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Virus-like particles in a summer bloom of *Emiliania huxleyi* in the North Sea

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ABSTRACT: The importance of viruses in controlling a bloom of the coccolithophorid *Emiliania huxleyi* in the North Sea was investigated during summer 1993. Viral infection of *E. huxleyi* was highest in the decaying phase of the bloom. Up to 50% of *E. huxleyi* cells were visibly infected. For *E. huxleyi*, 2 types of virus-like particles are reported. They differed in size and were occassionally found within the same cell. The infection level of the large virus-like particles was never higher than 25%. Viral lysis of *E. huxleyi* within the nitrogen-limited decaying phase of the bloom seemed an important source of organic carbon utilized by bacteria. In addition to infection in *E. huxleyi*, we also found severe viral infection in *Chrysochromulina* sp. Our results show that in natural ecosystems viruses can be a significant source of phytoplankton mortality, influencing phytoplankton (bloom) dynamics and the microbial food web.

KEY WORDS: *Emiliania huxleyi* bloom · Viruses · Viral lysis · Bacterial production

INTRODUCTION

The existence of virus-like particles has been reported for many eukaryotic phytoplankton species (Dodds 1979, Van Etten et al. 1991). However, only a few reports are known on the potential importance of viruses as agents of mortality for phytoplankton. Mayer (1977) was one of the first reporting established viral infection of the Prasinophyte *Micromonas pusilla*. Transfer of an aliquot of infected culture to an uninfected unialgal culture of *M. pusilla* resulted in complete lysis. Cottrell & Suttle (1991) showed that viruses which lysed *M. pusilla* were highly species-specific and geographically widespread. Suttle et al. (1991) documented 6 phytoplankton isolates of diverse taxonomy (including diatoms) that were susceptible to readily isolated viruses present in seawater. Furthermore, Suttle & Chan (1995) isolated a virus which caused lysis when added to exponentially growing cultures of the Prymnesiophyte *Chrysochromulina brevifilum*.

Studies on the role of viruses as control mechanisms on the phytoplankton population level are even more scarce (Sieburth et al. 1988, Bratbak et al. 1993, Nagasaki et al. 1994). Bratbak et al. (1993) studied viral importance during a bloom of *Emiliania huxleyi* in mesocosms enriched with nitrate and phosphate. In some cases, viral lysis could account for 25 to 100% of the net mortality of *E. huxleyi*. These results indicate that viruses have the potential to play an important role in phytoplankton dynamics. Viral cell lysis results in the release of the cellular components (e.g. proteins, nucleic acids) in addition to viruses themselves, which will influence the nutrient and energy cycling in the microbial food web (Fuhrman 1992, Bratbak et al. 1994). Fuhrman (1992) showed that inclusion of viruses in a food web model led to decreased availability of hosts to higher trophic levels, and to increased respiration rates.

The aim of this study was to clarify the importance of viruses as mortality agents of the coccolithophorid

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Emiliania huxleyi, blooming under natural conditions in the North Sea during summer 1993. We report on viral infection within the cells of *E. huxleyi* using electron microscopy and relate viral lysis to bacterial production. *E. huxleyi* has a world-wide distribution, may form extensive blooms and is a major producer of calcite (Balch et al. 1991), which makes it an important alga for studies on global biogeochemical cycles. Information on the viral mortality of *E. huxleyi* blooms is essential for a better understanding and modelling of carbon flows.

MATERIAL AND METHODS

Sampling site and chemical analyses. All samples were collected during a cruise aboard the RV 'Pelagia', from 28 June until 13 July 1993. Station locations are shown in Fig. 1. High levels of reflectance from the waters off the coast of Shetland, as observed by satellite images (Van der Wal et al. 1995), indicated large numbers of loose coccoliths during a bloom of *Emiliania huxleyi*. Based on level of reflectance we divided the stations into 3 regions: (A) region within the high reflectance area (Stns 4, 7, 15, 19S and 30); (B) borderline zone (Stns 12, 19N, 28 and 32); and (C) region outside the reflectance area (Stns 18 and 20–26). Vertical profiles of salinity and temperature were recorded for all stations with a Sea-Bird Electronic conductivity, temperature, depth profiles (CTD) (type SBE9+). Seawater samples were collected with a rosette sampler using 10.5 l NOEX bottles (Technicap, Cap d'Ail, France). Dissolved nutrients (ortho-phosphate, reactive silicate, ammonium-nitrogen, nitrate-nitrogen and nitrite-nitrogen) were measured in 0.2 µm Acrodisc filtered water samples on a TRAAC 800 autoanalyzer (Technicon, Buffalo Grove, IL, USA).

