Isolation of viruses responsible for the demise of an *Emiliania huxleyi* bloom in the English Channel

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This study used analytical flow cytometry (AFC) to monitor the abundance of phytoplankton, coccoliths, bacteria and viruses in a transect that crossed a high reflectance area in the western English Channel. The high reflectance area, observed by satellite, was caused by the demise of an *Emiliania huxleyi* bloom. Water samples were collected from depth profiles at four stations, one station outside and three stations inside the high reflectance area. Plots of transect data revealed very obvious differences between Station 1, outside, and Stations 2–4, inside the high reflectance area. Inside, concentrations of viruses were higher; *E. huxleyi* cells were lower; coccoliths were higher; bacteria were higher and virus:bacteria ratio was lower than at Station 1, outside the high reflectance area. This data can simply be interpreted as virus-induced lysis of *E. huxleyi* cells in the bloom causing large concentrations of coccoliths to detach, resulting in the high reflectance observed by satellite imagery. This interpretation was supported by the isolation of two viruses, *Eh*V84 and *Eh*V86, from the high reflectance area that lysed cultures of *E. huxleyi* host strain CCMP1516. Basic characterization revealed that they were lytic viruses approximately 170 nm–190 nm in diameter with an icosahedral symmetry. Taken together, transect and isolation data suggest that viruses were the major contributor to the demise of the *E. huxleyi* population in the high reflectance area. Close coupling between microalgae, bacteria and viruses contributed to a large organic carbon input. Consequent cycling influenced the succession of an *E. huxleyi*-dominated population to a more characteristic mixed summer phytoplankton community.

INTRODUCTION

Emiliania huxleyi (Haptophyta) is a unicellular alga found throughout the world's oceans. It is best known for its immense coastal and open ocean blooms at temperate latitudes that can cover 10,000 km^2 or more. The size and intensity of these blooms makes *E. huxleyi* important for nutrient and CO_2 cycling and biogenic sulphur production (in the form of dimethyl sulphide (Malin et al., 1994)) in the marine environment. Consequently it is a key species for current studies on global biogeochemical cycles and climate modelling (Charlson et al., 1987; Westbroek et al., 1993, 1994).

Emiliania huxleyi can be easily viewed by satellite imagery (Figure 1) (Holligan et al., 1993) since they are characterized by high light backscatter caused by coccoliths, elegant calcium carbonate scales, which *E. huxleyi* produces intracellularly and sequesters over its cell surface (Westbroek et al., 1984). Whilst *E. huxleyi* is blooming it is constantly producing and shedding coccoliths into the surrounding water. However, a bloom reveals itself most intensively on satellite images on its demise when the *E. huxleyi* cells in the bloom die and shed their remaining coccoliths. Subsequently, there is a vast export of coccoliths to the seabed (Ziveri et al., 2000), hence causing one of the largest long term sinks of inorganic carbon on earth.

One of the main mechanisms for the termination of *E. huxleyi* blooms is thought to be viral lysis. Several studies have shown that virus numbers typically increase following the demise of an *E. huxleyi*-dominated bloom (Bratbak et al., 1993, 1996; Brussaard et al., 1996; Castberg et al., 2001). It is clear from these investigations that viruses are intrinsically linked to the decline of *E. huxleyi* blooms. However, there have been no reports on the isolation of *E. huxleyi*-viruses, a necessary step in their characterization.

Viruses are abundant in aquatic environments (Bergh et al., 1989) and it is likely that most microbial organisms have viruses that infect them. Their contribution to nutrient (Wilhelm & Suttle, 1999) and biogeochemical (Fuhrman, 1999) cycling, as well as their roles in influencing structure and diversity of microbial and phytoplankton communities are widely accepted (Fuhrman, 1999; Wommack & Colwell, 2000). Viruses have also been observed to infect a wide range of aquatic algae (van Etten et al., 1991; van Etten & Meints, 1999), including bloom forming marine phytoplankton (Nagasaki et al., 1994; Jacobsen et al., 1996; Nagasaki & Yamaguchi, 1997; Sandaa et al., 2001).

In this paper we have used analytical flow cytometry to determine virus, bacteria, phytoplankton and coccolith concentrations through vertical profiles in a high reflectance area off the coast of Plymouth in the English Channel during July 1999. In addition, we describe the isolation of two *E. huxleyi*-viruses using seawater collected from this high reflectance area.
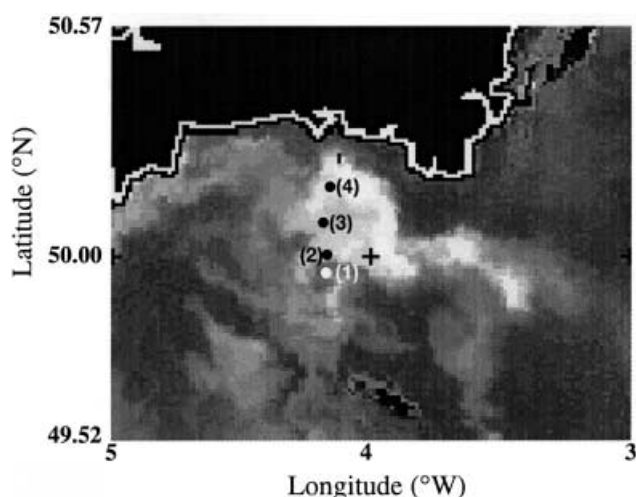
Figure 1. True colour satellite image of a high reflectance *E. huxleyi* bloom south of Plymouth, UK. Cruise track with approximate positions of Station 1 (white dot) and Stations 2–4 (black dots); 49°57.98′N 4°10.00′W (Station 1); 50°0.00′N 4°9.89′W (Station 2); 50°4.59′N 4°10.56′W (Station 3) and 50°13.79′N 4°9.59′W (Station 4). The cross in the middle of the figure (+) marks grid coordinate 50°N 4°W. Image courtesy of Plymouth Marine Laboratory, UK, Remote Sensing Group.

