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Seasonal Variations in Virus-Host Populations in Norwegian Coastal Waters: Focusing on the Cyanophage Community Infecting Marine *Synechococcus* spp.

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Viruses are ubiquitous components of the marine ecosystem. In the current study we investigated seasonal variations in the viral community in Norwegian coastal waters by pulsed-field gel electrophoresis (PFGE). The results demonstrated that the viral community was diverse, displaying dynamic seasonal variation, and that viral populations of 29 different sizes in the range from 26 to 500 kb were present. Virus populations from 260 to 500 kb and dominating autotrophic pico- and nanoeukaryotes showed similar dynamic variations. Using flow cytometry and real-time PCR, we focused in particular on one host-virus system: *Synechococcus* spp. and cyanophages. The two groups covaried throughout the year and were found in the highest amounts in fall with concentrations of 7.3×10^4 *Synechococcus* cells ml^{-1} and 7.2×10^3 cyanophage ml^{-1}. By using primers targeting the g20 gene in PCRs on DNA extracted from PFGE bands, we demonstrated that cyanophages were found in a genomic size range of 26 to 380 kb. The genetic richness of the cyanophage community, determined by denaturing gradient gel electrophoresis (DGGE) of PCR-amplified g20 gene fragments, revealed seasonal shifts in the populations, with one community dominating in spring and summer and a different one dominating in fall. Phylogenetic analysis of the sequences originating from PFGE and DGGE bands grouped the sequences into three groups, all with homology to cyanomyoviruses present in cultures. Our results show that the cyanophage community in Norwegian coastal waters is dynamic and genetically diverse and has a surprisingly wide genomic size range.

Viruses are the most numerous biological components of the marine environment, with concentrations typically around 10^7 particles ml^{-1} (4). They are normally 1 to 2 orders of magnitude more abundant than prokaryotes, and metagenome studies have shown that they are extremely diverse (5, 6, 7). As mortality agents of heterotrophic bacteria, cyanobacteria and phytoplankton viruses affect the abundance and diversity of host cell communities and play important roles in cycling carbon and nutrients in the sea (e.g., references 8, 43, 44, 49, and 50). In spite of their importance, knowledge is sparse regarding both the biodiversity and the functions of marine viruses. The fact that most hosts have not been cultured has severely limited studies of viral diversity. The fact that no single genetic element is shared by all viruses represents one further obstacle (37). However, whole-genome comparisons have shown that conserved genes, which are shared among all members within certain viral taxonomic groups, exist and can be used for studies of viral diversity.

One characteristic of viruses, which varies over a wide range and is readily determined, is the genome size. Reported viral genomes range from a few to several hundred thousand kilobases (9). Pulsed-field gel electrophoresis (PFGE) is a method that provides a separation over the full range of intact viral genome sizes. The method was first used for this purpose to analyze the bacteriophage community in sheep rumen, and it was then demonstrated that one PFGE band consisted of DNA from one single phage genotype (21, 47). Lately, this approach has been used in several studies to explore the dynamics in the communities of double-stranded DNA (dsDNA) viruses in the marine environment (10, 19, 22, 23, 33, 36, 41, 57). These studies have shown that the viral assemblage in the marine environment is distributed in a genome size range from approximately 20 to 560 kb. The most dominant populations have genome sizes between 20 and 100 kb (23, 33, 41, 57), which is also the size range of most cultured marine bacteriophages with dsDNA genomes (1, 19).

Marine unicellular cyanobacteria within the genus *Synechococcus* are abundant in the photic zone of the world's oceans and may be responsible for up to 25% of the total oceanic primary productivity, thus contributing significantly to global carbon cycling (34, 39, 54). Cyanophages are viruses that infect cyanobacteria and are, like their hosts, ubiquitous in marine environments. They are found in concentrations up to 10^6 ml^{-1} in coastal water during the summer and are considered a significant factor in determining the dynamics of *Synechococcus* sp. populations (45, 46, 55). All cyanophages described to date are tailed phages with dsDNA genomes belonging to the families *Myoviridae*, *Podoviridae*, and *Siphoviridae* (24), and the majority of isolated marine cyanophages are myoviruses with genomes ranging from 100 to 200 kb (24).

Primers specifically targeting the g20 gene, encoding the viral capsid structure for cyanomyoviruses, have been developed (18) and have been used in a variety of studies of cyanophage diversity in aquatic environments (14, 16, 27, 30, 40, 53, 56, 59). Application of these primers, either in combination with denaturing gradient gel electrophoresis (DGGE) or by cloning combined with sequencing, has revealed both temporal and spatial cyanophage community variations in a variety of

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aquatic environments (14, 27, 30, 40, 56, 59). However, as most *Synechococcus*-cyanophage research has been carried out in low-latitude temperate and tropical waters, little is yet known about the diversity of cyanophages and interactions between hosts and cyanophages in waters of latitudes above 60°.

The main objective of the current study was therefore to focus in detail on cyanophage dynamics and diversity as we also investigated seasonal changes in viral populations of Norwegian coastal waters in a broader sense. The temporal variation in the viral community in general, and cyanophages in particular, was linked to potential phytoplankton host organisms. We employed flow cytometry (FCM) to count *Synechococcus* bacteria, together with autotrophic nano- and picoeukaryotes, heterotrophic bacteria, and viruses. Viral community composition was investigated by PFGE, while cyanophage diversity and abundance were investigated by DGGE and real-time PCR, respectively, using primers targeting the g20 gene. The phylogenetic affiliations of the individual cyanophage populations were investigated by sequencing PCR products amplified from DNA extracted from PFGE and DGGE bands.

