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Spring phytoplankton bloom dynamics in Norwegian coastal waters: Microbial community succession and diversity

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Abstract

Most studies of spring bloom succession in Norwegian waters have employed light microscopy and accounted for species composition of phyto- and zooplankton. Flow cytometry and molecular tools enable us to extend such investigations to include smaller organisms like bacterio- and virioplankton. Here, we describe succession and diversity of algae, bacteria, and viruses in relation to environmental changes from 15 February to 27 April. The spring succession started with an increase in autotrophic picoeukaryotes and *Synechococcus* sp. The diatoms bloomed around the middle of March and caused nutrient depletion in the upper part of the water column. Upwelling in the beginning of April gave rise to a second bloom, consisting of diatoms and *Phaeocystis pouchetii.* Numerically, autotrophic picoeukaryotes and *Synechococcus* sp. dominated the periods between and after these two major blooms. Heterotrophic bacterial abundance increased throughout the experimental period and reached peak values during and after phytoplankton blooms. These bacteria were succeeded by viruses having low DNA fluorescence, whereas viruses with medium DNA fluorescence bloomed during or after blooms of autotrophic picoeukaryotes. High–DNA fluorescence viruses reached maximum concentrations during and after the diatom and *Phaeocystis* blooms. The diversity of the bacterial community remained relatively stable, whereas viral diversity varied more and increased after major phytoplankton blooms. Our investigation thus demonstrates how virioplankton are important elements of the total microbial diversity and how they are intimately linked to the rest of the microbial community and possibly act as an internal driving force in spring bloom successions.

Several studies describing phyto- and zooplankton successions in the coastal current off the Norwegian coast and in the fjords have been conducted over the years (e.g., Braarud et al. 1958; Matthews et al. 1978; Eilertsen et al. 1981; Erga and Heimdal 1984; Erga 1989). These investigations have focused on abundance and species composition of autotrophic eukaryotes and zooplankters in relation to physical and chemical parameters. Among other things these studies have revealed that the annual spring bloom, which usually occurs between February and April, is dominated by diatoms and that a *Phaeocystis pouchetii* (Hariot) Lagerheim bloom often succeeds the diatoms.

Heterotrophic bacteria constitute an important part of the pelagic food web by mineralizing dissolved organic carbon (DOC) and converting dissolved matter into particulate biomass available to higher trophic levels through the ''microbial loop'' (Azam et al. 1983), but because of methodological constraints, they were not included in the above-mentioned investigations. Bratbak et al. (1990) did, however, show how the culmination of a spring diatom bloom off the Norwegian west coast was followed by a peak in concentration of bacteria, thus confirming studies from other areas reporting that bacteria succeed phytoplankton blooms (e.g., Andersen and Sørensen 1986; Billen and Fontigny 1987; Li and Dickie 2001; Yager et al. 2001). In their study, which was based on transmission electron microscopy (TEM), Bratbak et al. (1990) also demonstrated that bacteriophage abundance increased as bacteria bloomed.

During the last 10–15 yr, an increasing amount of information indicating that viruses might control the densities that can be maintained within algae and bacteria populations has been gathered, and marine viruses have been recognized as important partners in pelagic food webs (*see* reviews in Fuhrman 1999; Suttle 2000; Wommack and Colwell 2000). Our knowledge of in situ relationships between viral and host communities in natural environments is, however, still limited (Wommack and Colwell 2000; Li and Dickie 2001). To improve our understanding of the ecological role of marine viruses, we need, among other things, to assess the picture of temporal and spatial distribution of viruses and to determine their abundance in relation to potential host organisms.

In previous mesocosm experiments, we have demonstrated how new technology can be used to obtain a detailed description of microbial communities in experimental systems (Larsen et al. 2001). The objective of the current study was to perform a similar investigation in a typical West Norwegian open fjord system in order to give a description of the seasonal spring bloom in which the smallest participants, bacteria and virus, were included. We used flow cytometry

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Table 1. Sampling frequency (marked with x) and depths for all analyses performed during a spring field study at a station in Raunefjorden, Norway, during spring 2000.

	February			March					April										
	15	22	29	9	14	21	27	30	4	7	12	14	16	17	19	21	23	25	27
Salinity*	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Temperature*	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Fluorescence*	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Nutrients (2, 20 m)	x	x	x	x	x	x	x	x	x	x	x	x	x	x					
LM counts (2, 10, 20 m)	x	x	x	x	x	x	x	x		x	x	x	x	x		x			
FCM (0, 2, 5, 10, 20 m; algae, total bacteria, viruses)	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
DGGE (2, 20 m)	x†	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
PFGE (2 m)	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x
POP (2, 20 m)	x	x	x			x	x	x		x	x‡								x
Fractionated Chl *a* (2, 20 m)			x			x	x	x		x	x‡								x
DOC (2, 20 m)			x			x	x	x		x	x‡								x
APA (2, 20 m)			x			x	x	x		x	x‡								x
BP (2, 20 m)			x			x	x	x		x	x‡								x

APA, alkaline phosphatase activity; BP, bacterial production; DGGE, denaturing gradient gel electrophoresis; DOC, dissolved organic carbon; FCM, flow cytometry; LM, light microscopy; PFGE, pulsed-field gel electrophoresis; POP, particulate organic phosphorus.
* Measurements were obtained continuously between 0 and 50 m.
† No 2-m sample obtained.
‡ No 20-m sample obtained.

(FCM) and light microscopy (LM) to detect and enumerate the most abundant groups of algae, bacteria, and virus. In this context, bacteria are heterotrophic bacteria, whereas autotrophic bacteria, like *Synechococcus* sp., are included in the algae group, which in this study includes microscopic phytoplankton only. Bacterial and viral community compositions were observed by denaturing gradient gel electrophoresis (DGGE) and pulsed-field gel electrophoresis (PFGE), respectively. We measured DOC, bacterial production (BP), alkaline phosphatase activity (APA), fractionated particulate phosphorous and fractionated chlorophyll *a* (Chl *a*) in order to get more detailed information of dynamics within and between the organism groups. We thus followed the succession and community dynamics of algae, bacteria, and viruses and related this to changes in the physical and chemical environment, in an attempt to fill some gaps in our knowledge of the role of virioplankton in spring blooms of temperate waters. Another objective was to test the hypothesis that blooms of *P. pouchetii* are terminated by viral lysis, as suggested by Jacobsen (2000).

Materials and methods

Sampling program and physical measurements—The sampling was carried out at a station in Raunefjorden (60°16.2′N, 5°12.5′E), south of Bergen, Norway (for a map, *see* Bratbak et al. 1990), from 15 February to 27 April 2000. Sampling frequency and depths chosen for the various analyses are summarized in Table 1.