MATERIALS AND METHODS

Study sites

The main study was conducted on the RV 'Squilla' on 30 July 1999 in an area of high reflectance associated with *Emiliania huxleyi*. The study area was located in the English Channel, off the South Devon/Cornwall coast (Figure 1). Samples were collected from four stations (Figure 1) along a northerly cruise transect with one station outside 49°57.98′N 4°10.00′W (Station 1) and three within the high reflectance area; 50°0.00′N 4°9.89′W (Station 2); 50°4.59′N 4°10.56′W (Station 3) and 50°13.79′N 4°9.59′W (Station 4). In addition, one depth profile was collected from Station L4 (50°15′N 04°13′W), south of Plymouth, UK, in the English Channel on 26 July 1999.

Sample collection

Seawater was collected from a depth profile down to 25 m, in 5 m intervals, using a rosette of 5-l Niskin bottles connected to a stainless steel cable operated by an on-deck winch. For each depth, a 1.5 ml aliquot of seawater was fixed in 0.5% (final concentration) glutaraldehyde for between 2 h and 7 h at 4°C for subsequent virus and bacteria enumeration. Samples were then stored frozen at −20°C until analysis one week after sampling. Samples for *E. huxleyi* and coccolith enumeration were collected from the rosette bottle sampler in sterile BlueMax™ 50-ml polypropylene conical centrifuge tubes (Becton Dickinson Labware, Franklin Lakes, USA) and maintained in the dark at ambient seawater temperature in covered flow-through tanks. Samples were returned to the laboratory and analysed immediately. Samples for transmission electron microscopy (TEM) (20 ml) were preserved in 2% glutaraldehyde then stored at 4°C until further analysis. Samples for virus isolation were pre-filtered through a 0.45 µm Supor (Gelman) filter and stored at 4°C in the dark.

Analytical flow cytometry (AFC) analysis

All analyses were conducted using a Becton Dickinson FACSort™ flow cytometer equipped with an air-cooled 15 mW laser providing 488 nm light with a standard filter set-up which measured chlorophyll fluorescence (>650 nm), phycoerythrin fluorescence (585 nm ±21 nm), forward scatter (light scattered in the same plane as the laser beam) and side scatter (light scattered at ninety degrees to the plane of the vertically polarized laser). Virus and bacteria enumeration was performed on each sample at the same time using methods adapted from Marie et al. (1999). Fixed frozen samples were defrosted at room temperature and diluted ten-fold in TE buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA) (pre-filtered through a 50 kDa VivaFlow 50 'flip-flow' system (Sartorius) then autoclaved) containing 0.1% (w/v) Triton X-100. Diluted samples were heated at 65°C for 15 min in the dark in the presence of SYBR Green I (Molecular Probes) at a final concentration of 10^{-4} of the commercial stock solution. Samples were analysed by AFC for two min at a flow rate of 12 µl min^{-1}. Data acquisition was triggered on green fluorescence and the detection threshold was adjusted to cut out most of the instrument noise from the blank. Viruses in *E. huxleyi* lysates were prepared as for seawater, with the exception that lysates were diluted 1000-fold prior to analysis. To prepare the blank, 10 kDa-filtered seawater was diluted ten-fold with TE buffer containing 0.1% Triton X-100 and SYBR Green I prior to AFC analysis. Measurements of side scatter and green fluorescence were made using CELLQuest™ software (Becton Dickinson) with log amplification on a four-decade scale. Data analysis was carried out using WinMDI 2.8 software (Joseph Trotter—freely available from http://facs.scripps.edu/). Scatter-plots of side-scatter vs green fluorescence were used to discriminate between different virus groups and bacteria. Large viruses could be easily discriminated from small viruses since there was at least an order of magnitude difference in their side scatter values (Wilson et al., in press).

Emiliania huxleyi and detached coccoliths were analysed separately by AFC using the same instrument as above. Data acquisition for *E. huxleyi* was triggered on chlorophyll fluorescence, using laboratory cultures to set rejection gates for background noise. Data acquisition for detached coccoliths was triggered on side scatter. Samples were analysed for three min at a flow rate of 91 (±1) µl min^{-1} and data was stored in listmode format then read using WinMDI 2.8 as above. Scatter-plots of side scatter vs chlorophyll fluorescence were produced for coccolithophores, and side scatter vs forward scatter for detached coccoliths. The scatter plots were also used to obtain particle counts for four other groups of phytoplankton (autotrophic dinoflagellates (<50 µm), cryptophytes, other nanophytoplankton and picoeukaryotes).