MATERIALS AND METHODS

Sample collection. Coastal water samples were collected at a station in Raunefjorden (60°16.2′N, 5°12.5′E), south of Bergen, Norway, from 3 March to 2 November 2004. A total volume of 30 liters was collected from a depth of 2 m nearly every second week from 3 June to 29 June; then on 10, 12, and 19 August; and thereafter every week from 21 September until 12 October. The final sample was obtained on 2 November. The samples were collected using a hand pump connected to a flask. Temperature was measured using an STD SAIV a/s SD 204 with a Sea Point fluorometer (SAIV A/S; Environmental Sensors and Systems, Bergen, Norway).

FCM. The abundances of autotrophic pico- and nanoeukaryotes, *Synechococcus* bacteria, heterotrophic bacteria, and viruses were determined by FCM. The autotrophs were analyzed on a FACSCalibur or a FACSAria flow cytometer (Becton Dickinson) equipped with lasers supplying 15 mW at 488 nm and 13 mW at 488 nm, respectively, with standard filter setup. Viruses and bacteria were counted in samples fixed with glutaraldehyde for 60 s at a viral event rate of between 100 and 1,000 s^{-1} using the FACSCalibur. Each sample was diluted at a minimum of two different dilutions from 50- to 200-fold before it was stained with SYBR green I. The flow cytometer instrumentation and the remaining methodology followed the recommendations of Marie et al. (26) and are described in more detail, for a similar study, by Larsen et al. (22).

Concentration of virus communities. Natural viral communities were concentrated from 25 liters of seawater by ultrafiltration. The samples were first filtered through a 0.45-μm low-protein-binding Durapore membrane filter of 142 mm in diameter (Millipore) to remove zooplankton, phytoplankton, and some of the bacteria. The filtered samples were then concentrated to a final volume of 150 to 250 ml, using a 30,000-molecular-weight-cutoff spiral-wound Millipore ultrafiltration cartridge (regenerated cellulose, PLTK Prep/scale TFF, 1 ft^2; Millipore Corporation, Bedford, MA). The concentrate was stored in the dark at 4°C until further processing.

One hundred forty-two milliliters of viral concentrate was concentrated further by ultracentrifugation (Beckman L8-M with SW-28 rotor; Beckman GmbH, Germany) for 2 h at 25,000 rpm at 10°C. The viral pellet was resuspended in 400 μl of SM buffer (0.1 M NaCl, 8 mM $MgSO_4 \cdot 7H_2O$, 50 mM Tris-HCl, 0.005% [wt/vol] glycerol). Two hundred microliters was stored at −20°C for DGGE and quantitative real-time PCR analysis, while 200 μl was used for PFGE analysis.

PFGE. Four virioplankton agarose plugs were made from the 200-μl concentrate, representing 7 liters of water sample (each plug representing 1.75 liters). The samples were run on a 1% (wt/vol) SeaKem GTG agarose (FMC, Rockland, ME) gel in 1× Tris-buffered EDTA (TBE) gel buffer using a Bio-Rad DR-II contour-clamped homogeneous electric field cell (Bio-Rad, Richmond, CA) electrophoresis unit (57). From each sample we used three of the plugs and ran them at three different pulse ramp conditions in order to separate a wide range of viral genome sizes: (i): 1- to 5-s switch time with 20-h run time for separation of small genome sizes (0 to 130 kb); (ii) 8- to 30-s switch time with 20-h run time for separation of medium genome sizes (130 to 300 kb); and (iii) 20- to 40-s switch time with 22-h run time for separation of large genome sizes (300 to 600 kb). A molecular size standard (lambda ladder and 5-kb ladder [Bio-Rad, Richmond, CA]) was employed on each side of the gel. Further details of the procedure may be found in the work of Larsen et al. (22). The gels were visualized and saved as computer files using the Fujifilm imaging system LAS-3000. The banding patterns were analyzed using a computer program, GEL2K (Svein Norland, Department of Microbiology, University of Bergen, Norway), which calculates the molecular weight of the different DNA fragments and the intensity of each of the DNA fragments and determines the presence or absence of bands. The intensity of a peak/band is calculated as the integrated pixel value (in the peak) over its background value. Clustering of the viral profiles was based on the simple matching algorithm, while the dendrogram was drawn using the complete link method.