Water samples were taken with a Ruttner water sampler or a hand pump. Global irradiance (J) during the sampling period was obtained from a monitoring station at the Institute of Geophysics in Bergen and was converted into photosynthetically active radiation (PAR, μmol m^{-2}) according to Skartveit and Olseth (1994). Temperature, salinity, and in situ fluorescence data were obtained with an STD SAIV a/s SD 204 with a Sea Point fluorometer (SAIV A/S, Environmental Sensors and Systems).

Chemical and biological parameters—Nutrients: Nitrate, phosphate, and silicate concentrations were determined with an autoanalyzer at Institute of Marine Research, Bergen, Norway, following their standard methods. The samples were preserved with 1% (v/v) chloroform and stored in the dark at 10°C until analysis.

Algae, viruses, and bacteria: Total numbers of diatoms, identification of some of the most common diatom species and genera, and *Phaeocystis pouchetii* concentrations were obtained by light microscopy on samples preserved either in Lugol glutaraldehyde (final concentration 1%) or neutralized formalin (final concentration 0.4%) by the sedimentation method of Utermöhl (1931).

Autotrophic picoeukaryotes, cyanobacteria, heterotrophic bacteria, and viruses were determined by FCM. All FCM analyses were performed with a FACSCalibur flow cytometer (Becton Dickinson) equipped with an air-cooled laser providing 15 mW at 488 nm and with a standard filter setup. For algal counts, fresh samples were analyzed for 90–300 s. Enumeration of viruses and bacteria was performed on samples fixed with glutaraldehyde for 60 s at a viral event rate between 100 and 1,000 s^{-1}. Each sample was diluted either 10- and 50-fold or 50- and 100-fold before they were stained with SYBR Green I. Flow cytometer instrumentation and methodology followed the recommendations of Marie et al. (1999) and is described in more detail for a similar study by Larsen et al. (2001).

Bacterial and viral diversity: We applied PCR-DGGE to depict bacterial diversity. Seawater samples (10-ml) were fil-

tered on 0.2-μm DynaGard hollow fiber syringe filters (Microgon) and stored at -70°C until further processing. DNA extraction and purification, PCR amplification with the primer set 338f (Lane 1991) with a 40 mer GC clamp in the 5′ end and PRU517r (Lane et al. 1985), PCR, DGGE, and photographic documentation followed the description in Larsen et al. (2001).

Viral diversity was determined by PFGE. Four virioplankton agarose plugs were made from a concentrate of 2-liter water samples and run on a 1% (wt/v) SeaKem GTG agarose (FMC) gel in 1× TBE (Tris/edatic acid/boric acid) gel buffer with a Bio-Rad DR-II CHEF Cell electrophoresis unit (Wommack et al. 1999). From each sample, we used three of the plugs and ran them at three different pulse ramp conditions in order to separate a large range of viral genome sizes: (1) 1–8-s switch time with 20-h run time for separation of small genome sizes (0–130 kbp); (2) 8–30-s switch time with 20-h run time for separation of medium genome sizes (130–300 kbp); (3) 20–40-s switch time with 22-h run time for separation of large genome sizes (300–600 kbp). Further details of the procedure are found in Larsen et al. (2001). We applied densitometry software and standards with known size and amount of DNA to determine relative abundance of each of the viruses with different genome size.

The banding patterns of the DGGE and the PFGE profiles define number and relative frequency of bacterial species and viral genome size classes, respectively. On the basis of these numbers and frequencies, diversities within the bacterial and the viral communities were calculated and presented as Shannon diversity indices (Shannon 1948).

Particulate organic phosphorus (POP) and fractionated Chl *a*: To determine the relative contributions of various size fractions to microbial organic biomass (POP) and autotrophic biomass (Chl *a*), the samples were fractionated in four size categories (10–60, 5–10, 1–5, and 0.2–1 μm). Subsamples (100 ml) were filtered in triplicates through 47-mm polycarbonate filters of 0.2, 1, 5, and 10 μm pore size mounted on top of each other (Thingstad et al. 1993). Prior to fractionation, the samples were prefiltered through 60 μm to remove mesozooplankton. POP was measured by wet oxidation in acid persulfate (Koroleff 1976), and Chl *a* was analyzed fluorometrically in a Perkin Elmer PE LS50B after extraction in 90% acetone (Holm-Hansen and Riemann 1978).

Dissolved organic carbon: The DOC content was determined following the procedure described in Zweifel et al. (1993) with some modifications. Samples (5 ml) were filtered through 1.2 μm Gelman support filters with a combusted Millipore all-glass filter holder, transferred directly into glass vials (small type, Shimadzu), acidified with 50 μl 0.5 mol L^{-1} HCl, and kept cold until analysis. The filters were carefully acid-rinsed with 1 mol L^{-1} HCl and washed with ultrapure water (Millipore Milli-Q) prior to filtration. DOC was measured from triplicate injections from three parallel samples with a high-temperature carbon analyzer (Shimadzu TOC 5000) calibrated with potassium hydrogen phthalate. Standard solutions were run between every second analysis to check for instrumental drift. Blanks were tested before each analysis by injection of ultrapure water. The average value of the blank was 29.4 ± 7.2 μmol C L^{-1}. Samples were not corrected for this blank.

Alkaline phosphatase activity: The APA assay is used as an indicator of phosphorus deficiency in phytoplankton and bacterioplankton. The alkaline phosphatases (APs) cleave organically bound phosphorus from phosphomonoesters, and elevated concentrations of such enzymes thus indicate that algal, bacterial, or both populations are phosphate limited. APA was measured fluorometrically in a Perkin Elmer fluorometer PE LS50B with methyl-fluorescein-phosphate as substrate (Perry 1972; Thingstad et al. 1993).

Bacterial production: Bacterial production was measured by uptake of tritiated leucine at a final concentration of 60 nmol L^{-1} (Kirchman et al. 1985; Smith and Azam 1992). The leucine uptake was converted to bacterial carbon production (BCP) according to Simon and Azam (1989) by the following equation.

$$\text{BCP (g C)} = \text{mol incorporated leucine} \times 1{,}797 \times 0.86 \times 2$$

The constant 1,797 is the grams protein produced per mole of incorporated leucine, 0.86 is the fraction of carbon in protein, and 2 is the correction factor for a twofold isotope dilution.

Results

Physical conditions—Daily irradiance increased during the experimental period, reaching >30 mol m^{-2} d^{-1} around the middle of March (Fig. 1A). At the beginning of the period, the water temperatures (Fig. 1B) reflected a typically winter situation, with cold water in the upper part of the water column and slightly increasing temperature with depth. The salinity profile (Fig. 1C) depicts stable water masses with an increase in salinity from 0 m to 40 m. In early April, an upwelling situation brought warmer and more saline water from deeper layers to the surface (Fig. 1B,C).

