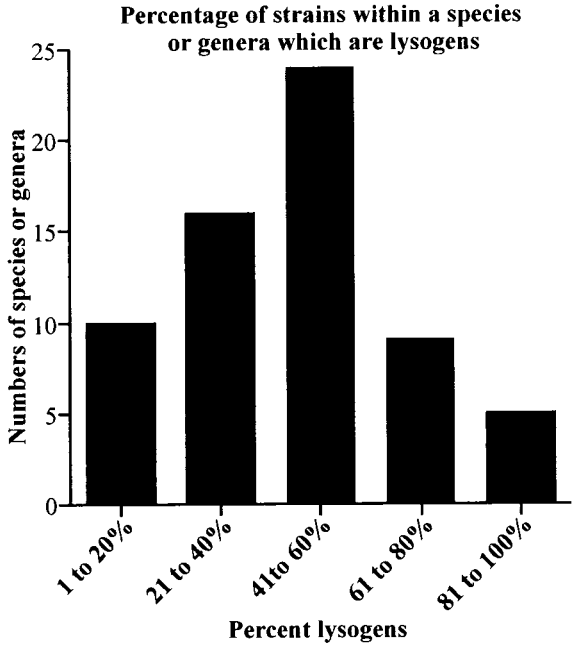

FIG. 4. Frequency of bacterial strains within a species or genus which are lysogens. Adapted from reference 2.

discovery by Ohki and Fujita (221), no cyanophages capable of lysogenizing a marine cyanobacterium were known. This discovery demonstrates the likelihood that all bacterial taxa contain member species or strains capable of carrying prophage. Since there is no means of reliably determining whether a free virus is temperate or virulent, many researchers have examined the incidence of lysogenic strains within collections of aquatic bacterial isolates. This assay involves treatment of a bacterial culture with prophage-inducing agents, such as UV irradiation (167, 289) or exposure to the antibiotic mitomycin C (14, 83, 147, 228). However, many chemical agents, such as aromatic and aliphatic hydrocarbons, can induce prophage (57, 135) (see reference 2 for a comprehensive listing of chemical and physical prophage-inducing agents). In general, mitomycin C has been observed to be a more potent inducing agent than UV irradiation (137, 350).

Through the assembly of numerous reports on the incidence of lysogeny within bacterial genera or species, Ackerman and DuBow (2) concluded that the frequency of lysogeny varies significantly among taxonomic groups. Their tabulated data are presented in Fig. 4. In general, for most taxonomic groups, ca. 21 to 60% of member species will be lysogens (Fig. 4). Studies directly examining the incidence of lysogeny among bacteria isolated from natural samples have found that similar proportions of lysogenic bacteria are present in collections of environmental bacterial strains.

Among 38 bacterial strains isolated from the nutrient-rich environment of the mammalian rumen, 9 were found to be lysogens (23.7% demonstrated lysogeny) (147). The results of two separate studies using a collection of marine bacterial isolates from southern Florida and Hawaii showed that ca. 40% of the marine bacterial strains assayed contained prophages (n = 167 bacterial isolates) (136, 137). The authors warned that these may be minimal estimates, since not all prophages are readily inducible with mitomycin C (136, 137, 147). The difficulty of extrapolating the frequency of lysogeny among bacterial isolates to the general prevalence of lysogens within bacterioplankton populations was illustrated by Ogunseitan et al. (220) using temperate bacteriophages of *Pseudomonas aeruginosa*. Using a combination of gene-probing and traditional bacterial culture methods, they demonstrated that a sizable proportion (45%) of susceptible *P. aeruginosa* cells in lake water mesocosms converted to carriers of phage UT1 within 12 h. In a broader study, they found that up to 40% of *P. aeruginosa* cells in lake water samples contained prophage homologous DNA, yet mitomycin C treatment revealed that only 3% of phage UT1-positive colonies liberated free bacteriophage (219).

In studies of marine bacterial strains, a greater incidence of lysogeny was noted for strains isolated from oligotrophic environments (136, 137). This finding is interesting since a higher frequency of lysogeny would be predicted for oligotrophic environments, which are characterized by a low density of slow-growing bacterioplankton. However, the possibility that lysogens from oligotrophic environments are more susceptible to the inducing effect of mitomycin C cannot be discounted. Differences in the incidence of lysogeny between environments has also been noted in studies comparing the prevalence of virulent and temperate phages in sewage and feces. From a compilation of the literature, it was apparent to Lenski (160) that sewage favors virulent phage, while in feces temperate phage predominate. He theorized that these differences reflect the rate at which new bacteria and phage populations invade the autochthonous consortia. In sewage, the constant influx of new susceptible bacterial hosts present in large numbers would support "blooms" of virulent phage populations while the relatively more stable populations of phage and bacteria in the gut would select for phage resistance and lysogeny. Alternatively, he suggested that physical and environmental constraints within the gut reduce host-virus encounters and thus select against lytic replication of virulent phage (160).

The enormous set of marine PHS collected by Moebus and Nattkemper (202) for the purpose of phage mapping offers insight into the prevalence of temperate phage in the marine environment. By careful examination of phage-host cross-reaction data, they found that lytic reactions (clear plaques) outnumbered lysogenic or inhibitory reactions (cloudy plaques) (67.3 and 32.3%, respectively). Additionally, 42.4% of the 217 phage isolates were presumably virulent, since this fraction produced only lytic reactions. After the original mapping study, Moebus (198) reexamined 300 phage strains and identified only 29 that were truly temperate. Most of the PHS (65%) were considered to be virulent.

Another, more direct approach to estimating the prevalence of lysogens within bacterioplankton populations has been to monitor VDC in water samples treated with an inducing agent. Most reported attempts of treatment with UV or sunlight, nutrient addition, and temperature failed to yield conclusive evidence of induction in water samples (37, 42, 350, 356). Generally, only treatment with mitomycin C has provided evidence of substantial increases in VDC, most probably a result of mass induction of lysogenic bacterioplankters. In water samples collected along an onshore-to-offshore transect in the Gulf of Mexico, treatment with mitomycin C always resulted in a significant increase in VDC over that in untreated control incubations. From the mitomycin C-induced change in VDC, it was calculated, based on minimum and maximum burst size estimates, that between 0.07 and 4.4% ($\bar{x}$ = 1.5%) of the bacterioplankton members were lysogens. Assuming that aquatic bacterioplankton populations double once per day, lysogen induction would account for between 0.14 and 8.8% of total bacterioplankton mortality in Gulf of Mexico waters (350). If the inducing agents stimulated phage release in a

majority of lysogens, the contribution of lysogenic production to the maintenance of Gulf of Mexico virioplankton populations would be small. Results of similar experiments using mitomyocin C or UV treatment of Lake Superior water samples also showed that only a small percentage of these bacterioplankton members (0.1 to 7.4%) harbor prophage (320). Nevertheless, a caveat for these experiments is that there is a possibility that mitomyocin C and UV-C radiation treatments affect only a small proportion of lysogens, thereby leading to a greatly underestimated incidence of lysogeny (350).

Using a more rigorous test of lysogenic induction from natural bacterioplankton (significant changes both in BDC [decrease] and VDC [increase]), Jiang and Paul (135) observed lysogenic induction in a majority of water samples collected from a variety of marine environments. For coastal and estuarine water samples, induction was recorded in 11 of 15 samples, while only 3 of 11 offshore water samples demonstrated significant levels of prophage induction. In a later study, estuarine waters of Tampa Bay showed a propensity to contain lysogens, i.e., 52% of the water samples showed significant levels of induction, with all induction-positive samples occurring during warm periods of the year (58). These findings indicate a greater degree of lysogenization among bacterioplankton populations in eutrophic environments and contradict the observation of a higher incidence of lysogeny among bacterial strains isolated from oligotrophic environments (136, 137). The authors suggest three explanations for these contradictory results: (i) that the greater metabolic activity of bacterioplankton in near-shore water samples made these lysogens more susceptible to chemical and physical inducing agents; (ii) that the toxicity of inducing agents was greater for bacterioplankton in oligotrophic water samples; (iii) that low viral abundance in oligotrophic waters, combined with the lower precision of counting viruses when the numbers are small, prevented the detection of significant changes in VDC. The frequency of lysogeny ranged greatly for these samples; however, the authors estimated that in estuarine environments an average of 34% of bacterial cells carry prophages. Interestingly, several common environmental pollutants, such as polychlorinated biphenyls, pesticides, and polyaromatic hydrocarbons, are potent inducing agents (57, 135). This suggests that besides their known mutagenic effects, these pollutants could have a significant impact on the composition and ecology of aquatic bacterioplankton.

The aforementioned studies, based on in situ induction, arrived at dramatically different estimates for the prevalence of lysogeny. The low estimates of Weinbauer and Suttle (350) suggest that even if every lysogen was eventually lysed via prophage induction, this form of phage production would contribute little to overall virioplankton production. The higher abundance of lysogens estimated both from the observations of Jiang and Paul (135) and from studies of bacterial culture collections indicate that even with a moderate level of induction, lysogenic production could contribute significantly to virioplankton production. However, the natural rate of spontaneous induction is low (10^{-2} to 10^{-5} phage produced per bacterium per generation) (2, 307). Using known or estimated values for the induction rate, bacterial growth rate, incidence of lysogeny, viral decay rate, virioplankton abundance, and bacterial abundance, Jiang and Paul (137) developed a predictive model for estimating the contribution of lysogenic production to total virioplankton production. At background levels of spontaneous induction, the contribution of lysogenic production is relatively low, ca. 0.02% of the daily virioplankton production. Lower viral decay rates, however, resulted in a significant input of spontaneous lysogenic production to overall virioplankton production (137). Under normal conditions, in aquatic environments, virioplankton production would be primarily lytic. However, in aquatic environments with low virioplankton decay rates or significant impacts of physical or chemical inducing agents, lysogenic production could be important. Finally, support for the importance of lytic production comes from the observations of Wilcox and Fuhrman (356), who found that changes in VDC in seawater mesocosms could be predicted entirely by the product of initial bacterial and viral abundances. Mesocosms with either low initial viral or bacterial titers failed to show significant increases in free virioplankton abundance. If lysogenic production were significant, virioplankton production would continue in mesocosms, unhindered by decreases in host abundance. Moreover, treatments with intense pulses of natural sunlight failed to stimulate virioplankton production (356).

(ii) Pseudolysogeny. The summation of in situ and laboratory-based studies suggests that even if ca. 40% of the bacterial cells contain prophage, the contribution of lysogenic production to total virioplankton production is low. However, the prevalence of lytic production appears to be incongruous in connection with the conditions of low host density and poor nutrient conditions that are found in many aquatic environments. Another possible explanation for the sustained production of virioplankton, despite adverse environmental conditions, is the phenomenon of pseudolysogeny. Simply stated, the characteristics of pseudolysogeny (a phage carrier state) resemble those of lysogeny, namely, that after infection bacteriophage can either enter a cryptic, intercellular phase or sustain rapid lytic infection (12). However, unlike true lysogeny, pseudolysogeny does not involve integration of host and phage genomic DNA and the phage DNA is not replicated and segregated equally into all progeny cells (2, 9, 119, 270).

While lysogeny is inherently a more stable association of phage and host (119), production of intercellular pseudolysogenic phage cannot be stimulated with chemical or physical inducing agents (9). Furthermore, pseudolysogens are relatively easily cured of their coexisting phage through repeated subcloning (276) or addition of phage antiserum to host culture media (2, 9, 12, 195). Barksdale and Arden (12) articulated three possible mechanisms for the pseudolysogenic state. The first two mechanisms involve either a limitation of available phage receptors on susceptible cells or a selective loss of phage receptors from the host population through action of a phage-encoded endolysin (164). The third mechanism predicts that virulent phage mutants capable of infecting lysogens frequently and spontaneously arise from prophage. In essence, lysogens encounter persistent episodes of viral infection and thus appear to have the less stable pseudolysogenic phenotype.

Altogether, the pseudolysogenic life cycle is an enigmatic one and is not easily recognized. Nevertheless, a few aquatic bacteriophages have been described as pseudolysogenic (196, 200, 220). Other PHS have demonstrated pseudolysogenic properties but have not been specifically identified as pseudolysogenic (22, 362). Among the earliest reports was that of phage Hs 1 infecting the halophilic archaebacterium, *Halobacterium salinarium* (267, 327). The association between Hs 1 and *H. salinarium* in culture was characterized by sporadic lysis of a majority of cells in batch cultures and an inability to subculture stable lysogenic clones. The infection dynamics of Hs 1 were observed to change dramatically with salt concentration. At the lower salinity limit for host survival (17.5% [wt/vol] NaCl), Hs 1 appeared to be virulent, lysing a majority of host cells. With increasing salt concentration (up to 30%), host survival improved dramatically and the majority of cells were phage carriers. In essence, conditions favoring phage and host

production were inverted. This relationship works to ensure phage survival under conditions deleterious to host growth (327). The life cycle characteristics of phage Hs 1 illustrate a feature of pseudolysogeny which is important for the maintenance of natural PHS, namely, that phage production is regulated by environmental conditions that dictate host growth and survival.

The influence of a single nutrient on phage replication has recently been demonstrated in cyanophage strain S-PM2 (362). In trials, adsorption (and presumably infection) of S-PM2 was unaffected by phosphate concentration while lysis rates and burst size were dramatically reduced under low-phosphate concentrations. Under phosphate-replete conditions, S-PM2 infection resulted in 100% lysis of the infected cells. These results indicate that phosphate concentration is a key factor responsible for the lysis-lysogeny decision in S-PM2 infection (362). However, the lysogenic state of S-PM2 was not sufficiently tested to determine if this relationship conformed to the characteristics of true lysogeny, i.e., stability and inducibility. The work with cyanophage S-PM2 demonstrates that environmental changes in nutrient dynamics may impact carbon flux, not only by affecting primary and secondary production but also by affecting the level of virioplankton production. Addition of amino acids to seawater mesocosms containing natural communities of aquatic microorganisms stimulated a significant increase in the number of viruses, while phosphate addition stimulated bacterial growth (328). It is possible that for marine heterotrophic bacteria the in situ concentration of nutrients, such as amino acids, which are sources of nitrogen, carbon, and energy, serve as metabolic triggers for lytic production of cryptic pseudolysogenic phages. For a pseudolysogenic cyanophage, such as S-PM2, phosphate is a nutrient more likely to stimulate lysis, since cyanobacteria are photoautotrophs and are often capable of fixing nitrogen.

Perhaps the most elegant study of the causal link between nutrient concentration, host growth state, and pseudolysogeny was conducted by Ripp and Miller (270). They hypothesized that "... due to the cell's highly starved condition, there is insufficient energy available for the phage to initiate either of these typical laboratory responses (lysis or lysogeny) to infection." This hypothesis predicts that once cellular energy becomes available, intracellular phages will either become true lysogens or initiate a lytic response (270). Noting the studies which have indicated the primacy of lytic production in aquatic environments, this response to nutrient addition is more likely. Employing chemostats and lakewater microcosms, they demonstrated that after 15 days at least 20% of *P. aeruginosa* cells contained phage UT1 DNA yet only a fraction of the cells that contained UT1 actually released the phage. Furthermore, when phage UT1 is a preprophage (i.e., in the pseudolysogenic state), it was passed in a random and haphazard fashion during cell division, supporting the earlier assertion that phage UT1 is indeed pseudolysogenic (220).

Long-term starvation experiments employing *P. aeruginosa* and phage UT1 gave the clearest demonstration of a link between nutrient concentration and phage production. After each nutrient addition, the fraction of cells releasing phage UT1 increased significantly for several days. Control mesocosms without nutrient addition showed consistently low levels of phage-producing cells. Finally, as the authors predicted, the outcome of infection with the pseudolysogenic phage UT1 was dramatically affected by nutrient conditions at the time of infection. Infection at high nutrient concentrations resulted in lytic growth, while infection at low nutrient concentrations elicited a pseudolysogenic response (270).

While the mechanistic details of pseudolysogeny are unknown, this phenomenon is nonetheless an attractive explanation for the maintenance of abundant aquatic virioplankton populations under conditions seemingly adverse to sustained lytic-virus production. From observations of hundreds of marine PHS, Moebus (200) concluded that pseudolysogeny is common in marine environments. Ackerman and DuBow (2) also believe, based on their extensive knowledge of bacteriophages, that pseudolysogeny is common among bacteriophages. Because pseudolysogeny is poorly understood, it is difficult to separate completely this phenomenon from the environmental conditions involved in the lysis-lysogeny decision of a temperate phage. It is well established that phage/host ratio and nutrient status influence the decision (364). However, the relationship between changes in environmental nutrient concentration and induction of lysogens is not well established. It is possible that the pseudolysogenic life cycle affords phage populations a means of quickly reacting to environmental changes; moreover, this life cycle may be a common evolutionary step toward the more stable and cryptic lysogenic life cycle. In conclusion, the pseudolysogenic life cycle clearly illustrates that the mode of phage replication significantly influences the impact of viral infection in aquatic microbial communities. If aquatic bacteriophages are primarily pseudolysogenic, influxes of limiting nutrients into aquatic environments could simultaneously stimulate both bacterial production and bacterial mortality through lytic production of preprophage.

Viral Infection and the Microbial Loop: Theoretical Considerations

The concept of bacterioplankton as a central component of the aquatic food web was established by Pomeroy over 20 years ago (126, 251). However, quantification of bacterioplankton involvement in the cycling of organic matter and nutrients through the aquatic environment was initially restricted by a lack of suitable in situ methods. Soon after direct-counting techniques revealed the true abundance and thus the biomass of bacteria in aquatic environments (127, 381), it was recognized that a significant proportion (10 to 50%) of primary production is incorporated by bacterioplankton (8). By utilizing dissolved products of primary production, aquatic bacterial populations recycle the primary production lost to higher-order consumers back into biomass. This process, known as the microbial loop (8), effectively makes a larger proportion of primary production available to the marine food web.

Examination and quantification of microbial loop processes remains an important focus of studies by marine microbial ecologists. Present global environmental concerns over the eutrophication of aquatic ecosystems and the real possibility of global warming have placed special emphasis on understanding the biogeochemical cycling of carbon and nitrogen. A significant proportion of biologically available carbon and nitrogen cycle through the oceanic food web. Therefore, determination of how the physiology and ecology of planktonic organisms affects global carbon and nitrogen cycling may improve our ability to predict and perhaps remediate the ecological impacts of large-scale anthropogenic carbon and nitrogen release.

The discovery of abundant virus populations in aquatic ecosystems had an immediate impact on the accepted dogma of the microbial loop. A conceptual model of viruses and viral lysis introduced into the aquatic food web is shown in Fig. 5, and demonstrates that viral lysis enhances the flux of bacterial biomass into the DOM pool. Viral lysis of algae and cyanobacteria may augment the flux of photosynthetically fixed carbon from phytoplankton biomass into the DOM pool. Indeed, all of

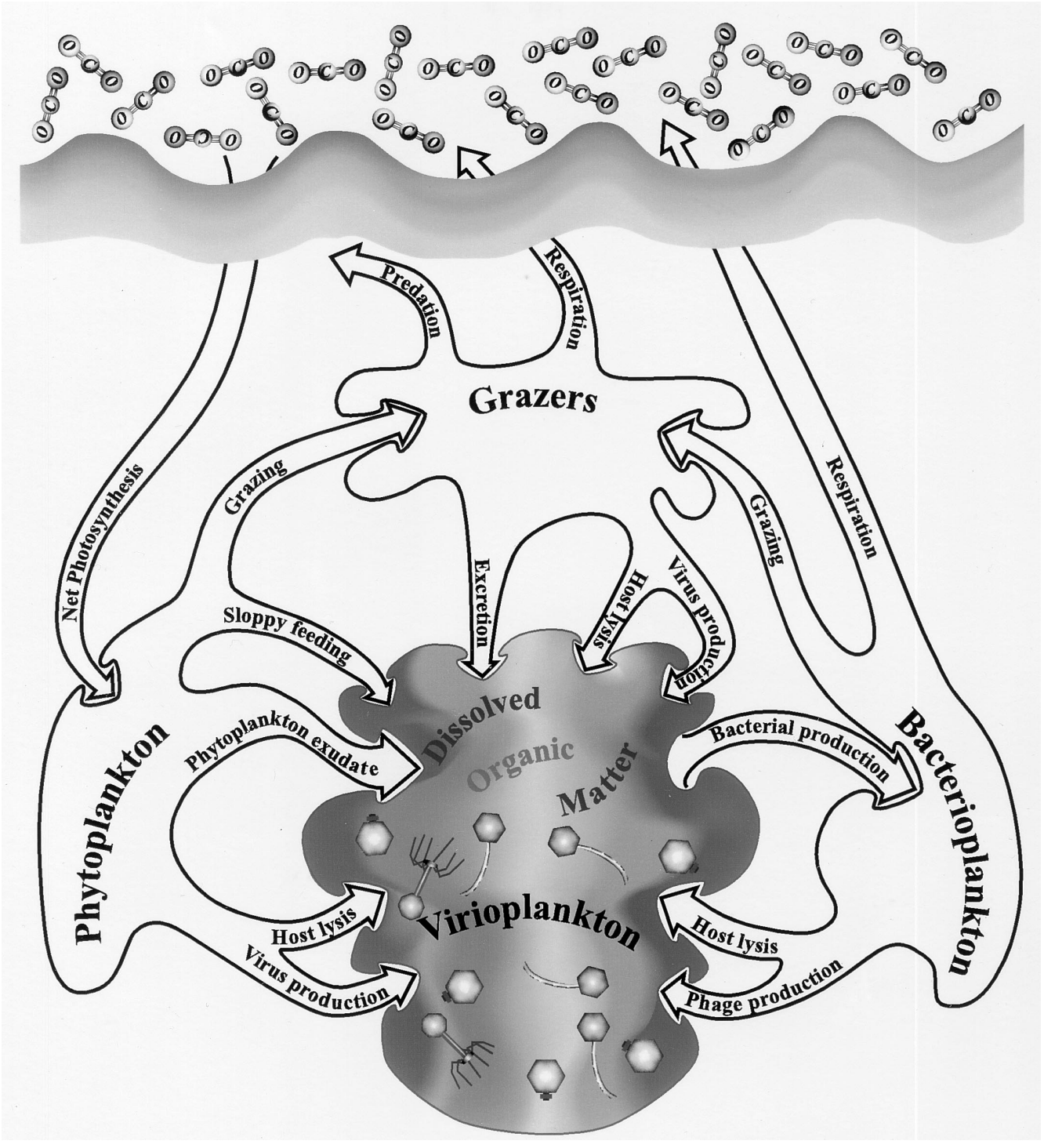

FIG. 5. Viruses and the microbial loop. A schematic diagram highlights the potential role of viral infection and lysis in the production of DOM in aquatic ecosystems.

the products of viral cell lysis, i.e., macromolecules, cell organelles, and virus particles, contribute to the DOM and POM pools (89). The theoretical effect of viral lysis is to divert carbon, fixed as phytoplankton and bacterial biomass, away from mesozooplankton consumers into the DOM pool (42, 89, 206, 323, 324). The potential impacts of viral activities on oceanic nutrient cycles have recently been reviewed by Wilhelm and Suttle (359). Another possible process of global significance mediated by viral infection is the recently recognized contribution of viral lysis to the production of dimethyl sulfide by marine phytoplankton communities. Dimethyl sulfide, an important natural source of submicron particles, is a critical component in the formation of clouds and in determination of the radiative qualities of the atmosphere (170, 365).