DGGE. PCR-DGGE analysis was performed using the primers CPS1 and CPS8 to amplify a 592-bp product of the g20 gene (59) with a 40-mer GC clamp in the 5′ end of the CPS8 primer. The 50-μl reaction mixture contained the following: sterile distilled water, PCR buffer (10× PCR buffer B; Promega, Madison, WI), deoxynucleoside triphosphates (200 nM each), primers (0.5 μM each), 1.5 mM MgCl, 2.5 U *Taq* DNA polymerase (Promega), and template amplicon (1 to 2 ng). We performed PCR amplification according to the protocol of Zhong et al. (59) and checked the 592-bp amplicon for length and purity on a 1.5% agarose gel. The DGGE was performed with a Dcode 16/16-cm gel system (Bio-Rad, Hertfordshire, United Kingdom). We used a gradient of DNA denaturant ranging from 20 to 45% and loaded the gel with 20 μl of PCR product together with 15 μl of a standard on each side. The standard consisted of a 1:1:1 mixture of PCR products using the phages S-PM2, S-BnM1, and S-WHM1 as template. The gels were run at 60°C at 60 V for 20 h in 1× TAE buffer (40 mM Tris [pH 7.4], 20 mM sodium acetate, 1 mM EDTA) and stained with SYBR green II (Molecular Probes, OR) before they were visualized and saved as computer files using the Fujifilm imaging system LAS-3000. The presence and absence of the different viral populations were recorded using the GEL2K software program (Svein Norland, Department of Microbiology, University of Bergen). Clustering of the DGGE profiles was based on the simple matching algorithm, while the dendrogram was drawn by applying the complete link method.

Sequencing DGGE and PFGE bands. DGGE bands to be sequenced were excised from the gel and eluted in 20 μl sterile distilled water overnight at 4°C. Two microliters of eluted DNA served as template in a PCR with the same primers (CPS1 and CPS8) and conditions as described above. PCR products were purified using the Microcon-PCR spin column (Millipore). PFGE bands to be sequenced were excised from the gel and frozen at −20°C before the DNA was extracted from the gel using the GeneClean Turbo kit (Bio 101) for extractions of large DNA fragments from the agarose gel, following the manufacturer's instructions, yielding approximately 10 ng/μl of DNA. The genomic DNA required for sequencing and PCR was produced by the GenomiPhi DNA amplification kit (Amersham Biosciences, Buckinghamshire, United Kingdom) according to the manufacturer's instructions, yielding between 4 and 17 μg of DNA. This DNA was used for PCR with the CPS1/CPS2 primers (18).

The different PCR products were sequenced by cycle sequencing according to the protocol from Perkin-Elmer (Foster City, CA), using the forward primers as a sequencing primer. Sequences were obtained on an ABI PRISM 3700 sequence analyzer (Perkin-Elmer). Analysis of DNA sequences was carried out by alignment to the closest relative in the GenBank database using TBLAST (2). Alignments were performed using CLUSTALX (51). Maximum parsimony and neighbor-joining (NJ) analysis were conducted on a nucleotide data set by using the PAUP* 4.0 b10 program (48). Supports for clades were estimated by means of bootstrap analysis, as implemented in PAUP* using 1,000 replicates. The trees were viewed using the TreeView program and rooted with the gp20 gene of *Escherichia coli* phage T4 as an outgroup.

Estimation of abundance of cyanophages. The abundance of cyanophages was estimated using real-time PCR with the primers CPS1 and CPS2 amplifying a 165-bp product of the g20 gene. The assay was run directly on the viral concentrates without any DNA extraction step (18, 56). For the detection of the CPS1/CPS2 product, a SYBR green I real-time PCR assay was used (Molecular Probes, Eugene, OR). The 20-μl master mix contained the following: Mastermix solution (2× Mastermix, DyNAmoHS; Finnzymes, Espoo, Finland), primers (0.5 μM each), sterile distilled water, and template amplicon (1-μl viral sample). The PCR was optimized using the cyanophage S-BnM1 as template. Sterile water was used as a negative control. The optimized cycling protocol was as follows. After an initial denaturation step at 95°C for 15 min, the reaction mixture was run for 35 cycles at 95°C for 10 s, 53.5°C for 30 s, and 72°C for 30 s, followed by 72°C for 10 min. Thereafter, a melting curve from 72 to 95°C with an increase of 0.2°C per s was run in order to check the specificity of the PCR. The assays were run on an MJ Research Opticon real-time PCR machine (Bio-Rad). Each sample was

assayed at three different concentrations and repeated three times. Concentration values were obtained using serial dilutions from 1 to 10^5 phages μl^{-1} of S-BnM1 containing one copy of the g20 gene. The serial dilutions were made fresh every time, and the number of phages in the lysate was determined by epifluorescence microscopy at a 1,000× magnification, after staining with a 400-fold dilution of SYBR green I (Molecular Probes) as described by Noble and Fuhrman (31).

Nucleotide sequence accession numbers. The nucleotide sequences reported in this paper have been submitted to GenBank (DQ354990 to DQ355010).

RESULTS

Succession of viruses, bacteria, and algae. In general, the abundance of *Synechococcus*, cyanophages, total bacteria, and viruses increased throughout the sampling period (Fig. 1A and B). Total viral numbers rose from 6.0×10^6 ml^{-1} in the spring to 2.0×10^7 ml^{-1} in the early fall, before declining to 1.4×10^7 ml^{-1} in the late fall (Fig. 1). The total bacterial number, which was 5.6×10^5 ml^{-1} on the first sampling day, peaked three times during the sampling period (Fig. 1A): in April (9×10^5 ml^{-1}), July (1.6×10^6 ml^{-1}), and October (1.8×10^6 ml^{-1}). Cyanophage abundance ranged from $<5.0 \times 10$ ml^{-1} in early spring to 7.2×10^3 ml^{-1} in late fall (Fig. 1B) and revealed two distinct peaks: a minor one on 25 May and a major one on 12 October. *Synechococcus* also peaked twice, with a minor peak on 15 June (3.5×10^4 cells ml^{-1}) and a major one on 21 September (7.3×10^4 cells ml^{-1}) (Fig. 1B).