Nutritional conditions—We recorded initial nitrate, phosphate, and silicate concentrations of 7, 0.5, and 4 μmol L^{-1}, respectively, at both 2 and 20 m (Fig. 2). At 2 m, the nutrient concentration decreased substantially from 14 to 27 March, before the upwelling situation in early April led to transient replenishment of nutrients. At 20 m, the decreases in nutrient concentrations were not as pronounced. At 2 m, two obvious peaks in APA were observed (Fig. 3A). The first peak appeared around 27–30 March, and a second one appeared at 27 April. At 20 m, APA stayed low until the last sampling day (27 April), at which time it increased substantially.

Two main points can be read from the DOC measurements (Fig. 3B): The concentration of DOC was always higher at 2 m than at 20 m, and at both depths, substantially higher DOC concentrations were measured on 21 March than during the rest of the sampling period.

Distribution and succession of algae, bacteria, and viruses—Using FCM, we determined two numerically dominat-

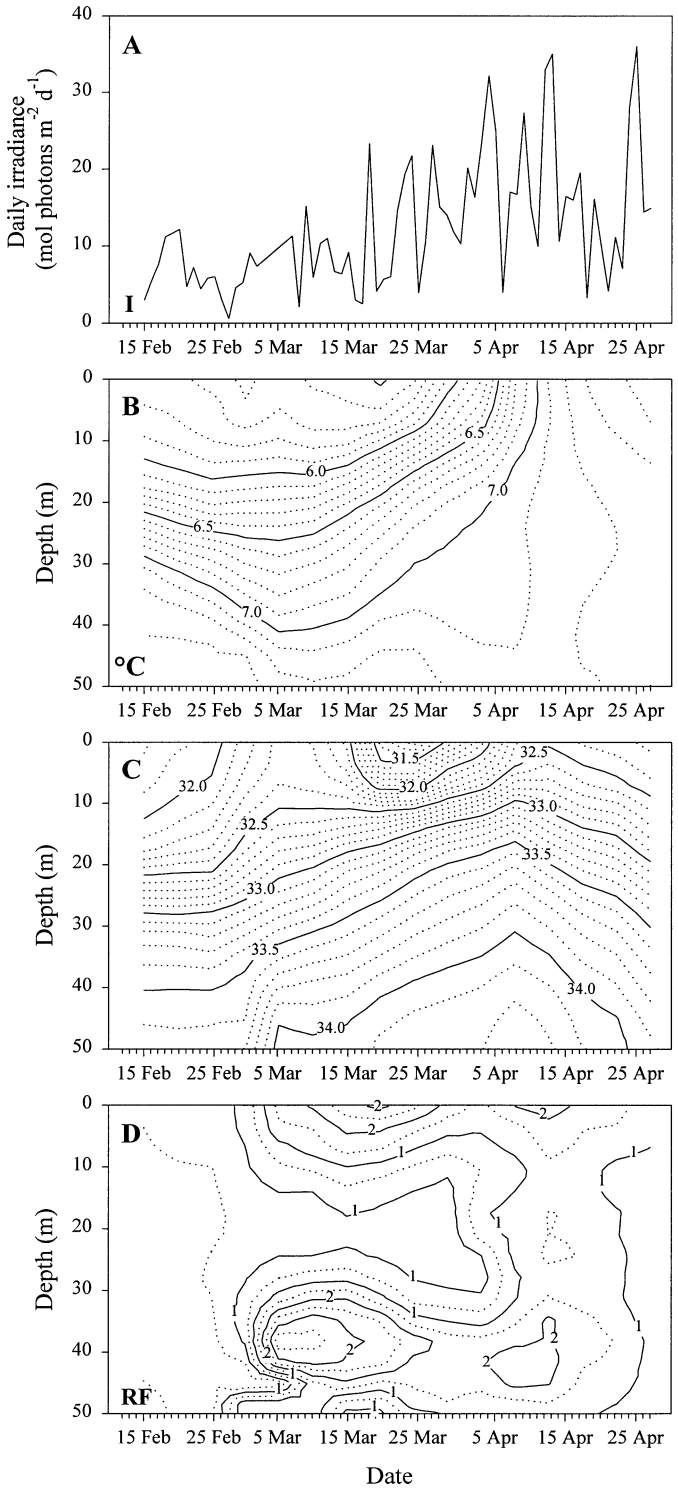

Fig. 1. (A) Irradiance (I), measured as daily photosynthetically active radiance (μmol m^{-2}), and isopleth diagrams for (B) water temperature (°C), (C) salinity, and (D) relative fluorescence (RF) at a station in Raunefjorden, Norway, during spring 2000.

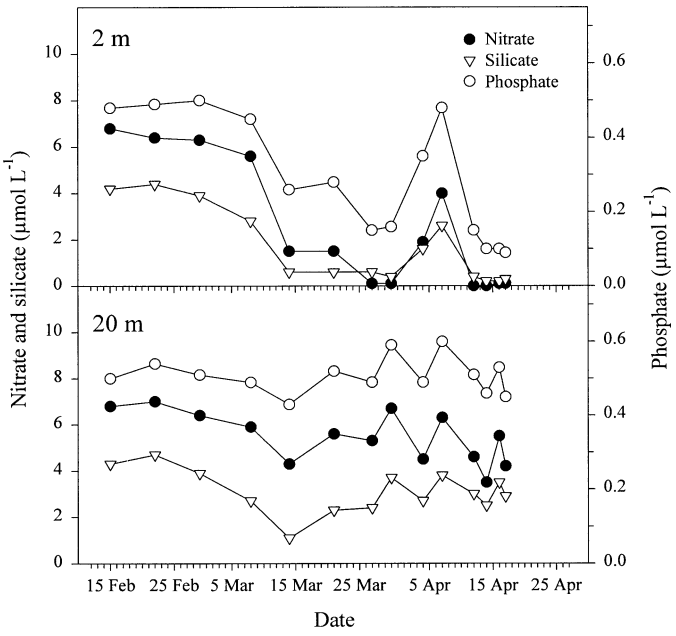

Fig. 2. Nitrate, silicate, and phosphate concentrations (μmol L^{-1}) at 2 m and 20 m, measured at a station in Raunefjorden, Norway, during spring 2000.

ing autotrophic algal populations (*Synechococcus* sp., autotrophic picoeukaryotes), three virus populations (V1, low–DNA fluorescence viruses; V2, medium–DNA fluorescence viruses; V3, viruses with high DNA fluorescence combined