Detailed modeling of viral involvement in the aquatic microbial food web has been limited to only a few studies. Nevertheless, these simulations have resulted in useful hypotheses of how viral infection may change current views of the aquatic microbial food web. First, in an indirect way, bacteriophage lysis may impact our current understanding of the microbial loop by contribution to experimental error. Recognition of the ubiquity of viruses in aquatic environments calls into question some of the methods used to assess microbial loop processes. The majority of microbial loop studies have relied on estimation of in situ bacterial production through measurement of the incorporation of a radiolabeled nucleotide substrate, usually [^{3}H]thymidine, into bacterioplankton nucleic acid (90, 92). Heldal and Bratbak (120) pointed out that some iterations of the [^{3}H]thymidine method cannot distinguish between labeled DNA in the viral, cellular, or dissolved pools.

In essence, viral infection is a new mechanism for the flux of microbial biomass into DOM. This mechanism of DOM release is quantitatively different from other well-studied mechanisms, such as "sloppy feeding" by grazers, in that all of the cell contents are moved into the DOM pool. Fuhrman (89) theorized that a single virus produced in a burst event probably represents 1% or less of the cell biomass released. To continue the cycle of virus production and thereby the steady-state condition, only one virus needs to survive to infect another host cell. In this scenario, 99% of the original host cell contents are moved into the DOM pool. Thus, viral lysis represents an extremely efficient means of biomass-to-DOM conversion (89). Based on such logic, an early hypothesis of Bratbak et al. (38) was that phage lysis of bacterioplankton represents a source of nutrient-rich growth substrate for bacterial production. They proposed that viral infection could result in the rapid recycling of organic material between bacterial biomass and the DOM pool. Each turn of the DOM-to-bacterial-biomass cycle results in both respiratory loss and sustained bacterial production at higher levels than would be possible without phage-mediated DOM release. Ironically, the results of active phage lysis are higher levels of bacterial production and less transfer of organic matter to higher trophic levels.

To demonstrate this point, Fuhrman (89) compared two hypothetical marine food webs, with and without a viral lysis component. Both were steady-state models (stable biomass within each compartment) with carbon fixation balanced by CO_2 input. In the virus model, bacterial mortality is divided equally between viral lysis and grazing by nanozooplankton. Comparison of carbon flux through mathematical models indicated that viral lysis (at a level of 50% of total bacterial mortality) resulted in a 27% increase in bacterial carbon mineralization and production rates and a 37% decrease in export of DOM to nanozooplankton grazers (net loss of 25% in nanozooplankton production) (89). Later, Fuhrman and Suttle (96) expanded this model to include both viral infection of phytoplankton and loss of virioplankton to consumption by nanozooplankton (110). Using published estimates for model parameters, levels of virus-mediated mortality were set at 10% of total phytoplankton mortality and loss of viruses to grazing was set at 13% of viral production. The result was a 33% increase in bacterial production and respiration and a 20% reduction in nanozooplankton production (96).

While the ultimate impact of viral infection on in situ macrozooplankton production would be exceedingly difficult to test experimentally, it is possible to examine empirically the effects of viral lysis on the flux and composition of the DOM pool. In mesocosm experiments it was found that phage lysis products stimulated bacterial growth and metabolic activity, principally by introducing available phosphorus (183). These experiments demonstrated that viral lysis can contribute to a futile loop in which a portion of available nutrients cycle between the DOM pool and bacterial biomass without contributing to higher trophic levels (183). Viral lysis of a chrysophyte alga (*Aureococcus anophagefferens*) was responsible for significant changes in the partitioning of important nutrients, i.e., C, N, P, Fe, and Se, between dissolved and particulate phases. Extrapolation of experimental observations to viral lysis of a typical *Aureococcus anophagefferens* bloom predicted significant enhancement of carbon, phosphorus, and iron in the DOM pool (109). Finally, changes in DOM composition with viral lysis have been demonstrated in mesocosm experiments. Slight (i.e., 2.5-fold) increases in virioplankton concentration resulted in measurable changes in the chemical characteristics of the DOM, compared to unamended controls (346). These changes were presumably due to increased incidence of viral lysis in virus-enriched mesocosms.

The most thorough theoretical consideration of the impact of viruses on the aquatic microbial food web compared three food web models which differed by the number of trophic levels involved in the transfer of bacterioplankton biomass to mesozooplankton (206). In the models, three input variables (growth efficiency, recycling efficiency, and virus-induced mortality) were examined under two environmental nutrient regimes (oligotrophic and mesotrophic). Focusing only on accepted standard values for growth and recycling efficiency (40 and 20%, respectively), maximal levels of viral infection (100% of bacterial mortality) resulted in a 5 to 15% reduction in mesozooplankton production (206). At all levels of viral mortality, the greatest impact of viral infection on mesozooplankton production occurred under oligotrophic conditions. Murray and Eldridge (206) proposed that bacteriophage infection demonstrates the greatest interference with mesozooplankton production in environments where recycling of organic matter predominates. Such conditions exist for oligotrophic environments in which biomass consists primarily of bacteria (95) and bacterial production accounts for 15 to 25% of mesozooplankton nutrition (206). In mesotrophic environments, where a greater proportion of primary production is directly available for mesozooplankton nutrition, bacteriophage-mediated mortality was predicted to cause minor reductions in mesozooplankton production (1.2 to 7.4%) for the range of lysis levels tested (25 to 100% of bacterial mortality). Altogether, Murray and Eldridge's food web models represent an excellent resource for interpreting in situ measurements of microbial loop processes.

Demonstration of In Situ Virus-Mediated Mortality

Abundant virus-like particles seen in TEM direct counts of water samples have been suggested to arise from soil runoff. Another explanation was that virus particles were long-lived in natural waters and that the high titers merely represented a buildup of recalcitrant virus particles. Both of these explanations predict that natural virioplankton populations would generally consist of noninfectious particles. An obvious test of the infectivity of natural virioplankton is simply to observe, in mesocosm incubations, the effect of increased virioplankton density on bacterioplankton abundance and production. Several studies have used concentrates of virus-sized particles between 2 and 200 nm in diameter (high-molecular-weight concentrates [HMWC]) obtained from natural water samples to raise the abundance of virioplankton in seawater incubations. Subsequent measurement of microbiological parameters has nearly always revealed a reduction in the abundance of both phytoplankton and bacterioplankton. The negative effects

of HMWC on phytoplankton abundance and primary production have been well documented (245, 310, 315, 316, 346).

Early experiments with axenic cultures of five species representing different classes of phytoplankton showed that addition of virioplankton concentrates resulted in immediate loss of cells (indicated by reduction of in vivo fluorescence) compared to control treatments (315, 316). In trials with natural seawater samples, addition of HMWC caused significant reduction in primary production rates ([^{14}C]bicarbonate uptake) as well as in situ fluorescence (310). The level of HMWC addition necessary for significant inhibitory effects was modest. Suttle (310) found that increases in the virioplankton size fraction of as little as 20% resulted in a 50% reduction in both phytoplankton abundance and primary production rate. Other reports indicate that even slight increases of 2.5-fold, well within the range of natural virioplankton abundance, cause significant changes in phytoplankton abundance (245, 246, 346). Moreover, microscopic observations indicated that size groups of phytoplankton were differentially inhibited by addition of HMWC, supporting the contention that the negative effects of the HMWC are caused by selective viral infection (310).

One common observation of long-term incubations (48 to 500 h) with added HMWC is an immediate and significant reduction in phytoplankton abundance followed by a slow increase in phytoplankton abundance to levels equal to or greater than those in control incubations (245, 246, 310, 346). The simplest explanation for this observation is that initial declines in host abundance arise from an epidemic of viral infection and that the subsequent recovery of phytoplankton density is due to outgrowth of virus-resistant strains. Recovery of phytoplankton abundance to levels exceeding those in control incubations could be the result of stimulation of phytoplankton growth by release of nutrients during lysis. In each study, researchers have cautioned that the inhibitory effects of HMWC could also be caused by bioactive toxins. However, viruses were the only component to increase significantly in number in the HMWC (254, 346). Most importantly these studies demonstrate that natural bioactive components within the virus-rich, 2- to 200-nm fraction of seawater can have a significant impact on in situ primary production (310, 315). If, as many have assumed, the bioactive component is made up of viruses, natural virioplankton populations can, by analogy, influence phytoplankton abundance and productivity.

While data on the effects of HMWC on natural phytoplankton populations and pure cultures of phytoplankton species are convincing, observations of the effects of increased virioplankton concentrations on bacterioplankton populations are less clear. In virioplankton-enriched seawater incubations of >24 h, a 25 to 40% decline in bacterioplankton abundance has generally been observed (246, 254, 257, 346). Employing longer incubations (up to 208 h), Weinbauer and Peduzzi (346) found that at four of the six sample time points the percentage of visibly infected bacterioplankton was significantly greater in HMWC-enriched incubations. However, the sudden and significant declines seen in phytoplankton abundance with the addition of HMWC have not been reported for bacterioplankton populations. Indeed, in one report analyzing the effect of virioplankton enrichment on simulated algal blooms, the abundance of bacterioplankton was initially stimulated (0 to 150 h) and then repressed (150 to 550 h) in mesocosms with added viruses (246). To our knowledge, there are no reports on the effect of virioplankton enrichment on rates of bacterial secondary production.

Measurement of In Situ Virus-Mediated Mortality

Frequency of visibly infected cells. The earliest and most convincing indications that viral infection could be quantitatively significant in aquatic microbial communities came from studies examining the incidence of intracellular viruses within a unicellular alga (293), cyanobacteria, and heterotrophic bacteria (256) collected from seawater samples. By thin-sectioning picoplankton cells, it is possible to enumerate the fraction of cells which are virus infected within aquatic microbial consortia. This approach has demonstrated in situ infection of a number of planktonic algae (33, 209, 211, 263, 293, 314) and heterotrophic nanoflagellates (102, 210) and has been used to estimate the virioplankton production rate. Proctor and Fuhrman (256) demonstrated that intracellular viruses could be detected in cyanobacteria and heterotrophic bacteria collected from several marine environments, ranging from mesotrophic coastal sites (Long Island Sound) to the oligotrophic Sargasso Sea. The incidence of cells containing viruses ranged from 0.9 to 4.3% of the heterotrophic bacteria and 0.8 to 2.8% of cyanobacteria (Table 4). In a separate study that examined bacterial populations associated with sinking particles, a similar FVIC (0.7 to 3.7%) was recorded, indicating that bacterial populations within this unique marine niche may also be controlled by viral lysis (255).

The FVIC within the bacterioplankton has also been determined simply through TEM examination of whole bacterial cells at a high accelerating voltage (80 kV) (342). While this method requires significantly less effort than thin sectioning of bacterioplankton, concern has been expressed over its accuracy (93, 304). A recent study found that whole-cell estimations of FVIC were an average of 79% of estimates based on thin sections (345) (Table 4). Therefore, whole-cell estimation consistently underestimates the level of virus-mediated mortality.

Bacterioplankton which contains mature phages represents only a small proportion of the virus-infected population, since intact viral capsids are visible only late in the infection cycle. To satisfy the objective of estimating the overall level of virus-mediated lysis in situ, it is necessary to convert FVIC values to the proportion of the entire bacterioplankton community which is virus infected. This conversion requires knowledge of the length of the delay between initial virus infection and the first appearance of viral capsids within the infected cell. In essence, the FVIC conversion factor is the inverse product of the percentage of the latent period during which intracellular viruses are visible. In their initial studies, Proctor and Fuhrman (256) chose a single conversion factor of 10 based on observations of phage development in the marine bacterium *Cytophaga marinoflava* (331). However, utilization of a single conversion factor relies on the unrealistic assumption that all bacteriophages have identical development patterns. In subsequent studies, many investigators have chosen to use a range of conversion factors for analyzing FVIC data (Table 4).

It is easy to understand the importance of the conversion factor in interpreting FVIC data. High conversion factors lead to overestimation of the impact of viral infection on bacterioplankton populations. To determine a more appropriate range of conversion factors for marine bacteriophages, Proctor et al. (258) monitored the development of two *Vibrio* bacteriophage. Based on the combination of their empirical data and published data on the development cycles of other marine bacteriophages, they hypothesized that infected heterotrophic marine bacteria exhibit mature phage after 73 to 86% of the latent period has passed. Therefore, the total abundance of infected cells is expected to exceed the number of visibly infected cells by ca. 3.7- to 7.14-fold.

TABLE 4. Virus-mediated mortality based on the FVIC method

Geographical location (reference)	Method	FVIC (%)	Conversion factor[a]	Burst size	Host mortality			Comments
					FIC (%)	Median FIC (%)	Virus mediated (%)	
Marine								
Sargasso and Caribbean Seas (256)	TS[b]	3.2	10		32			Heterotrophic bacteria
	TS	1.5	10		15			Cyanobacteria
Pacific Ocean (255)	TS	0.7–3.7	2.33–10		2–37	19.5		Particle-associated bacteria
Pacific Ocean (258)	TS	0.9–4.3	3.70–7.14		3–31	17	6–62	Free-living bacteria; empirical determination of conversion factor for FVIC to FIC; recalculation based on FVIC values reported previously (256)
Pacific Ocean (258)	TS	0.7–3.7	3.70–7.14		3–26	14.5	6–52	Particle-associated bacteria; recalculation based on FVIC values reported previously (255)
Northern Adriatic Sea								
Mesotrophic (342)	WC[c]	≅1	3.70–7.14	16–20	3.5–7.3	5.4	3.5–24	VTt[d] = 3.8–35.6 days (mesotrophic conditions)
Eutrophic (342)	WC	1.9–2.7	3.70–7.14	30–32	7–19.5	13.25	7.0–64.3	VTt = 2–12.8 days (eutrophic conditions)
Northern Adriatic Sea (348)	WC	max. 4.2	3.70–7.14		15.5–30	23		
Santa Monica, Calif. (93)	TS[e]	3.3–4.6	3.70–7.14	20	24–66	45		Viral production rate method also employed
Bering and Chukchi Seas (304)	WC[e]	0.2–3.3		50		19	2–36	VTt = 0.38 to 9.8 days; also used viral production rate method FVIC-positive correlation with both bacterial production and growth rate ($P = 0.75$)
Solar salterns (37–372‰) (113)	WC	0–3.76	3.70–7.14	6–35			<20	For halophilic square archaea, burst size was 200 and 1.2–10% of cells were visibly infected
Freshwater								
Danube River (176)	WC[e]	1–4	3.70–7.14	17–36	5.4–21	13.2	11–43	Also used viral loss rate method
Lake Constance, Germany (121)	TS	0–1.7	10	21–121	0–17	9	24–34	
Lake Plußsee (345)	WC TS	1.3–2.5 1.5–3.3	3.70–7.14				7.7–97.3	Compared impact of viral lysis in different layers of stratified water column

[a] Dimensionless conversion factor relating FVIC to FIC.
[b] Frequency of infected cells determined from TEM examination of thin sections.
[c] Frequency of infected cells determined from TEM examination of whole cells.
[d] Virus turnover time.
[e] Study utilized two methods for estimation of virus-mediated mortality.

From data presented in Table 4, the general incidence of visibly infected bacteria in aquatic microbial communities can be calculated as 1 to 4% of the total bacterioplankton. Using conversion factors ranging from 2.33 to 10, i.e., visible phage after 57 to 90% of the latent period, it is evident that between 2 and 40% of aquatic bacterioplankton are at some stage of phage infection. Considering the combined inaccuracies of scoring visibly infected cells and the conversion factor itself, estimates of the phage-infected bacterial population range over an order of magnitude. Therefore, it is a reasonable assumption that the estimate of total infected bacteria prepared from FVIC data will be a range around the actual value. A notion of the general level of bacteriophage infection within bacterioplankton communities can be developed by examining median values of the ranges, as shown in Table 4. The range of median values for total bacteriophage infection predicted from FVIC data is 5.4 to 45%, with a mean of 17%. Therefore, based on FVIC data, an average of 17% of the bacteria comprising bacterioplankton communities from a variety of aquatic environments will be phage infected.

While the FVIC data enable an estimation of the overall level of virus infection and strongly support the hypothesis that viruses are produced in situ, the data cannot be directly translated into the proportion of bacterial mortality attributable to phage lysis on a daily basis. This requires knowledge of the relationship between the length of the latent period of the phage infection cycle and host generation time (255, 258). To enable estimation of bacterial mortality from FIC, it is first necessary to assume that the latent period equals the host generation time. Along with this assumption, Proctor and Fuhrman (256) reasoned that to maintain a steady-state population of bacterioplankton, it is necessary that half the bacteria from each generation divide to form the next generation. Thus, an agent which causes 50% of the population to die is responsible for 100% of the allowable mortality in a steady-state system. This has been described as the factor-of-two rule (24), in that estimation of total phage-mediated mortality is simply a matter of doubling the value for the number of phage-infected bacterioplankton. While the argument supporting the factor-of-two rule seems sound, it has been recently shown to be mathematically incorrect (24). Additionally, inherent in the factor-of-two rule is the implied assumption that all infected cells are ultimately killed through lysis or, alternatively, no infected cells are lost to bactivory. In spite of this unrealistic assumption, several authors have used the factor-of-two rule when calculating daily bacterial mortality rates ascribable to phage infection (113, 121, 176, 255, 258, 304).

Binder (24) recently tested the assumptions of the factor-of-two rule using mathematical models which account for grazing of infected cells and the relationship between latent period and host growth rate (generation time). Recalculations based on published FVIC data indicate that the factor-of-two rule systematically overestimated the frequency of mortality due to viral lysis (FMVL) by an average of 24%. Rather than a factor of two, the relationship of FMVL to FVIC is best described by the equation $\mathrm{FMVL} \approx \mathrm{FVIC}/[\gamma \ln(2)(1 - \varepsilon - \mathrm{FVIC})]$, where, γ is the latent period host generation time ratio and ε is the fraction of the latent period prior to formation of intracellular

TABLE 5. Virus-mediated mortality based on direct viral production rate measurement

Geographical location (reference)	Method	Viral production (10^9 ml^{-1} day^{-1})	Conversion factor[c]	Burst size	Host mortality rate (% day^{-1})	Viroplankton turnover time (days)	Comments
Bering and Chukchi Seas (304)	VPR[a,b]	0.5–4.2	2×10^{21} virus (mol of TdR[d])[1]	50	1.9–12	1.2–15	FVIC method also used
Southern California Bight (306), nearshore	VPR	12–230	1.25×10^{20} virus (mol of Pi[e])$^{-1}$	10–300	0.97–40	0.57–3.9	Nearshore stations
Southern California Bight (306), offshore	VPR	0–2.8	1.25×10^{20} virus (mol of Pi)$^{-1}$	10–300	6–8	8.9–30	Offshore stations
Santa Monica, Calif. (93)	VPR[b]	24–46	6.17×10^{20} virus (mol of TdR)$^{-1}$	20	29–67	0.46–0.6	Loss of bacteria to grazing and lysis is similar; FVIC method also used
Lake Hoare, Taylor Valley, Antarctica (144)	VPR	49	6.17×10^{20} virus (mol of TdR)$^{-1}$			0.3	

[a] Viral production rate (direct estimation of in situ virus production through incorporation of radiolabeled substrate).
[b] Study utilized two methods for estimation of virus-mediated mortality.
[c] Conversion factor to estimate the number of viruses produced per mole of radiolabeled substrate incorporated into the viroplankton fraction.
[d] [^{3}H]thymidine-radiolabeled substrate.
[e] ^{32}P-radiolabeled substrate.

virus particles. While these models revealed significant errors in the factor-of-two rule, perhaps the most important finding is that calculation of viral mortality from FVIC data is extremely sensitive to changes in the FVIC-to-FIC conversion factor (ε) and the ratio of latent period to host generation time (γ). Perhaps the greatest gains toward more accurate estimation of FMVL from FVIC data will come through a better understanding of these parameters in natural communities (24).

While the simplicity of equating latent period with generation time is attractive, there is not a great deal of evidence to support this assumption (258). Pure-culture data for PHS show that in general, the latent period lengthens when host growth slows (79, 113, 258). However, the results of experiments employing a *Vibrio* PHS showed that as the generation time increased, the latent period shortened under nutrient-limiting conditions (258). In testing the relationship between latent period and generation time, Guixa-Boixareu et al. (113) used data for several marine and halophilic bacterial and archael hosts. Regression analysis results showed that host doubling time and phage latent period were significantly correlated ($r^2 = 0.68$), with a slope of ca. 1.

Only two examples can be cited that incorporate the generation time of bacterioplankton into the calculation of virus-mediated mortality rate. By dividing the estimated fraction of infected bacterioplankton by generation times of 0.3 and 1 day, Weinbauer et al. (342) calculated the potential loss of bacterioplankton to phage lysis on a daily basis in the northern Adriatic Sea (Table 4). In a study of virioplankton in the hypersaline environment of a solar saltern, Guixa-Boixareu et al. (113) used the in situ bacterial doubling rate (calculated from [^{3}H]thymidine incorporation data) to calculate the virus-mediated mortality of bacterial and archaeal hosts. While this approach relies on an assumption of the equality of generation time and latent period, it accounts for the intrinsic growth rate of the host community. Finally, using FVIC data, it is possible to estimate the time required for turnover of virioplankton standing stock, i.e., multiplying the number of bacteria lysed per day by an assumed burst size for each lytic event. FVIC data have been used in two studies to estimate virioplankton turnover time (304, 342) (Table 4). These estimates indicate that virioplankton populations may turn over every few days or once a month. The estimated turnover times are shorter for eutrophic, coastal waters, where viral productivity rates, estimated from FVIC, are higher (342).

The possibly inaccurate assumption of equating latent period and host generation time (24) and the wide range of FVIC-to-FIC conversion factors used to calculate virus-mediated mortality (Table 4) indicate that these measurements are, at best, estimates. Nevertheless, the FVIC data suggest that bacteria in the final stage of bacteriophage infection are present in a variety of aquatic environments. Despite these uncertainties, data obtained in a variety of approaches suggest that ca. 10 to 20% of bacterioplankton members are lysed through phage infection per day (311).

Radiotracer incorporation. In addition to direct observation of intracellular viruses, strong evidence for in situ production of bacteriophage comes from studies which monitored radiolabeled tracer incorporation into virioplankton biomass (Table 5). In an approach similar to measuring bacterial production (90, 145), Steward et al. (305, 306) developed methods for estimating in situ virus production based on incorporation of [^{3}H]thymidine or [^{32}P]orthophosphate into virioplankton nucleic acid. A necessary assumption associated with these methods is that nucleotides used in bacteriophage synthesis are derived from the host cell nucleic acid pool or exogenous uptake and not de novo synthesis. Results of studies examining genomic DNA synthesis during the replication of three marine bacteriophages support this assumption. In trials, it was determined that the metabolic strategy consistently used for phage genome synthesis involved recycling of host nucleic acids and not de novo synthesis (360).

Converting the amount of tracer incorporated into virioplankton nucleic acid to the number of viruses produced relies on a conversion factor, obtained empirically, using either a purified marine PHS (305, 360) or data from natural seawater incubation experiments in which changes in viral abundance and label incorporation are monitored simultaneously (305).