Autotrophic pico- and nanoeukaryotes varied substantially throughout the sampling period. The autotrophic picoeukaryotes reached highest concentrations during spring and summer (13 April, 2.1×10^4 cells ml^{-1}, and 15 June, 2.0×10^4 cells ml^{-1}) (Fig. 1C) but peaked three times in the course of the fall as well, with the highest cell number on 6 October (1.4×10^4 cells ml^{-1}). The autotrophic nanoeukaryotes peaked three times during the sampling period, twice in spring (13 April, 9.6×10^3 cells ml^{-1}, and 11 May, 1.4×10^4 cells ml^{-1}) and once in summer (19 August; 8.2×10^3 cells ml^{-1}) (Fig. 1C). The abundance subsequently decreased and fluctuated around 1.6×10^3 cells ml^{-1} for the rest of the period. The water temperature rose from 5.2 to 15.4°C from March to early September before falling again to 9.8°C (Fig. 1C).

Seasonal changes within the virioplankton community. The virioplankton PFGE banding pattern revealed 29 viral populations of different genome sizes ranging from 26 to 500 kb (Fig. 2A). The most abundant populations were those of 31, 38, 44, 46, 67, 75, 95, 140, 200, and 240 kb. All samples comprised viral populations in the size range from 26 to 240 kb. One population with a size of 95 kb was present in all samples. From 13 April to 21 September we also detected viral populations with sizes from 260 to 500 kb. On average 13 PFGE bands were detected in each sample, with the lowest number of bands (five) in the sample collected on 12 August and the highest (18) on 29 June.

The cluster analysis resulted in a dendrogram consisting of two main groups (I and II). Samples from March to 21 September clustered in one group (II), whereas samples from late September to November clustered together in the other (I). Group II consisted of three subgroups: (i) samples from April and May; (ii) samples from June and August; and (iii) samples from March, 12 August, and 21 September (Fig. 2B).

Cyanophage diversity. Fifteen out of 25 PFGE bands gave a positive signal with the CPS1 and CPS2 primers when DNA extracted from the bands was used in a PCR on products amplified with the GenomiPhi DNA amplification kit (Table 1). These PFGE bands ranged from 26 to 380 kb. Between 2 and 13 bands were detected when PCR products amplified with the CPS1 and CPS8 primers were run on a DGGE gel (data not shown). A dendrogram based on the fingerprints grouped the samples into two main groups (I and II) (Fig. 3). Group II comprises samples from 2 March to 15 June, while group I consists of samples from 12 August until the end of the sampling period (Fig. 3).

Phylogenetic analysis of the g20 gene was carried out in PAUP* using both maximum parsimony and an NJ analysis. The two methods gave similar results, and we present those emerging from the NJ analysis (Fig. 4). All g20 sequences amplified from the DGGE/PFGE bands cluster within the cultured *Synechococcus* phage group (40, 59) (Fig. 4). One group displays highest similarity with the cyanophage S-BnM1 and consists of sequences from four DGGE bands (D-7a, D-9b, D-10b, and D-11b). Another group comprises sequences from three DGGE bands (D-9a, D-11a, and D-5b) and clusters within clade III of the cultured phage of *Synechococcus* (CPS) group. These two groups are composed of DNA sequences originating from DGGE bands which dominated the samples throughout the entire experimental period, with the exception of band D-5b, which originated from 25 May. All sequences from the PFGE bands, which were in the size range from 26 to 380 kb (P-S6, P-S7, P-5b, P-3b, P-6b, P-7b, P-6c2, P-4c2, P-5c2, P-S8, P-S2, and P-S5) fell within CPS group II, showing closest homology to the cyanophages S-RIM20 and S-RIM17. Sequences from three DGGE bands (D-6b, D-7b, and D-8b) clustered within clade I, with highest homology to S-PM2. These sequences originated from DGGE bands that appeared only in samples collected in the fall.

DISCUSSION

Viruses are widely distributed, and they can have an enormous impact on ecosystem dynamics (3, 8, 17, 35, 44, 49, 58). It has been hypothesized that viruses have a large influence on shaping the host community and thus on species diversity. Most studies so far have focused on changes in the virioplankton community, without linking these alterations to changes in the host community structure (19, 41, 57). One objective of this study was to improve our understanding of the seasonal dynamics of viral populations in Norwegian coastal waters and to link shifts in the viral populations to changes in host community abundance.

The study demonstrates a pronounced seasonal dynamic in viral populations and shows how this dynamic correlates with changes in the abundance of possible hosts. Viral richness was low in late winter and late summer (12 August) as were the numbers of possible hosts (bacteria and autotrophic pico- and nanoeukaryotes). From mid-April, an increase in the number of viral populations in the size range of 26 to 102 kb and 260 to 500 kb accompanied the increase of abundance of bacteria and autotrophic pico- and nanoeukaryotes. In late September, several viral populations in the size range of 240 to 500 kb disappeared concurrently with a fall in autotrophic nanoeukaryote