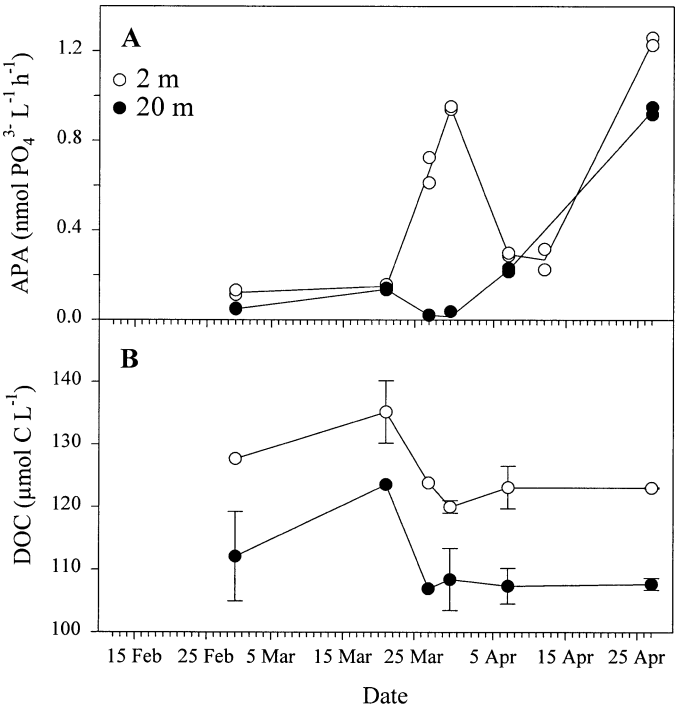

Fig. 3. (A) Alkaline phosphatase activity (APA, nmol PO_4^{3-} L^{-1} h^{-1}) at 2 m and 20 m and (B) dissolved organic carbon (DOC, μmol L^{-1}) at 2 m and 20 m at a station in Raunefjorden, Norway, during spring 2000.

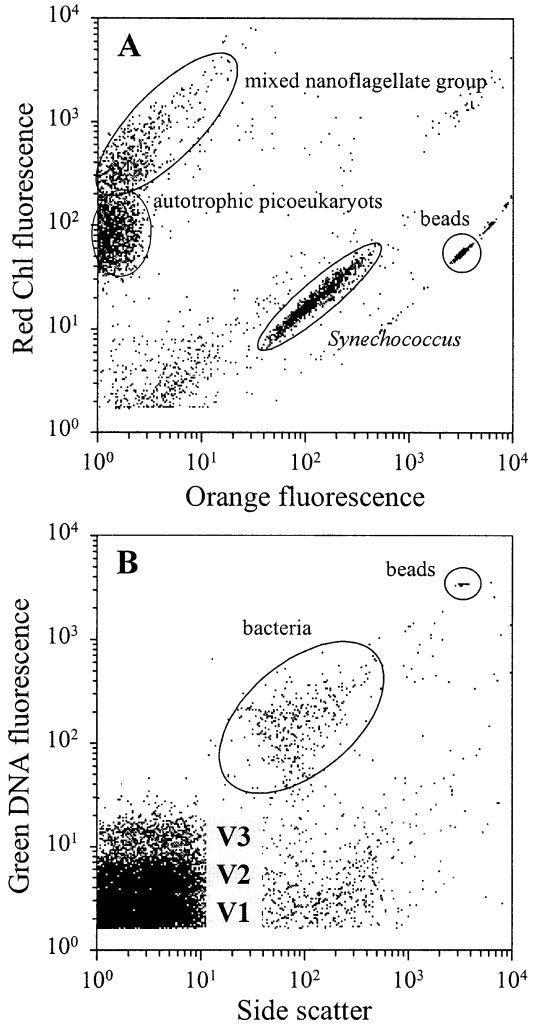

Fig. 4. Biparametric flow cytometry plots showing characteristic populations of algae, viruses and bacteria. (A) Orange versus red fluorescence, showing the major algal subpopulations (autotrophic picoeukaryotes and *Synechococcus* sp.), together with a mixed group of nanoflagellates, including *P. pouchetii* (*see* further explanation in the text). (B) Side-scatter versus green fluorescence demonstrating bacterial, V1, V2, and V3 populations. See further explanation in the text.

with high side scatter signal), in addition to total bacterial abundance (Fig. 4).

The development of FCM-determined algal, bacterial, and viral groups is depicted in Fig. 5, together with the development of the two dominant algal populations determined by light microscopy (i.e., the haptophyte *Phaeocystis pouchetii* and diatoms, dominated by *Skeletonema costatum, Thalassiosira nitzchioides, Leptocylindrus danicus, L. minimus,* and *Thalassiosira* spp.). The figure illustrates a typical