Phytoplankton abundance and bacterial production. The number of *Emiliania huxleyi* coccospheres (living and dead) were obtained from Van der Wal et al. (1995, pers. comm.). Chlorophyll *a* (chl *a*) concentration was determined fluorimetrically according to Holm-Hansen et al. (1965). Samples for chl *a* were gently filtered through Whatman GF/F filters and extracted in 90% acetone after homogenization. For several main stations (Stns 7, 12, 15, 19S) we divided algal biomass (obained from flow-cytometer countings of algal size classes in the upper 25 m of the water column; M. J. W Veldhuis pers. comm.) by the corresponding chl *a* concentration. The estimated average phytoplankton C:chl *a* ratio of 30 ± 4 was used throughout this study. Our estimated ratio equalled the C:chl *a* ratio for *E. huxleyi*, which was derived from the literature assuming 0.2 pg chl *a* $cell^{-1}$ as reported by Paasche & Klaveness (1970) and Kristiansen (1987) and 6 pg C $cell^{-1}$ as reported by Conte et al. (1995).

Bacterial production was determined with ^{3}H-leucine incorporation into bacterial biomass according to Simon & Azam (1989).

Virus-like particles. At 6 stations (Stns 7, 12, 15, 18, 19N, 19S) we took samples of 10 to 130 l at 15 to 25 m depth (chlorophyll maximum if present) for thin sectioning of cells. Samples were preserved with 2% formaldehyde and stored until use at 4°C. After centrifugation [first step at 12 000 × *g* with a continuous centrifugation (LWA 205), and second step 10 min at 4000 rpm (2600 × *g*) with a Sigma (St. Louis, MO, USA) 2M centrifuge], the concentrated cells were washed in cacodylate buffer (0.1 M, pH = 7.4) for 30 min and postfixed with 2% OsO_4 in cacodylate buffer (Fluka, Switzerland) for 2 h at 4°C. After dehydration in an alcohol series, cells were embedded in Epon (Fluka, Switzerland) for 24 h at 60°C using BEEM capsules (Biorad, Richmond, CA, USA) and sectioned on a Reichert OM U3 ultamicrotome. Thin sections were poststained with uranyl acetate (3.5%; 5 min) followed by Reynolds

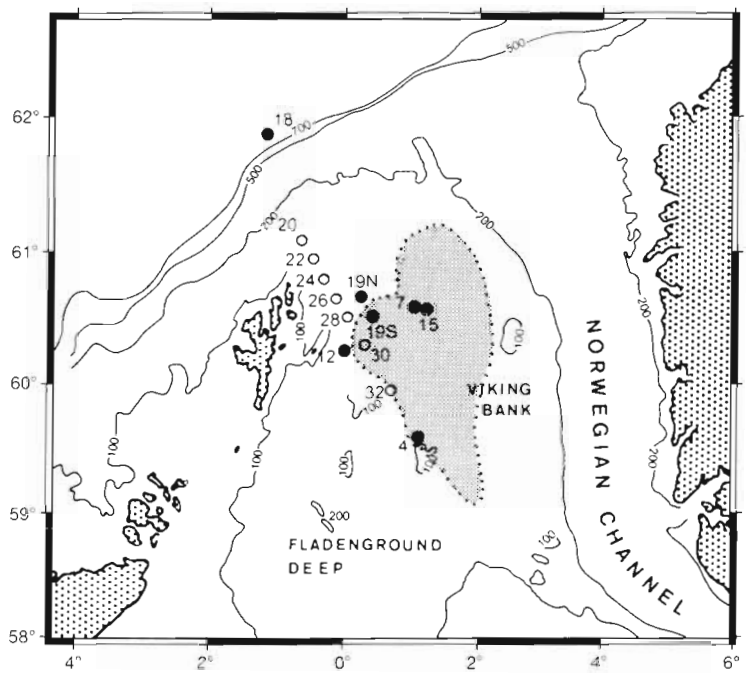

Fig. 1 Map of study area, showing the position of the stations investigated during a cruise off the coast of Shetland (North Sea) in June/July 1993. The high reflectance area (decaying phase of the *Emiliania huxleyi* bloom studied), as observed by satellite images, is shaded. Location of main stations (Stns 4, 7, 12, 15, 18, 19N, 19S) (○); location of transect Stns 20–32 (●)

Pb-citrate (5 min), and viewed at 60 kV using a Philips CM 10 transmission electron microscope. The number of cell sections examined for intracellular virus-like particles (VLPs) ranged between 100 and 250 in each sample. Special care was taken so as to count a sectioned cell only once. Particles were identified as viruses on the basis of size, staining properties and shape.

Free large VLPs (>120 nm) were counted in samples preserved with 1% glutaraldehyde (final conc.) according to Bratbak et al. (1990). Unfortunately, due to technical failure of the refrigerator used for storage, most samples were unsuitable for further use. We have only counts for transect Stns 20 to 32.