At present, there is no consensus on an appropriate conversion factor. As shown in Table 5, a lower conversion factor was used by Fuhrman and Nobel (93) to estimate virus production in California coastal water whereas a higher conversion factor was used to convert incorporation data for Bering and Chukchi sea samples (304) and Lake Hoare, Antarctica, water samples (144). The reasons for choosing a specific conversion factor were not stated. Nevertheless, results of the comparison of virus-mediated mortality estimates from radiotracer and FVIC data were nearly identical for Santa Monica, Calif., water samples and showed considerable overlap for Bering and Chukchi

sea samples (Tables 4 and 5). In the latter study, FVIC estimates of virus-induced bacterial mortality were usually greater than [^{3}H]thymidine estimates (304).

The combination of extensive observations of viruses within bacterioplankton cells and incorporation of a radiolabeled tracer into virioplankton provides direct evidence that viral infection and lysis occur in the aquatic environment. Of these approaches, the former may be a preferred means of demonstrating viral infection of bacterioplankton in oligotrophic environments. In water samples collected from oligotrophic offshore stations, the radiotracer techniques detected significant low-level virus production in only two of eight samples tested (306). However, an advantage of the radiotracer approach is that it provides a more direct measurement of virus production rates and, thereby, useful data for modeling the flux of bacterioplankton biomass into virioplankton standing stocks.

Viral loss or decay and contact rates. As presented in an earlier section, infectious viruses in aquatic ecosystems are lost or destroyed at measurable rates. Because virioplankton abundance is relatively stable over seasonal scales, it is intuitive that to maintain the standing stock, viral production must roughly equal the loss of free viruses. Using the assumption of steady state, along with a burst size conversion factor, several investigators have estimated the degree of virus-mediated bacterioplankton mortality, based on viral decay and loss rates (Table 6). The virioplankton loss rate has commonly been estimated by observing the reduction in virioplankton abundance in seawater incubation after the addition of a cellular poison to prevent further virus production (120). Results of the application of this technique to Scandinavian coastal seawater samples yielded extreme virioplankton production rates between 30 and 54% h^{-1} (39, 120). Such rapid virioplankton production requires that between 2 and 24% of the bacterioplankton members be lysed per hour. With these estimates, Bratbak et al. (39) attempted to incorporate viral production into carbon flux through the microbial loop. From their measurements, algal release and bacterial consumption were roughly equal; however, predation and viral lysis rate estimates exceeded bacterial production by factors of 2 and 6, respectively.

While these results were significant in establishing viral lysis as a component of carbon flux, they indicate that measurements of microbial loop processes (i.e., predation, production, and lysis) are broadly less than precise. The obvious possibility that viral loss is enhanced by addition of the cell poison cyanide has been disproven (120, 318). Other factors influencing estimates of virioplankton production, such as the precision of VDC and burst size conversion factors, would need to be grossly incorrect to affect subsequent virus-mediated mortality estimates based on the viral decay rate (39). Subsequent studies utilizing the cyanide technique have indicated much lower virioplankton loss rates of between 1 and 6% h^{-1} in river water and oligotrophic sea water (Table 6) (97, 176). These estimates are in closer agreement with those based on loss of infectivity of specific viruses (Table 3) and suggest an upper limit of ca. 30% of bacterial production being consumed by viral lysis. The reason for the large discrepancy between virioplankton loss/production rates in Scandinavian waters and other water samples is not clear.

Virioplankton production and the amount of virus-mediated mortality has also been estimated by monitoring short-term changes in virioplankton abundance in an enclosed water sample. New viral production can be calculated as the difference between actual values of virioplankton abundance and those predicted from known viral decay rates. In turn, through the use of a burst size conversion factor, the level of bacterial mortality necessary to support new virioplankton production can be estimated. The most thorough application of this method was demonstrated by Jiang and Paul (136) for a 200-liter mesocosm of Tampa Bay water. Their calculations utilized four decay rate values (0.0042, 0.096, 0.3, and 1.1 h^{-1}) and two burst sizes (50 and 185) to estimate that between 3 and 53% of bacterioplankton members were lysed per day (Table 6). This approach was also applied to a defined mass of Adriatic Sea water, where average estimates of daily bacterioplankton loss were between 39 and 212% at 5 m and between 20 and 157% at 22 m (343) (Table 6). In both instances, the investigators found that median viral decay rates of 0.096 and 0.3 h^{-1} gave the most realistic estimates of daily virus-mediated mortality, corresponding to 5.5 and 37% for Tampa Bay and 36.5 and 105% for the Northern Adriatic Sea. Direct, empirical measurements of viral decay under in situ marine conditions, carried out by Suttle and Chen (318), indicated that in the absence of sunlight, viral infectivity is lost at an average rate of 0.41 day^{-1}. To support this decay rate at a burst size of 50 viruses per infection, between 4 and 13% of the bacterioplankton would have to be infected daily (Table 6). Assuming that bacterioplankton cells double once daily (256), viral infection would cause between 8 and 26% of the bacterioplankton cells to be lysed daily.

Finally, it is possible to predict the potential limit for viral infection simply by the rate at which specific hosts and viruses encounter each other in situ. In their groundbreaking report of high viral abundance in aquatic ecosystems, Bergh et al. (19) used VDC data and hypothetical estimates of adsorption rate and burst size to demonstrate that virus-induced lysis could be quantitatively important in aquatic microbial communities. However, the use of contact rate data to estimate virus-mediated mortality has been most successful in instances where the specific titers of virus and host can be accurately measured. From in situ titers of viable cyanophage and direct counts of *Synechococcus* cells, Waterbury and Valois (341) were able to calculate the rate of cyanophage-*Synechococcus* contact in water samples. Assuming that each encounter resulted in infection, for the time points and locations sampled, between 0.005 and 3.2% of *Synechococcus* cells would be infected daily (Table 6) (341). Such low predictions demonstrate that viral infection will have only a minor impact on marine *Synechococcus* populations.

These low estimates were not supported in a subsequent study of *Synechococcus*-cyanophage contact rates in Texas Gulf coast waters. In an approach identical to that of Waterbury and Valois (341), Suttle and Chan (312) found that for inshore water, between 5 and 14% of marine *Synechococcus* cells would be infected daily. Offshore stations, with significantly lower titers of infective cyanophage, yielded greatly decreased contact incidence. Interestingly, due to the abundance of cyanophages and *Synechococcus* cells at inshore stations, a majority (up to 83%) of *Synechococcus* would have contact with a cyanophage each day. However, to maintain the standing stock of cyanophage, only 1 to 3% of these contacts needed to result in infection (101, 312). The authors proposed that this low level of infection supports the observation of widespread cyanophage resistance among *Synechococcus* strains reported by Waterbury and Valois (341). The level of potential *Synechococcus* infection calculated from contact rates was further supported by estimations of virus-mediated mortality based on cyanophage decay rates. Using a sophisticated model, which incorporated the impact of incident solar radiation on cyanophage decay, Suttle and Chan (312) estimated that even with a large burst size of 250, between 2 and 7% of marine *Synechococcus* would be infected daily (Table 6).

The discrepancy between these two studies is probably due

TABLE 6. Predicted levels of virus-mediated mortality based on calculations utilizing virioplankton decay or loss rates and contact rate

Geographical location (reference)	Method	Viral loss rate/production rate	Burst size	Host mortality		Comments
				Virus mediated[d]	FIC (%)	
Raunefjorden, Norway (120)	VLR[a]	0.54 h^{-1}	50	2–24% h^{-1}	8–13	Burst size and FIC determined with streptomycin treatment
Lake Kalandsvannet, Norway (120)	VLR	0.44 h^{-1}	50	2–24% h^{-1}	2–16	
Aarhus Bay, Denmark (39)	VLR	0.3 h^{-1}	100	72%	12–29	Estimated carbon flux through microbial loop
Bergen, Norway (33)	VLR[c]	0.4 day^{-1}	500	25–100%		Estimates specific to coccolithophorid host, *Emiliania huxleyi*; also reported FVIC data
California (97)	VLR	6.5% h^{-1} (light) 4.5% h^{-1} (dark)	50 or 100	9–33% day^{-1}		
Danube River (176)	VLR[c]	1.2–3.0% h^{-1}	17–36	16–30%		Calculated VDR for range of temperatures; also used FVIC method
Gulf of Mexico, Tex. (318)	VDR[b]	0.41 day^{-1}	50		4–13	Avg rate of viral decay in dark conditions
Tampa Bay, Fla (136)	VDR	0.0042–1.1 h^{-1}	50–185	10–53% day^{-1}		Range of known decay rates
Northern Adriatic Sea (343)	VDR	0.0042–1.1 h^{-1}	50	39–212% day^{-1} (5 m), 19–157% day^{-1} (22 m)		Range of known decay rates
Woods Hole, Mass. (341)	Contact rate				0.005–3.2	Adsorption coeficient of 2.4×10^{-6} ml/min used in calculation; estimates specific for marine *Synechoccus* host community
Gulf of Mexico, Tex. (312)	VDR[c]	0.12 day^{-1} (inshore), 2.1 day^{-1} (offshore)	250	2–7% day^{-1}		Estimates specific for marine *Synechoccus* host community
Gulf of Mexico, Tex. (312)	Contact rate[c]		92–324	5–14% day^{-1}		Adsorption coeficient of 2.4×10^{-6} ml/min used in calculation; burst size needed to balence decay rate
Inshore, Gulf of Mexico, Tex. (62)	VDR	0.82 day^{-1}	72	2–10% day^{-1}		Empirically determined adsorption coeficient of 1.4×10^{-9} ml/min used in calculation; viral production rate (0.79 day^{-1}) calculated from net change in *Mp*V abundance minus *Mp*V decay rate
Gulf of Mexico, Tex. (101)	VDR	0.5–0.75 day^{-1}	81	1–8% day^{-1}		Specific for cyanophage; 1–3% of cyanophage-*Synechoccus* contacts result in infection

[a] Viral loss rate method (viral loss rates based on reduction in VDC after addition of cyanide).
[b] Viral decay rate method.
[c] Study utilized two methods for estimation of virus-mediated mortality.
[d] Net bacterioplankton mortality unless otherwise indicated.

to differences in cyanophage abundance. Titers of cyanophage in Texas seawater samples which infected the *Synechococcus* indicator strain DC2 were ca. 10^4 ml^{-1} (312), whereas the cyanophage titers obtained using infection of marine *Synechococcus* strain WH8012 as the detection agent were 10^3 ml^{-1} or lower (341). This illustrates an important point. If estimates of virus-mediated mortality based on contact rate are to be reasonably accurate, a high degree of precision in enumerating both phage and host cells is required. Ideally, this technique should employ a virus-host system for which the titers of a single specific host and its viruses can be measured.

Perhaps the most thorough examination of in situ dynamics of a single virus-host system was that using viruses (*Mp*V) infecting the cosmopolitan prasinophyte alga *Micromonas pusilla*. In this case, host and virus titers and burst size could be determined accurately. Cottrell and Suttle (62) were able to measure rates of *Mp*V production (i.e., net change in *Mp*V titer minus the *Mp*V decay rate) and *M. pusilla* host cell lysis in situ. A critical variable in this model, the adsorption rate, was both empirically and theoretically determined. Values reported in Table 6 are calculated from the empirical adsorption rate, which was 5 times lower than the theoretical rate. In total, for the months of February and March, between 2 and 10% of *M. pusilla* growth each day was lost to viral infection (62). The only other study to examine in situ dynamics of a specific marine virus and host was conducted with seawater mesocosms and viruses of the coccolithophorid alga *Emiliania huxleyi* (33). Compared to the previously cited study, this lacked precise measurements of decay rate, burst size, and adsorption rate (33). Nevertheless, assuming a modest viral decay rate of 0.4 day^{-1}, viral lysis could account for between 25 and 100% of *E. huxleyi* mortality (Table 6). This high estimate, as well as evidence from other observations, was interpreted to mean that viral infection was instrumental in the collapse of *E. huxleyi* blooms (33, 41). In the future, studies examining in situ dynamics of specific virus-host systems may provide the most quantitative measurements of virus-mediated mortality. Ultimately, the cumulative effect of individual virus-host interactions determines the overall level of virus-mediated mortality. It has been suggested that the inaccuracies inherent to all measurements of microbial loop fluxes (i.e., predation, production, and lysis) may occur because these processes are the net product of smaller, more specific interactions within the complex microbial consortia (89).

Method comparisons and conclusions. Despite the shortcomings of methods for quantitation of virus-mediated mor-

tality, researchers interested in the impact of viral lysis on microbial communities must use one of the approaches presented above. At present, no single one of these methods can be said to be preferable to the others, because each approach has pitfalls. Virus-mediated mortality estimates based on FVIC data are satisfying because they involve direct examination of ongoing viral production. However, in all instances, only a small proportion (<5%) of host cells observed are visibly infected. Because these percentages are low and variable, it is not possible to use FVIC values directly as a measure of total viral infection within microbial populations. The low occurrence of visibly infected cells is not unexpected, since even at the end of a virus-induced collapse of a monospecific phytoplankton bloom, FVIC values do not exceed 5% (33, 334). Comparisons between samples or environments based on FVIC data must be made on estimates of total infection. This requires both a conversion factor (the proportion of the latent period during which virons are visible) and the assumption that the host generation time and virus latent period are of similar duration. While there is some support for equating the host generation time and latent period, empirical evidence for conversion factor values are limited; unfortunately, lysis estimates from FVIC data are highly influenced by the conversion factor. Waterbury and Valois (341) argued that if, as in the case of a few cyanobacterial viruses, assembled virons are visible for 50 or 60% of the latent period, the estimates for virus-mediated mortality based on FVIC data would be significantly lower. Similarly, Mathias et al. (176) found that estimates of host lysis determined from virioplankton loss rates were in better agreement with those determined from FVIC data if virons were assumed to be visible during a greater proportion of the latent phase.

Like the FVIC method, the radiotracer method provides direct evidence of in situ virioplankton production by incorporation of [^{3}H]thymidine into virioplankton biomass. Comparisons between values of virus-mediated mortality estimated by these two methods have generally been overlapping, with the FVIC-based values usually being greater than those calculated from [^{3}H]thymidine incorporation (93, 304). Steward et al. (304) found agreement between these methods supportive of the assumptions necessary for interpreting FVIC data; however, they warned that this agreement was tenuous because conversion of radiotracer incorporation data to bacterioplankton mortality could be affected significantly by the burst size used. Similarly, viral decay/loss methods, which assume steady-state conditions for calculating estimates of in situ viral production rates, can be affected by the burst size conversion factor used in the calculations. Suttle and Chan (312) found that with a large in situ burst size of 250 phage cell^{-1}, the level of cyanophage-mediated *Synechococcus* mortality, predicted from viral decay rates, was below that theoretically possible according to the cyanophage-host contact rate. At lower burst sizes, 100 and 50 phage cell^{-1}, cyanophage-mediated mortality would either approximate or exceed, respectively, the theoretical limit set by the contact rate.

Altogether, studies of in situ virioplankton-mediated mortality, whose results are listed in Tables 4 to 6, reveal that measurable levels of mortality caused by viral infection exist for all unicellular host groups, e.g., eukaryotic algae, cyanobacteria, and bacteria, found in plankton. However, to appreciate the effect of viral lysis on host mortality, it must be compared to the other major mortality mechanism, i.e., predation. Prior to the discovery of the abundance of viruses in seawater, grazing by heterotrophic nanoflagellates was believed to be primarily responsible for controlling bacterioplankton abundance (181). Multiple-regression analysis of temporal changes in the abundance of heterotrophic nanoflagellates, virio- and bacterioplankton in the Northern Adriatic Sea indicated that a greater proportion of the variability in bacterioplankton abundance could be explained by changes in virioplankton populations (343, 348), further suggesting that at high bacterial concentrations, loss of bacterioplankton cells to viral lysis could exceed that to flagellate grazing (348).

Direct estimates of bacterioplankton losses to both grazing and lysis have shown that viruses and flagellates are responsible for similar amounts of bacterial mortality. Steward et al. (304) recorded a broad range of lysis-to-grazing ratios, i.e., from 0.08 to 12, in a group of water samples collected at a number of depths and locations. Overall, no statistically significant trend was evident; however, it was apparent that lysis exceeded grazing (ratio, >1) above 30 m while between 50 and 250 m grazing exceeded lysis (ratio, <1) (304). Similarly, using large seawater mesocosms in replicate, Fuhrman and Noble (93) reported that lysis and grazing contributed almost equally to accountable bacterioplankton mortality. However, in neither study did lysis and grazing together account for 100% of bacterioplankton mortality. Support for an equal contribution of lysis and grazing to in situ bacterioplankton mortality has not been universal. Observations of microbial loop processes over a range of moderate- and high-salinity environments showed that, when present (<150‰ saline), predation greatly exceeded lysis (113). In environments where grazing was recorded, bacterial production and loss rates were roughly equal. In higher-salinity environments (150 to 370‰), without bactivory, production estimates were ca. two- to fourfold greater than loss estimates indicating that in the absence of grazing, lysis does not account for a significant proportion of bacterioplankton mortality (113). Finally, recent studies using water samples from the highly stratified and eutrophic Lake Plußsee demonstrated that the relative contribution of lysis and grazing to bacterioplankton mortality changes dramatically with depth (344, 345). Within warmer, oxygenated surface waters, i.e., the epilimnion (0 to 5 m deep), grazing was predominant, while in the anoxic hypolimnion (10 to 25 m deep), viral lysis accounted for most for the bacterial mortality. Moreover, FVIC data indicated that the impact of viral lysis was greatest for larger bacterioplankton members, leading the investigators to suggest that viral predation may act as a selective pressure toward smaller cell size in bacterioplankton communities (344). Therefore, while measurable levels of virus-mediated mortality exist in a number of environments, it is likely that the contribution of lysis to overall bacterioplankton and phytoplankton mortality will depend greatly on environmental conditions and host community structure.

Finally, the severity of in situ viral lysis may be different among plankton groups. Suttle (311) concluded that between 10 and 20% of heterotrophic bacterial populations are lost to viral infection daily whereas considerably fewer cyanobacteria (ca. 3% day^{-1}) are killed by viral infection. It is hoped that future investigations will clarify which component groups, even species, within aquatic microbial communities are affected by viral infection. At present, the number of aquatic environments examined is too few and the precision of measurements of in situ virus-mediated mortality is too low to allow us to draw specific conclusions on the impact of viral infection in different aquatic environments. Nevertheless, it appears that the levels of virus-mediated bacterioplankton mortality may be remarkably similar, ca. 15 to 20% day^{-1}, across a wide range of environments.

VIRAL INFECTION AND THE MAINTENANCE OF HOST COMMUNITY DIVERSITY

Results of both field and mesocosm studies suggest that ca. 20% or less of bacterioplankton and phytoplankton mortality is attributable to viral infection. Thus, viruses play a modest role in controlling the population density of bacteria, algae, and protists within healthy aquatic microbial communities. Many investigators have speculated that another important impact of virus infection on aquatic microbial communities may be its influence on the diversity and clonal composition of bacterioplankton and phytoplankton populations (33, 42, 89, 91, 96, 161, 223, 304, 311, 315, 323, 335, 341, 371, 372). Additionally, viruses may influence diversity at the population genetic level, since it has been demonstrated that natural virioplankton consortia can mediate genetic exchange among bacterial strains via transduction (53, 163, 284; see references 184, 187, 232, and 281 for reviews). Widespread and universal viral infection within aquatic microbial communities may serve as an explanation for the paradoxical situation noted by Hutchinson (129) more than 35 years ago, i.e., why, in the relatively homogenous aquatic environment, are there diverse assemblages of coexisting phytoplankton species (and by extension bacterioplankton strains) competing for similar resources, when theory would predict there to be only a few dominant species (33, 96)? The specific nature of viral predation, along with the importance of host cell concentration relative to viral infection and replication, can be envisioned to exert selective mortality on species which exceed a defined stoichiometric density limit.

Horizontal Gene Transfer: Transduction

Genetic exchange within bacterial populations differs in important ways from genetic exchange within populations of sexually reproducing organisms. In the bacterial world, genetic recombination appears to be a relatively rare event in which a small amount of genetic material (one or a few alleles) is exchanged between two species. This occurs without the requirement that the two species be close genetic relatives (59, 140). Because bacterial populations in active growth may exhibit short generation times and fast growth, even rare genetic recombination events can quickly alter (increase or decrease) the diversity of genotypes within a community. The mechanisms by which genetic exchange occurs in bacterial populations, principally conjugation, transformation, and transduction, are well understood from results of laboratory experiments and have become valuable tools for microbial geneticists. However, the same processes which provide the technology to construct bacterial strains exhibiting novel and economically useful phenotypes also provide the means by which genetic material from genetically engineered microorganisms (GEMs) can be introduced into the multitude of genetic backgrounds found in natural microbial communities (184). Concern about the release of GEMs continues to stimulate the examination of genetic exchange processes in aquatic environments. More recently, whole-genome sequencing of bacteria has revealed that lateral transfer may also be an important factor in the evolution of bacterial species (45, 84, 216, 247).

Studies done to determine how effective gene transfer actually is in the aquatic environment have focused principally on conjugation and transformation (232, 285). Examination of limited data from in situ incubation studies suggests that these exchange mechanisms generally show higher frequency of transfer than transduction does (88). Transduction has been assumed to be the least prevalent form of natural genetic transfer because of its dependence on an autonomous transducing phage and the general observation that most bacteriophages are restricted to infection of a narrow range of bacterial hosts (187). Moreover, transduction is a reductive process, in that the genetic donor is killed (lysis) in the process of producing a transducing phage particle (186). Alternatively, it has been speculated that the phage-as-carrier aspect of transduction provides specific advantages over transformation and conjugation, namely, that transducing phage particles can be long-lived in the environment, provide for both protection of DNA and dispersal of genetic material, and eliminate the requirement for cell-to-cell contact (248, 285). The discovery of abundant virus particles in natural waters and recent data showing high transduction frequency in marine environments (53, 54; H. X. Chiura, H. Yamamoto, K. Kenji, H. Akira, and M. Younosuke, Abstr. Eighth Int. Symp. Microb. Ecol., p. 124, 1998) indicate that transduction may be an underappreciated means of horizontal gene transfer within aquatic bacterial populations. Finally, a noteworthy reminder of the power of bacteriophage-mediated horizontal gene transfer events is provided by numerous findings that the acquisition of virulence determinants by disease-causing bacteria, such as *Vibrio cholerae* (23, 337), has been associated with transduction and lysogenic conversion (see reference 48 for a review).

Transduction begins as a serendipitous event, in which the host DNA (plasmid or chromosomal) is mistakenly packaged into the capsid during the production of phage particles. The resultant defective phage particle (released upon host cell lysis) is able to adsorb and introduce DNA contained within the capsid into a new host cell; however, because the phage does not contain a complete phage genome, no progeny phage are produced (154). Two types of transduction are possible, generalized and specialized, according to the phenotype of the bacteriophage mediating the genetic transfer. Generalized transducing phages can carry, usually with equal frequency, any DNA contained within the host cell regardless of location (271, 284). Both temperate and virulent phages can perform generalized transduction, and these phages have common attributes with respect to phage genome replication and DNA packaging (154, 184). Conversely, specialized transducing phages are solely temperate bacteriophages which integrate within the host chromosome and are capable of transferring only specific chromosomal genes located close to the phage integration site. Because of its indiscriminant nature, all studies of transduction within aquatic environments have focused on the incidence of generalized transduction.