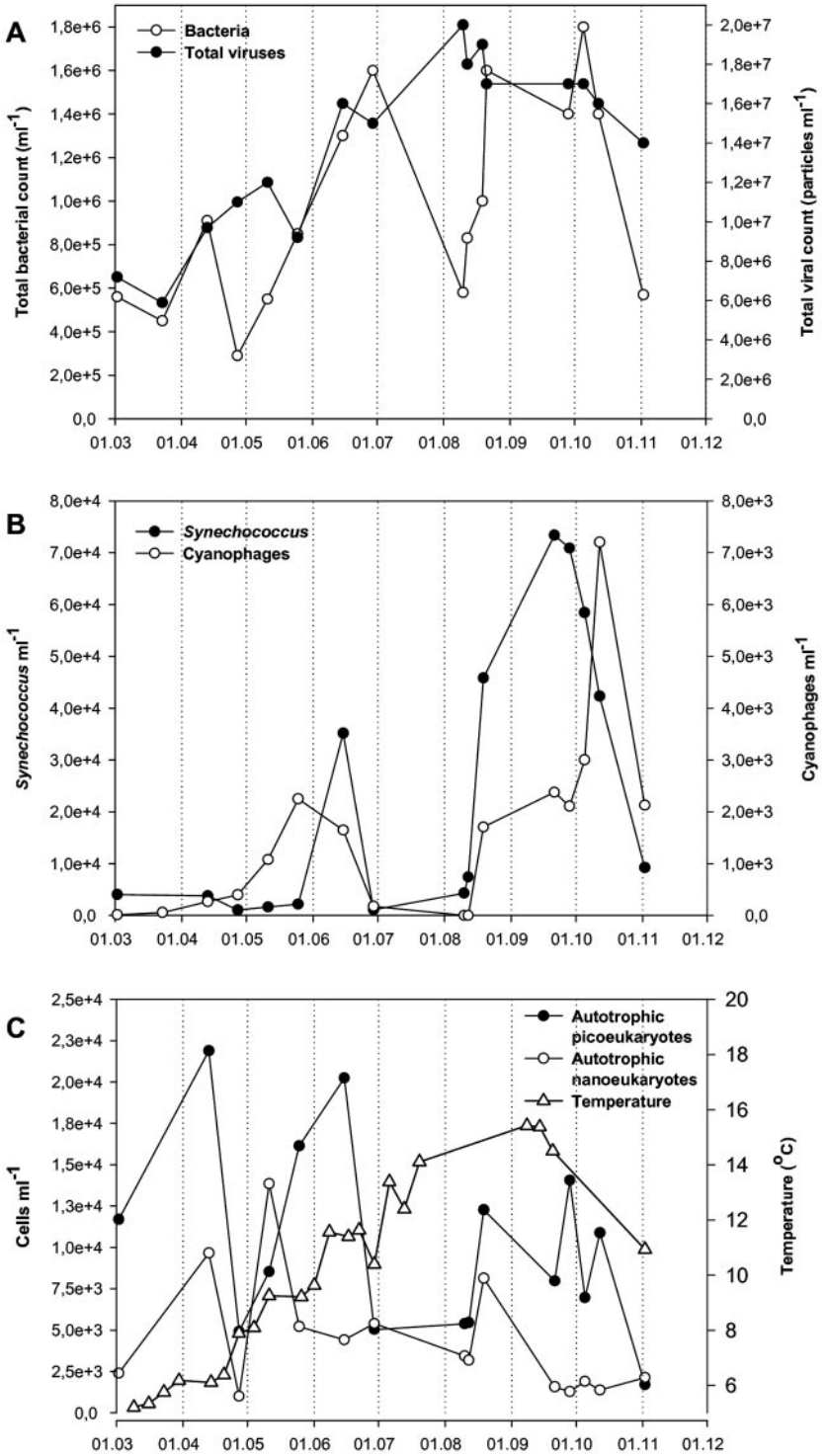

FIG. 1. Development of bacteria and viruses (A) and algae belonging to the nano- and picoeukaryote groups (B) as measured by FCM. (C) *Synechococcus* (measured by FCM) and cyanophages (measured in real time using primers against the g20 gene). Panel C also shows water temperature measured at the sampling site in Raunefjorden, Norway, from March to November 2004.

abundance. Algal viruses often have genome sizes between 200 and 600 kb (11, 38, 52), and the occurrence of additional bands in the size range between 260 and 500 kb might thus be linked to the increase of these nanoeukaryotes. Most viruses in the sea are assumed to be phages (7), and the dominant viral populations found by investigations of the viral assemblage by PFGE have sizes between 20 and 100 kb (23, 33, 41, 57). The increase in the number of bands below 102 kb, from mid-April,