RESULTS

Hydrographical and chemical conditions

Our study area is influenced by the inflow of ocean water from the North Atlantic between Scotland and Norway, and the outflow of North Sea water along the Scandinavian coast (Lee 1970). The latter has a lower salinity as a result of mixing with fresh water run-off from the land and low salinity water entering the North Sea from the Baltic. Stratification is well known to occur in this area and can be due to heating of the surface water during spring and summer or to a strong inflow of low-saline water. A low salinity layer (34 to 35 ppt, top 20 m) at Stns 4, 7 and 15 indicated such an inflow of low-saline water and subsequent stratification. The impact of Atlantic water was clearly recorded for Stn 18, with constant temperature and salinity of 10.6°C and 35.3 ppt, respectively, over the entire water column. All other stations showed a thermal stratification. Temperature in the mixed layer (depth 30 to 40 m) ranged from 10.4 to 11°C. Salinity was between 35.2 and 35.3 ppt over the entire water colunm. The pH ranged between 7.9 and 8.1 at all stations.

All nutrient depth profiles show enhanced concentrations below the thermocline. Concentrations of silicate ranged between 0.3 and 2 µM in the mixed layer. Highest concentrations of phosphate, nitrate and nitrite in the mixed layer (Fig. 2) were measured in the region outside the high reflectance area. For phosphate and nitrate minimum values of 0.06 and 0.07 µM were recorded, respectively. Nitrite concentrations ranged between 0 and 0.15 µM. Concentrations of ammonium in the mixed layer were usually between 0.3 and 1.6 µM.

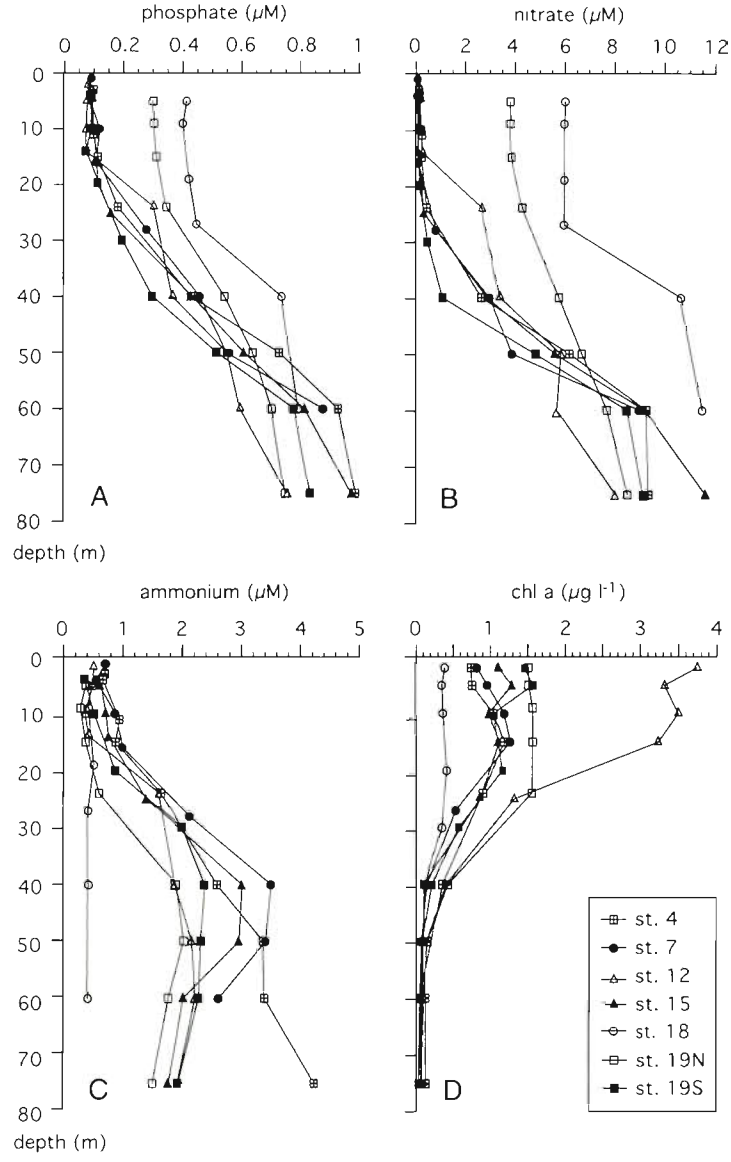

Fig. 2. Depth profiles of concentrations (µM) of (A) phosphate, (B) nitrate, (C) ammonium, and (D) concentration (µg l^{-1}) of chlorophyll *a* at the main stations during June/July 1993

Phytoplankton occurrence and bacterial production

For most stations chl *a* concentration in the mixed layer varied between 0.75 and 2.3 µg l^{-1} (Fig. 2; main stations). Exceptions were Stn 12, with values up to 3.7 µg chl *a* l^{-1}, and Stns 18 and 32, with values as low as 0.35 to 0.45 µg chl *a* l^{-1}. At all stations, cyanobacteria (*Synechococcus* sp.) were an important contributor of the algal community in terms of numbers. The North Atlantic station (Stn 18) was further characterised by high numbers of pico-eukaryotes, whereas diatoms were usually found in the borderline zone. Highest numbers of *Emiliania huxleyi* coccospheres (max. 1.8×10^6 l^{-1}) were found within the high reflectance area.