Transmission electron microscope analysis

Gluteraldehyde-preserved samples were examined by TEM following ultracentrifugation of the particles directly onto formvar-coated copper grids (Børsheim et al., 1990). Centrifuge tubes were modified by moulding a flat supporting bottom of two-component epoxy glue (slow

setting Araldite®) by centrifuging the glue onto the bottom of the tube then leaving it to set. Modified centrifuge tubes were filled with preserved water and formvar-coated grids attached to nitro-cellulose filter paper were submersed below the surface and left to sink with the copper grids upwards. The samples were centrifuged in a Beckman L8-70M ultracentrifuge using a swing-out SW40 rotor at 30,000 rpm (160,000*g*) for 1 h at 10°C. Following centrifugation, the supernatant was poured off and the grid was air-dried. For examination of viruses from lysed cultures, a drop of lysate was added to a formvar-coated grid and left for approximately 30 min. Excess liquid was then wicked off and left to air dry. All grids were post-stained with a saturated solution (approximately 3% w/v) of uranyl acetate. TEM analysis was conducted on a JEOL JEM 200CX TEM operated at 160 kV at magnifications ranging from ×20,000 to ×100,000.

### Scanning electron microscope (SEM) analysis

To view viruses attached to host *E. huxleyi* strain CCMP1516 cells, virus/host suspensions were fixed in 5% glutaraldehyde, sedimented by centrifugation, dehydrated in an acetone series and critical point dried using acetone in a Polaron 3000 apparatus. Dried infected cells were shaken onto double-sided sticky tape on a specimen support stub. Specimens were sputter-coated with gold in an EMscope coating unit. Viruses attached to cells were viewed in a JEOL JSM 35C scanning electron microscope operated at 20 kV.

Virus isolation and plaque purification

Virus lysates were obtained by adding 1 ml of 0.45 μm-filtered seawater, collected from a range of depths from Station 4 (50°13.79′N 4°9.59′W), to exponentially growing host *E. huxleyi* strain CCMP1516 (http://ccmp.bigelow.org/) in f/2 medium (Guillard, 1975). Once clearing of the host culture was observed, the lysate was passed through a 0.2 μm syringe filter (Gelman). A virus clone was obtained by serial dilution to extinction three times. The highest dilution to lyse the host culture was passed through a 0.2 μm syringe filter (Gelman) for use in subsequent inoculations.

The dilution to extinction virus lysate was further purified by three-rounds of a plaque purification assay. Serial dilutions (100 μl) of the lysate were added to 0.9 ml of a 100× concentration of exponentially growing *E. huxleyi* cells and incubated at 15°C under constant illumination for two hours to allow viruses to adsorb. The virus-host suspension was mixed in 3 ml of molten 0.4% (w/v) electrophoresis grade agar (40°C), made in f/2 medium, and poured onto 1.5% (w/v) electrophoresis grade agarose plates made in f/2 medium. Plaques were usually visible after four-days incubation at 15°C in a cooled illuminated incubator (Sanyo) set on a 16:8 h L:D cycle. A 1-mm diameter plug containing a single plaque was lifted from the plate using a sterile Pasteur pipette, resuspended in 0.5 ml f/2 medium and used for subsequent inoculations. Virus stocks (either fresh 0.2 μm-filtered lysates or plaque resuspensions) were stored at 4°C in the dark.