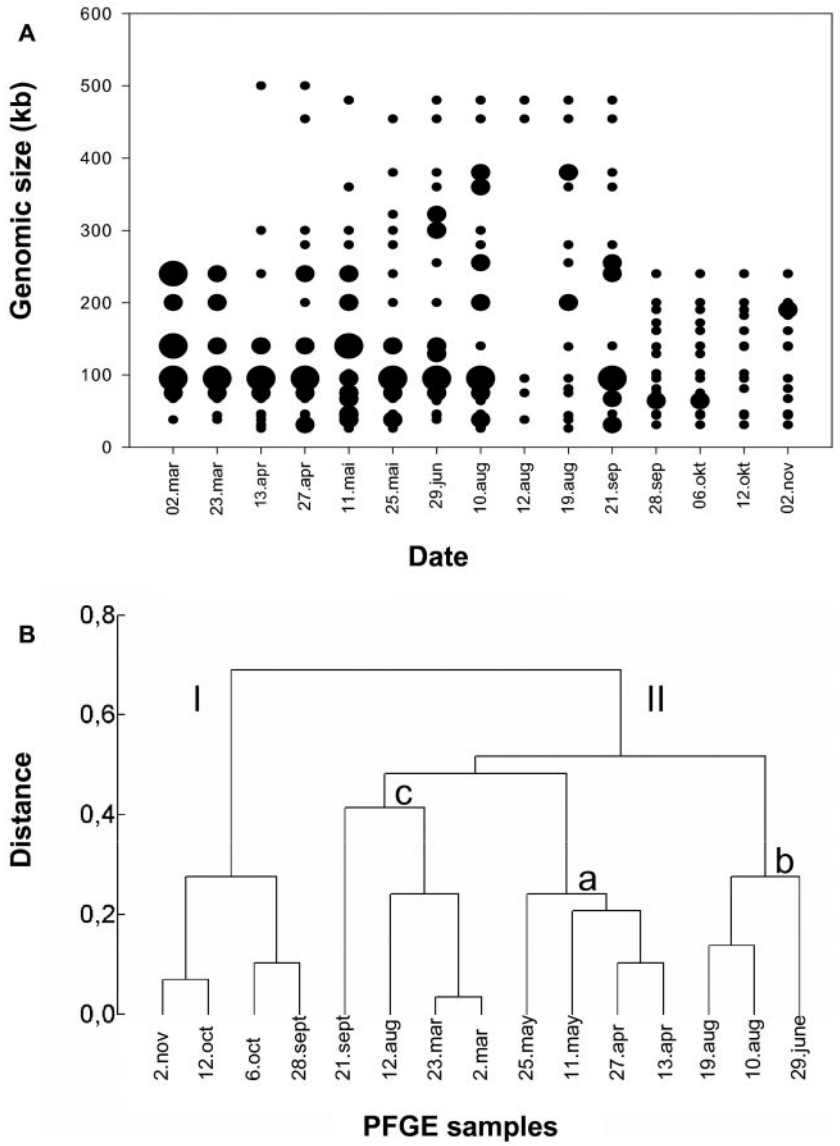

FIG. 2. (A) Schematic outline of the relative abundance (indicated by the size of the dots) of viral populations determined by PFGE. Viral populations are defined by genome size, and the outline is based on three different electrophoresis runs for each viral concentrate. (B) Cluster analysis of the total viral populations from individual samples. The dendrogram is constructed from a binary matrix of similarity values, using a distance calculation algorithm based on the absence or presence of bands. Clustering is based on the simple matching algorithm, while the dendrogram was drawn using the complete link method.

might therefore be linked to the increase in the abundance of bacteria. During the fall, several viral populations in the size range of 161 to 190 kb and at 139 kb appeared in our samples. The occurrence of these viral populations correlates with a decline in *Synechococcus* and an increase in the abundance of cyanophages. As the majority of *Synechococcus* phages in culture have genome sizes from 100 to 200 kb (24), it is tempting to suggest that some of these bands are of cyanophage origin. It should be noted, however, that both bacteria and picoeukaryotes were found in relatively high numbers in the same period.

Temporal studies of the viral community in natural marine waters have mostly been performed over limited periods of time, e.g., during spring blooms (23, 36). These studies report a surprisingly high stability, as opposed to our study, which shows that the viral community changes according to season. However, one of the groups in cluster II, c, consisted of samples from different periods. These samples might represent transient situations that arise between changes in the host community, e.g., before/after a major phytoplankton bloom. The viral community in such periods seems to be dominated by a few core viral populations, as a viral population of 95 kb was present in all the samples and another band of 38 kb was present in 90% of the samples in cluster II. The viral population of 95 kb is probably a cyanophage, as it gave a positive product when amplified both with primers against the g20 gene and with primers against the photosynthetic genes *psbA* and *psbD* (data not shown) that lately have been detected in cyano-

TABLE 1. Size range and sampling dates of viral populations (PFGE bands) investigated for the presence of the g20 gene

Viral band	Date of sampling[a] (2004)	Genome size (kb)	Presence of g20 gene[b]
1-b	29 June	322	−
2-b	29 June	300	+
3-b	10 Aug.	255	+
4-b	10 Aug.	200	+
5-b	19 Aug.	380	+
6-b	9 June	140	+
7-b	11 May	140	+
8-b	11 May	200	−
1C2	13 Apr.	75	+
2C2	13 Apr.	67	−
3C2	27 Apr.	44	−
4C2	27 Apr.	38	+
5C2	11 May	31	+
6C2	13 Apr.	26	+
S1	2 Nov.	240	−
S2	2 Nov.	200	+
S5	2 Nov.	110	+
S6	2 Nov.	140	+
S7	2 Nov.	139	+
S8	2 Nov.	95	+
S9	2 Nov.	67	−
S10	6 Oct.	64	−
S11	6 Oct.	46	−
S12	12 Oct.	44	−
S13	28 Sept.	31	−

[a] Abbreviations: Aug., August; Apr., April; Nov., November; Oct., October; Sept., September.
[b] +, present; −, absent.

phages (29). The other viral population (38 kb) could well be a T7-like phage, as it is in the same size range as all known T7-like podophages (6). This bacteriophage group is widely distributed in the environment, occurring in numbers up to 700 per 10^6 phage particles in marine systems (6). In the current study, the viral community structure during and after the bloom of *Synechococcus* (cluster I) showed 70% differences from the other samples. This probably reflects the major changes in the host community structure taking place when *Synechococcus* "enters the scene" before, between, and after blooms of larger phytoplankton species (23).