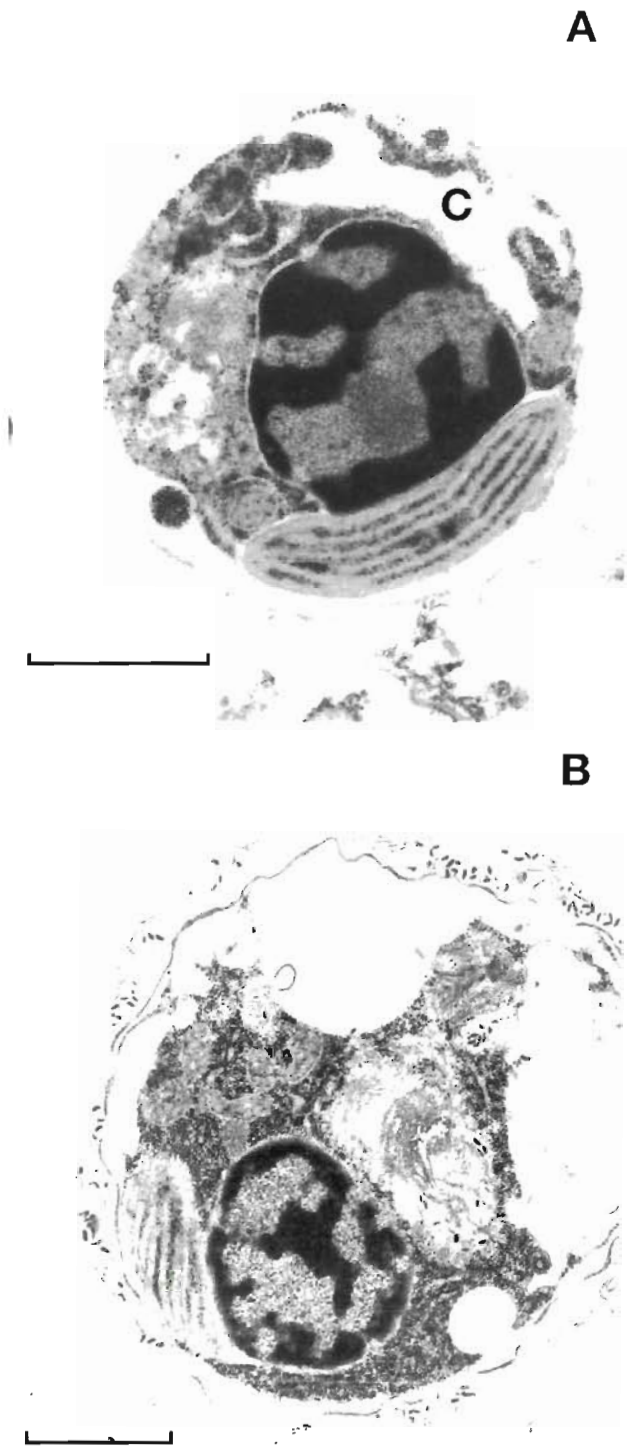

Fig. 3. Transmission electron micrographs of (A) a coccolith-producing cell of *Emiliania huxleyi* (C-cell) and (B) what could possibly be an organic scales-bearing cell of *E. huxleyi* (S-cell; J. C. Green pers. comm.). The densely stained material around the cell has been described for *E. huxleyi* S-cells by Van der Wal (1985). Note in the micrograph of the *E. huxleyi* C-cell the intracellular outline (coccoliths have been lost during the preparative procedure) of the coccolith (C). Scale bar = 1 µm

Total numbers of *E. huxleyi* cells at the other stations were lower, with maximum values of 0.6×10^6 l^{-1}. Within the high reflectance area *E. huxleyi* made up 20 to 45% of total phytoplankton biomass as chl *a* in the mixed layer and only <5% outside the high reflectance area. Using transmission electron microscopy (TEM), we found in addition to the coccolith producing cells (C-cells) significant numbers of what could possibly be organic scale-bearing cells (S-cells, Klaveness 1972, Van der Wal 1985, J. C. Green pers. comm.). It is difficult to identify the cells with certainty from TEM micrographs (Fig. 3), but the size of the cells and the form and diameter of the scales (in general 0.3 µm, and occasionally up to 0.5 µm) were comparable to the S-cells described by Klaveness (1972). The occurrence of these cells only within the centre of the bloom and the fact that only they contained exactly the same type of VPLs as the C-cells furthermore suggest that we found S-cells of *E. huxleyi* in our natural samples. If so, the actual biomass of *E. huxleyi* was higher than mentioned above because with the method used to count *E. huxleyi* cells (Van der Wal et al. 1995) only coccospheres can be detected.

Bacterial production correlated significantly to chl *a* ($p < 0.05$, $n = 39$). Bacterial production (Fig. 4; main stations) within the mixed layer ranged between 0.1 and 2 µg C l^{-1} d^{-1}, with highest values at the stations inside the high reflectance area. Maximum rates up to 2 µg C l^{-1} d^{-1} were measured at Stn 19S. Bacterial growth rates varied between 0.02 and 0.32 d^{-1}, with highest rates estimated again at Stn 19S (data not shown).