Significant contributions to the understanding of transduction in aquatic environments have come from the collective efforts of Miller and colleagues and their examination of transduction rates between strains of *Pseudomonas aeruginosa* in lake water (204, 268, 269, 271, 283, 284; see reference 186 for a review). Several combinations of incubation methods have been used in these studies: nonlysogenic recipient cells and cell-free lysates of a generalized transducing phage (usually phage F116 [185] or variants thereof [283]), a lysogenic donor and a nonlysogenic recipient, a nonlysogenic donor and a lysogenic recipient, and two lysogenic strains (one of which carries the transduction marker). From the results of their earliest studies, it was evident that the highest frequencies of in situ transduction resulted from incubations with lysogenic donor cells as the source of transducing particles. It was found that 1-h transduction frequencies were 5×10^{-6} transductant per recipient with an F116 cell-free lysate and 1.4×10^{-5} with an F116 lysogenic donor (204). From the results of a subsequent study, the conclusion was heightened by the observation that measurable levels of in situ transduction were found only when a lysogenic recipient and a nonlysogenic donor were incubated

together in a microcosm (283). Intuitively, this genetic exchange requires several steps: production of phage from the recipient, infection of donor cells and production of transducing particles, and successful reinfection of the lysogenic recipient. A conceptual model for the passage of genetic material from an introduced nonlysogenic GEM to an environmental lysogen is provided by Saye and Miller (281).

Moreover, in microcosm studies, Saye et al. (284) observed reciprocal transduction of chromosomal genes between lysogens and nonlysogens as well as between two different lysogens. Again, lysogenized strains were judged to be better recipients because they showed a higher transduction frequency (10- to 100-fold) than did nonlysogens. A logical explanation for this observation rests on the protective nature of superinfection immunity. In effect, lysogens are open to introduction of foreign DNA from a transducing particle but are protected (via superinfection immunity) from infection and subsequent lysis by wild-type bacteriophages (184). Data from these and other studies led Miller (186) to conclude that the "... most likely reservoir of environmental transducing bacteriophages is the lysogenized members of the natural microbial population."

Several external factors are acknowledged to influence transduction frequency between *P. aeruginosa* strains in situ. Incubations done in the presence of a natural bacterial community showed undetectable levels of transduction, since donor and recipient cells were quickly lost (283). However, the presence of suspended particulate matter greatly enhanced transduction, as well as the production of phage particles, in lake water microcosms (269). It is not entirely surprising that particulate surfaces provide hot spots for transducing activity, since it is well known that bacteria attached to surfaces demonstrate markedly different metabolic characteristics from those of planktonic bacteria. The relative concentrations of donor, recipient, and transducing phage also influence transduction frequency. An excess of donor cells, up to an ideal donor/recipient ratio of 20:1, greatly enhanced transduction frequency during in situ incubations (283). Conversely, in chemostats the concentration of recipients and the ratio of free bacteriophage to bacterial cells both had positive effects on the apparent transduction rate (268).

Thus, the results of these studies serve to establish that transduction may contribute significantly to the horizontal transfer of genes in aquatic bacterial communities; however, none addresses the central question whether transduction serves to maintain an introduced, selectively disadvantaged phenotype within a bacterial population (186). The answer to this question is critical in assessment of risks that may be associated with the release of GEMs and in estimations of the contribution of transduction to host biodiversity. In an elegant chemostat study using various *P. aeruginosa* strains, Replicon et al. (268) conclusively demonstrated that via transduction, a less fit phenotype was maintained within a chemostat population compared to the situation in control experiments where transduction was effectively blocked. These results agreed with the predictions of a two-component mathematical model which incorporated the separate effects of selection and transduction on gene frequency. On the basis of these observations, the authors concluded that even strong negative selective pressures may not prevent the introduction of a genetic determinant into natural bacterial communities.

The use of *P. aeruginosa*, a normal constituent of freshwater environments (219), as a model system was, no doubt, critical to revealing the efficacy of transduction in freshwater environments. Considerably less is known about the incidence of transduction in marine environments. The earliest indications that transduction could occur in a marine environment came through uncontrolled studies of phage-mediated transmission of antibiotic resistance between strains of *Vibrio parahaemolyticus* in oysters (15). More recently, Jiang et al. (134) addressed the lack of a suitable model system for studies of transduction in marine environments by isolating several temperate bacteriophage host systems from Hawaiian coastal waters. Two strains, HSIC and D1B, and their corresponding temperate phages were chosen to assay for transduction frequency of an antibiotic resistance plasmid. In the assays, using either wild-type strains or concentrates of natural marine bacterial communities as recipients, low transduction frequencies ranging from 1.3×10^{-7} transductant/PFU (for laboratory assays) to 3.7×10^{-8} transductant/PFU (for natural communities) were found (133). Considering the collective observations of transduction between *P. aeruginosa* strains detailed above, these low frequencies may not be surprising, because in all the assays, cell-free lysates instead of lysogenic donors were used as the source of transducing particles. Nevertheless, using simple mathematical models, transduction is predicted to have a significant impact on horizontal gene transfer even at low transduction frequencies (36, 133). For an estuary of the size (volume) of Tampa Bay, up to 1.3×10^{14} transduction events are estimated to occur annually (133).

All of the studies reported above employed a specific donor strain for the analysis of transduction frequency. An alternative approach, reported by Chiura et al; (53, 54; Chiura et al., Abstr. Eighth Int. Symp. Microb. Ecol. 1998), indicates these estimates of in situ transduction frequency may be extremely conservative. In their studies, auxotrophic *Escherichia coli* or *Bacillus subtilis* recipient strains were restored to prototrophy after incubation with either VLPs released from pure cultures of various marine bacteria or concentrates of VLPs collected from marine waters (54) or geothermal hot springs (Chiura et al., Abstr. Eighth Int. Symp. Microb. Ecol. 1998). Transduction frequencies ranged from 2.6×10^{-3} to 3.9×10^{-5} transductants per VLP for pure-culture VLPs and from 5.6×10^{-5} to 10^{-6} transductants per VLP for environmental VLPs. Remarkably, the some of the highest previously reported transduction frequencies are approximately equal to the lowest transduction frequencies reported in these studies. In no case were the VLPs proven to be capable of infecting the recipient host, yet each collection of VLPs demonstrated bacteriocidal effects by the significant reduction in plating efficiency of the *E. coli* recipient strain (54). Beyond the implication that transduction may occur with high frequency in marine environments, these results challenge the assumption that narrow host range prevents wide dissemination of genetic material by generalized transduction.

Perhaps mechanisms of phage-host specificity exist which prevent only replication and production of heterologous phages in other hosts and not the introduction of DNA from a broad range of bacteriophage strains. For bacterial populations, such a mechanism would provide a significant evolutionary advantage. In essence, this could prevent the negative aspect of viral infection, namely, cell death from lysis, while encouraging the positive aspect of genetic transfer via generalized transduction. In summary, from a variety of experimental approaches, the results indicate that transduction provides a significant mechanism for maintaining and/or controlling genetic diversity via genetic recombination.

Model for Viral Control of Host Community Diversity

To explain more completely the involvement of viral infection in the maintenance of host community diversity, we have developed a conceptual model (Fig. 6) depicting changes in the

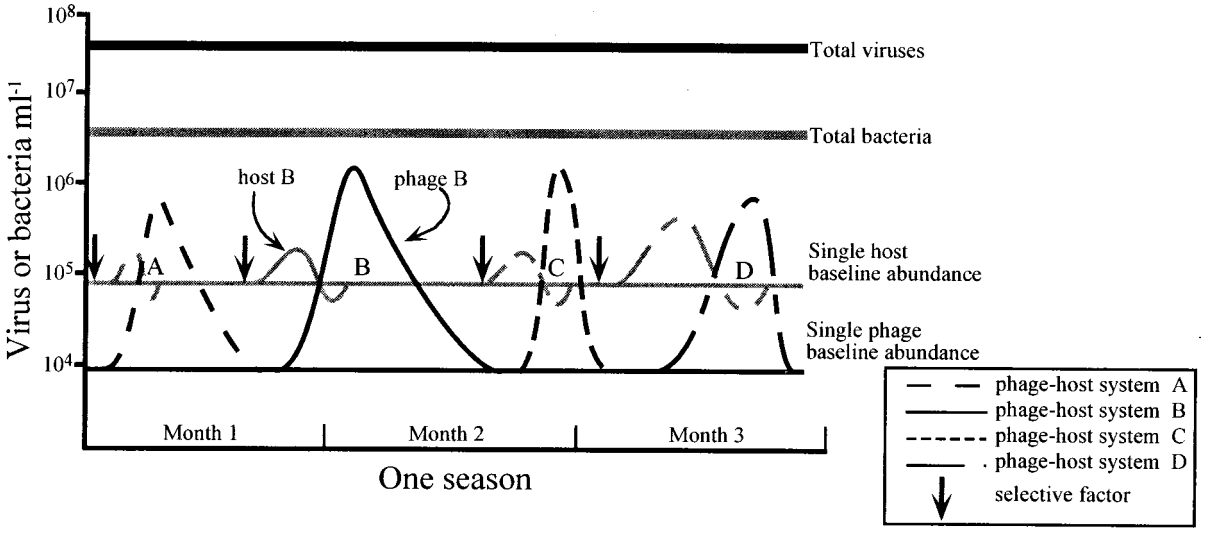

FIG. 6. Model of virioplankton control of host community diversity. For each PHS, a selective factor stimulates the growth of a specific host. An epidemic of phage infection begins at a critical host cell density, and the abundance of a specific phage increases. Phage lysis causes the abundance of host cells to decline to baseline levels and thus prevents excessive dominance of a single host species. At the end of the epidemic, numbers of infective phage decline to a baseline level at a decay rate specific for each phage. It is also possible that the PHS shown are temperate. Stimulation of host growth, from a selective event, causes curing of lysogeny and thus a release of phage. While the abundances of specific hosts and phages change rapidly, the overall abundance of virioplankton and bacterioplankton is stable over longer, seasonal scales. A and D, moderate burst size (10 to 50); B and C, large burst size (100 to 500); A and B, slow decay; C and D, fast decay. Adapted from reference 371.

in situ abundance of four phage-host systems within an aquatic microbial consortium. The model assumes, as many studies have demonstrated, that within a given season, total viral and total bacterial abundance remain relatively stable, with the former usually exceeding the latter by a factor of 10. The hypothetical community diagrammed in Fig. 6 represents one that is found in eutrophic estuarine waters, where the total bacterial and viral abundances are ca. 10^6 and 10^7 ml^{-1}, respectively. This community contains at least 10 to 50 different bacterial species (the average abundance of each individual species is ca. 10^5 ml^{-1}) and 100 to 300 different bacteriophage strains (the average abundance of a single phage type is ca. 10^4 ml^{-1}). The degree to which these numbers and abundances of specific phage and bacterial species reflects a typical microbial consortium in estuarine waters is not presently known. However, Thingstad and others have speculated, based on a simple model which considers relevant parameters (i.e., viral decay rate, burst size, and host-phage encounter) governing the abundance of virus and bacterial hosts, that with reasonable parameter values a typical pelagic food web would support 100 coexisting virus-host systems (19, 40, 322). Our model assumes that within a typical aquatic microbial community, there is a subset of active bacterial species which account for the majority of the total bacterial abundance. It is possible that the actual number of bacterial species within the consortium will be much higher if many species are present at low ($>10^3$ ml^{-1}) density. The strength of our assumptions concerning the constitution of specific host and viral abundance cannot be judged because suitable methods to obtain unbiased assessment of the diversity and abundance of individual bacterial and bacteriophage species within a complex natural community are only now being developed.

The proposed mechanism by which viral infection influences host community diversity is selective destruction of only bacterial strains at high concentration undergoing fast growth. This process of selectively "killing the winner populations" was recently modeled by Thingstad and Lignell (324). We envision that an event such as the influx of a particular limiting nutrient creates favorable growth conditions for a single bacterial species (species A). In response to increased nutrient availability, species A undergoes rapid growth and quickly increases in population density. Eventually, the density of species A reaches a critical threshold at which an epidemic of phage (ΦA) infection ensues. Rapid infection and replication results in a dramatic increase in the titer of ΦA, and eventually the epidemic of phage infection reduces the density of sensitive host cells to an abundance well below the threshold necessary to maintain phage production. At the height of the epidemic, ΦA could reach a concentration 10 to 50 times that of its host and possibly 100 times its usual, nonepidemic density. Nevertheless, the increased densities of phage and host shown in Fig. 6 would not cause a detectable change in total counts, since ΦA and species A comprise a small proportion of the total virioplankton and bacterioplankton abundance. The epidemic of ΦA infection ceases once the frequency of ΦA-species A encounters no longer supports viral production at a level necessary to replace the loss of infectious virions.

Examination of this conceptual model in light of the biology of PHS offers several scenarios for an outbreak of viral production. The rates of phage production and decline at the beginning and end of the epidemic are influenced by traits particular to each PHS. In Fig. 6, we depict four PHS with different rates of decay and different burst sizes. Changes in the abundance of a particular virioplankton strain in situ will be greatly influenced by these parameters. Our model includes the possibility that during an epidemic, free phage are produced either directly through infection and lysis (virulent bacteriophage) or via induction of prophage from lysogenic bacteria (temperate bacteriophage). Because we assume that most aquatic bacteriophage strains are released through cell lysis, we do not include viral production through leakage or budding, a process limited to a few rare bacteriophage groups (2). It has been demonstrated that in some instances, lysogens possess a competitive growth advantage over nonlysogens (77, 78, 165) and that sudden changes in the growth rate of a lysogen, as a result of improved growth conditions, can cause the induction

of prophages and subsequent host cell lysis (174). Therefore, the individual bacterial blooms (Fig. 6) could easily represent subpopulations of lysogenic bacteria which, upon reaching high growth rates, are lysed through the induction of prophages.

The phenomenon of bursts in phage production with changes in nutrient status or environmental quality is perhaps best demonstrated by the PHS identified as pseudolysogenic (220, 270, 327, 362). Indeed, if many aquatic bacteriophages are capable of pseudolysogeny, as has been suggested (2, 200), this would support many aspects of the model presented in Fig. 6. However, the results of studies directly examining the effect of nutrient input on virioplankton production have been unequivocal. In both short-term (<2-h) seawater incubations (40) and in situ observations of specific bacteria (329), nutrient input did not stimulate an increase in the abundance of virioplankton. In longer (48-h) mesocosm incubations of Norwegian coastal seawater, virioplankton production was stimulated by carbon and energy input, i.e., amino acid addition, but not phosphate addition (328).

Our suggestion that selective events can lead to monospecific blooms within aquatic microbial communities is supported by the common observation of monospecific phytoplankton blooms. It is less clear whether heterotrophic bacterial communities undergo similar bursts in the abundance of a single strain. Ephemeral and significant blooms (up to 80% of the total bacterial abundance) of single bacterioplankton strains have been observed in studies using taxonomically specific 16S rRNA probes (262). More commonly, however, studies utilizing immunofluorescent probes have found that target strains rarely reach an abundance that would account for 1% of the total bacterioplankton community (65, 329, 338, 339). Nonetheless, from observations of changes in the abundance of specific bacterial species, Tuomi et al. (329) found that short-lived blooms (1 to 3 weeks) did occur. Involvement of viral infection in phytoplankton bloom dynamics has been suspected for some time (see the reviews by Padan and Shilo [229] and Zingone [382]). There is strong circumstantial evidence implicating virus-mediated lysis in bloom collapse. Increases in the numbers of both free and intracellular viruses concomitant with the decline of a natural or stimulated monospecific algal bloom have been documented for several important bloom-forming algae (33, 188, 209, 211, 293). The fact that one of these organisms, *Emiliania huxleyi*, which accounts for a significant proportion of global phytoplankton biomass, could be limited by viral infection was cited as potential evidence that widespread viral infection may cause selective, background extinction of abundant and widespread plankton species (80). Involvement of viral lysis in phytoplankton bloom dynamics has also been supported on theoretical grounds. Beltrami and Carroll (17) found that inclusion of viral disease in mathematical models of phytoplankton abundance predicted bloom events at cycles and magnitudes similar to those observed in situ.

The Problem of Resistance

A well-founded argument against widespread and significant levels of viral infection in aquatic microbial communities is the tendency for fast-growing clonal organisms, such as bacteria, to quickly acquire resistance to cooccurring parasites. It has been demonstrated that newly isolated *Synechococcus* clones are generally resistant to cyanophages found within the same environment (341). Similarly, a culture collection of activated-sludge bacteria was found to be composed largely of bacterial strains which were phage resistant (114). By extension, it can be assumed that bacterioplankton members are, in general, largely resistant to cooccurring bacteriophages. The ability of bacteria to gain resistance quickly to a virulent phage strain has best been demonstrated in long-term chemostat studies. In general, these studies have shown that after the acquisition of resistance, host concentrations are 10 to 100 times greater than the virulent-phage density (160). Nevertheless, both theoretical predictions and empirical investigations indicate that despite the predominance of resistant hosts, small populations of susceptible hosts (>0.001% of total host abundance) and virulent phage survive (47, 160–162). A possible explanation for this paradoxical behavior is that phage resistance has a physiological cost; in essence, susceptible hosts maintain a growth advantage over resistant hosts (47, 108, 300).

If natural bacterioplankton communities are composed primarily of phage-resistant strains, what prevents the rapid extinction of cooccurring bacteriophage populations? Several possible explanations have been proposed. First, the evolutionary response of a virulent bacteriophage to phage resistance (acquired principally through alteration in the structure or number of phage receptor molecules) often consists of selection for phage mutants with the ability to use alternate receptors. Termed host range mutants, these phages are capable of infecting hosts which are resistant to the parent wild-type phage, by attaching to a different receptor on the bacterial cell surface (160). Host range mutant populations within the virioplankton could quickly grow by exploiting the large population of formerly phage-resistant bacterioplankton hosts. Such a situation would predict coevolving populations of bacteria and phages with endless cycles of resistance and counterresistance mutations. In considering this possibility, Lenski (159) concluded that the structural constraints of altered host range are greater for a bacteriophage than is the physiological cost of changes in receptor efficiency or function for a bacterial host, thus resulting in a fundamental asymmetry in the coevolutionary potential of bacteria and phages (159). A later chemostat experiment supported this conclusion by finding that evolution of a resistant host strain was inevitable while corresponding evolution of a host range mutant phage was rare (161). Therefore, endless, rapid cycles of host and phage mutation in aquatic microbial communities do not seem likely; however, this mechanism could be responsible for the sudden collapse of large monospecific populations.

A second and more likely possibility is that virulent phages survive by scavenging on the rare sensitive cells within the bacterioplankton (341). Conversely, sensitive cells survive in the presence of virulent phages due to their rarity and the resulting low incidence of phage-host contact (47). Chao et al. (47) hypothesized that within the chemostat, the stable relationship between virulent phage and sensitive host is maintained because the growth of phage populations is prevented by the continuous loss of virulent phage to dilution. While this property is unique to the chemostat environment, it is possible that within natural aquatic environments, phage decay is a surrogate for loss of viable phages to dilution.

The presumption that bacterioplankton populations are largely resistant to their cooccurring bacteriophages seems at odds with observations of fast virioplankton turnover and high abundances of VLPs in aquatic ecosystems. If most bacterioplankton are resistant to phage infection, how are abundant and dynamic virus populations maintained? Recently, Fuhrman (91) speculated that perhaps phage resistance is not pervasive among aquatic bacterioplankton, especially in nutrient-depleted waters. Abortive infections in which a virus attaches to and injects its genetic material into an incompatible host could be viewed as a rich source of nutrients in an oligotrophic environment. Thus, increases in nutritional fitness through dis-

play of a range of receptors for incompatible phages may outweigh the risk of death through lysis. Additionally, food web models demonstrate that bacterioplankton production actually increases with viral infection (89); therefore, viral infection and lysis may be a positive, "community level" selection factor for the bacterioplankton. Unfortunately, these arguments, while intriguing, are highly speculative. Until data become available which address this paradox of the virioplankton, it seems necessary to accept the experimentally well-founded argument that most bacteria will be resistant to cooccurring bacteriophages.

Assuming that each bacterial host species has acquired resistance to infection by most of its cooccurring bacteriophages, the conceptual model for viral control of host community diversity (Fig. 6) predicts that there are between 10 and 30 different phage strains capable of infecting each bacterial host. This predicted redundancy in the number of virus species capable of infecting a single host supports the observation that the abundance of free viruses greatly outnumbers that of host cells and ensures that virioplankton populations can respond quickly to outgrowth of competitively dominant bacteria.

From studies utilizing sensitive molecular methods, there is some indication that, indeed, within the virioplankton there exist several phage strains capable of infecting a given single bacterial host. In a series of studies, Chen and colleagues (49–52), using a PCR-based method for selectively amplifying the DNA polymerase gene (*pol*) of algal viruses, found that at least four genotypically different *Mp*V strains exist in samples of Gulf of Mexico seawater. The choice of *pol* as a taxonomic marker for algal and other eukaryotic viruses was appropriate because polymorphisms within *pol* correlated well with the loose taxonomic groups that are based on host range and were sensitive enough to distinguish closely related algal viruses infecting *Chlorella* strains, *Micromonas pusilla*, or *Chrysochromulina* spp. (50). Similarly, recent work indicates that the genetic homologues of the T4 capsid assembly protein, g20, in cyanophages may be useful in detection, quantification, and phylogenetics of this virioplankton group (98).

Using the accepted molecular taxonomic methods of restriction fragment length polymorphism and hybridization analysis, Kellogg et al. (143) were able to identify six OTU groupings within a collection of 60 phages infecting the cosmopolitan bacterioplankter *Vibrio parahaemolyticus*. Common restriction patterns were found in *V. parahaemolyticus* phages isolated from geographically disparate regions, suggesting a broad distribution of phage strains. A similar analysis of five cyanophage isolates infecting the phage-sensitive marine *Synechococcus* strain WH7803 found that each phage was a unique strain, yet they all had significant genetic similarity. Based on their results, the investigators suggested that single strains of marine cyanophage may have broad ranges within the North Atlantic (363). Finally, an investigation which utilized reciprocal hybridization of viral genomic DNA found a wide range of genetic similarity, 13 to 70%, among eight *Mp*V clones isolated from a variety of locations. Interestingly, the level of similarity among the *Mp*V strains was not related to their geographic proximity (63). Altogether, the results of these studies of viral genetic diversity based on specific groups of viruses which infect the same host indicate that within the virioplankton populations there probably exist several virus strains for each phytoplankton or bacterioplankton host species.

Evidence of Viral Control of Host Community Diversity

Many aspects of our model for viral control of host community diversity reflect the observations and conclusions of both empirical and field studies. However, obtaining direct evidence that the species diversity of bacterioplankton and phytoplankton communities is affected by viral lysis is exceedingly difficult. A few reports providing observations of planktonic communities imply that viral lysis has an influence in shaping bacterioplankton and phytoplankton community composition. Hara et al. (115) observed that both small bacterioplankton cells and high VBRs occurred in water samples from the deep ocean. They hypothesized, based on diffusional transport theory (208), that an increased propensity for virus-host contact (high VBR) would select for bacterioplankton strains with smaller cell sizes. Similarly, in incubations of seawater with various nutrients added, it was noted that a decrease in bacterioplankton morphological diversity was accompanied by low VBRs, indicating that the bacterioplankton community was possibly dominated by a few phage-resistant strains (328). Supporting this conclusion, Weinbauer and Peduzzi (347) found, based on FVIC data, that morphological groups (i.e., cocci, rods, and spirilla) within the bacterioplankton were differentially affected by bacteriophage infection. Finally, long-term (ca. 550-h) mesocosm experiments, in which phytoplankton blooms had been artificially stimulated, demonstrated that virioplankton enrichment dramatically changed the successional patterns of planktonic groups during the bloom (246).