Infection kinetics of virus clones were determined by adding dilutions of the virus to exponentially growing
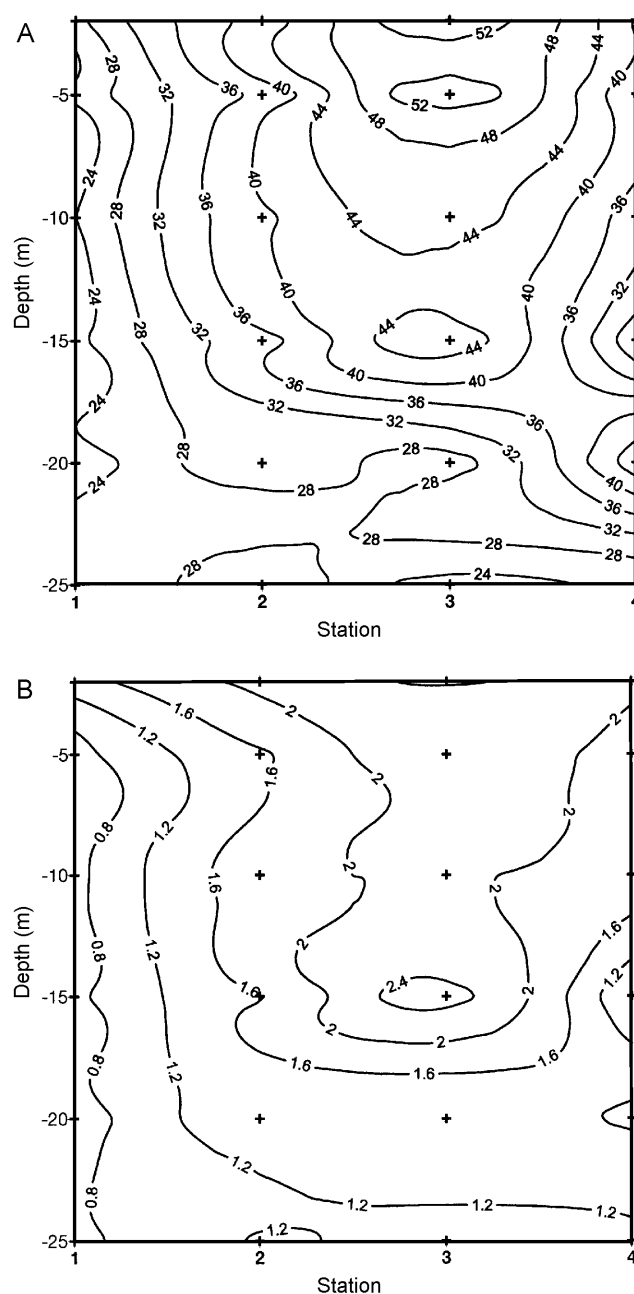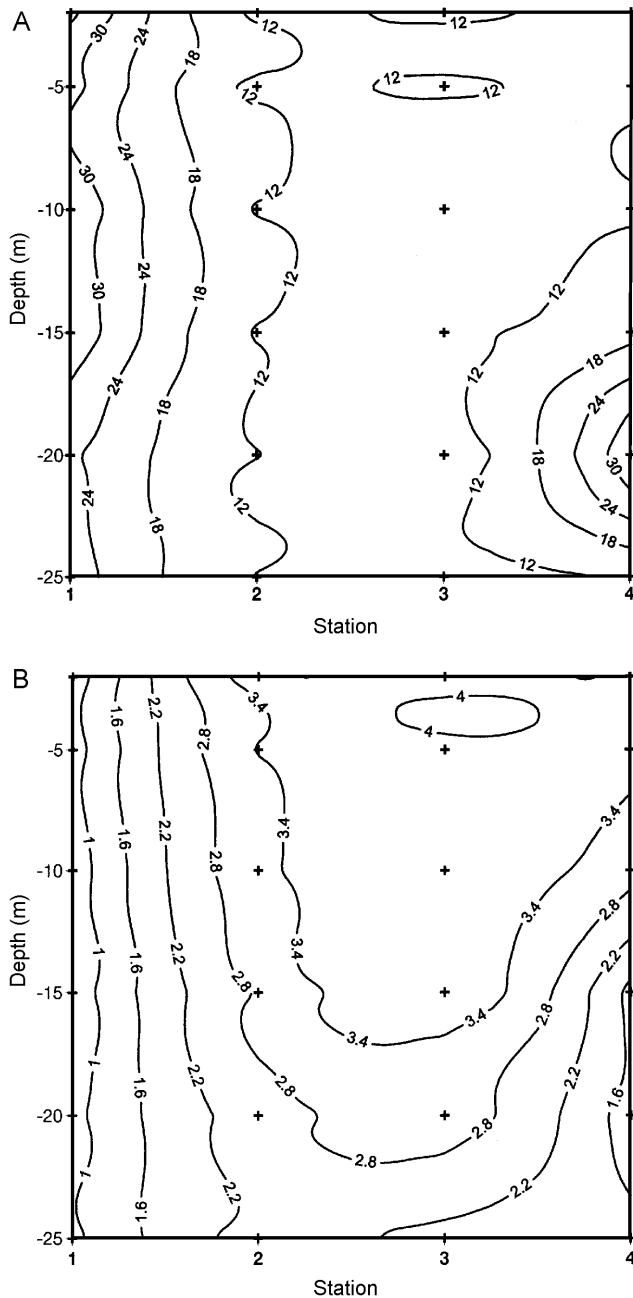
Figure 2. Contour plots of virus concentrations through the water column from Stations 1–4 on the cruise transect, determined by AFC. (A) Total virus ($\times 10^6$ virus particles ml^{-1}); and (B) large virus ($\times 10^6$ virus particles ml^{-1}) concentrations. Crosses indicate the depth at which samples were collected.