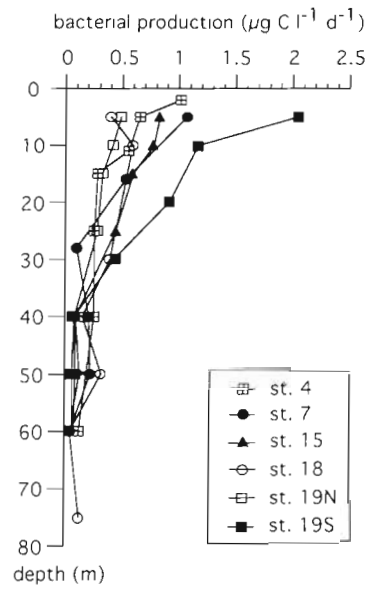

Fig. 4. Depth profiles of bacterial production (µg C l^{-1} d^{-1}) at the main stations during a cruise in the North Sea during summer 1993

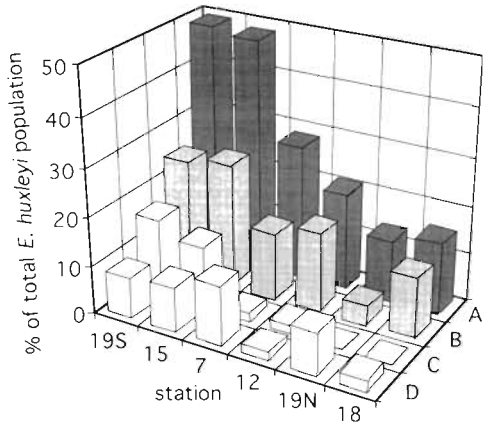

Fig. 5. Distribution of VLP-infected *Emiliania huxleyi* cells in June/July 1993 near the Shetland Islands (North Sea) as percentage of the total *E. huxleyi* population. A: Fraction of *E. huxleyi* cells visibly infected with VLPs; B: fraction of *E. huxleyi* cells containing only small VLPs; C: fraction of *E. huxleyi* cells containing both VLP size classes within the same cell; D: fraction of *E. huxleyi* cells containing only large VLPs

Virus-like particles

Cells of *Emiliania huxleyi* infected with VLPs were observed at all stations checked, although the level of infection differed markedly (Fig. 5) Highest percentages of *E. huxleyi* cells visibly infected were recorded within the high reflectance area, with maximum values of almost 50% at Stns 15 and 19S. An interesting feature was the detection of 2 types of VLPs in *E. huxleyi* (Fig. 6). Both types were hexagonal, suggesting icosahedral symmetry in 3-dimensional morphology (Caspar & Klug 1962), but the size was different. The largest VLP was 185 to 200 nm in diameter, and the smallest VLP was at least 3 times smaller (50 to 60 nm). Regularly, both size classes were present in the same cell. The small VLPs caused the higher degree of infection in *E. huxleyi*. The fraction of *E. huxleyi* cells containing both types of VLPs within the same cell was highest at Stns 15 and 19S. From Fig. 5 it becomes clear that the total level of infection caused by large VLPs (from cells with only large VLPs and with both types of VLPs) was never higher than 25%. Lowest levels (<10%) were recorded outside the high reflectance area. The average number of VLPs (both for large and small VLPs) per *E. huxleyi* cell section was always <10, with highest numbers within the high reflectance area. Maximum numbers of large VLPs (± 25) and small VLPs (± 30) per cell section were also found within this area. Stages of complete disruption of the organelles and lysis of the cells were frequently observed. Free large VLPs, counted for transect Stns 20 to 32, ranged between 1.5 and 6×10^7 l^{-1}, 2 orders of magnitude higher than the cell number of *E. huxleyi* at these stations