Ultimately, however, the most promising approaches for investigating the effects of viruses on community diversity are those which utilize sensitive and nonselective techniques to examine in situ temporal changes in the abundance of specific virioplankton or bacterioplankton strains. Three recent studies, using novel methodological approaches, provide insight into virioplankton population dynamics. Hennes et al. (123) monitored the population density of a putative *Vibrio natriegens* strain, PWH3a, introduced into a seawater mesocosm. Titers of PWH3a were monitored using phage PWH3a-P1 which had been fluorescently labeled with the nucleic acid dye YOYO-1. Unambiguous identification and enumeration of PWH3a by ELM was possible because fluorescently labeled PWH3a-P1 attached only to PWH3a cells. After inoculation of the mesocosm, PWH3a titers quickly rose to ca. 40% of the total abundance and subsequently declined to $<2\%$ after incubation for 2 days. Conversely, titers of phages infecting PWH3a rose throughout the incubation period from undetectable levels to concentrations in excess of 10^8 ml^{-1}, or ca. 70% of the total virioplankton abundance (123). These data indicate that natural virioplankton strains present at low titers can selectively limit the population density of a sensitive bacterial strain within the bacterioplankton. Furthermore, this result illustrates the mechanism, proposed in our model and by others (324), through which viruses can control aquatic microbial community structure.

The conceptual model shown in Fig. 6 makes two predictions which, through the use of sensitive molecular techniques, can be readily tested. First, the overall structure of virioplankton consortia should be dynamic, with the abundance of dominant virus species changing both temporally and spatially. The second prediction, which relates to the first, is that peaks in the abundance of specific virioplankton strains should be ephemeral. Using pulsed-field gel electrophoresis (PFGE), it is possible to analyze the community structure of virioplankton populations (148, 319). Through PFGE analysis of virus consortia, individual virus strains within a virioplankton concentrate are electrophoretically separated according to genome size. After PFGE, virioplankton DNA within the agarose gel is stained with a fluorescent dye, resulting in a banding pattern in which each DNA band represents a subpopulation of viruses with a particular genome size. The resultant virioplankton PFGE

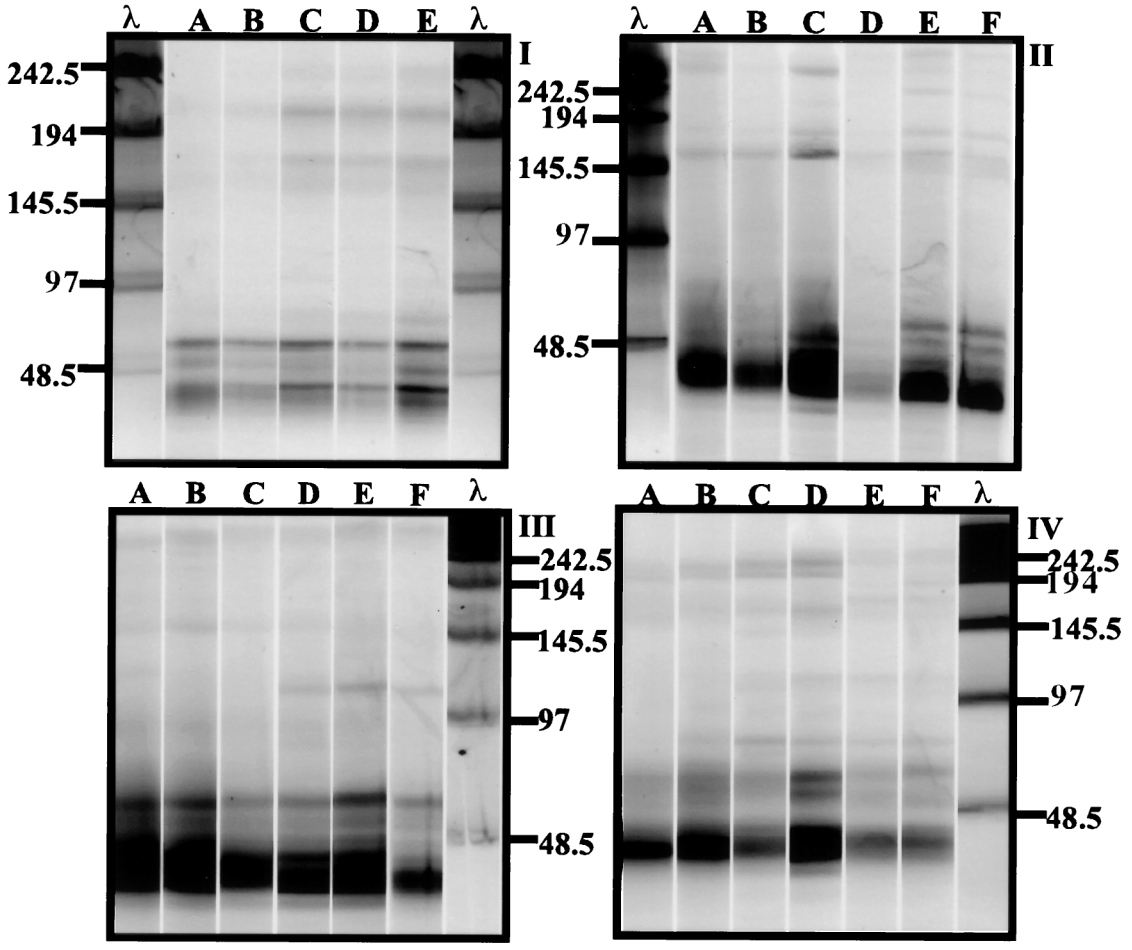

FIG. 7. PFGE of virioplankton genomes from Chesapeake Bay water samples. August 1995 water samples (I) from stations 858, 845, 818, 744, and 724 are shown in lanes A to E, respectively. The May 1996 (II), June 1996 (III), and July 1996 (IV) water sample station lane designations are identical: 908 (A), 858 (B), 845 (C), 818 (D), 744 (E), and 724 (F). Molecular size markers (in kilobases) specific for each pulsed-field gel are shown in lanes λ Adapted from reference 372.

banding pattern is referred to as a virioplankton PFGE fingerprint (372; K. E. Wommack, J. Ravel, R. T. Hill, and R. R. Glwell, Abstr. 97th Gen. Meet. Am. Soc. Microbiol. 1997, abstr. N-53, p. 391, 1997). An example of virioplankton PFGE fingerprints is shown in Fig. 7.

Recently Wommack et al. (372; Wommack et al., Abstr. 97th Gen. Meet. Am. Soc. Microbiol. 1997) used this approach to examine Chesapeake Bay virioplankton populations. Densitometric data obtained using virioplankton PFGE fingerprints revealed that the abundance of viruses within particular genome size groups changed dramatically over the course of monthly samplings within the Chesapeake Bay. In all water samples examined, viruses with 23- to 97-kb genomes comprised the majority of the total virioplankton abundance, supporting an earlier suggestion that the majority of Chesapeake Bay virioplankters are bacteriophages (368, 372; Wommack et al., Abstr. 97th Gen. Meet. Am. Soc. Microbiol. 1997). Bacteriophages with genome sizes ranging from 25 to 100 kb have been found in German coastal waters (353). Cluster and principal-component analysis of virioplankton PFGE fingerprint patterns definitively showed that the virioplankton community structure was more alike within sampling dates than between sampling dates. Moreover, the degree of similarity between virioplankton populations was directly related to the distance between the stations where the water sample was collected (372; Wommack et al., Abstr. 97th Gen. Meet. Am. Soc. Microbiol. 1997). Finally, changes in virioplankton community structure were sensitive to physical environmental parameters, such as time and degree of water column stratification. Overall, PFGE observations of virioplankton community structure supported the prediction that natural virioplankton populations are spatially and temporally dynamic.

To test further the validity of the conceptual model presented in Fig. 6, it is necessary to monitor changes in the in situ abundance of individual PHS over time. In a subsequent study, the abundance of specific viruses within Chesapeake Bay virioplankton populations was examined using hybridization analysis (371). Virioplankton PFGE fingerprints (Fig. 7) were analyzed with gene probes specific to single virus strains or particular genome size groups. In each instance, titers (inferred from the signal intensity of the bound radiolabeled probe) of single viruses or genome size groups were highly localized to particular stations within the Bay. Furthermore, titers changed over time, increasing to peak abundance and then slowly declining to undetectable levels. The dynamics of single-virus abundance observed in the hybridization analysis resembled those predicted in Fig. 6 for a bacteriophage involved in controlling a bloom of a single host strain (371).

Perhaps the strongest support for virus-mediated control of host community diversity is provided by recent data from a

study examining changes in the community structure of plankton in large lakewater mesocosms after a virus-induced cyanobacterial lysis event (335). Using denaturing gradient gel electrophoresis analysis of microbial-community stable RNAs, van Hannen et al. (335) showed dramatic changes in the bacterioplankton community structure immediately following the lysis event. Interestingly, bacterial species which appeared soon after the mass lysis of cyanobacteria belonged to the *Cytophagles* and *Actinomycetes*, phylogenetic groups known for their ability to metabolize complex biopolymers. Altogether, the combination of results obtained from controlled chemostat studies, observations of aquatic microbial communities, and direct evidence indicated that viral lysis is a factor in controlling the structure of bacterioplankton and phytoplankton host communities.

CONCLUSION: VIRIOPLANKTON AS AN ACTIVE AND IMPORTANT COMPONENT OF AQUATIC MICROBIAL COMMUNITIES

The discovery about 10 years ago (19) that viruses are highly abundant in natural waters initiated renewed research on the impact of viral infection and lysis on aquatic microorganisms and gave significance to a previously underappreciated plankton class: the virioplankton. In the ensuing years, numerous studies on a wide variety of aquatic environments have documented that viruses are generally 10 times more abundant than the next most abundant class, the bacterioplankton. Unlike other plankton classes, virioplankton populations exhibit extreme variability in abundance, ranging from between less than 10^4 and more than 10^8 viruses ml^{-1} in different aquatic environments. From long-term observations, it can be concluded that virioplankton abundance changes seasonally in accordance with cycles of primary and secondary productivity. Some of the most dramatic changes in virioplankton abundance have been noted over the course of seasonal algal bloom events, with peaks in virioplankton numbers following those of phytoplankton and bacterioplankton abundance. Diel changes in virioplankton abundance have also been observed. However, consistent daily cycles of abundance have not been shown. Interestingly, significant changes in viral abundance have been noted for time intervals of as little as 20 min, suggesting that viral production may occur through a series of synchronous lysis events (40).

Overall, enumeration data demonstrated the dynamic nature of virioplankton populations and provided the first indication that abundant virus-like particles seen in transmission electron micrographs were, indeed, autochthonously produced. Intuitively, however, the abundance of an obligate parasite is necessarily tied to the abundance of its host. If aquatic viruses are active within aquatic microbial communities, virioplankton abundance should be highly correlated with the in situ concentration of the host community. For aquatic viruses, abundance has, in nearly every case that has been examined, correlated most strongly with bacterioplankton concentration. Furthermore, it appears that larger virioplankton populations are found under conditions of high bacterial productivity. Many authors have speculated that bacteriophages comprise the majority of virioplankton populations. Thus, it is not surprising that the abundance of aquatic viruses is closely correlated with the abundance and activity of bacterioplankton.

For the vast majority of known virus-host systems, production of free viruses occurs in a single burst event which destroys the host cell. Thus, virioplankton production augments grazing as a factor in the mortality of phytoplankton and bacterioplankton cells. Inclusion of viral lysis into models of carbon flux through aquatic microbial communities results in a diversion of bacterial production away from higher trophic levels toward the DOM pool. Ironically, this "viral shunt" may actually augment bacterioplankton production by increasing the amount of labile DOM available for bacterioplankton growth. Direct demonstration of the impact of viral infection on the flux of carbon between biomass and DOM pools would be very difficult. However, several methods for estimation of virus-mediated mortality of bacterioplankton and phytoplankton cells have been developed. Observations of visibly infected cells and the incorporation of radiolabeled substrates into virioplankton biomass have directly demonstrated that viral production occurs in situ and that a measurable amount of daily bacterial and phytoplankton production is lost to viral lysis. In a less direct approach, an indication of the level of virus-mediated mortality can be made from estimates of viral decay and loss, assuming that under steady-state conditions viral production is equivalent to viral loss. Virus-mediated mortality estimates based on this approach have generally agreed with those from more direct methods and have provided insight into the mechanisms responsible for the destruction of infective viruses in situ. Overall, estimates of virus-mediated mortality indicate that ca. 20% of heterotrophic bacteria and ca. 3 to 5% of phytoplankton cells are lost to viral lysis each day (311).

It is possible that the impact of viral infection on the mortality of host populations is incidental to another important role of viruses in aquatic microbial communities, namely, the maintenance of phytoplankton and bacterioplankton community structure. The regulatory role of viral infection is theorized to occur through selective predation of host strains which are numerically abundant. Based on the findings of chemostat studies, it is likely that fast-growing, dominant hosts are either phage-susceptible strains or lysogens. In either case, it is easily anticipated that natural selective events, such as nutrient input, stimulate rapid growth of a specific bacterial strain. Once this subpopulation achieves a critical density or growth rate, an epidemic of viral infection will occur, selectively limiting the abundance of the fast-growing host and preventing the excessive dominance of a single strain within the community. Recent evidence, obtained using novel microscopic and molecular techniques, strongly indicates that autochthonous viruses are capable of selectively lysing abundant bacterial strains within the bacterioplankton (123). Furthermore, temporal changes in Chesapeake Bay virioplankton community structure (372; Wommack et al., Abstr. 97th Gen. Meet. Am. Soc. Microbiol. 1997) and the ephemeral presence of specific virus strains within Chesapeake Bay virioplankton populations (371) support the behavior predicted for a virioplankton population that is involved in selectively influencing host community diversity.

ACKNOWLEDGMENT

During the editing stages of this work, K.E.W. was supported through a National Research Council postdoctoral fellowship.

REFERENCES

1. **Ackermann, H. W.** 1996. Frequency of morphological phage descriptions in 1995. Arch. Virol. **141:**209–218.
2. **Ackermann, H. W., and M. S. DuBow.** 1987. Viruses of prokaryotes: general properties of bacteriophages. CRC Press, Inc., Boca Raton, Fla.
3. **Ackermann, H. W., and M. S. DuBow.** 1987. Viruses of prokaryotes: natural groups of bacteriophages. CRC Press, Inc., Boca Raton, Fla.
4. **Adams, M. H.** 1959. Bacteriophages. Wiley-Interscience, New York, N.Y.
5. **Akin, E. W., W. H. Benton, and W. F. Hill.** 1971. Enteric viruses in ground and surface waters: A review of their occurrence and survival, p. 59–73. *In* V. L. Snoeyink and V. L. Griffin (ed.), Virus and water quality: occurrence and control. Champaign Engineering Publications, Urbana, Ill.
6. **Akin, E. W., J. W. F. Hill, G. B. Cline, and W. H. Benton.** 1976. The loss of poliovirus 1 infectivity in marine waters. Water Res. **10:**59–63.

7. **American Public Health Association.** 1989. Standard methods for the examination of water and wastewater, 16th ed ed. American Public Health Association, Washington, D.C.
8. **Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer Reil, and F. Thingstad.** 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. **10:**257–263.
9. **Baess, I.** 1971. Report on a pseudolysogenic mycobacterium and a review of the literature concerning pseudolysogeny. Acta Pathol. Microbiol. Scand. Sect. B **79:**428–434.
10. **Bamford, D. H., J. Caldentey, and J. K. Bamford.** 1995. Bacteriophage PRD1: a broad host range dsDNA tectivirus with an internal membrane. Adv. Virus Res. **45:**281–319.
11. **Barinaga, M.** 1996. A shared strategy for virulence. Science **272:**1261–1263.
12. **Barksdale, L., and S. B. Arden.** 1974. Persisting bacteriophage infections, lysogeny, and phage conversions. Annu. Rev. Microbiol. **28:**265–299.
13. **Baross, J. A., J. Liston, and R. Y. Morita.** 1978. Ecological relationship between *Vibrio parahaemolyticus* and agar-digesting vibrios as evidenced by bacteriophage susceptibility patterns. Appl. Environ. Microbiol. **36:**500–505.
14. **Baross, J. A., J. Liston, and R. Y. Morita.** 1978. Incidence of *Vibrio parahaemolyticus* bacteriophages and other *Vibrio* bacteriophages in marine samples. Appl. Environ. Microbiol. **36:**492–499.
15. **Baross, J. A., R. Y. Morita, and J. Liston.** 1974. Some implications of genetic exchange among marine vibrios, including *Vibrio parahaemolyticus*, naturally occurring in the Pacific oyster, p. 129–137. *In* T. Fujino (ed.), First International Symposium on *Vibrio parahaemolyticus*. Saikon Publishing Co., Ltd., Tokyo, Japan.
16. **Beebee, T. J. C.** 1991. Analysis, purification and quantification of extracellular DNA from aquatic environments. Freshwater Biol. **25:**525–532.
17. **Beltrami, E., and T. O. Carroll.** 1994. Modeling the role of viral disease in recurrent phytoplankton blooms. J. Math. Biol. **32:**857–863.
18. **Benzer, S.** 1955. Fine structure of a genetic region in bacteriophage. Proc. Natl. Acad. Sci. USA **41:**344–354.
19. **Bergh, O., K. Y. Borsheim, G. Bratbak, and M. Heldal.** 1989. High abundance of viruses found in aquatic environments. Nature (London) **340:**467–468.
20. **Bernstein, C.** 1981. Deoxyribonucleic acid repair in bacteriophage. Microbiol. Rev. **45:**72–98.
21. **Berry, S. A., and B. G. Norton.** 1976. Survival of bacteriophages in seawater. Water Res. **10:**323–327.
22. **Bigby, D., and A. M. B. Kropinski.** 1989. Isolation and characterization of a *Pseudomonas aeruginosa* bacteriophage with a very limited host range. Can. J. Microbiol. **35:**630–635.
23. **Bik, E. M., A. E. Bunschoten, R. D. Gouw, and F. R. Mooi.** 1995. Genesis of the novel epidemic *Vibrio cholerae* O139 strain: evidence for horizontal transfer of genes involved in polysaccharide synthesis. EMBO J. **14:**209–216.
24. **Binder, B.** 1999. Reconsidering the relationship between virally induced bacterial mortality and frequency of infected cells. Aquat. Microb. Ecol. **18:**207–215.
25. **Bird, D. F., and R. Maranger.** 1993. Palmer LTER: aquatic virus abundances near the Antarctic Peninsula. Antarct. J. U. S. **28:**234–235.
26. **Bitton, G.** 1975. Adsorption of viruses onto surfaces in soil and water. Water Res. **9:**473–484.
27. **Bitton, G., and R. Mitchell.** 1974. Effect of colloids on the survival of bacteriophages in seawater. Water Res. **8:**227–229.
28. **Boehme, J., M. E. Frischer, S. C. Jiang, C. A. Kellogg, S. Pichard, J. B. Rose, C. Steinway, and J. H. Paul.** 1993. Viruses, bacterioplankton, and phytoplankton in the southeastern Gulf of Mexico: distribution and contribution to oceanic DNA pools. Mar. Ecol. Prog. Ser. **97:**1–10.
29. **Børsheim, K. Y.** 1993. Native marine bacteriophages. FEMS Microbiol. Ecol. **102:**141–159.
30. **Børsheim, K. Y., G. Bratbak, and M. Heldal.** 1990. Enumeration and biomass estimation of planktonic bacteria and viruses by transmission electron microscopy. Appl. Environ. Microbiol. **56:**352–356.
31. **Boyer, H. W.** 1971. DNA restriction and modification mechanisms in bacteria. Annu. Rev. Microbiol. **25:**153–176.
32. **Bradley, D. E., and E. L. Rutherford.** 1975. Basic characterization of a lipid-containing bacteriophage specific for plasmids of the P, N, and W compatibility groups. Can. J. Microbiol. **21:**152–63.
33. **Bratbak, G., J. K. Egge, and M. Heldal.** 1993. Viral mortality of the marine alga *Emiliania huxleyi* (Haptophyceae) and termination of algal blooms. Mar. Ecol. Prog. Ser. **93:**39–48.
34. **Bratbak, G., O. H. Haslund, M. Heldal, A. Nœss, and T. Røeggen.** 1992. Giant marine viruses? Mar. Ecol. Prog. Ser. **85:**201–202.
35. **Bratbak, G., and M. Heldal.** 1993. Total count of viruses in aquatic environments, p. 135–138. *In* P. F. Kemp (ed.), Handbook of methods in aquatic microbial ecology CRC Press, Inc., Boca Raton, Fla.
36. **Bratbak, G., and M. Heldal.** 1995. Viruses—the new players in the game: their ecological role and could they mediate genetic exchange by transduction? p. 249–264. *In* I. Joint (ed.), Molecular ecology of aquatic microbes, vol. 38. Springer-Verlag KG, Berlin, Germany.
37. **Bratbak, G., M. Heldal, A. Naess, and T. Roeggen.** 1993. Viral impact on microbial communities, p. 299–302. *In* R. Guerrero and C. Pedros-Alio (ed.), Trends in microbial ecology Spanish Society for Microbiology, Barcelona.
38. **Bratbak, G., M. Heldal, S. Norland, and T. F. Thingstad.** 1990. Viruses as partners in spring bloom microbial trophodynamics. Appl. Environ. Microbiol. **56:**1400–1405.
39. **Bratbak, G., M. Heldal, T. F. Thingstad, B. Riemann, and O. H. Haslund.** 1992. Incorporation of viruses into the budget of microbial C-transfer. A first approach. Mar. Ecol. Prog. Ser. **83:**273–280.
40. **Bratbak, G., M. Heldal, T. F. Thingstad, and P. Tuomi.** 1996. Dynamics of virus abundance in coastal seawater. FEMS Microbiol. Ecol. **19:**263–269.
41. **Bratbak, G., M. Levasseur, S. Michaud, G. Cantin, E. Fernandez, B. R. Heimdal, and M. Heldal.** 1995. Viral activity in relation to *Emiliania huxleyi* blooms: a mechanism of DMSP release? Mar. Ecol. Prog. Ser. **128:**1–3.
42. **Bratbak, G., F. Thingstad, and M. Heldal.** 1994. Viruses and the microbial loop. Microb. Ecol. **28:**209–221.
43. **Brenner, S., F. Jacob, and M. Meselson.** 1961. An unstable intermediate carrying information from genes to ribosomes for protein synthesis. Nature (London) **190:**576–581.
44. **Brussaard, C. P. D., R. S. Kempers, A. J. Kop, R. Riegman, and M. Heldal.** 1996. Virus-like particles in a summer bloom of *Emiliania huxleyi* in the North Sea. Aquat. Microb. Ecol. **10:**105–113.
45. **Bult, C. J., O. White, G. J. Olsen, L. Zhou, R. D. Fleischmann, G. G. Sutton, J. A. Blake, L. M. FitzGerald, R. A. Clayton, J. D. Gocayne, A. R. Kerlavage, B. A. Dougherty, J. F. Tomb, M. D. Adams, C. I. Reich, R. Overbeek, E. F. Kirkness, K. G. Weinstock, J. M. Merrick, A. Glodek, J. L. Scott, N. S. M. Geoghagen, J. F. Weidman, J. L. Fuhrmann, J. C. Venter, et al.** 1996. Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii*. Science **273:**1058–1073.
46. **Carlucci, A. F., and D. Pramer.** 1960. An evaluation of factors affecting the survival of *Escherichia coli* in sea water. IV. bacteriophages. Appl. Microbiol. **8:**254–256.
47. **Chao, L., B. R. Levin, and F. M. Stewart.** 1977. A complex community in a simple habitat: An experimental study with bacteria and phage. Ecology **58:**369–378.
48. **Cheetham, B. F., and M. E. Katz.** 1995. A role for bacteriophages in the evolution and transfer of bacterial virulence determinants. Mol. Microbiol. **18:**201–208.
49. **Chen, F., and C. A. Suttle.** 1995. Amplification of DNA polymerase gene fragments from viruses infecting microalgae. Appl. Environ. Microbiol. **61:**1274–1278.
50. **Chen, F., and C. A. Suttle.** 1996. Evolutionary relationships among large double-stranded DNA viruses that infect microalgae and other organisms as inferred from DNA polymerase genes. Virology **219:**170–178.
51. **Chen, F., and C. A. Suttle.** 1995. Nested PCR with three highly degenerate primers for amplification and identification of DNA from related organisms. BioTechniques **18:**609–612.
52. **Chen, F., C. A. Suttle, and S. M. Short.** 1996. Genetic diversity in marine algal virus communities as revealed by sequence analysis of DNA polymerase genes. Appl. Environ. Microbiol. **62:**2869–2874.
53. **Chiura, H. X.** 1997. Generalized gene transfer by virus-like particles from marine bacteria. Aquat. Microb. Ecol. **13:**75–83.
54. **Chiura, H. X., B. Velimirov, and K. Kogure.** Virus-like particles in microbial population control and horizontal gene transfer in the aquatic environment. *In* C. R. Bell, M. Brylinsky, and P. Johnson-Green (ed.), Proceedings of the Eighth International Symposium on Microbial Ecology, in press. Atlantic Canada Society for Microbial Ecology, Halifax, Canada.
55. **Reference deleted.**
56. **Cochlan, W. P., J. Wikner, G. F. Steward, D. C. Smith, and F. Azam.** 1993. Spatial distribution of viruses, bacteria, and chlorophyll a in neritic, oceanic and estuarine environments. Mar. Ecol. Prog. Ser. **92:**77–87.
57. **Cochran, P. K., C. A. Kellogg, and J. H. Paul.** 1998. Prophage induction of indigenous marine lysogenic bacteria by environmental pollutants. Mar. Ecol. Prog. Ser. **164:**125.
58. **Cochran, P. K., and J. H. Paul.** 1998. Seasonal abundance of lysogenic bacteria in a subtropical estuary. Appl. Environ. Microbiol. **64:**2308.
59. **Cohan, F. M.** 1996. The role of genetic exchange in bacterial evolution. ASM News **62:**631–636.
60. **Colwell, R. R.** 1997. Microbial diversity: the importance of exploration and conservation. J. Ind. Microbiol. Biotechnol. **18:**302–307.
61. **Colwell, R. R., P. R. Brayton, D. J. Grimes, D. B. Roszak, S. A. Huq, and L. M. Palmer.** 1985. Viable, but non-culturable, *Vibrio cholerae* and related pathogens in the environment: Implications for release of genetically engineered microorganisms. Bio/Technology **3:**817–820.
62. **Cottrell, M. T., and C. A. Suttle.** 1995. Dynamics of a lytic virus infecting the photosynthetic marine picoflagellate *Micromonas pusilla*. Limnol. Oceanogr. **40:**730–739.
63. **Cottrell, M. T., and C. A. Suttle.** 1995. Genetic diversity of algal viruses which lyse the photosynthetic picoflagellate *Micromonas pusilla* (Prasinophyceae). Appl. Environ. Microbiol. **61:**3088–3091.
64. **Curtis, P., and K. Nealson.** 1973. The use of enrichment techniques to study