Another objective was to gain information about the seasonal variation and genetic diversity of specific viruses and their hosts, and here we report the first data on seasonal variation in *Synechococus*-cyanophage abundance from latitudes above 60°. The *Synechococcus* cyanophage dynamics observed were somewhat different from those in other coastal water studies, where the maximum abundance occurs earlier in summer (27, 53). The water temperature being highest in late August and September is a probable explanation for the late bloom of *Synechococcus* and its phage in Norwegian coastal waters, as correlations between high water temperature and high abundances of *Synechococcus* and cyanophages have previously been reported (54). Plaque assays on solid media or the most probable number method in liquid culture has previously yielded cyanophage numbers that ranged from undetectable to 10^6 cyanophages per ml^{-1} seawater (27, 45, 53, 55). By using real-time PCR we obtained cyanophage numbers in the same range as these numbers. This is somewhat surprising, as the primers targeting the g20 gene were originally designed to amplify cyanomyoviruses (18) and recent studies have shown that cyanophages belonging to other taxonomic groups such as *Siphoviridae* and *Podoviridae* are important in coastal seawater as well (27). Our numbers may therefore underestimate cyanophage abundance. On the other hand, the specificity of the g20 primers has been questioned (40), and there is thus a chance that the amplified product may represent bacteriophages other than cyanophages and that our cyanophage numbers may be overestimates. However, as the number of *Synechococcus* bacteria detected in this study was 1 order of magnitude higher than the cyanophage number, it is more likely that we underestimated the true abundance of cyanophages in the samples.

The *Synechococcus* bloom in the fall preceded a peak in the cyanophage numbers, supporting the idea of a link between these two groups. The classical explanation for seasonal succession of marine phytoplankton is that the different species vary in their response to environmental conditions, such as variation in nutrient supply, light, temperature, and grazing. Our data, which show increased cyanophage abundance and diversity after the *Synechococcus* bloom, suggest that cyanophages are also important for the seasonal succession of *Synechococcus* populations. Data on the genetic diversity on the *Synechochoccus* populations could shed future light on this matter, but the impact of viruses on host communities can to a certain extent be inferred from viral community diversity, as viral taxonomy can provide insight into the hosts that viruses infect. Seasonal changes in the cyanophage community have also been demonstrated by other authors (30, 53), and the diversity of the cyanophage in these studies was highest during the annual maximum of *Synechococcus* abundance.

The elevated concentrations of *Synechococcus* and cyanophages in early summer did not, however, follow a typical predator-prey relationship such as the one observed in fall. This could be due to the previously mentioned nonspecificity of the g20 primers, which might result in amplified products representing bacteriophages other than cyanophages (40). Alternatively, nanoflagellates, known to graze on *Synechococcus*

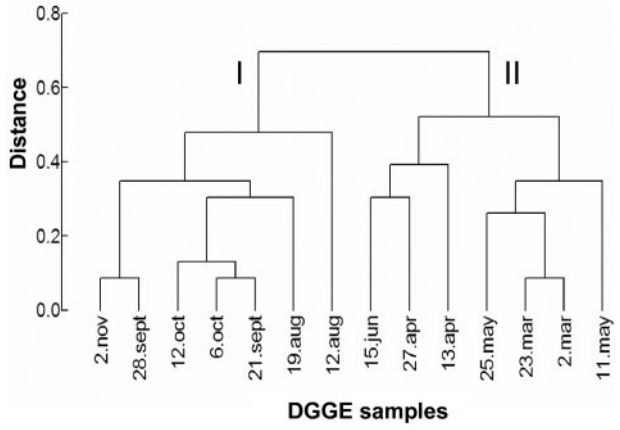

FIG. 3. Cluster analysis of the DGGE profiles of amplified g20 gene fragments from individual samples. The dendrogram is constructed from a binary matrix of similarity values, using a distance calculation algorithm based on the absence or presence of bands. Clustering is based on the simple matching algorithm, while the dendrogram was drawn using the complete link method.