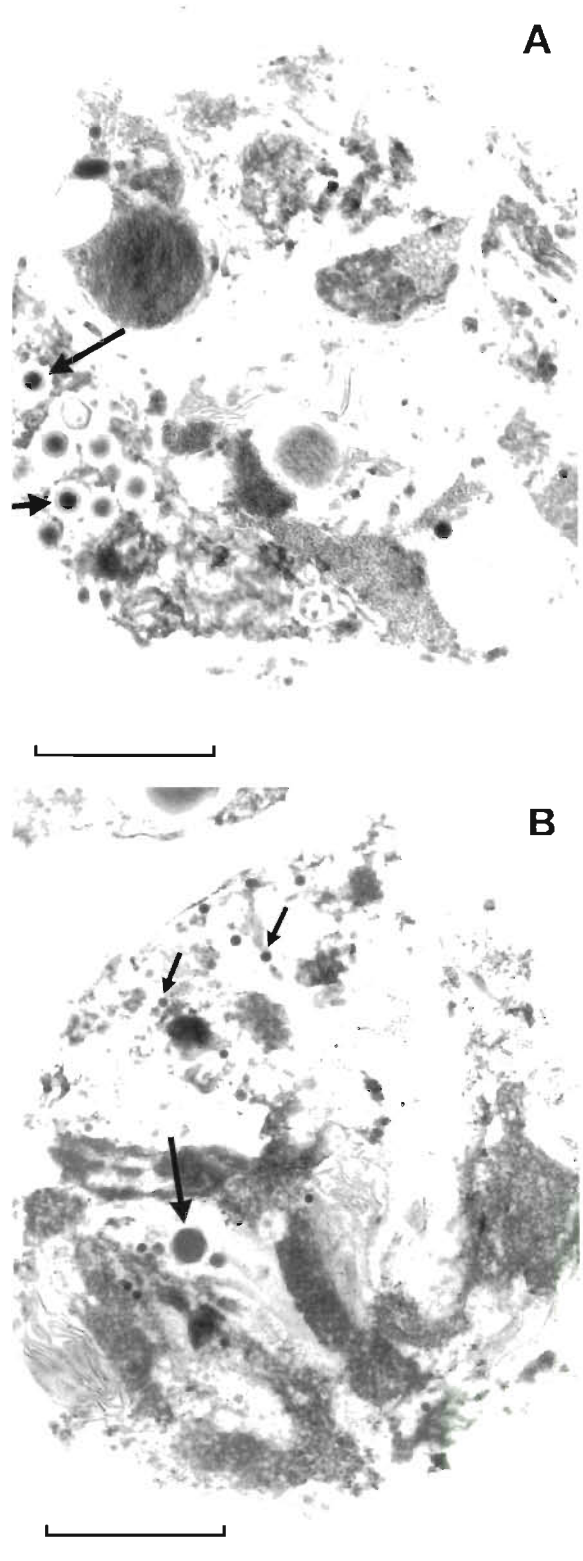

Fig. 6. *Emiliania huxleyi*. Electron micrographs of ultrathin sections showing *E. huxleyi* containing VLPs. (A) Infected cell with large VLPs (large arrows), and (B) infected cell with both VLP size classes (small VLPs indicated with small arrows). Note the moribund appearance of the cells. Scale bar = 1 µm

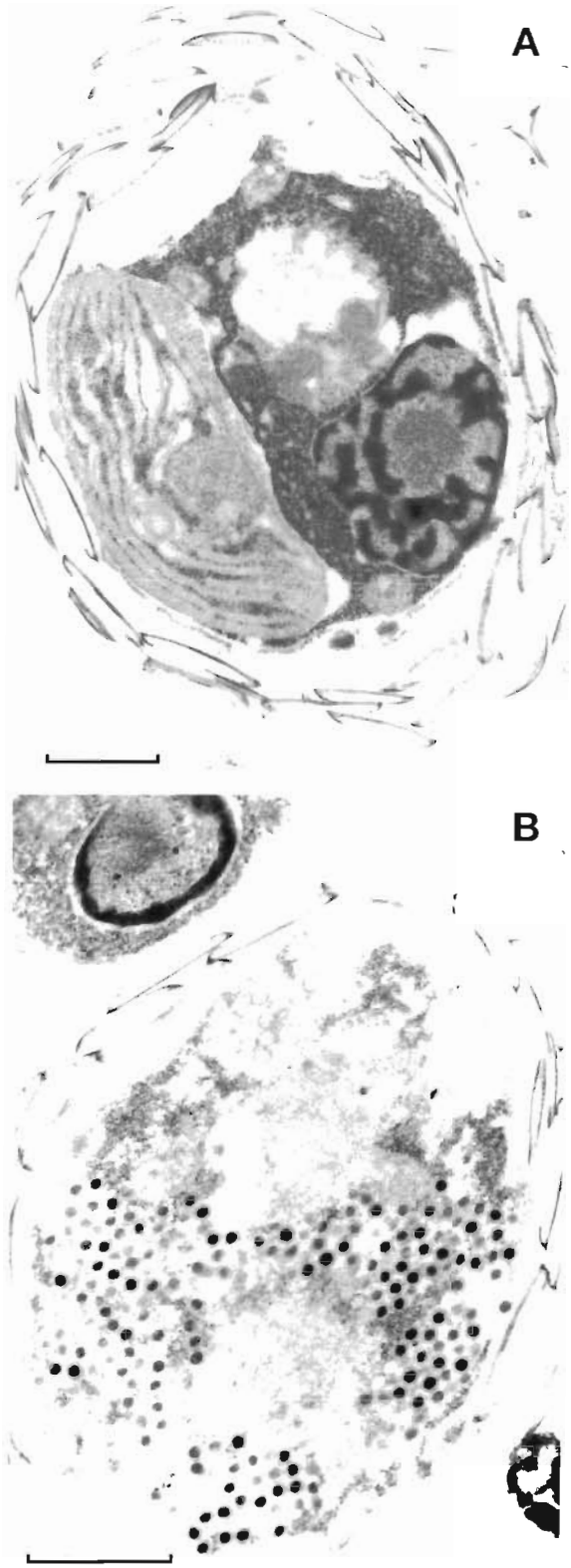

Fig. 7. *Chrysochromulina* sp. Electron micrograph of a *Chrysochromulina* sp. cell (A) without and (B) with intracellular VLPs. Note the high number of VLPs in a single thin section of an infected cell. Scale bar = 1 µm

(0.05 to 0.6 × 10^6 l^{-1}). However, free large VLPs are produced not only by *E. huxleyi*, as other organisms are also known to have VLPs in this size class (Dodds 1979, Van Etten et al. 1991, Reisser 1993, this study).