marine bacterial viruses. Biol. Bull. **145:**431.
65. **Dahle, A. B., and M. Laake.** 1982. Diversity dynamics of marine bacteria studied by immunofluorescent staining on membrane filters. Appl. Environ. Microbiol. **43:**169–176.
66. **DeFlaun, M. F., and J. H. Paul.** 1989. Detection of exogenous gene sequences in dissolved DNA from aquatic environments. Microb. Ecol. **18:** 21–28.
67. **DeFlaun, M. F., J. H. Paul, and D. Davis.** 1986. Simplified method for dissolved DNA determination in aquatic environments. Appl. Environ. Microbiol. **52:**654–659.
68. **DeFlaun, M. F., J. H. Paul, and W. H. Jeffrey.** 1987. Distribution and molecular weight of dissolved DNA in subtropical estuarine and oceanic environments. Mar. Ecol. Prog. Ser. **38:**65–73.
69. **DeLong, E. F.** 1992. Archaea in coastal marine environments. Proc. Natl. Acad. Sci. USA **89:**5685–5689.
70. **DeLong, E. F., K. Y. Wu, B. B. Prezelin, and R. V. Jovine.** 1994. High abundance of *Archaea* in Antarctic marine picoplankton. Nature (London) **371:**695–697.
71. **Demuth, J., H. Neve, and K. P. Witzel.** 1993. Direct electron microscopy study on the morphological diversity of bacteriophage populations in Lake Plußsee. Appl. Environ. Microbiol. **59:**3378–3384.
72. **Ducklow, H. W., D. A. Purdie, P. J. LeB. Williams, and J. M. Davies.** 1986. Bacterioplankton: a sink for carbon in a coastal marine plankton community. Science **232:**863–867.
73. **Duckworth, D. H.** 1987. History and basic properties of bacterial viruses, p. 1–43. *In* S. M. Goyal, C. P. Gerba, and G. Britton (ed.), Phage ecology. John Wiley & Sons, Inc., New York, N.Y.
74. **Dulbecco, R.** 1950. Experiments on photoreactivation of bacteriophages inactivated with ultraviolet radiation. J. Bacteriol. **59:**329–347.
75. **Dulbecco, R.** 1949. Reactivation of ultraviolet-inactivated bacteriophage by visible light. Nature (London) **163:**949–950.
76. **Edlin, G.** 1978. Alteration of *Escherichia coli* outer membrane proteins by prophages. A model for benevolent virus-cell interaction, p. 1–14. *In* J. G. Stevens, G. J. Todaro, and C. F. Fox (ed.), Persistent viruses, vol. 11. Academic Press, New York, N.Y.
77. **Edlin, G., L. Lin, and R. Bitner.** 1977. Reproductive fitness of phage P-1 lysogen phage P-2 lysogen and phage Mu lysogen of *Escherichia coli*. J. Virol. **21:**560–564.
78. **Edlin, G., L. Lin, and R. Kudrna.** 1975. Lambda lysogens of *Escherichia coli* reproduce more rapidly than non-lysogens. Nature (London) **255:**735–737.
79. **Ellis, E. L., and M. Delbrück.** 1939. The growth of bacteriophage. J. Gen. Physiol. **22:**365–384.
80. **Emiliani, C.** 1993. Extinction and viruses. BioSystems **31:**155–159.
81. **Farrah, S. R., C. P. Gerba, C. Wallis, and J. L. Melnick.** 1976. Concentration of viruses from large volumes of tap water using pleated membrae filters. Appl. Environ. Microbiol. **31:**221–226.
82. **Farrah, S. R., D. R. Preston, G. A. Toranzos, M. Girard, G. A. Erdos, and V. Vasuhdivan.** 1991. Use of modified diatomaceous earth for removal and recovery of viruses in water. Appl. Environ. Microbiol. **57:**2502–2506.
83. **Ferreirós, C. M., M. J. Souto, M. T. Criado, and P. Suarez.** 1991. Phage typing and phage induction in carrier and invasive *Staphylococcus epidermidis* isolates. J. Hosp. Infect. **18:**293–299.
84. **Fleischmann, R. D., M. D. Adams, O. White, R. A. Clayton, E. F. Kirkness, A. R. Kerlavage, C. J. Bult, J. F. Tomb, B. A. Dougherty, J. M. Merrick, et al.** 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science **269:**496–512.
85. **Flint, K. P.** 1996. A method for the enumeration of *Aeromonas* bacteriophage in aquatic environments. Lett. Appl. Microbiol. **22:**244–248.
86. **Frank, H., and K. Moebus.** 1987. An electron microscopic study of bacteriophages from marine waters. Helgol. Meeresunters. **41:**385–414.
87. **Freifelder, D.** 1983. Molecular biology: a comprehensive introduction to prokaryotes and eukaryotes. Science Books International, Boston, Mass.
88. **Fry, J. C., and M. J. Day.** 1993. Overview of gene transfer in aquatic habitats, p. 315–318. *In* R. Guerrero and C. Pedros-Alio (ed.), Trends in microbial ecology. Spanish Society for Microbiology, Barcelona.
89. **Fuhrman, J.** 1992. Bacterioplankton roles in cycling of organic matter: the microbial food web, p. 361–383. *In* P. G. Falkowski and A. D. Woodhead (ed.), Primary productivity and biogeochemical cycles in the sea. Plenum Press, New York, N.Y.
90. **Fuhrman, J., and F. Azam.** 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica and California. Appl. Environ. Microbiol. **39:**1085–1096.
91. **Fuhrman, J. A.** 1999. Marine viruses and their biogeochemical and ecological effects. Nature (London) **399:**541–548.
92. **Fuhrman, J. A., and F. Azam.** 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar. Biol. **66:**109–120.
93. **Fuhrman, J. A., and R. T. Noble.** 1995. Viruses and protists cause similar bacterial mortality in coastal seawater. Limnol. Oceanogr. **40:**1236–1242.
94. **Fuhrman, J. A., and C. C. Ouverney.** 1998. Marine microbial diversity studied via 16S rRNA sequences: cloning results from coastal waters and counting of native archaea with fluorescent single cell probes. Aquat. Ecol. **32:**3–15.
95. **Fuhrman, J. A., T. D. Sleeter, C. A. Carlson, and L. M. Proctor.** 1989. Dominance of bacterial biomass in the Sargasso Sea and its ecological implications. Mar. Ecol. Prog. Ser. **57:**207–217.
96. **Fuhrman, J. A., and C. A. Suttle.** 1993. Viruses in marine planktonic systems. Oceanography **6:**50–62.
97. **Fuhrman, J. A., R. M. Wilcox, R. T. Noble, and N. C. Law.** 1993. Viruses in marine food webs, p. 295–298. *In* R. Guerrero and C. Pedros-Alio (ed.), Trends in microbial ecology. Spanish Society for Microbiology, Barcelona.
98. **Fuller, N. J., W. H. Wilson, I. R. Joint, and N. H. Mann.** 1998. Occurrence of a sequence in marine cyanophages similar to that of T4 g20 and its application to PCR-based detection and quantification techniques. Appl. Environ. Microbiol. **64:**2051–2060.
99. **Furuse, K.** 1987. Distribution of coliphages in the environment: general considerations, p. 87–124. *In* S. M. Goyal, C. P. Gerba, and G. Britton (ed.), Phage ecology. John Wiley & Sons, Inc., New York, N.Y.
100. **Furuta, M., J. O. Schrader, H. S. Schrader, T. A. Kokjohn, S. Nyaga, A. K. McCullough, R. S. Lloyd, D. E. Burbank, D. Landstein, L. Lane, and J. L. Van Etten.** 1997. *Chlorella* virus PBCV-1 encodes a homolog of the bacteriophage T4 UV damage repair gene *denV*. Appl. Environ. Microbiol. **63:**1551–1556.
101. **Garza, D. R., and C. A. Suttle.** 1998. The effect of cyanophages on the mortality of *Synechococcus* spp. and selection for UV resistant viral communities. Microb. Ecol. **36:**281.
102. **Garza, R. D., and C. A. Suttle.** 1995. Large double-stranded DNA viruses which cause the lysis of a marine heterotrophic nanoflagellate (*Bodo* sp.) occur in natural marine viral communities. Aquat. Microb. Ecol. **9:**203–210.
103. **Gerba, C. P.** 1987. Phage as indicators of fecal pollution, p. 197–209. *In* S. M. Goyal, C. P. Gerba, and G. Britton (ed.), Phage ecology. John Wiley & Sons, Inc., New York, N.Y.
104. **Gerba, C. P., J. B. Rose, and S. N. Singh.** 1985. Waterborne gastroenteritis and viral hepatitis. Crit. Rev. Environ. Control **15:**213–236.
105. **Gerba, C. P., and G. Schaiberger.** 1975. Effect of particulates on virus survival in seawater. J. Water Pollut. Control Fed. **47:**93–103.
106. **Gerba, C. P., and G. E. Schaiberger.** 1975. Aggregation as a factor in loss of viral titer in seawater. Water Res. **9:**567–571.
107. **Gersberg, R. M., S. R. Lyon, R. Brenner, and B. V. Elkins.** 1987. Fate of viruses in artificial wetlands. Appl. Environ. Microbiol. **53:**731–736.
108. **Gill, M. L., and K. Nealson.** 1972. Isolation and host range studies of marine bacteriophage. Biol. Bull. **143:**463–464.
109. **Gobler, C. J., D. A. Hutchins, N. S. Fisher, E. M. Cosper, and S. A. Sanudo-Wilhelmy.** 1997. Release and bioavailability of C, N, P, Se, and Fe following viral lysis of a marine chrysophyte. Limnol. Oceanogr. **42:**1492–1504.
110. **Gonzalez, J. M., and C. A. Suttle.** 1993. Grazing by marine nanoflagellates on viruses and virus-sized particles: ingestion and digestion. Mar. Ecol. Prog. Ser. **94:**1–10.
111. **Gowing, M. M.** 1993. Large virus-like particles from vacuoles of phaeodarian radiolarians and from other marine samples. Mar. Ecol. Prog. Ser. **101:**33–43.
112. **Goyal, S. M.** 1984. Viral pollution of the marine environment. Crit. Rev. Environ. Control **14:**1–32.
113. **Guixa-Boixareu, N., J. I. Calderon-Paz, M. Heldal, and G. Bratbak.** 1996. Viral lysis and bacterivory as prokaryotic loss factors along a salinity gradient. Aquat. Microb. Ecol. **11:**215–227.
114. **Hantula, J., A. Kurki, P. Vuoriranta, and D. H. Bamford.** 1991. Ecology of bacteriophages infecting activated sludge bacteria. Appl. Environ. Microbiol. **57:**2147–2151.
115. **Hara, S., I. Koike, K. Terauchi, H. Kamiya, and E. Tanoue.** 1996. Abundance of viruses in deep oceanic waters. Mar. Ecol. Prog. Ser. **145:**269–277.
116. **Hara, S., K. Terauchi, and I. Koike.** 1991. Abundance of viruses in marine waters: assessment by epifluorescence and transmission electron microscopy. Appl. Environ. Microbiol. **57:**2731–2734.
117. **Hastings, J. W., A. Keynan, and K. McCloskey.** 1961. Properties of a newly isolated bacteriophage of luminescent bacteria. Biol. Bull. **121:**375.
118. **Havelaar, A. H., M. vanOlphen, and Y. C. Drost.** 1993. F-specific RNA bacteriophages are adequate model organisms for enteric viruses in fresh water. Appl. Environ. Microbiol. **59:**2956–2962.
119. **Hayes, W.** 1968. The genetics of bacteria and their viruses. John Wiley & Sons Inc., New York, N.Y.
120. **Heldal, M., and G. Bratbak.** 1991. Production and decay of viruses in aquatic environments. Mar. Ecol. Prog. Ser. **72:**205–212.
121. **Hennes, K. P., and M. Simon.** 1995. Significance of bacteriophages for controlling bacterioplankton growth in a mesotrophic lake. Appl. Environ. Microbiol. **61:**333–340.
122. **Hennes, K. P., and C. A. Suttle.** 1995. Direct counts of viruses in natural waters and laboratory cultures by epifluorescence microscopy. Limnol. Oceanogr. **40:**1050–1055.
123. **Hennes, K. P., C. A. Suttle, and A. M. Chan.** 1995. Fluorescently labeled virus probes show that natural virus populations can control the structure of marine microbial communities. Appl. Environ. Microbiol. **61:**3623–3627.

124. **Herrmann, J. E., J. K. D. Kostenbader, and D. O. Oliver.** 1974. Persistence of enteriviruses in lake water. Appl. Microbiol. **28:**895–896.
125. **Hershey, A. D., and M. Chase.** 1952. Independent functions of viral protein and nucleic acid in growth of bacteriophage. J. Gen. Physiol. **36:**39–56.
126. **Hobbie, J. E.** 1994. The state of the microbes: a summary of a symposium honoring Lawrence Pomeroy. Microb. Ecol. **28:**113–116.
127. **Hobbie, J. E., R. J. Daley, and S. Jasper.** 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. **33:**1225–1228.
128. **Holligan, P. M., R. P. Harris, R. C. Newell, D. S. Harbour, R. N. Head, E. A. S. Linley, M. I. Lucas, P. R. G. Tranter, and C. M. Weekley.** 1984. Vertical distribution and partitioning of organic carbon in mixed, frontal and stratified waters of the English Channel. Mar. Ecol. Prog. Ser. **14:**111–127.
129. **Hutchinson, G. E.** 1961. The paradox of the plankton. Am. Nat. **95:**137–145.
130. **Inoue, T., S. Matsuzaki, and S. Tanaka.** 1995. A 26-kDa outer membrane protein, OmpK, common to *Vibrio* species is the receptor for a broad-host-range vibriophage, KVP40. FEMS Microbiol. Lett. **125:**101–105.
131. **Inoue, T., S. Matsuzaki, and S. Tanaka.** 1995. Cloning and sequence analysis of *Vibrio parahaemolyticus ompK* gene encoding a 26-kDa outer membrane protein, OmpK, that serves as receptor for a broad-host-range vibriophage, KVP40. FEMS Microbiol. Lett. **134:**245–249.
132. **Jensen, E. C., H. S. Schrader, B. Rieland, T. L. Thompson, K. W. Lee, K. W. Nickerson, and T. A. Kokjohn.** 1998. Prevalence of broad-host-range lytic bacteriophages of *Sphaerotilus natans*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. **64:**575–580.
133. **Jiang, S. C., and J. H. Paul.** 1998. Gene transfer by transduction in the marine environment. Appl. Environ. Microbiol. **64:**2780.
134. **Jiang, S. C., C. A. Kellogg, and J. H. Paul.** 1998. Characterization of marine temperate phage-host systems isolated from Mamala Bay, Hawaii. Appl. Environ. Microbiol. **64:**535–542.
135. **Jiang, S. C., and J. H. Paul.** 1996. Occurrence of lysogenic bacteria in marine microbial communities as determined by prophage induction. Mar. Ecol. Prog. Ser. **142:**27–38.
136. **Jiang, S. C., and J. H. Paul.** 1994. Seasonal and diel abundance of viruses and occurrence of lysogeny/bacteriocinogeny in the marine environment. Mar. Ecol. Prog. Ser. **104:**163–172.
137. **Jiang, S. C., and J. H. Paul.** 1997. Significance of lysogeny in the marine environment: studies with isolates and a model of lysogenic phage production. Microb. Ecol. **35:**235–243.
138. **Jiang, S. C., and J. H. Paul.** 1995. Viral contribution to dissolved DNA in the marine environment as determined by differential centrifugation and kingdom probing. Appl. Environ. Microbiol. **61:**317–325.
139. **Jiang, S. C., J. M. Thurmond, S. L. Pichard, and J. H. Paul.** 1992. Concentration of microbial populations from aquatic environments by vortex flow filtration. Mar. Ecol. Prog. Ser. **80:**101–107.
140. **Jones, D., and P. H. Sneath.** 1970. Genetic transfer and bacterial taxonomy. Bacteriol. Rev. **34:**40–81.
141. **Kapuscinski, R. B., and R. Mitchell.** 1980. Processes controlling virus inactivation in coastal waters. Water Res. **14:**363–371.
142. **Karl, D. M., and M. D. Bailiff.** 1989. The measurement and distribution of dissolved nucleic acids in aquatic environments. Limnol. Oceanogr. **34:**543–558.
143. **Kellogg, C. A., J. B. Rose, S. C. Jiang, J. M. Thurmond, and J. H. Paul.** 1995. Genetic diversity of related vibriophages isolated from marine environments around Florida and Hawaii, USA. Mar. Ecol. Prog. Ser. **120:**89–98.
144. **Kepner, R. L., R. A. Wharton, and C. A. Suttle.** 1998. Viruses in Antarctic lakes. Limnol. Oceanogr. **43:**1754.
145. **Kirchman, D., E. K'Nees, and R. Hodson.** 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Appl. Environ. Microbiol. **49:**599–607.
146. **Kjelleberg, S., M. Hermansson, P. Marden, and G. W. Jones.** 1987. The transient phase between growth and nongrowth of heterotrophic bacteria, with emphasis on the marine environment. Annu. Rev. Microbiol. **41:**25–49.
147. **Klieve, A. V., J. F. Hudman, and T. Bauchop.** 1989. Inducible bacteriophages from ruminal bacteria. Appl. Environ. Microbiol. **55:**1630–1634.
148. **Klieve, A. V., and R. A. Swain.** 1993. Estimation of ruminal bacteriophage numbers by pulsed-field gel electrophoresis and laser densitometry. Appl. Environ. Microbiol. **59:**2299–2303.
149. **Klut, M. E., and J. G. Stockner.** 1990. Virus-like particles in an ultra-oligotrophic lake on Vancouver Island, British Columbia. Can. J. Fish. Aquat. Sci. **47:**725–730.
150. **Koch, A. L.** 1981. Growth measurements, p. 197–209. *In* P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Kreig, and G. B. Phillips (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
151. **Koga, T., S. Toyoshima, and T. Kawata.** 1982. Morphological varieties and host ranges of *Vibrio parahaemolyticus* bacteriophages isolated from seawater. Appl. Environ. Microbiol. **44:**466–470.
152. **Kogure, K., and I. Koike.** 1987. Particle counter determination of bacterial biomass in seawater. Appl. Environ. Microbiol. **53:**274–277.
153. **Koike, I., S. Hara, K. Terauchi, and K. Kogure.** 1990. Role of submicrometre particles in the ocean. Nature (London) **345:**242–244.
154. **Kokjohn, T. A.** 1989. Transduction: Mechanism and potential for gene transfer in the environment, p. 73–93. *In* S. B. Levy and R. V. Miller (ed.), Gene transfer in the environment. McGraw-Hill, New York, N.Y.
155. **Reference deleted.**
156. **Kokjohn, T. A., G. S. Sayler, and R. V. Miller.** 1991. Attachment and replication of *Pseudomonas aeruginosa* bacteriophages under conditions simulating aquatic environments. J. Gen. Microbiol. **137:**661–666.
157. **Kolter, R., D. A. Siegele, and A. Tormo.** 1993. The stationary phase of the bacterial life cycle. Annu. Rev. Microbiol. **47:**855–874.
158. **LaBelle, R., and C. P. Gerba.** 1982. Investigations into the protective effect of estuarine sediment on virus survival. Water Res. **16:**469–478.
159. **Lenski, R. E.** 1984. Coevolution of bacteria and phage: are there endless cycles of bacterial defenses and phage counterdefenses? J. Theor. Biol. **108:**319–325.
160. **Lenski, R. E.** 1988. Dynamics of interactions between bacteria and virulent bacteriophage. Adv. Microb. Ecol. **10:**1–44.
161. **Lenski, R. E., and B. R. Levin.** 1985. Constraints on the coevolution of bacteria and virulent phage: a model, some experiments, and predictions for natural communities. Am. Nat. **125:**585–602.
162. **Levin, B. R., F. M. Stewart, and L. Chao.** 1977. Resource limited growth, competition, and predation: A model and experimental studies with bacteria and bacteriophage. Am. Nat. **111:**3–24.
163. **Levisohn, R., J. Moreland, and K. H. Nealson.** 1987. Isolation and characterization of a generalized transducing phage for the marine luminous bacterium *Vibrio fischeri* MJ-1. J. Gen. Microbiol. **133:**1577–1582.
164. **Li, K., L. Barksdale, and L. Garmise.** 1961. Phenotypic alterations associated with the bacteriophage carrier state of *Shigella dysenteriae*. J. Gen. Microbiol. **24:**355–367.
165. **Lin, L., R. Bitner, and G. Edlin.** 1977. Increased reproductive fitness of *Escherichia coli* lambda lysogens. J. Virol. **21:**554–559.
166. **Lo, S., J. Gilbert, and F. Hetrick.** 1976. Stability of human enteroviruses in estuarine and marine waters. Appl. Environ. Microbiol. **32:**245–249.
167. **Lwoff, A., L. Siminovitch, and N. Kjeldgaard.** 1950. Induction de la production de bacteriophages chez une bactérie lysogène. Ann. Inst. Pasteur **79:**815–859.
168. **Lycke, E., S. Magnusson, and E. Lund.** 1965. Studies on the nature of the virus inactivating capacity of sea water. Arch. Gesamte Virusforsch. **17:**409–413.
169. **MacLeod, R. A.** 1965. The question of the existence of specific marine bacteria. Bacteriol. Rev. **29:**2–23.
170. **Malin, G., W. H. Wilson, G. Bratbak, P. S. Liss, and N. H. Mann.** 1998. Elevated production of dimethylsulfide resulting from viral infection of cultures of *Phaeocystis pouchetii*. Limnol. Oceanogr. **43:**1389–1393.
171. **Maranger, R., and D. F. Bird.** 1995. Viral abundance in aquatic systems: a comparison between marine and fresh waters. Mar. Ecol. Prog. Ser. **121:**1–3.
172. **Maranger, R., D. F. Bird, and S. K. Juniper.** 1994. Viral and bacterial dynamics in Arctic sea ice during the spring algal bloom near Resolute, N.W.T., Canada. Mar. Ecol. Prog. Ser. **111:**121–127.
173. **Marie, D., C. P. D. Brussard, R. Thyrhaug, G. Bratbak, and D. Vaulot.** 1999. Enumeration of marine viruses in culture and natural samples by flow cytometry. Appl. Environ. Microbiol. **65:**45–52.
174. **Marsh, P., and E. M. H. Wellington.** 1994. Phage-host interactions in soil. FEMS Microbiol. Ecol. **15:**99–107.
175. **Maruyama, A., M. Oda, and T. Higashihara.** 1993. Abundance of virus-sized non-DNase-digestible DNA (coated DNA) in eutrophic seawater. Appl. Environ. Microbiol. **59:**712–717.
176. **Mathias, C. B., A. K. T. Kirschner, and B. Velimirov.** 1995. Seasonal variations of virus abundance and viral control of the bacterial production in a backwater system of the Danube river. Appl. Environ. Microbiol. **61:**3734–3740.
177. **Matin, A., E. A. Auger, P. H. Blum, and J. E. Schultz.** 1989. Genetic basis of starvation survival in nondifferentiating bacteria. Annu. Rev. Microbiol. **43:**293–316.
178. **Matossian, A. M., and G. A. Garabedian.** 1967. Virucidal action of sea water. J. Epidemiol. **85:**1–8.
179. **Matsuzaki, S., T. Inoue, and S. Tanaka.** 1992. Evidence for the existence of a restriction-modification system common to several species of the family Vibrionaceae. FEMS Microbiol. Lett. **94:**191–194.
180. **Matsuzaki, S., S. Tanaka, T. Koga, and T. Kawata.** 1992. A broad-host-range vibriophage, KVP40, isolated from seawater. Microbiol. Immunol. **36:**93–97.
181. **McManus, G. B., and J. A. Fuhrman.** 1988. Control of marine bacterioplankton populations: measurement and significance of grazing. Hydrobiologia **159:**51–62.
182. **Metcalf, T. G., J. L. Melnick, and M. K. Estes.** 1995. Environmental virology: from detection of virus in sewage and water by isolation to identification by molecular biology—a trip of over 50 years. Annu. Rev. Microbiol. **49:**461–87.
183. **Middelboe, M., N. O. G. Jorgensen, and N. Kroer.** 1996. Effects of viruses