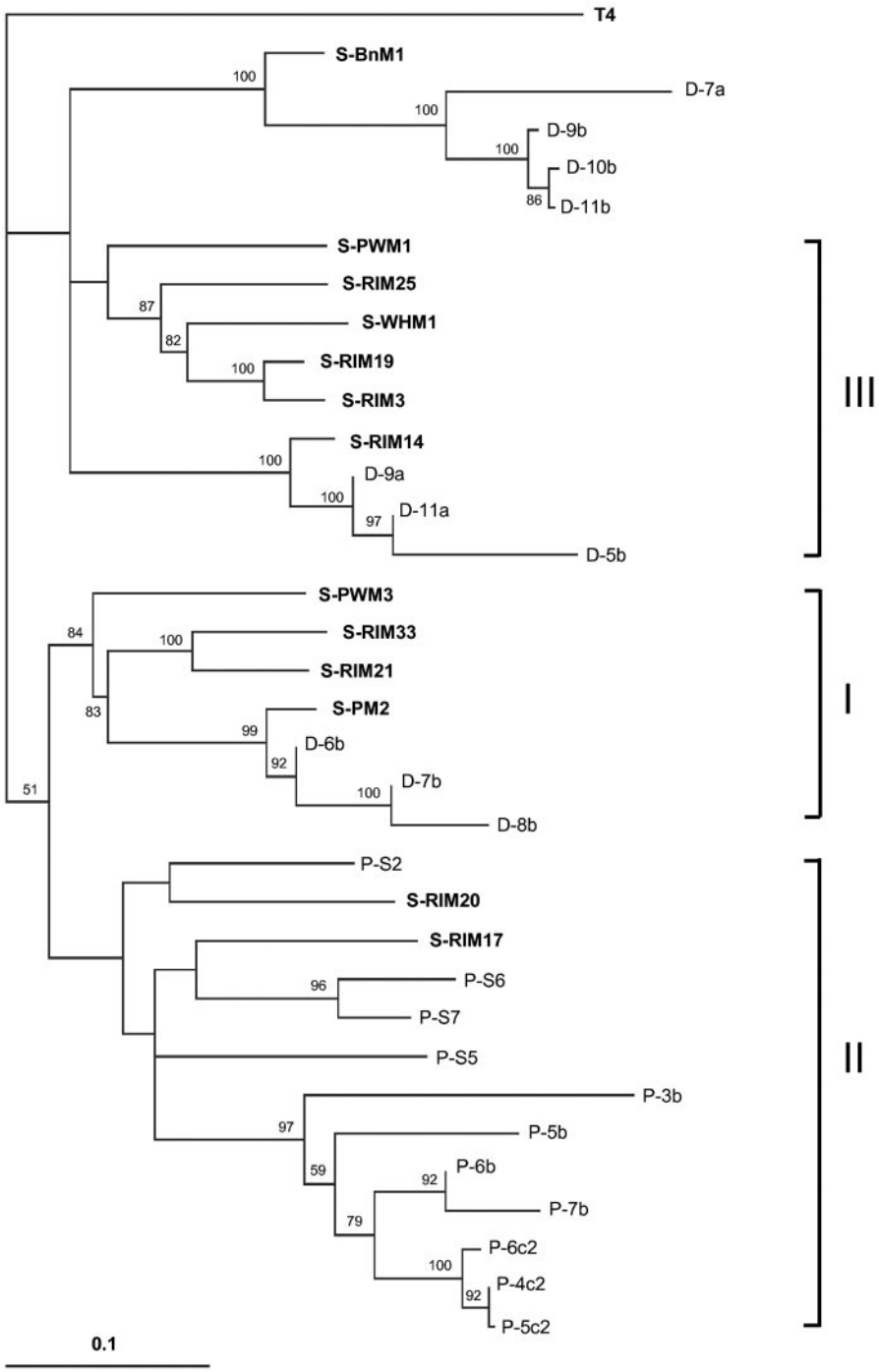

FIG. 4. Phylogenetic analysis using neighbor-joining analysis (PAUP*) of sequences from DGGE and PFGE bands and representative g20 sequences retrieved from the GenBank (NCBI) database. Groups I, II, and III refer to the cyanophage classification by Zhong et al. (59). Boldface indicates g20 sequences from GenBank. Bootstrap values were generated with 1,000 replicates; values of <50 are not shown. The scale bar represents 0.1 substitution per site.

(13), may also have interrupted a typical relationship between host and phage. A third explanation could be that the cyanophage community during the spring and summer period comprises viruses that are lysogenic and therefore do not follow the host community dynamic. Lysogeny may well be a common feature of marine phages (15, 28, 32), and a tyrosine site-specific recombinase (int) gene encoding a protein that enables temperate phages to integrate their genomes into the host genome was recently reported in the cyanophage P-SSP7 (42). This phage was isolated at the end of summer stratification when nutrients were extremely limited (42). The cyanophage peak at the end of May occurred after the bloom of autotro-

phic pico- and nanoeukaryotes, and as blooms reduced the nutrient concentrations in the seawater, this may have triggered phages with lysogenic cycles.

We have detected the g20 gene in viral bands within a wide size range, supporting the idea of a promiscuous horizontal gene transfer of gene modules within a common cyanophage gene pool. A hypothesis about transfer of photosynthetic genes (*psbA* and *psbD*) between cyanophages and even to their host has recently been put forward (29). It has been suggested that all dsDNA phages are mosaics and have access by horizontal exchange to a common gene pool (20). Sequence analysis of the cyanophage S-PM2 has shown mosaicism and proved that it carries a set of structural genes corresponding to genes found in T4 (25). Likewise, the sequence analysis of *Synechococcus* podovirus P60 has shown gene arrangements similar to those of coliphage T7 (12).

The g20 sequences produced in our study originated from viral populations with sizes from 26 to 380 kb. As mentioned above, the specificity of the g20 primers has been questioned (40), but all the sequences in the present study displayed homology to isolated cyanophages, grouping into the main clades I, II, and III and together with S-BnM1 inside the CPS group (40, 59). This favors the presumption that these viral populations are cyanomyoviruses. We thus need to evaluate our thinking on cyanomyoviruses and consider the possibility that this group of viruses is much more diverse, and is found in a wider genomic size range, than has been reported hitherto.

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