E. huxleyi strain CCMP1516 host cultures and following their growth compared to non-infected controls. *E. huxleyi* in the cultures were enumerated in a Neubauer improved haemocytometer.
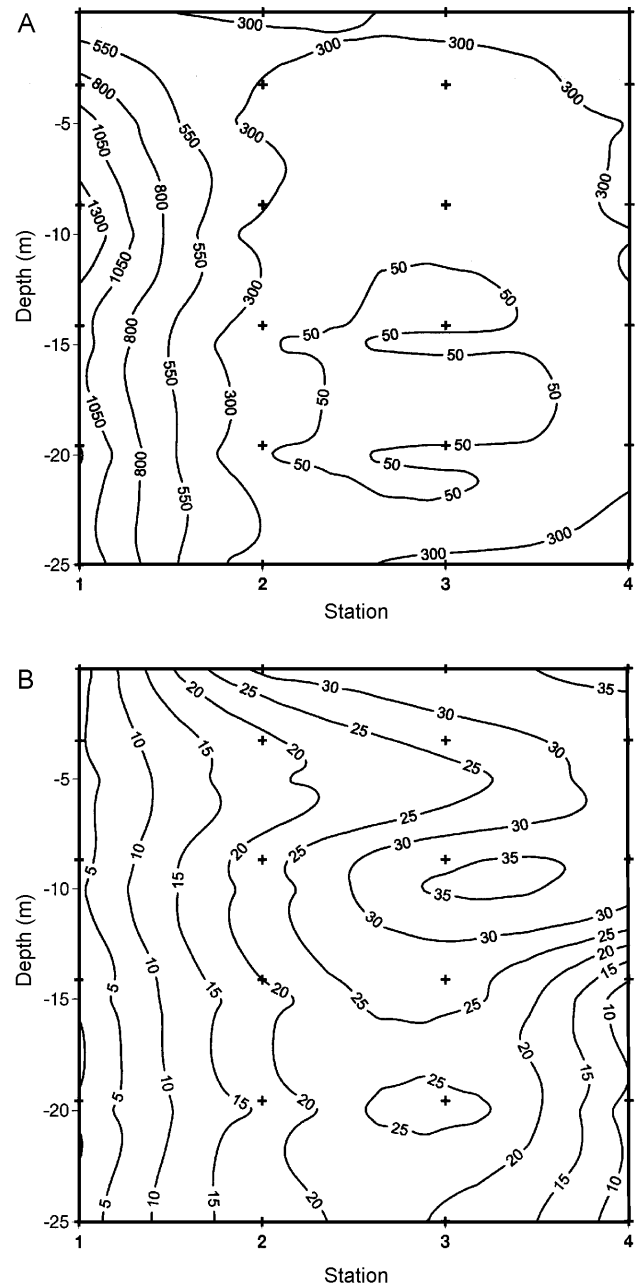
RESULTS

Transect data

The high reflectance area was milky in appearance. The 32 km transect started at Station 1 just south of the high reflectance area, approximately 48 km south of Plymouth, UK, where the milky appearance of the surface water was less apparent. Surface chlorophyll concentrations inferred

Figure 3. (A) Contour plots of virus:bacteria ratios and (B) bacteria concentrations ($\times 10^6$ cells ml^{-1}) through the water column determined by AFC, from Stations 1–4 on the cruise transect. Crosses indicate the depth at which samples were collected.

Figure 4. (A) Contour plots of *E. huxleyi* (cells ml^{-1}) and (B) coccolith ($\times 10^4$ liths ml^{-1}) concentrations through the water column determined by AFC, from Stations 1–4 on the cruise transect. Crosses indicate the depth at which samples were collected.

from satellite imagery ranged from 1.4 μg l^{-1} at Station 1 to 2–7.5 μg l^{-1} at Stations 2–4 (data not shown). Details of the physical structure of the bloom will be described elsewhere (T. Smyth, personal communication). Briefly, Station 1 had a deep mixed layer (>30 m) compared to Stations 2–4 which had a thermocline at approximately 25 m. In addition, the surface water temperature increased by approximately 1°C towards the northern end of the transect.

Total virus concentrations ranged from 2.1$\times 10^7$ ml^{-1} to 5.5$\times 10^7$ ml^{-1} with the lowest concentrations (2.1–2.7$\times 10^7$ ml^{-1}) being observed at Station 1 (Figure 2A). A similar pattern was observed for large-virus-like particles (LVLP), with concentrations ranging from 5.6 to

14.0$\times 10^5$ ml^{-1} at Station 1, increasing to 8.0 to 25.4$\times 10^5$ ml^{-1} inside the high reflectance area (Figure 2B). At Stations 2–4 virus concentrations were higher in the surface layers and the highest concentrations were observed at Station 3. There was a large difference in virus to bacteria ratios between Station 1, which averaged 33.0 (± 5.2) through the water column, and Stations 2–4, which averaged 13.9 (± 5.9) (Figure 3A). Bacteria concentrations were also high inside the high reflectance area (1.3–4.0$\times 10^6$ ml^{-1}) compared to Station 1 (6.4–8.6$\times 10^5$ ml^{-1}) (Figure 3B).