Apart from *Emiliania huxleyi*, we also found viral infection in a species of the genus *Chrysochromulina* (Fig. 7; J. C. Green pers. comm.). Although *Chrysochromulina* sp. was recorded at Stns 12, 15, 18, and 19S, infected cells were found only at Stns 15 and 19S. The VLPs were 100 to 110 nm in diameter and the average number was 170 VLPs per cell section. Only recently, Suttle & Chan (1995) isolated viruses which lysed *Chrysochromulina brevifilum* and *C. strobilus*. The high number of VLPs in a single thin section in both studies is remarkable. No viral infections in other algae were observed in the thin sections. However, we did find large VLPs in several heterotrophic organisms.

DISCUSSION

The bloom

The high reflectance area (Fig. 1) corresponded to the input of run-off water, which facilitated stratification suitable for the development of *Emiliania huxleyi* blooms (Balch et al. 1991). Both chl *a* concentration (0.7 sto 1.5 µg l^{-1}) and *E. huxleyi* cell numbers in the bloom (max. 1.8 × 10^6 ml^{-1}) are comparable to values reported for *E. huxleyi* blooms under natural conditions by Balch et al. (1991) and Malin et al. (1993). The low ratio of living to dead *E. huxleyi*, combined with a high number of detached coccoliths (Van der Wal et al. 1995), indicate that the bloom was already at an advanced stage. Within the high reflectance area (decaying phase of the *E. huxleyi* bloom), nitrogen concentrations were low. According to Eppley et al. (1969), *E. huxleyi* becomes growth-limited on either nitrate or ammonium at concentrations below 1 µM. Riegman et al. (1992) and Egge & Heimdal (1994) found *E. huxleyi* a poor competitor on nitrogen. Nitrogen limitation seem to have limited the growth of *E. huxleyi* within the high reflectance area, which is confirmed by W. Stolte (pers. comm.), who found phytoplankton within the decaying region to be nitrogen limited based on the ratio of intracellular glutamine to glutamate (an index for nitrogen limitation; Flynn 1990). No nitrogen limitation could be determined outside the bloom area.

Viral interactions

The highest level of visible viral infection in *Emiliania huxleyi* was found in the decaying phase of the

bloom (inside the high reflectance area). The higher infection level at Stn 15 as compared to Stn 7 corresponds well with the lower ratio of living to dead *E. huxleyi* coccospheres found at this station by Van der Wal et al. (1995). Up to 50% of *E. huxleyi* had mature VLPs (both types) in the cell, which is quite high compared to most reports on viral infection of phytoplankton (Dodds 1979, Proctor & Fuhrman 1990, Bratbak et al. 1993, Reisser 1993). High levels of viral infection within algal cells are reported for the green alga *Platymonas* sp. (30%; Pearson & Norris 1974), the scale-bearing freshwater alga *Paraphysomonas* (75%; Preisig & Hibberd 1984) and the red tide alga *Heterosigma akashiwo* (11.5%; Nagasaki et al. 1994). However, these results were obtained either from cultured algae or from natural samples only after incubation in the laboratory. As far as we are aware, this is the first study reporting on high levels of visible viral infection of phytoplankton under natural conditions. The North Atlantic station (Stn 18, which is located far outside the area of the *E. huxleyi* bloom) was sampled for reference. Surprisingly, we did not find a low level of visible viral infection in the low numbers of *E. huxleyi* present. A possible reason for this could lie in the paradoxal situation of high nutrient and low chl *a* concentrations, indicative of possible iron-limited growth (K. Timmermans pers. comm.).

The occurrence of 2 types of VLPs within the same cell has also been observed in *Pyramimonas orientalis* (Moestrup & Thomsen 1974), *Cryptomonas* sp. (Pienaar 1976), and *Chromophysomonas cornuta* (Preisig & Hibberd 1984). For *Emiliania huxleyi* only the occurrence of large VLPs has previously been recorded (Bratbak et al. 1993). The percentage of *E. huxleyi* cells in thin sections visibly infected with the large type of VLPs was between 15 and 25% in the decaying phase of the bloom, which again is high compared to the 3% during the collapse of an *E. huxleyi* bloom in mesocosms reported by Bratbak et al. (1993). These authors stated that their results may have been seriously underestimates, because cells containing large VLPs lysed during centrifugation and cells with only a few large VLPs may have escaped their attention. Although the first possible source of error also applies to our data, the frequently found moribund cells suggest no great loss of the delicate cells. Our thorough screening of the thin sections would seem to rule out the latter source of error.