on nutrient turnover and growth efficiency of noninfected marine bacterioplankton. Appl. Environ. Microbiol. **62:**1991–1997.
184. **Miller, R. V.** 1988. Potential for transfer and establishment of engineered genetic sequences. Trends Biotechnol. **6:**S23–S27.
185. **Miller, R. V., J. M. Pemberton, and A. J. Clark.** 1977. Prophage F116—evidence for extrachromosomal location in *Pseudomonas aeruginosa* strain PAO. J. Virol. **22:**844–847.
186. **Miller, R. V., S. Ripp, J. Replicon, O. A. Ogunseitan, and T. A. Kokjohn.** 1992. Virus-mediated gene transfer in freshwater environments, p. 51–62. *In* M. J. Gauthier (ed.), Gene transfers and environment. Springer-Verlag KG, Berlin, Germany.
187. **Miller, R. V., and G. S. Sayler.** 1992. Bacteriophage-host interactions in aquatic systems, p. 176–193. *In* E. M. H. Wellington and J. D. van Elsas (ed.), Genetic interactions among microorganisms in the natural environment. Pergamon Press, New York, N.Y.
188. **Milligan, K. L. D., and E. M. Cosper.** 1994. Isolation of virus capable of lysing the brown tide microalga, *Aureococcus anophagefferens*. Science **266:** 805–807.
189. **Mitchell, J. G., A. Okubo, and J. A. Fuhrman.** 1985. Microzones surrounding phytoplankton form the basis for a stratified marine microbial ecosystem. Nature (London) **316:**58–59.
190. **Mitchell, R.** 1971. Destruction of bacteria and viruses in seawater. J. Sanit. Eng. Div. Proc. Am. Soc. Civil Eng. **97:**425–432.
191. **Mitchell, R., and H. W. Jannaasch.** 1969. Processes controlling virus inactivation in seawater. Environ. Sci. Technol. **3:**941–943.
192. **Mitra, S. N., S. Kar, R. K. Ghosh, S. Pajni, and A. Ghosh.** 1995. Presence of lysogenic phage in the outbreak strains of *Vibrio cholerae* O139. J. Med. Microbiol. **42:**399–403.
193. **Moebus, K.** 1987. Ecology of marine bacteriophages, p. 137–155. *In* S. M. Goyal, C. P. Gerba, and G. Britton (ed.), Phage ecology. John Wiley & Sons, Inc., New York, N.Y.
194. **Moebus, K.** 1992. Further investigations on the concentration of marine bacteriophages in the waters around Helgoland, with reference to the phage-host systems encountered. Helgol. Meeresunters. **46:**275–292.
195. **Moebus, K.** 1997. Investigations of the marine lysogenic bacterium H24.1. General description of the phage-host system. Mar. Ecol. Prog. Ser. **148:** 217–228.
196. **Moebus, K.** 1997. Investigations of the marine lysogenic bacterium H24.2. Development of pseudolysogeny in nutrient rich broth. Mar. Ecol. Prog. Ser. **148:**229–240.
197. **Moebus, K.** 1992. Laboratory investigations on the survival of marine bacteriophages in raw and treated seawater. Helgol. Meeresunters. **46:**251–273.
198. **Moebus, K.** 1983. Lytic and inhibition responses to bacteriophages among marine bacteria, with special reference to the origin of phage-host systems. Helgol. Meeresunters. **36:**375–391.
199. **Moebus, K.** 1996. Marine bacteriophage reproduction under nutrient-limited growth of host bacteria. 1. Investigations with six phage-host systems. Mar. Ecol. Prog. Ser. **144:**1–12.
200. **Moebus, K.** 1996. Marine bacteriophage reproduction under nutrient-limited growth of host bacteria. 2. Investigations with phage-host system [H3: H3/1]. Mar. Ecol. Prog. Ser. **144:**13–22.
201. **Moebus, K.** 1980. A method for the detection of bacteriophages from ocean water. Helgol. Meeresunters. **34:**1–14.
202. **Moebus, K., and H. Nattkemper.** 1981. Bacteriophage sensitivity patterns among bacteria isolated from marine waters. Helgol. Meeresunters. **34:** 375–385.
203. **Morona, R., and U. Henning.** 1984. Host range mutants of bacteriophage Ox2 can use two different outer membrane proteins of *Escherichia coli* K-12 as receptors. J. Bacteriol. **159:**579–582.
204. **Morrison, W. D., R. V. Miller, and G. S. Sayler.** 1978. Frequency of F116-mediated transduction of *Pseudomonas aeruginosa* in a freshwater environment. Appl. Environ. Microbiol. **36:**724–730.
205. **Muramatsu, K., and H. Matsumoto.** 1991. Two generalized transducing phages in *Vibrio parahaemolyticus* and *Vibrio alginolyticus*. Microbiol. Immunol. **35:**1073–1084.
206. **Murray, A. G., and P. M. Eldridge.** 1994. Marine viral ecology: Incorporation of bacteriophage into the microbial planktonic food web paradigm. J. Plankton Res. **16:**627–641.
207. **Murray, A. G., and G. A. Jackson.** 1993. Viral dynamics II: a model of the interaction of ultraviolet light and mixing processes on virus survival in seawater. Mar. Ecol. Prog. Ser. **102:**105–114.
208. **Murray, A. G., and G. A. Jackson.** 1992. Viral dynamics: a model of the effects of size, shape, motion and abundance of single-celled planktonic organisms and other particles. Mar. Ecol. Prog. Ser. **89:**103–116.
209. **Nagasaki, K., M. Ando, I. Imai, S. Itakura, and Y. Ishida.** 1994. Virus-like particles in *Heterosigma akashiwo* (Raphidophyceae): a possible red tide disintegration mechanism. Mar. Biol. **119:**307–312.
210. **Nagasaki, K., M. Ando, I. Imai, S. Itakura, and Y. Ishida.** 1995. Virus-like particles in unicellular apochlorotic microorganisms in the coastal water of Japan. Fish. Sci. **61:**235–239.
211. **Nagasaki, K., M. Ando, S. Itakura, I. Imai, and Y. Ishida.** 1994. Viral mortality in the final stage of *Heterosigma akashiwo* (Raphidophyceae) red tide. J. Plankton Res. **16:**1595–1599.
212. **Nathans, D., and H. O. Smith.** 1975. Restriction endonuclease in the analysis and restructuring of DNA molecules. Annu. Rev. Biochem. **44:**273–293.
213. **Niemi, M.** 1976. Survival of *Escherichia coli* phage T7 in different water types. Water Res. **10:**751–755.
214. **Noble, R. T., and J. A. Fuhrman.** 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat. Microb. Ecol. **14:**113–118.
215. **Noble, R. T., and J. A. Fuhrman.** 1997. Virus decay and its causes in coastal waters. Appl. Environ. Microbiol. **63:**77–83.
216. **Nowak, R.** 1995. Bacterial genome sequence bagged. Science **269:**468–470.
217. **O'Brien, R. T., and J. S. Newman.** 1977. Inactivation of polioviruses and coxsackieviruses in surface water. Appl. Environ. Microbiol. **33:**334–340.
218. **Officer, C. B., R. B. Biggs, J. L. Taft, L. E. Cronin, M. A. Tyler, and W. R. Boynton.** 1984. Chesapeake Bay anoxia: Origin, development, and significance. Science **223:**22–26.
219. **Ogunseitan, O. A., G. S. Sayler, and R. V. Miller.** 1992. Application of DNA probes to analysis of bacteriophage distribution patterns in the environment. Appl. Environ. Microbiol. **58:**2046–2052.
220. **Ogunseitan, O. A., G. S. Sayler, and R. V. Miller.** 1990. Dynamic interactions of *Pseudomonas aeruginosa* and bacteriophages in lake water. Microb. Ecol. **19:**171–185.
221. **Ohki, K., and Y. Fujita.** 1996. Occurrence of a temperate cyanophage lysogenizing the marine cyanophyte *Phormidium persicinum*. J. Phycol. **32:** 365–370.
222. **Okazaki, R. T., K. Okazaki, K. Sakabe, K. Sugimoto, and A. Sugino.** 1968. Mechanism of DNA chain growth. I. Possible discontinuity and unusual secondary structure of newly synthesized chains. Proc. Natl. Acad. Sci. USA **59:**598–603.
223. **Olofsson, S., and S. Kjelleberg.** 1991. Virus ecology. Nature (London) **351:**612–613.
224. **Olsen, G. J.** 1994. Microbial ecology: Archaea, Archaea, everywhere. Nature (London) **371:**657–658.
225. **Olson, R. J., S. W. Chisholm, E. R. Zettler, M. A. Altabet, and J. A. Dusenberry.** 1990. Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic Ocean. Deep Sea Res. Ser. A **37:**1033–1051.
226. **Olson, R. J., S. W. Chisholm, E. R. Zettler, and E. V. Armbrust.** 1990. Pigments, size, and distribution of *Synechococcus* in the North Atlantic and Pacific Oceans. Limnol. Oceanogr. **35:**45–58.
227. **Oren, A., G. Bratbak, and M. Heldal.** 1997. Occurrence of virus-like particles in the Dead Sea. Extremophiles **1:**143.
228. **Otsuji, N., M. Sekiguchi, T. Iijima, and Y. Takagi.** 1959. Induction of phage formation in the lysogenic *Escherichia coli* K-12 by mitomyosin C. Nature (London) **184:**1079–1080.
229. **Padan, E., and M. Shilo.** 1973. Cyanophages-viruses attacking blue-green algae. Bacteriol. Rev. **37:**343–370.
230. **Pajni, S., N. R. Chowdhury, A. Ghosh, S. Kar, and R. K. Ghosh.** 1995. Characterization of phage phi O139, a *Vibrio cholerae* O139 temperate bacteriophage with cohesive DNA termini. FEMS Microbiol. Lett. **131:**69–74.
231. **Patrick, J. R., D. E. Brabham, and P. M. Achey.** 1981. Photoreactivation of UV-B damage in bacteriophage ΦX-174 DNA. Photochem. Photobiol. **33:**769–771.
232. **Paul, J. H.** 1999. Microbial gene transfer: an ecological perspective. J. Mol. Microbiol. Biotechnol. **1:**45–50.
233. **Paul, J. H., W. H. Jeffrey, A. W. David, M. F. DeFlaun, and L. H. Cazares.** 1989. Turnover of extracellular DNA in eutrophic and oligotrophic freshwater environments of southwest Florida. Appl. Environ. Microbiol. **55:** 1823–1828.
234. **Paul, J. H., L. H. Cazares, A. W. David, M. F. DeFlaun, and W. H. Jeffrey.** 1991. The distribution of dissolved DNA in an oligotrophic and a eutrophic river of Southwest Florida. Hydrobiologia **218:**53–63.
235. **Paul, J. H., and A. W. David.** 1989. Production of extracellular nucleic acids by genetically altered bacteria in aquatic-environment microcosms. Appl. Environ. Microbiol. **55:**1865–1869.
236. **Paul, J. H., M. F. DeFlaun, W. H. Jeffrey, and A. W. David.** 1988. Seasonal and diel variability in dissolved DNA and in microbial biomass and activity in a subtropical estuary. Appl. Environ. Microbiol. **54:**718–727.
237. **Paul, J. H., W. H. Jeffrey, and J. P. Cannon.** 1990. Production of dissolved DNA, RNA, and protein by microbial populations in a Florida reservoir. Appl. Environ. Microbiol. **56:**2957–2962.
238. **Paul, J. H., W. H. Jeffrey, A. W. David, M. F. DeFlaun, and L. H. Cazares.** 1989. Turnover of extracellular DNA in eutrophic and oligotrophic freshwater environments of Southwest Florida. Appl. Environ. Microbiol. **55:** 1823–1828.
239. **Paul, J. H., W. H. Jeffrey, and M. F. DeFlaun.** 1987. Dynamics of extracellular DNA in the marine environment. Appl. Environ. Microbiol. **53:**170–179.
240. **Paul, J. H., S. C. Jiang, and J. B. Rose.** 1991. Concentration of viruses and dissolved DNA from aquatic environments by vortex flow filtration. Appl. Environ. Microbiol. **57:**2197–2204.