Emiliania huxleyi concentrations were highest at Station 1 ranging from 390 to 1500 cells ml^{-1} with increasing depth.
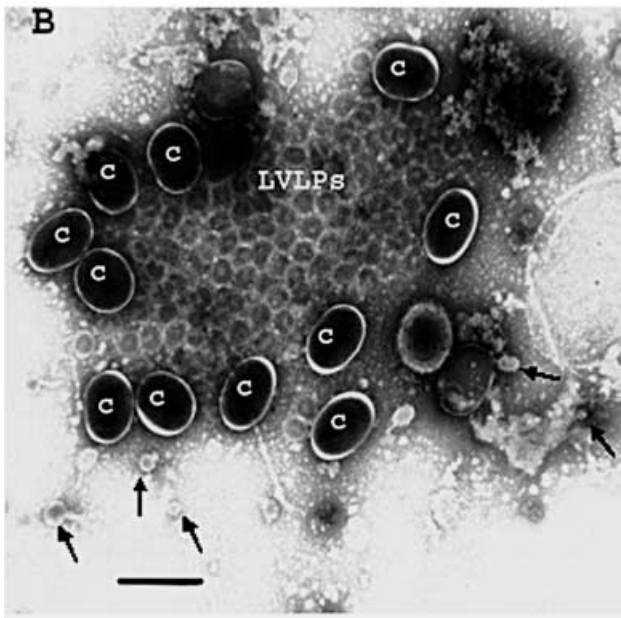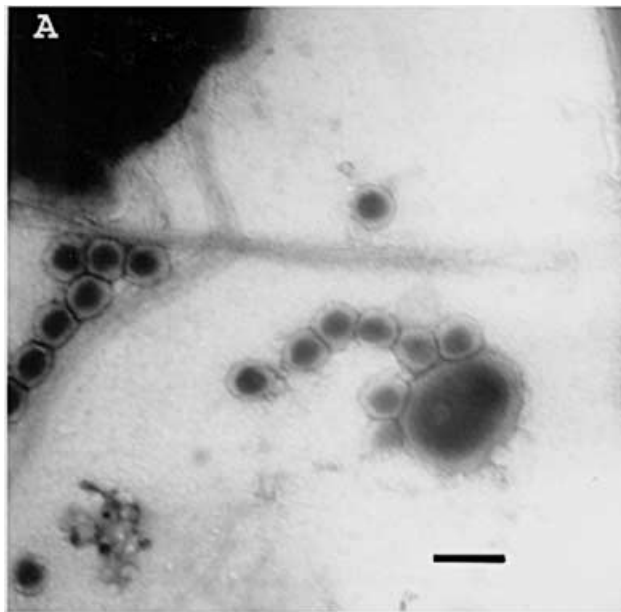
Figure 5. Transmission electron microscope analysis of fixed seawater samples collected from Station 4 (50°13.79′N 4°9.59′W) of the cruise transect. (A) Numerous large virus-like particles (LVLP) approx 150 nm–200 nm in size were observed. (B) Large VLPs bursting from what we assumed was an *Emiliania huxleyi* cell surrounded by coccoliths (c). Small arrows indicate smaller VLPs that were also abundant in all water samples. Scale bars: A, ∼250 nm; B, ∼500 nm.

However, these concentrations dropped significantly inside the high reflectance area, ranging from less than 100 to a maximum of 400 cells ml^{-1} (Figure 4A). Conversely, detached coccolith concentrations were low outside the bloom in Station 1 (1–3×10^4 coccoliths ml^{-1}) and an order of magnitude higher inside the bloom (0.7–4×10^5 coccoliths ml^{-1}) (Figure 4B). *Emiliania huxleyi* dominated the phytoplankton community outside the high reflectance area at Station 1. Their numerical abundance was on average 48% (±13%) of the total phytoplankton community through the water column, compared to an average of
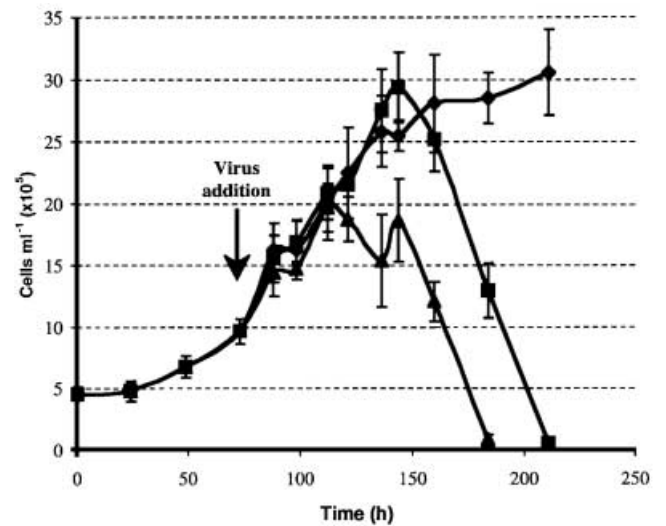
Figure 6. Infection kinetics of *Eh*V86 propagated on *Emiliania huxleyi* strain CCMP1516 at multiplicity of infections of approximately 0.1 (▲) and 0.001 (■) compared to a non-infected control (◆).

7.8% (±7.7%) at Stations 2–4. Other phytoplankton determined by AFC included autotrophic dinoflagellates (<50 μm), other nanophytoplankton, cryptophytes and picoeukaryotes, whose concentrations all increased between Station 1 and Stations 2–4 inside the high reflectance area (data not shown).

Transmission electron microscope analysis of preserved samples collected from all stations revealed there was a large morphological diversity of virus like particles (VLPs) present. The most abundant VLPs were in the 40 nm–100 nm size range (see arrows in Figure 5B). In addition, there was a higher number of larger particles in the 120 nm–200 nm size range (Figure 5A), consistent with the high concentrations observed by AFC (Figure 2B). In some fields of view LVLPs were observed bursting from *E. huxleyi* cells, since they were surrounded by what appeared to be oval shaped coccoliths (Figure 5B). In each of these images between 120 and 150 LVLPs were associated with each *E. huxleyi* cell, however, on closer inspection it looked as if there was more than one layer of LVLPs, suggesting burst sizes >300.