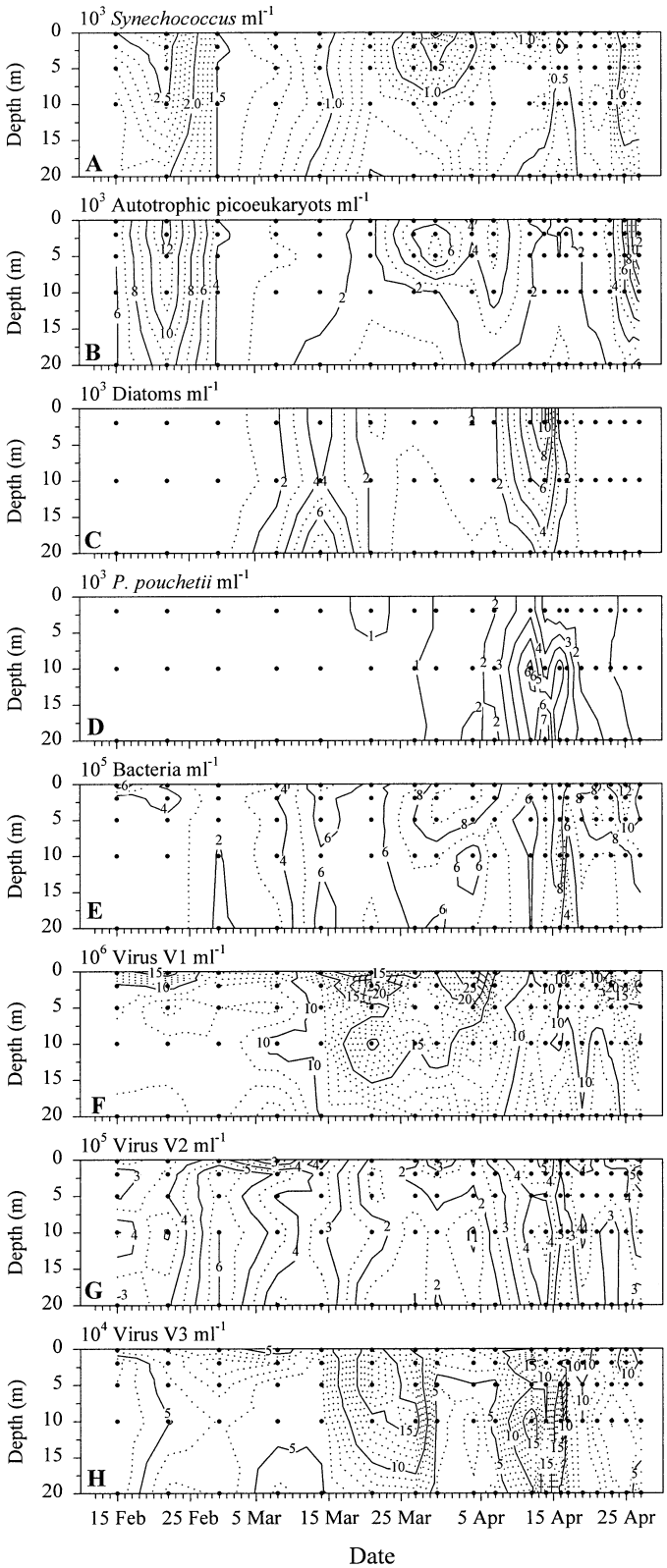

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Fig. 5. Development of algae, viruses, and bacteria from 15 February to 27 April 2000 at a station in Raunefjorden, Norway, as determined by flow cytometry (FCM) and light microscopy (LM). (A) *Synechococcus* sp. (ml^{-1}, FCM), (B) autotrophic picoeukaryotes (ml^{-1}, FCM), (C) diatoms (ml^{-1}, LM), (D) *P. pouchetii* (ml^{-1}, LM), (E) bacteria (ml^{-1}, FCM), (F) V1 (ml^{-1}, FCM), (G) V2 (ml^{-1}, FCM), (H) V3 (ml^{-1}, FCM). Note that the scales are different.

spring bloom situation with diatoms found in elevated concentrations around the middle of March, reaching cell numbers around 8×10^3 cells ml^{-1} (Fig. 5C), as also indicated by a concurrent increase of in situ fluorescence values (Fig. 1D). A second diatom bloom developed during the first half of April and took place simultaneously with a bloom of *P. pouchetii*. Maximum diatom cell numbers during this second bloom (1×10^4 cells ml^{-1}) appeared close to the surface, whereas the *P. pouchetii* maximum (8×10^3 cells ml^{-1}) was registered at 20 m (Fig. 5C,D). These observations were in accordance with in situ fluorescence maxima in mid-April, which demonstrated peak values at the surface and close to 20-m depth (Fig. 1D).

The succession of the phytoplankton community started prior to the first diatom bloom, with a doubling of autotrophic picoeukaryotes from 15 to 21 February (from 6×10^3 cells ml^{-1} to 12×10^3 cells ml^{-1}, Fig. 5B). These algae also numerically dominated the periods between and after the major blooms, with maximum cell numbers around 7×10^3 cells ml^{-1} and 13.5×10^3 cells ml^{-1}, respectively. The *Synechococcus* sp. development followed a similar pattern, but maximum values did not exceed 2.8×10^3 cells ml^{-1} (Fig. 5A).

Bacterial abundance increased from $\sim 3 \times 10^5$ cells ml^{-1} to $\sim 10 \times 10^5$ cells ml^{-1} throughout the experimental period and reached peak values during and after phytoplankton blooms (Fig. 5E).

Increases in bacterial numbers were succeeded by higher concentrations of V1 viruses (Fig. 5F), whereas V2 viruses flourished during or after blooms of autotrophic picoeukaryotes (Fig. 5G). The abundance of V1 viruses varied between 5.3 and 30×10^6 viruses ml^{-1}, and the numbers of V2 viruses were found in a range from 0.9 to 6.7×10^5 viruses ml^{-1}. A third group of viruses, V3, was found in numbers from 0.2 to 1.9×10^5 viruses ml^{-1}, with the most marked increase in numbers found after or during the diatom and the diatom/*P. pouchetii* blooms (Fig. 5H).

In addition to the algal populations described above, we detected (by FCM) one population with a higher red fluorescence signal than the autotrophic picoeukaryotes (Fig. 4A). The FCM signature of this population resembles that of *P. pouchetii* (and other nanoflagellates), and beyond the *P. pouchetii* bloom, this group did not contribute significantly to the algal community (results not shown). We therefore conclude that the majority of cells in this group were *P. pouchetii* and that our light microscopic data account for the development of these algae. Moreover, molecular data obtained from sequencing a band of the PCR-DGGE 18S rDNA eukaryotic profile support this assumption (results not shown).

The contribution of the largest size fraction (10–60 μm) to total Chl *a* (Fig. 6) reflects how the relative importance of the diatoms changed throughout. By 12 April, the contribution of the 5–10-μm size fraction was equally important as the largest size fraction, mirroring an incipient bloom of *P. pouchetii* (only measured at 2 m) whose cell sizes varies from 5 μm to 7 μm (Jacobsen 2000). By 27 April, the Chl *a* 1–5-μm size fraction proportion had increased to approximately 50% of total Chl *a* (at 2 m), an increase co-occurring in time with a marked increase in autotrophic picoeukaryote abundance in the upper part of the water column. The smallest size fraction (0.2–1 μm) constituted from 0.4 to 2.8% of the total Chl *a* biomass, and its dynamic reflected that of *Synechococcus* sp. abundance at all times.