A high percentage of cells containing intracellular VLP can be the result of a high viral infection rate or of synchronized induction of temperate viruses carried by a lysogenic host population. A bloom situation with a high host density will increase viral proliferation. However, it has been suggested that most viruses are temperate rather than virulent (Freifelder 1987, Bratbak et al. 1990), so the viral production rate will then depend on the induction rate in lysogenic host cells. In our study, the highest levels of viral infection were recorded in the nitrogen-limited decaying phase of the *Emiliania huxleyi* bloom. Enhanced viral production under low nitrogen concentrations has already been reported for *E. huxleyi* blooming in mesocosms (Bratbak et al. 1993). Since high levels of visible viral infection will lead to high lysis rates of the host cells, we conclude that viral lysis was an important factor in controlling the bloom of *E. huxleyi* in the North Sea during summer 1993.

Viral lysis

The 50 to 70 nm thin sections contain about 2.5% of the cell content of an *Emiliania huxleyi* cell. Using average numbers of 4 large VLPs (with a diameter of 185 to 200 nm) and 9 small VLPs (50 to 60 nm diameter) per cell section, a total burst size of roughly 400 VLPs per cell was estimated for *E. huxleyi* in the decaying phase of the bloom. Comparable values were reported for *E. huxleyi* (Bratbak et al. 1993) and *Synechococcus* spp. (Suttle & Chan 1993). Combined with the high level of viral infection, it is clear that viruses were an important source of mortality for *E. huxleyi* (Proctor & Fuhrman 1990). In order to estimate the amount of released algal cellular carbon due to viral lysis, we assumed that all visibly infected *E. huxleyi* cells lysed within 24 h. This assumption is based on the frequent occurrence of moribund, infected *E. huxleyi* cells and free VLPs in the ultrathin sections of stations within the decaying phase of the bloom, suggesting that *E. huxleyi* was already at a late stage of the latent period (Proctor et al. 1993). Viral lysis of *E. huxleyi* was highest within the high reflectance area, with rates of 2 to 4 µg cellular C l^{-1} d^{-1}. In the borderline zone and outside the high reflectance area rates were always <0.5 µg C l^{-1} d^{-1}.

The clear relationship found between bacterial production and phytoplankton abundance indicates that phytoplankton (by excretion and/or lysis) was the dominant source of organic matter for bacteria. Assuming a bacterial growth conversion efficiency of 0.3 (an intermediate value obtained on phytoplankton lysis products and detritus by Newell et al. 1981, Biddanda 1988 and Van Wambeke 1994), we estimated the bacterial carbon demands at VLP sampling depths (15 to 25 m) to be 1 to 3 µg C l^{-1} d^{-1}. Our data suggest that viral lysis of *Emiliania huxleyi* within the decaying part of the bloom may have been an important source of organic carbon for bacteria. The nutrient-rich organic components released after cell lysis are very likely utilized quickly by bacteria. For a better

understanding of the role of viral lysis of phytoplankton in the microbial food web we need more research on the level of viral infection within algae varying with depth.

CONCLUSIONS

From our data we conclude that viruses can be important agents for *Emiliania huxleyi* bloom decay under natural conditions. The severe viral infection of *Chrysochromulina* sp. reported in this study is another example of the regulating effect of viruses on population dynamics. Our results furthermore suggest that viral lysis of *E. huxleyi* can significantly influence the microbial food web under natural blooming conditions by introducing nutrient-rich organic components into the system. This study seems to confirm the results of the modified model of Fuhrman & Suttle (1993), in which the authors included not only the viral infection of bacteria, but also of phytoplankton. With a phytoplankton mortality rate due to viruses of only 10%, they observed an increased bacterial production which was 33% above the level without viruses. Although our study clearly shows that viral infection should be included in models concerning carbon cycling, we want to stress that more knowledge on whether viruses are lytic or lysogenic is needed. And, if the viruses are lysogenic, the factors which induce viral lysis of *E. huxleyi* and phytoplankton in general need to be known. Also, the occurrence of what possibly were S-cells of *E. huxleyi* raises the question of whether or not the different stages are equally vulnerable to viral infection or induction. Further study is needed of hypotheses such as: (1) S-cells are more easily infected by viruses than C-cells, because they have no coccoliths to protect them; or (2) the transition from C-cell to S-cell, seen during senescence (Klaveness 1972), induces virus proliferation.

With the inclusion of viruses in models, we should keep in mind that, although small VLPs are generally classified as bacteriophages (Cochlan et al. 1993), small VLPs are also regularly recorded in phytoplankton (Van Etten et al. 1991, this study). If a significant fraction of viruses or VLPs from eukaryotes are in the size range of <70 nm, the viral mortality of the bacterial fraction has often been overestimated. This may, for example, partly explain the lack of balance in the C-budget of Bratbak et al. (1992)

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