Virus isolation

Seawater samples from all depths caused lysis of *E. huxleyi* strain CCMP1516 host cultures within ten days. Lysis was evident when the culture turned from a normal healthy milky-green colour to a milky white colour. In addition, a characteristic sulphurous odour was also indicative of culture lysis. Serial dilutions of these lysed cultures down to 10^{-7} ml^{-1} lysed fresh exponentially growing *E. huxleyi* strain CCMP1516 host cultures, which indicated that lysed cultures contained at least 10^7 infectious virus particles ml^{-1}. Two clonal isolates were identified by dilution to extinction assays; *Eh*V84, which was originally isolated from a seawater sample collected at a depth of 15 m from Station L4 (50°15′N 04°13′W) on 26 July 1999; and *Eh*V86, which was originally isolated from a seawater sample collected at a depth of 15 m from Station 4 of the transect (50°13.79′N 4°9.59′W) on 30 July 1999. Complete
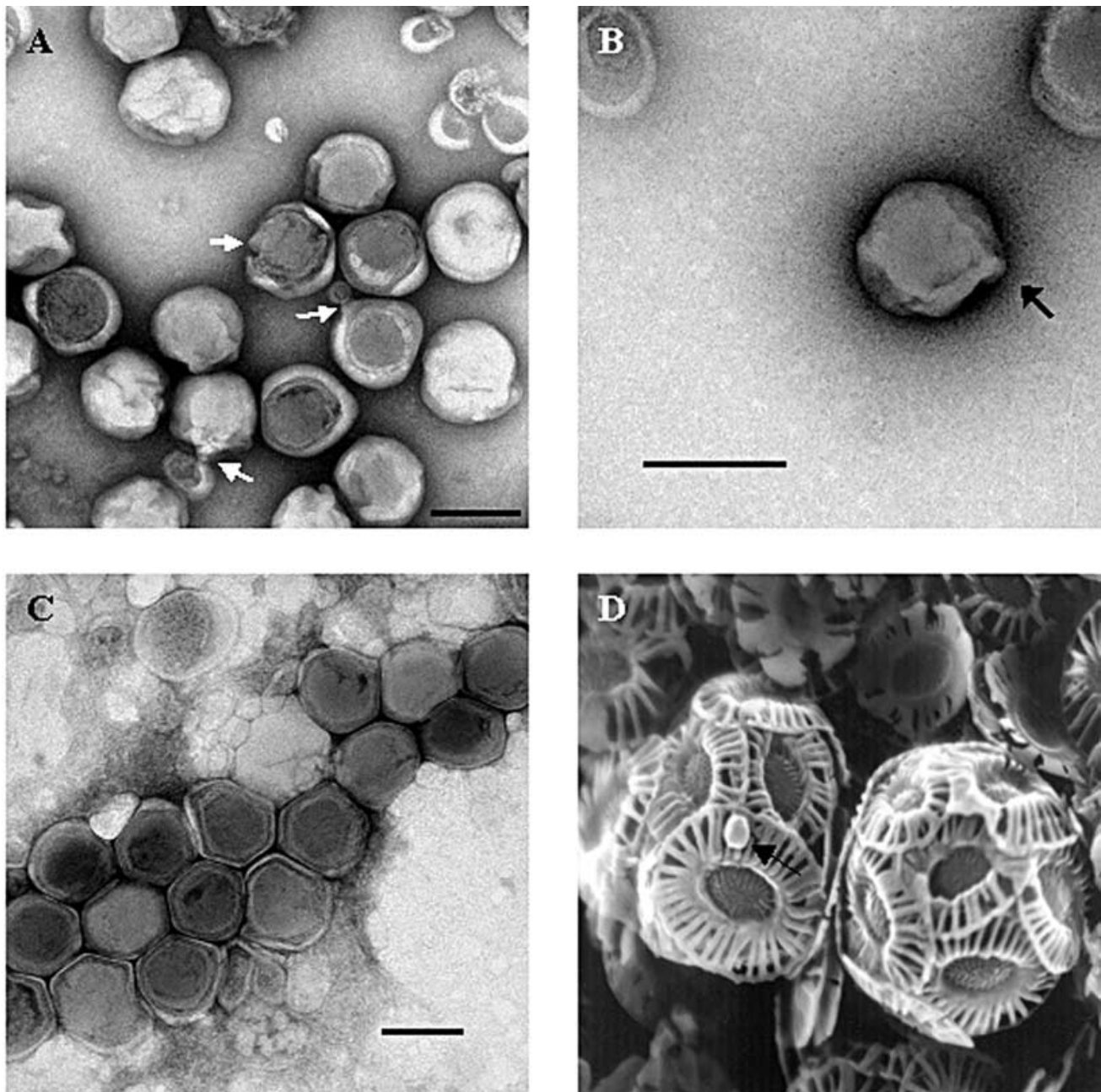
Figure 7. Transmission electron microscope (TEM) and scanning electron microscope (SEM) analysis of *Emiliania huxleyi*-specific virus isolates. (A) and (B) TEMs of *Eh*V84, the arrows indicate possible tail stubs that may be involved in attachment. (C) TEM of *Eh*V86. (D) SEM of *Eh*V86 (arrowed) attached to an *E. huxleyi* cell. Scale bars: A–C, ∼190 nm; D, no scale information available.