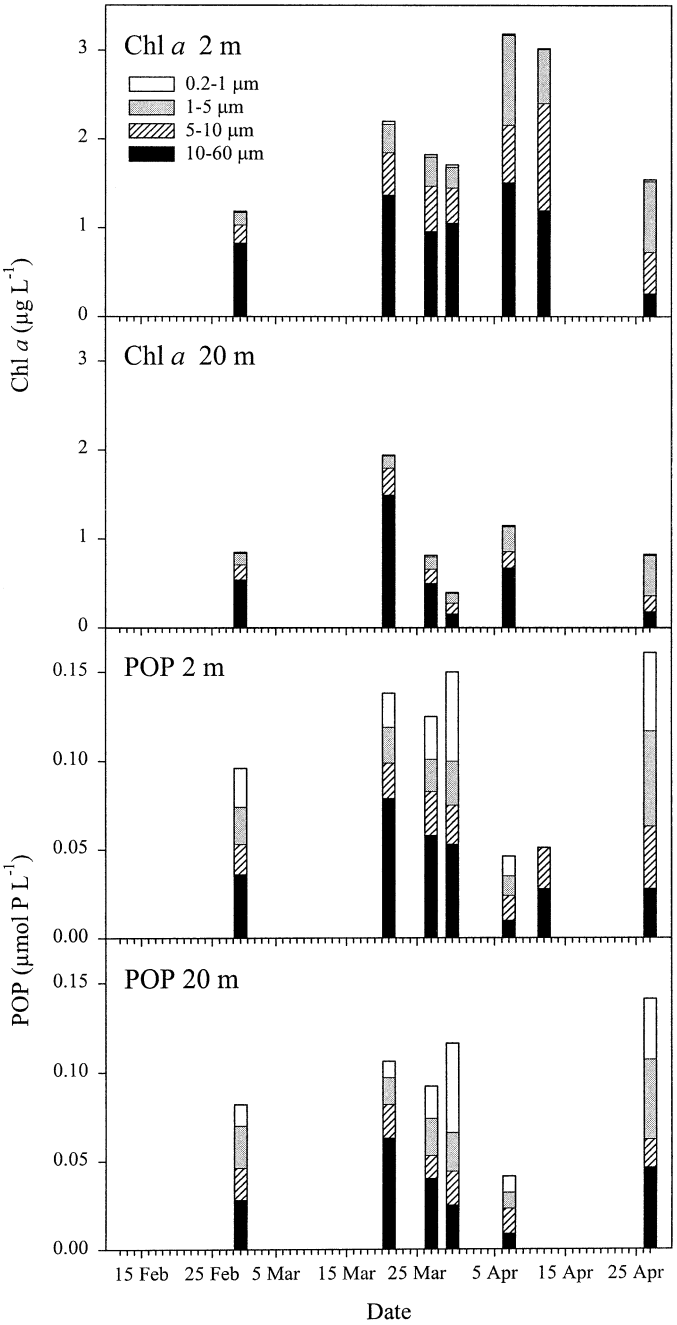

Fig. 6. Fractionated Chl *a* and particulate organic phosphorus (POP) measured at 2 m and 20 m at a station in Raunefjorden, Norway, during spring 2000. Size fraction is indicated by gray scales.

On most sampling days, the largest size fraction (10–60 μm) dominated the POP (Fig. 6), and the relative importance of this size group varied concurrently with the observed diatoms dynamics with peaks around 21 March and 12 April. The remaining POP was more equally distributed between

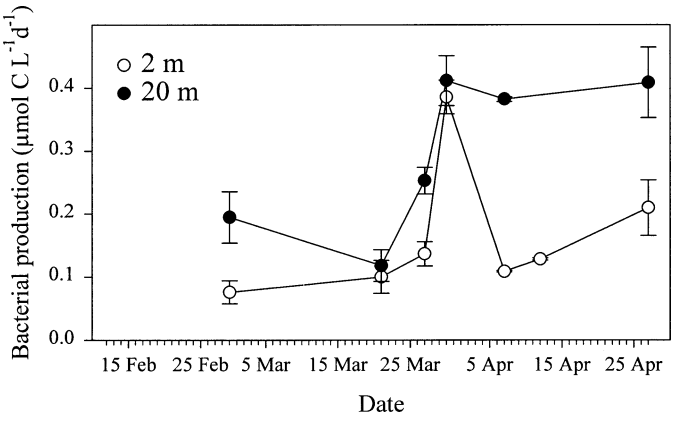

Fig. 7. Bacterial production (μmol C m^{-3} d^{-1}) measured at 2 m and 20 m at a station in Raunefjorden, Norway, during spring 2000.

the smallest size fractions than was the Chl *a*, thus reflecting the importance of microheterotrophs in these size groups. A marked increase in the importance of the smallest size fraction (0.2–1 μm) occurred after the first diatom bloom (from 21 to 30 March), mirroring the increase in bacterial abundance. On the last sampling day, the 1–5-μm size group constituted ~30% of the total POP, reflecting the increase in autotrophic picoeukaryotes.

BP was higher at 20 m than at 2 m on all sampling days (Fig. 7). At 20 m, BP decreased from 29 February to 21 March, after which it increased significantly to 411 μmol C m^3 d^{-1} on 30 March and stayed high (around 400 mmol C m^3 d^{-1}) for the rest of the period. At 2 m, a corresponding increase took place between 27 and 30 March. We subsequently observed a significant decrease followed by a new increase.

Viral and bacterial communities—The virus : bacteria ratios (VBR) given in Fig. 8A varied between 10 and 52, with a distinct peak 21 April. Our results from the PCR-DGGE analysis (Fig. 8A,B) documented only moderate changes within the bacterial community during the spring bloom. Shannon diversity indices varied from 1.8 to 2.4 for bacteria, which is slightly lower than values reported from the Catalan coast (2.5–3.1; Schauer et al. 2000) but higher than Shannon indices reported from a seasonal study in a eutrophic lake (0.2–0.9; Höfle et al. 1999). Two bands (1 and 2) out of 12 dominated the DGGE profiles during the whole period (Fig. 8B; data for 20 m are not shown). Bands 3, 4, 5, and 6 were also found in the profile throughout the whole period. Bands 7, 8, and 9 were present from mid-February when the investigation started but thereafter gradually disappeared. Bands 10, 11, and 12 appeared in early April and were present during the rest of the period. The banding pattern at 20 m resembled that of the 2-m pattern (results not shown), but most of the bands were generally somewhat less distinct, except bands 1 and 2, which dominated also at 20 m.

Viral diversity as determined by PFGE varied more than bacterial diversity with Shannon index values ranging from 0.7 to 2.0 (Fig. 8A). The highest values were obtained during the blooms of autotrophic picoeukaryotes/*Synechococcus* sp.