241. **Paul, J. H., C. A. Kellogg, and S. C. Jiang.** 1994. Viruses and DNA in marine environments, p. 119–128. *In* R. R. Colwell, U. Simidu, and K. Ohwada (ed.), Microbial diversity in space and time. Plenum Press, New York, N.Y.
242. **Paul, J. H., and B. Myers.** 1982. Fluorometric determination of DNA in aquatic microorganisms by use of Hoechst 33258. Appl. Environ. Microbiol. **43:**1393–1399.
243. **Paul, J. H., J. B. Rose, S. C. Jiang, C. A. Kellogg, and L. Dickson.** 1993. Distribution of viral abundance in the reef environment of Key Largo, Florida. Appl. Environ. Microbiol. **59:**718–724.
244. **Paul, J. H., J. B. Rose, S. C. Jiang, P. London, X. Xhou, and C. Kellogg.** 1997. Coliphage and indigenous phage in Mamala Bay, Oahu, Hawaii. Appl. Environ. Microbiol. **63:**133–138.
245. **Peduzzi, P., and M. G. Weinbauer.** 1993. Effect of concentrating the virus-rich 2-200-nm size fraction of seawater on the formation of algal flocs (marine snow). Limnol. Oceanogr. **38:**1562–1565.
246. **Peduzzi, P., and M. G. Weinbauer.** 1993. The submicron size fraction of seawater containing high numbers of virus particles as bioactive agent in unicellular plankton community successions. J. Plankton Res. **15:**1375–1386.
247. **Pennisi, E.** 1998. Genome data shake tree of life. Science **280:**672–674.
248. **Pickup, R. W.** 1992. Detection of gene transfer in aquatic environments, p. 145–164. *In* E. M. H. Wellington and J. D. van Elsas (ed.), Genetic interactions among microorganisms in the natural environment. Pergamon Press, New York, N.Y.
249. **Pina, S., A. Creus, N. Gonzalez, R. Girones, M. Felip, and R. Sommaruga.** 1998. Abundance, morphology and distribution of planktonic virus-like particles in two high-mountain lakes. J. Plankton Res. **20:**2413.
250. **Pitt, T. L., and M. A. Gaston.** 1995. Bacteriophage typing. Methods Mol. Biol. **46:**15–26.
251. **Pomeroy, L. R.** 1974. The ocean's food web, a changing paradigm. BioScience **24:**499–504.
252. **Primrose, S. B., and M. Day.** 1977. Rapid concentration of bacterophages from aquatic habitats. J. Appl. Bacteriol. **42:**417–421.
253. **Proctor, L. M.** 1997. Advances in the study of marine viruses. Microsc. Res. Tech. **37:**136–161.
254. **Proctor, L. M., and J. A. Fuhrman.** 1992. Mortality of marine bacteria in response to enrichments of the virus size fraction from seawater. Mar. Ecol. Prog. Ser. **87:**283–293.
255. **Proctor, L. M., and J. A. Fuhrman.** 1991. Roles of viral infection in organic particle flux. Mar. Ecol. Prog. Ser. **69:**133–142.
256. **Proctor, L. M., and J. A. Fuhrman.** 1990. Viral mortality of marine bacteria and cyanobacteria. Nature (London) **343:**60–62.
257. **Proctor, L. M., J. A. Fuhrman, and M. C. Ledbetter.** 1988. Marine bacteriophages and bacterial mortality. Eos **69:**1111–1112.
258. **Proctor, L. M., A. Okubo, and J. A. Fuhrman.** 1993. Calibrating estimates of phage-induced mortality in marine bacteria: ultrastructural studies of marine bacteriophage development from one-step growth experiments. Microb. Ecol. **25:**161–182.
259. **Propst-Ricciuti, C.** 1976. The effect of host-cell starvation on virus-induced lysis by MS2 bacteriophage. J. Gen. Virol. **31:**323–330.
260. **Propst-Ricciuti, C.** 1972. Host-virus interactions in *Escherichia coli*: effect of stationary phase on viral release from MS2-infected bacteria. J. Virol. **10:**162–165.
261. **Ptashne, M.** 1991. The genetic switch. Blackwell Scientific Publications and Cell Press, Cambridge, Mass.
262. **Rehnstam, A. S., S. Bäckman, D. C. Smith, F. Azam, and Å. Hagström.** 1993. Blooms of sequence-specific culturable bacteria in the sea. FEMS Microbiol. Ecol. **102:**161–166.
263. **Reisser, W.** 1993. Viruses and virus-like particles of freshwater and marine eukaryotic algae—a review. Arch. Protistenkd. **143:**257–265.
264. **Reisser, W., B. Becker, and T. Klein.** 1986. Studies on ultrastructure and host range of a *Chlorella* attacking virus. Arch. Hydrobiol. **135:**162–165.
265. **Reisser, W., S. Grein, and C. Krambeck.** 1993. Extracellular DNA in aquatic ecosystems may in part be due to phycovirus activity. Hydrobiologica **252:**199–201.
266. **Reisser, W., and S. Vietze.** 1993. Soluble DNA in freshwater: the role of algal viruses, p. 308–310. *In* R. Guerrero and C. Pedros-Alio (ed.), Trends in microbial ecology. Spanish Society for Microbiology, Barcelona.
267. **Reiter, W. D., W. Zillig, and P. Palm.** 1988. Archaebacterial viruses. Adv. Virus Res. **34:**143–188.
268. **Replicon, J., A. Frankfater, and R. V. Miller.** 1995. A continuous culture model to examine factors that affect transduction among *Pseudomonas aeruginosa* strains in freshwater environments. Appl. Environ. Microbiol. **61:**3359–3366.
269. **Ripp, S., and R. V. Miller.** 1995. Effects of suspended particulates on the frequency of transduction among *Pseudomonas aeruginosa* in freshwater environments. Appl. Environ. Microbiol. **61:**1214–1219.
270. **Ripp, S., and R. V. Miller.** 1997. The role of pseudolysogeny in bacteriophage-host interactions in a natural freshwater environment. Microbiology **143:**2065–2070.
271. **Ripp, S., O. A. Ogunseitan, and R. V. Miller.** 1994. Transduction of a freshwater microbial community by a new *Pseudomonas aeruginosa* generalized transducing phage, UT1. Mol. Ecol. **3:**121–126.
272. **Robb, F. T., and R. T. Hill.** Bacterial viruses and hosts: influence of culturable state. *In* R. R. Colwell and D. J. Grimes (ed.), Nonculturable microorganisms in the environment, in press. ASM Press, Washington, D.C.
273. **Robb, S. M., D. R. Woods, and F. T. Robb.** 1978. Phage growth characteristics on stationary phase *Achromobacter* cells. J. Gen. Virol. **41:**265–272.
274. **Robb, S. M., D. R. Woods, F. T. Robb, and J. K. Struthers.** 1977. Rifampicin-resistant mutant supporting bacteriophage growth on stationary phase *Achromobacter* cells. J. Gen. Virol. **35:**117–123.
275. **Robertson, B. R., and D. K. Button.** 1989. Characterizing aquatic bacteria according to population, cell size, and apparent DNA content by flow cytometry. Cytometry **10:**70–76.
276. **Romig, W. R., and A. M. Brodetsky.** 1961. Isolation and preliminary characterization of bacteriophages for *Bacillus subtilis*. J. Bacteriol. **82:**135–141.
277. **Roszak, D. B., and R. R. Colwell.** 1987. Survival strategies of bacteria in the natural environment. Microbiol. Rev. **51:**365–379.
278. **Safferman, R. S., T. O. Diener, P. R. Desjardins, and M. E. Morris.** 1972. Isolation and characterization of AS-1, a phycovirus infecting the blue-green algae, *Anacystis nidulans* and *Synechococcus cedrorum*. Virology **47:**105–13.
279. **Safferman, R. S., and M. E. Morris.** 1963. Algal virus: isolation. Nature (London) **140:**679–680.
280. **Safferman, R. S., I. R. Schneider, R. L. Steere, M. E. Morris, and T. O. Diener.** 1969. Phycovirus SM-1: a virus infecting unicellular blue-green algae. Virology **37:**386–395.
281. **Saye, D. J., and R. V. Miller.** 1989. The aquatic environment: consideration of horizontal gene transmission in a diversified habitat, p. 223–259. *In* S. B. Levy and R. V. Miller (ed.), Gene transfer in the environment. McGraw-Hill, New York, N.Y.
282. **Reference deleted.**
283. **Saye, D. J., O. Ogunseitan, G. S. Sayler, and R. V. Miller.** 1987. Potential for transduction of plasmids in a natural freshwater environment: effect of plasmid donor concentration and a natural microbial community on transduction in *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. **53:**987–995.
284. **Saye, D. J., O. A. Ogunseitan, G. S. Sayler, and R. V. Miller.** 1990. Transduction of linked chromosomal genes between *Pseudomonas aeruginosa* strains during incubation in situ in a freshwater habitat. Appl. Environ. Microbiol. **56:**140–145.
285. **Schicklmaier, P., and H. Schmieger.** 1995. Frequency of generalized transducing phages in natural isolates of the *Salmonella typhimurium* complex. Appl. Environ. Microbiol. **61:**1637–1640.
286. **Schrader, H. S., J. O. Schrader, J. J. Walker, T. A. Wolf, K. W. Nickerson, and T. A. Kokjohn.** 1997. Bacteriophage infection and multiplication occur in *Pseudomonas aeruginosa* starved for 5 years. Can. J. Microbiol. **43:**1157–63.
287. **Seeley, N. D., and S. B. Primrose.** 1979. Concentration of bacteriophages from natural waters. J. Appl. Bacteriol. **46:**103–116.
288. **Seeley, N. D., and S. B. Primrose.** 1982. The isolation of bacteriophages from the environment. J. Appl. Bacteriol. **53:**1–17.
289. **Seyedirashti, S., C. Wood, and J. M. Akagi.** 1991. Induction and partial purification of bacteriophages from *Desulfovibrio vulgaris* (Hildenborough) and *Desulfovibrio desulfuricans* ATCC 13541. J. Gen. Microbiol. **137:**1545–1549.
290. **Shaffer, J. J., L. M. Jacobsen, J. O. Schrader, K. W. Lee, E. L. Martin, and T. A. Kokjohn.** 1999. Characterization of *Pseudomonas aeruginosa* bacteriophage UNL-1, a bacterial virus with a novel UV-A-inducible DNA damage reactivation phenotype. Appl. Environ. Microbiol. **65:**2606–2613.
291. **Shuval, H. I., A. Thompson, B. Fattal, S. Cymbalista, and Y. Wiener.** 1971. Natural virus inactivation processes in seawater. J. Sanit. Eng. Div. Proc. Am. Soc. Civil Eng. **97:**587–600.
292. **Sieburth, J. M.** 1979. Sea microbes. Oxford University Press, New York, N.Y.
293. **Sieburth, J. M., P. W. Johnson, and P. E. Hargraves.** 1988. Ultrastructure and ecology of *Aureococcus anophagefferens* gen. et sp. nov. (Chrysophyceae) the dominant picoplankter during a bloom in Narragansett Bay Rhode Island USA summer 1985. J. Phycol. **24:**416–425.
294. **Siegele, D. A., and R. Kolter.** 1992. Life after log. J. Bacteriol. **174:**345–348.
295. **Singh, R. N., and P. K. Singh.** 1967. Isolation of cyanophages from India. Nature (London) **216:**1020–1021.
296. **Smith, D. C., G. F. Steward, F. Azam, and J. T. Hollibaugh.** 1992. Virus and bacteria abundances in the Drake Passage during January and August 1991. Antarct. J. U. S. **27:**125–126.
297. **Smith, E. M., C. P. Gerba, and J. L. Melnick.** 1978. Role of sediment in the persistence of enteroviruses in the estuarine environment. Appl. Environ. Microbiol. **35:**685–689.
298. **Sommaruga, R., M. Krössbacher, W. Salvenmoser, J. Catalan, and R. Psenner.** 1995. Presence of large virus-like particles in a eutrophic reservoir. Aquat. Microb. Ecol. **9:**305–308.
299. **Sommaruga, R., and R. Psenner.** 1995. Trophic interactions within the microbial food web in Piburger See (Austria). Arch. Hydrobiol. **132:**257–278.

300. **Spanakis, E., and M. T. Horne.** 1987. Co-adaptation of *Escherichia coli* and coliphage lambda vir in continuous culture. J. Gen. Microbiol. **133:**353–360.
301. **Spencer, R.** 1960. Indigenous marine bacteriophages. J. Bacteriol. **79:**614.
302. **Spencer, R.** 1955. A marine bacteriophage. Nature (London) **175:**690–691.
303. **Stent, G. S.** 1963. Molecular biology of bacterial viruses. W. H. Freeman & Co., San Francisco, Calif.
304. **Steward, G. F., D. C. Smith, and F. Azam.** 1996. Abundance and production of bacteria and viruses in the Bering and Chukchi seas. Mar. Ecol. Prog. Ser. **131:**287–300.
305. **Steward, G. F., J. Wikner, W. P. Cochlan, D. C. Smith, and F. Azam.** 1992. Estimation of virus production in the sea. I. Method development. Mar. Microb. Food Webs **6:**57–78.
306. **Steward, G. F., J. Wikner, W. P. Cochlan, D. C. Smith, and F. Azam.** 1992. Estimation of virus production in the sea. II. Field results. Mar. Microb. Food Webs **6:**79–90.
307. **Stewart, F. M., and B. R. Levin.** 1984. The population biology of bacterial viruses: why be temperate? Theor. Pop. Biol. **26:**93–117.
308. **Suttle, C. A.** 1997. Community structure: viruses, p. 272–277. *In* C. J. Hurst, G. R. Knudsen, M. J. McInerney, L. D. Stetzenbach, and M. V. Walter (ed.), Manual of environmental microbiology. ASM Press, Washington, D.C.
309. **Suttle, C. A.** 1993. Enumeration and isolation of viruses, p. 121–134. *In* P. F. Kemp (ed.), Handbook of methods in aquatic microbial ecology CRC Press, Boca Raton, Fla.
310. **Suttle, C. A.** 1992. Inhibition of photosynthesis in phytoplankton by the submicron size fraction concentrated from seawater. Mar. Ecol. Prog. Ser. **87:**105–112.
311. **Suttle, C. A.** 1994. The significance of viruses to mortality in aquatic microbial communities. Microb. Ecol. **28:**237–243.
312. **Suttle, C. A., and A. M. Chan.** 1994. Dynamics and distribution of cyanophages and their effect on marine *Synechococcus* spp. Appl. Environ. Microbiol. **60:**3167–3174.
313. **Suttle, C. A., and A. M. Chan.** 1993. Marine cyanophages infecting oceanic and coastal strains of *Synechococcus*: abundance, morphology, cross-reactivity and growth characteristics. Mar. Ecol. Prog. Ser. **92:**99–109.
314. **Suttle, C. A., and A. M. Chan.** 1995. Viruses infecting the marine Prymnesiophyte *Chrysochromulina* spp.: isolation, preliminary characterization and natural abundance. Mar. Ecol. Prog. Ser. **118:**275–282.
315. **Suttle, C. A., A. M. Chan, and M. T. Cottrell.** 1990. Infection of phytoplankton by viruses and reduction of primary productivity. Nature (London) **347:**467–469.
316. **Suttle, C. A., A. M. Chan, and M. T. Cottrell.** 1991. Use of ultrafiltration to isolate viruses from seawater which are pathogens of marine phytoplankton. Appl. Environ. Microbiol. **57:**721–726.
317. **Suttle, C. A., A. M. Chan, C. Feng, and D. R. Garza.** 1993. Cyanophages and sunlight: a paradox, p. 303–307. *In* R. Guerrero and C. Pedros-Alio (ed.), Trends in microbial ecology. Spanish Society for Microbiology, Barcelona.
318. **Suttle, C. A., and F. Chen.** 1992. Mechanisms and rates of decay of marine viruses in seawater. Appl. Environ. Microbiol. **58:**3721–3729.
319. **Swain, R. A., J. V. Nolan, and A. V. Klieve.** 1996. Natural variability and diurnal fluctuations within the bacteriophage population of the rumen. Appl. Environ. Microbiol. **62:**994–997.
320. **Tapper, M. A., and R. E. Hicks.** 1998. Temperate viruses and lysogeny in Lake Superior bacterioplankton. Limnol. Oceanogr. **43:**95–103.
321. **Tartera, C., and J. Jofre.** 1987. Bacteriophages active against *Bacteroides fragilis* in sewage-polluted waters. Appl. Environ. Microbiol. **53:**1632–1637.
322. **Thingstad, T. F., G. Bratbak, M. Heldal, and I. Dundas.** 1997. Trophic interactions controlling the diversity in pelagic microbial food webs, p. 107–114. *In* M. T. Martins (ed.), Progress in microbial ecology. Brazilian Society for Microbiology, São Paulo.
323. **Thingstad, T. F., M. Heldal, G. Bratbak, and I. Dundas.** 1993. Are viruses important partners in pelagic food webs? Trends Ecol. Evol. **8:**209–212.
324. **Thingstad, T. F., and R. Lignell.** 1997. Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand. Aquat. Microb. Ecol. **13:**19–27.
325. **Toranzo, A. E., J. L. Barja, and F. M. Hetrick.** 1983. Mechanism of poliovirus inactivation by cell-free filtrates of marine bacteria. Can. J. Microbiol. **29:**1481–1486.
326. **Torrella, F., and R. Y. Morita.** 1979. Evidence by electron micrographs for a high incidence of bacterophage particles in the waters of Yaquina Bay, Oregon: ecological and taxonomical implications. Appl. Environ. Microbiol. **37:**774–778.
327. **Torsvik, T., and I. D. Dundas.** 1980. Persisting phage infection in *Halobacterium salinarium* str. 1. J. Gen. Virol. **47:**29–36.
328. **Tuomi, P., K. M. Fagerbakke, G. Bratbak, and M. Heldal.** 1995. Nutritional enrichment of a microbial community: the effects on activity, elemental composition, community structure and virus production. FEMS Microbiol. Ecol. **16:**123–134.
329. **Tuomi, P., T. Torsvik, M. Heldal, and G. Bratbak.** 1997. Bacterial population dynamics in a meromictic lake. Appl. Environ. Microbiol. **63:**2181–2188.
330. **Tuttle, J. H., R. B. Jonas, and T. C. Malone.** 1987. Origin, development and significance of Chesapeake Bay anoxia, p. 442–472. *In* S. K. Majumdar, L. W. Hall, Jr., and H. M. Austin (ed.), Contaminant problems and management of living Chesapeake Bay resources. Pennsylvania Academy of Sciences Press, Philadelphia.
331. **Valentine, A. F., and G. B. Chapman.** 1966. Fine structure and host-virus relationship of a marine bacterium and its bacteriophage. J. Bacteriol. **92:**1535–1554.
332. **Valentine, A. F., P. K. Chen, R. R. Colwell, and G. B. Chapman.** 1966. Structure of a marine bacteriophage as revealed by the negative-staining technique. J. Bacteriol. **91:**819–822.
333. **Van Etten, J. L., D. E. Burbank, A. M. Schuster, and R. H. Meints.** 1985. Lytic viruses infecting a *Chlorella*-like alga. Virology **140:**135–143.
334. **Van Etten, J. L., L. C. Lane, and R. H. Meints.** 1991. Viruses and viruslike particles of eukaryotic algae. Microbiol. Rev. **55:**586–620.
335. **Van Hannen, E. J., G. Zwart, M. P. Van Agterveld, H. J. Gons, J. Ebert, and H. J. Laanbroek.** 1999. Changes in bacterial and eukaryotic community structure after mass lysis of filamentous cyanobacteria associated with viruses. Appl. Environ. Microbiol. **65:**795–801.
336. **Vilagines, P., B. Sarrette, and R. Vilagines.** 1982. Preformed magnesium hydroxide precipitate for second-step concentration of enteroviruses from drinking and surface waters. Can. J. Microbiol. **28:**783–787.
337. **Waldor, M. K., and J. J. Mekalanos.** 1996. Lysogenic conversion by a filamentous phage encoding cholera toxin. Science **272:**1910–1914.
338. **Ward, B. B., and A. R. Cockcroft.** 1993. Immunofluorescence detection of the denitrifying strain *Pseudomonas stutzeri* (ATCC 14405) in seawater and intertidal sediment environments. Microb. Ecol. **25:**233–246.
339. **Ward, B. B., and A. F. Carlucci.** 1985. Marine ammonia- and nitrite-oxidizing bacteria: serological diversity determined by immunofluorescence in culture and in the environment. Appl. Environ. Microbiol. **50:**194–201.
340. **Ward, R. L., and C. S. Ashley.** 1976. Inactivation of poliovirus in digested sludge. Appl. Environ. Microbiol. **31:**921–930.
341. **Waterbury, J. B., and F. W. Valois.** 1993. Resistance to co-occurring phages enables marine *Synechococcus* communities to coexist with cyanophages abundant in seawater. Appl. Environ. Microbiol. **59:**3393–3399.
342. **Weinbauer, M. G., D. Fuks, and P. Peuzzi.** 1993. Distribution of viruses and dissolved DNA along a coastal trophic gradient in the northern Adriatic Sea. Appl. Environ. Microbiol. **59:**4074–4082.
343. **Weinbauer, M. G., D. Fuks, S. Puskaric, and P. Peduzzi.** 1995. Diel, seasonal, and depth-related variability of viruses and dissolved DNA in the Northern Adriatic sea. Microb. Ecol. **30:**25–41.
344. **Weinbauer, M. G., and M. G. Hofle.** 1998. Size-specific mortality of lake bacterioplankton by natural virus communities. Aquat. Microb. Ecol. **15:** 103–113.
345. **Weinbauer, M. G., and M. G. Höfle.** 1998. Significance of viral lysis and flagellate grazing as factors controlling bacterioplankton production in a eutrophic lake. Appl. Environ. Microbiol. **64:**431–438.
346. **Weinbauer, M. G., and P. Peduzzi.** 1995. Effect of virus-rich high molecular weight concentrates of seawater on the dynamics of dissolved amino acids and carbohydrates. Mar. Ecol. Prog. Ser. **127:**245–253.
347. **Weinbauer, M. G., and P. Peduzzi.** 1994. Frequency, size and distribution of bacteriophages in different marine bacterial morphotypes. Mar. Ecol. Prog. Ser. **108:**11–20.
348. **Weinbauer, M. G., and P. Peduzzi.** 1995. Significance of viruses versus heterotrophic nanoflagellates for controlling bacterial abundance in the northern Adriatic Sea. J. Plankton Res. **17:**1851–1856.
349. **Weinbauer, M. G., and C. A. Suttle.** 1997. Comparison of epifluorescence and transmission electron microscopy for counting viruses in natural marine waters. Aquat. Microb. Ecol. **13:**225–232.
350. **Weinbauer, M. G., and C. A. Suttle.** 1996. Potential significance of lysogeny to bacteriophage production and bacterial mortality in coastal waters of the Gulf of Mexico. Appl. Environ. Microbiol. **62:**4374–4380.
351. **Weinbauer, M. G., S. W. Wilhelms, C. A. Suttle, and D. R. Garza.** 1997. Photoreactivation compensates for UV damage and restores infectivity to natural marine virus communities. Appl. Environ. Microbiol. **63:**2200–2205.
352. **Wells, M. K., and E. D. Goldberg.** 1991. Occurrence of small colloids in sea water. Nature (London) **353:**342–344.
353. **Wichels, A., S. S. Biel, H. R. Gelderblom, T. Brinkhoff, G. Muyzer, and C. Schutt.** 1998. Bacteriophage diversity in the North Sea. Appl. Environ. Microbiol. **64:**4128–4133.
354. **Wiebe, W. J., and J. Liston.** 1968. Isolation and characterization of a marine bacteriophage. Mar. Biol. **1:**244–249.
355. **Wiggins, B. A., and M. Alexander.** 1985. Minimum bacterial density for bacteriophage replication: implications for significance of bacteriophages in natural ecosystems. Appl. Environ. Microbiol. **49:**19–23.
356. **Wilcox, R. M., and J. A. Fuhrman.** 1994. Bacterial viruses in coastal seawater: lytic rather than lysogenic production. Mar. Ecol. Prog. Ser. **114:**35–45.
357. **Wilhelm, S. W., M. G. Weinbauer, C. A. Suttle, and W. H. Jeffrey.** 1998. The role of sunlight in the removal and repair of viruses in the sea. Limnol. Oceanogr. **43:**586.
358. **Wilhelm, S. W., M. G. Weinbauer, C. A. Suttle, R. J. Pledger, and D. L. Mitchell.** 1998. Measurements of DNA damage and photoreactivation im-

ply that most viruses in marine surface waters are infective. Aquat. Microb. Ecol. **14:**215–222.
359. **Wilhelm, S. W., and C. A. Suttle.** 1999. Viruses and nutrient cycles in the sea. Bioscience **49:**781–788.
360. **Wilkner, J., J. J. Vallino, G. F. Steward, D. C. Smith, and F. Azam.** 1993. Nucleic acids from the host bacterium as a major source of nucleotides for three marine bacteriophages. FEMS Microbiol. Ecol. **12:**237–248.
361. **Williams, N.** 1996. Phage transfer: a new player turns up in cholera infection. Science **272:**1869–1870.
362. **Wilson, W. H., N. G. Carr, and N. H. Mann.** 1996. The effect of phosphate status on the kinetics of cyanophage infection in the oceanic cyanobacterium *Synechococcus* sp. WH7803. J. Phycol. **32:**506–516.
363. **Wilson, W. H., I. R. Joint, N. G. Carr, and N. H. Mann.** 1993. Isolation and molecular characterization of five marine cyanophages propagated on *Synechococcus* sp. strain WH7803. Appl. Environ. Microbiol. **59:**3736–3743.
364. **Wilson, W. H., and N. H. Mann.** 1997. Lysogenic and lytic viral production in marine microbial communities. Aquat. Microb. Ecol. **13:**95–100.
365. **Wilson, W. H., S. Turner, and N. H. Mann.** 1998. Population dynamics of phytoplankton and viruses in a phosphate-limited mesocosm and their effect on DMSP and DMS production. Estuarine Coastal Shelf Sci. **46:**49.
366. **Reference deleted.**
367. **Wommack, K. E., R. T. Hill, and R. R. Colwell.** 1995. A simple method for the concentration of viruses from natural water samples. J. Microbiol. Methods **22:**57–67.
368. **Wommack, K. E., R. T. Hill, M. Kessel, E. Russek-Cohen, and R. R. Colwell.** 1992. Distribution of viruses in the Chesapeake Bay. Appl. Environ. Microbiol. **58:**2965–2970.
369. **Wommack, K. E., R. T. Hill, T. A. Muller, and R. R. Colwell.** 1996. Effects of sunlight on bacteriophage viability and structure. Appl. Environ. Microbiol. **62:**1336–1341.
370. **Reference deleted.**
371. **Wommack, K. E., J. Ravel, R. T. Hill, and R. R. Colwell.** 1999. Hybridization analysis of Chesapeake Bay virioplankton. Appl. Environ. Microbiol. **65:**241–250.
372. **Wommack, K. E., J. Ravel, R. T. Hill, and R. R. Colwell.** 1999. Population dynamics of Chesapeake Bay virioplankton: total community analysis using pulsed field gel electrophoresis. Appl. Environ. Microbiol. **65:**231–240.
373. **Reference deleted.**
374. **Wong, F. H., and L. E. Bryan.** 1978. Characteristics of PR5, a lipid-containing plasmid-dependent phage. Can. J. Microbiol. **24:**875–882.
375. **Woods, D. R.** 1976. Bacteriophage growth on stationary phase *Achromobacter* cells. J. Gen. Virol. **32:**45–50.
376. **Xenopoulos, M. A., and D. F. Bird.** 1997. Virus a la sauce Yo-Pro: microwave-enhanced staining for counting viruses by epifluorescence microscopy. Limnol. Oceanogr. **42:**1648–1650.
377. **Yates, M. V., C. P. Gerba, and L. M. Kelley.** 1985. Virus persistence in groundwater. Appl. Environ. Microbiol. **49:**778–781.
378. **Zachary, A.** 1976. Physiology and ecology of bacteriophages of the marine bacterium *Beneckea natriegens*: salinity. Appl. Environ. Microbiol. **31:**415–422.
379. **Zhang, Y. P., D. E. Burbank, and J. L. Van Etten.** 1988. Chlorella viruses isolated in China. Appl. Environ. Microbiol. **54:**2170–2173.
380. **Zillig, W., D. Prangishvilli, C. Schleper, M. Elferink, I. Holz, S. Albers, D. Janekovic, and D. Gotz.** 1996. Viruses, plasmids and other genetic elements of thermophilic and hyperthermophilic *Archaea*. FEMS Microbiol. Rev. **18:**225–236.
381. **Zimmerman, R., and L. A. Meyer-Reil.** 1974. A new method for fluorescence staining of bacteria populations on membrane filters. Kiel. Meeresforsch. **30:**24–27.
382. **Zingone, A.** 1995. The role of viruses in the dynamics of phytoplankton blooms. G. Bot. Ital. **129:**415–423.
383. **Zobell, C. E.** 1946. Marine microbiology. Chronica Botanica Press, Waltham, Mass.