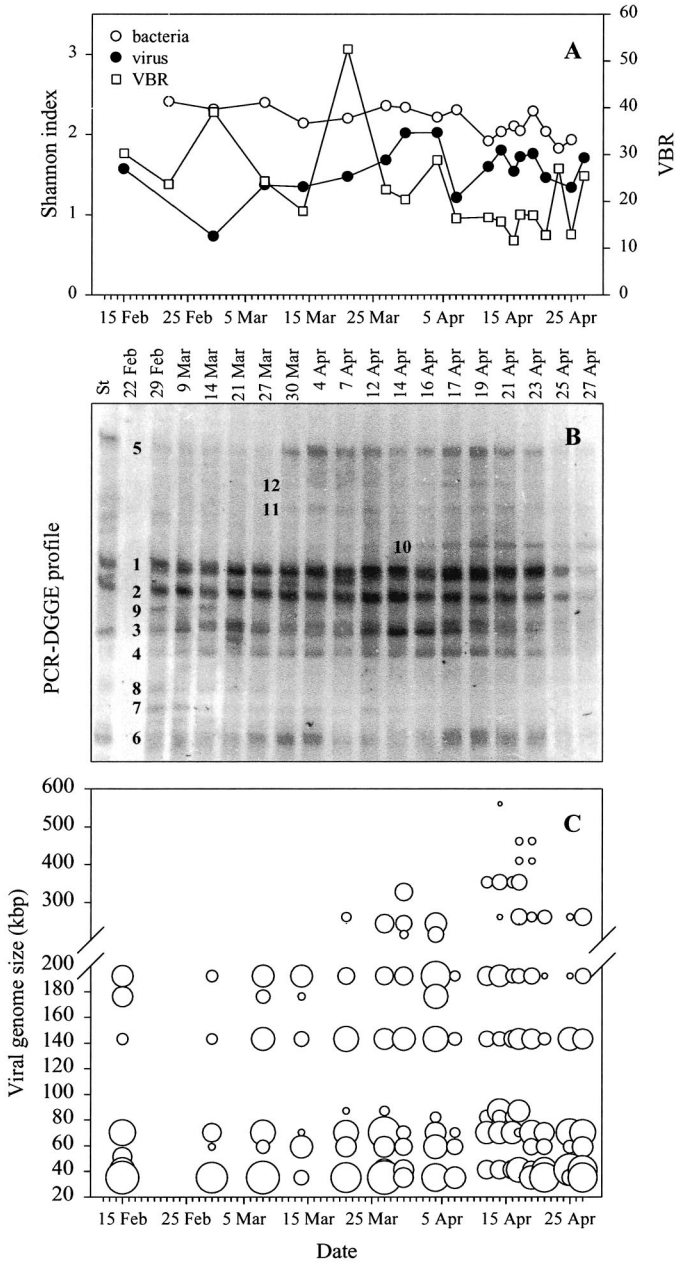

Fig. 8. Virus : bacteria ratios and diversity within the bacterial and viral communities at 2-m depth at a station in Raunefjorden, Norway, during spring 2000. (A) Shannon index for the bacterial and viral communities and virus : bacteria ratio (VBR). (B) PCR-DGGE profile from analysis of prokaryotic rDNA. The bands discussed in the text are indicated with numbers on the picture. (C) Schematic outline of the relative abundance (indicated by the size of the bullet) of distinct viral populations determined by PFGE. Populations are defined by genome sizes, and the outline is based on profiles emerging from the three different electrophoretic runs for each viral concentrate (*see Material and methods*).

(middle of February, beginning of April, end of April) and during the combined diatom bloom/*Phaeocystis* bloom (middle of April). By 29 February and 7 April, the indices decreased to 0.7 and 1.2, respectively. At these dates, we also observed a decrease in V1 (Fig. 5; by 29 February, this

decrease only took place in the upper layers, from where samples for PFGE analyses also were taken). These concurrent decreases in viral abundance and diversity could have been caused by an exchange of surface water in these periods (Fig. 1). Figure 8C shows that the viral populations with genome size <200 kbp appeared more frequently and in higher concentrations than populations with genome sizes from 200 kbp to 600 kbp. These populations of low–DNA fluorescence viruses were dominated by four size classes (190, 145, 70, and 35 kbp), two of which were present on all sampling days (190 and 145 kbp). Virus populations with genome size >200 kbp were only found during the second half of the period. Some populations (215, 245, and 330 kbp) peaked in early April, some around the middle of April (355, 410, 460, and 560 kbp), and one (260 kbp) in both these periods.

Discussion

The objective of the current study was to improve our knowledge of temporal and spatial distribution of marine pelagic viruses in the Norwegian coastal and fjord waters. We therefore determined abundance and diversity of autotrophic, bacterial, and viral populations during the spring bloom and illustrated the appearance of viruses in relation to potential host organisms. We did not aim to give a complete carbon flow budget and have not, therefore, included all possible organism groups (e.g., heterotrophic flagellates, some groups of autotrophic flagellates, ciliates) in our data set but, rather, those that dominated numerically.

The spring bloom took a course typical of Norwegian coastal waters, with diatoms blooming as the light increased (Erga and Heimdal 1984) and a *P. pouchetii* bloom appearing sometime later (Eilertsen et al. 1981 and references therein; Jacobsen 2000). Both diatoms and *P. pouchetii* reached numbers that are normally observed during spring bloom in this region (Eilertsen et al. 1981; Erga and Heimdal 1984; Jacobsen 2000). We observed, however, that *Synechococcus* sp. and autotrophic picoeukaryotes numerically dominated the algal community before, between, and after these blooms. Maximum numbers of both groups were within typical ranges of coastal waters of this latitude at this time of year (Bratbak et al. 1990; Li and Dickie 2001). During or after blooms of autotrophic picoeukaryotes, the abundance of medium–DNA fluorescence virus (V2) increased, whereas high–DNA fluorescence virus (V3) proliferated during diatom/*P. pouchetii* blooms. Increased concentrations of low–DNA fluorescence virus (V1) followed the peaks of heterotrophic bacteria, which in turn succeeded the phytoplankton blooms. The bacterial community composition was quite stable throughout the period, whereas the diversity within the viral community varied somewhat more. In general, abundance of heterotrophic bacteria increased during the sampling period.

Close to the surface, nutrient concentrations decreased substantially after the first diatom bloom, and it is therefore reasonable to believe that nutrient depletion was responsible for the termination of the bloom. High alkaline phosphatase activity confirms that organisms appearing after the diatom bloom (i.e., autotrophic picoeukaryotes, *Synechococcus* sp., or heterotrophic bacteria) were phosphate limited. At 20 m, neither nitrate, phosphate, nor APA levels indicated N- or P-limited algae, but the silicate concentration was low, pointing to Si as the primary limiting nutrient. The diatom numbers were lower close to the surface than at 20 m, whereas the highest relative fluorescence (RF) values were recorded at 0–5 m. This might reflect that the bloom developed in surface waters and that senescent cells (thus containing less fluorescence per cell), which were sinking out, dominated the diatom population at 20 m. This assumption is supported by the observation that more nutrients were used close to the surface.

A wind-driven overturn in early April, probably caused by a predominant longshore north wind stress typical for this area at this time of the year (Svendsen 1981; Erga and Heimdal 1984), led to a transient replenishment of nutrients. A second bloom of diatoms, which in time, although not in space, co-occurred with the *P. pouchetii* bloom, followed the renewal of the nutrients. We thus agree with Peperzak et al. (1998), who did not find empirical support for the earlier proposed hypothesis (Egge and Aksnes 1992) that *Phaeocystis* spring blooms develop only after depletion of Si by diatoms. At 2 m, all nutrients decreased dramatically, and the diatom bloom subsequently collapsed. The *P. pouchetii* population, which reached maximum concentrations at 20 m, also decreased dramatically, although the nutrient values at 20 m did not indicate nutrient limitation. We observed a simultaneous increase of V3 at all depths and the appearance of a virus population with a genome size of ~460 kbp at 2-m depth (not investigated at 20 m). The FCM signature of V3 was similar to that of a *P. pouchetii* virus (PpV) (pers. obs.), and the size of a PpV isolated from Norwegian waters is close to 460 kbp (between 445 kbp and 545 kbp; pers. obs.). It is thus tempting to suggest a causal relation between the observed viral population (V3/460 kbp) and the decline of the *P. pouchetii* bloom. Ciliates might be important grazers on *P. pouchetii* (Peperzak et al. 1998), and we cannot rule out that they accounted for the decrease in the *P. pouchetii* population abundance. Thus, we do not present unambiguous evidences showing that *P. pouchetii* blooms in general are terminated by viral lysis (Jacobsen 2000). PpV-like viruses were a dynamic part of the microbial community, however, and it is reasonable to argue that a combination of grazing and viral activity might have prevented *P. pouchetii* from reaching levels high enough to use all nutrients still available.

Our FCM data give a relatively coarse classification of the viral populations because they are based on only two parameters (side scatter and green fluorescence). It is therefore most likely that V3, which also was found in high concentrations after the first diatom bloom, consisted of more than one viral population. This assumption is supported by the elevated concentration of several viral populations (in the size ranges between 200 kbp and 300 kbp and from 350 kbp to 560 kbp) during the two periods with high V3 abundance that were not present at all times. Furthermore, the V3 population was found in high numbers in the whole-water column during the combined diatom/*Phaeocystis* bloom, although *P. pouchetii* were found in highest concentration at

20 m. We can therefore conclude that although V3 probably contain PpV, viruses specific to other species or groups of organisms might also well be a part of it. Diatoms might, on the basis of their appearance at the time, be connected to these co-occurring viruses. To date, there are no confirmed observations of a virus infecting a diatom, and it is therefore speculative, although tempting, to suggest a connection between either of these organism groups and the V3 population.

As shown by the FCM data, the fractionated Chl *a* and the POP measurements, autotrophic picoeukaryotes and *Synechococcus* sp. started the spring bloom season, and these organisms peaked three times during the sampling period. The two last peaks built up when nitrate and phosphate concentrations were low, indicating that these small organisms compete well when nutrients are limiting. Nutrient data are not available for the last part of the period, but high APA measured at both 2 m and 20 m indicate that algal, bacterial, or both populations were phosphate limited at the time. The two first *Synechococcus* sp./autotrophic picoeukaryote blooms ceased while nutrient levels were high. Grazing might account for this. High abundance in the FCM-determined V2 population coincided, however, with all three peaks and so did the abundance of several small- and medium-sized viral populations as determined by PFGE. In their description of viruses as partners in a spring bloom, Bratbak et al. (1990) also show that autotrophic picoeukaryotes preceded the diatom bloom. They were uncertain, however, whether to link any viral populations to this organism group. During our investigation, we repeatedly observed a coinciding increase between autotrophic picoeukaryotes/*Synechococcus* sp., and the V2 viruses, and we find it reasonable to argue that this strong temporal coupling indicates that the viruses are one important mechanism controlling the activity of the smallest groups of photosynthesizing organisms.

V1 were found from 5.3 to 30 $\times$ 10^6 viruses ml^{-1} and thus reached concentrations 10- to 100-fold higher than those of V2 and V3. The majority of viruses within this group were thus most likely bacteriophages (Marie et al. 1999; Wommack and Colwell 2000; Larsen et al. 2001; Culley and Welschmeyer 2002). Moreover, the numbers are comparable to bacteriophage abundance reported from coastal areas elsewhere (Wommack and Colwell 2000). V1 increased considerably shortly after the bacterial blooms in mid-March and in early and late April. These coinciding peaks of bacteria and viruses support the idea that bacteriophages cause bacterial lysis and changes in bacterial abundance, leading to a short circuit of bacterial production (Bratbak et al. 1990). Assuming that the majority of V1 were bacteriophages, we used V1 numbers to calculate VBR ratios, which ranged from 10 to 52. Normally, such ratios fall between 3 and 10, but are higher for nutrient-rich productive areas (Wommack and Colwell 2000). VBR values <1 are thought to indicate low levels of virus-mediated bacterial mortality, whereas ratios >10 are conditions favoring bacterial lysis (Wilcox and Fuhrman 1994). The ratios observed in our material might thus indicate that bacteriophages accounted for much of the bacterial mortality.

Enhanced bacterial production and abundance characterized phytoplankton postbloom phases. An increase in both BP and bacterial numbers followed the increase in DOC concentration that was measured after the first diatom bloom. Superimposed on the general increase in bacteria concentrations throughout the spring season, we also found that peaks in bacterial numbers succeeded the declines in other autotrophic populations. A similar succession pattern between algae, DOC, and bacteria was observed during a lysis-induced decline of a *Phaeocystis* spring bloom in the North Sea (van Boekel et al. 1992), and Bratbak et al. (1998) showed experimentally that algal carbon released upon viral lysis was converted efficiently to bacterial biomass. As opposed to observations during a mesocosm diatom bloom and its decline (Riemann et al. 2000), an increase in bacterial activity was not, however, accompanied by major changes in bacterial community composition. In contrast, the bacterial diversity was very stable. Certainly some bands of the DGGE profile appeared and other disappeared, but the bands at the same position dominated throughout the sampling period at both depths. These persistent bands are not likely to be of eukaryotic chloroplast origin because the organisms dominating the eukaryotic community changed several times during the period. The banding pattern thus suggests a bacterial community that did not vary much. Similarly, Riemann and Middelboe (2002) found surprisingly stable bacterial community compositions across temporal or spatial gradients in Danish coastal waters despite major variations in productivity. Their PFGE profiles also documented a stable viral community with high similarity in banding patterns throughout the spring bloom period, whereas the current investigation documents substantial changes in viral diversity after the major phytoplankton blooms. One reason for this apparent discrepancy could be that Riemann and Middelboe (2002) focused their investigation on viruslike genome sizes $<$200 kbp, whereas we used electrophoresis conditions that allowed for separation of larger DNA. Our profiles therefore include algal viruses, which can have genome sizes between 200 kbp and 600 kbp (van Etten and Meints 1999; Sandaa et al. 2001; Castberg et al. 2002) to a much larger extent. Even though the current study supports studies claiming that algal viruses do not contribute significantly to total viral abundance (Wommack and Colwell 2000; Culley and Wellschmeyer 2002), we have demonstrated that their activity does not seem to be correspondingly insignificant. Moreover, we have demonstrated their importance for total microbial aquatic diversity.

Several problems need to be solved in order to obtain a complete picture of the importance of viral activity in the ocean. Examples of such are whether the host virus interaction mechanisms are similar for algal viruses and bacteriophages and whether one of the most important groups of algae, the diatoms, experience viral attacks. Similarly, the ongoing work of developing species-specific molecular probes for both host and virus will improve our understanding of how important viral activity might be in the regulation of specific algal and bacterial populations. Our study has not answered the above-mentioned problems. It has, however, consistent with, and maybe more clearly than earlier studies (Li and Dickie 2001), demonstrated how virioplankton are intimately linked to the rest of the microbial community and

possibly act as an internal driving force in spring bloom successions.

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