lysis of *E. huxleyi* strain CCMP1516 host cultures by *Eh*V86 was observed four days (multiplicity of infection (MOI) 0.1) or five days (MOI 0.001) after virus addition (Figure 6). The TEM analysis of *Eh*V84 and *Eh*V86 revealed they were morphologically similar, icosahedral particles with diameters of 191 nm (±13 nm) (*Eh*V84) (Figure 7A,B) and 170 nm (±17 nm) (*Eh*V86) (Figure 7C). On some VLPs a small tail stub was visible (arrowed in Figure 7A,B) indicating a potential attachment mechanism. The SEM analysis of an *Eh*V/host suspension revealed several images of VLPs of the correct approximate size attached to the surface of an *E. huxleyi* cell (Figure 7D). However, it cannot be ruled out that these particles were binding to the surface of the *E. huxleyi* cells non-specifically.

Visual inspection of the plates during the plaque purification procedure revealed that *E. huxleyi* grew uninhibited in the plates, looking almost clear on day one to dark green after 2–3 weeks. Circular plaques approximately 1 mm diameter were observed after 4-d incubation (results not shown) for both *Eh*V84 and *Eh*V86. The size of the plaques gradually increased to approximately 8 mm diameter after 2–3 weeks incubation or when the cells

stopped growing on the agar plate. A 1 mm plaque was shown to contain approximately 10^4 plaque forming units (pfu) following further propagation.

DISCUSSION

The occurrence of a high reflectance bloom in the English Channel (Figure 1), only a few kilometres from our laboratory, presented an ideal opportunity to determine if viruses were involved in its dying stages. The high reflectance of such blooms, which allows them to be visualized by satellite imagery, is caused by backscattering of light by coccoliths detached from the *Emiliania huxleyi* cells (Gordon & Du, 2001). Coccoliths form an elaborate shell around the cells. They are produced intracellularly in a specialized vacuole (Westbroek et al., 1984), then extruded and incorporated into an extracellular coccosphere. Coccoliths are shed continuously, however, when the cell dies or lyses, large concentrations of coccoliths are detached and the surrounding water turns a characteristic milky white and it is during this period the bloom can be easily observed by satellite imagery. A vertical flux then follows as coccoliths sink to the seabed (Steinmetz, 1991; Ziveri et al., 2000). Consequently, high reflectance areas of any bloom are likely to be comprised of dead or dying *E. huxleyi* cells and the aim of this study was to determine if viruses are the cause of death.

Comparison of biological parameters, determined by analytical flow cytometry (AFC), between data collected outside and inside the high reflectance area led us to conclude that viruses were indeed responsible for the collapse of this bloom. Although such conclusions from similar data sets are not new (Bratbak et al., 1993, 1996; Brussaard et al., 1996; Castberg et al., 2001), there have been no reports of *E. huxleyi*-specific viruses being isolated and maintained in culture from *E. huxleyi* blooms. Bratbak et al. (1996) reported the initial isolation of a virus by plaque assay, however they were unable to propagate it further for characterization.

Transect data

Contour plots of transect data revealed very obvious differences between Station 1, outside and Stations 2–4, inside the high reflectance area (Figures 2–4). Briefly summarized: inside the high reflectance area concentrations of viruses were higher (Figure 2); *E. huxleyi* cells were lower (Figure 4A); coccoliths were higher (Figure 4B); bacteria were higher (Figure 3B) and virus to bacteria ratio (VBR) was lower (Figure 3A) than at Station 1, outside the high reflectance area. These data can simply be interpreted as virus-induced lysis of *E. huxleyi* cells in the bloom causing large concentrations of coccoliths to detach resulting in the high reflectance observed by satellite imagery (Figure 1). This interpretation is supported by TEM images of fixed samples from inside the high reflectance area where large virus-like particles (LVLP) ranging from 120 nm to 200 nm in size were clearly abundant (e.g. Figure 5A). In addition, LVLPs were observed bursting from *E. huxleyi* cells (Figure 5B) suggesting that viruses were being actively propagated inside the high reflectance area.

Virus concentrations in this study are at least an order of magnitude higher than those measured in a developing *E. huxleyi* bloom in the northern North Sea only one month earlier (Wilson et al., in press). These workers suggested that microzooplankton grazing contributed more significantly than viruses to *E. huxleyi* loss processes during the developing stages of a bloom, particularly since Archer et al. (2001) calculated that grazing accounted for between 22%–39% of *E. huxleyi* turnover in that same bloom. During a parallel study in the Plymouth bloom, Fileman et al. (2002) demonstrated that protozoan abundances were higher in the high reflectance area than Station 1. Moreover, these workers calculated that microzooplankton accounted for 61%–64% of total phytoplankton community turnover in the high reflectance area. However, more significantly, Fileman et al. (2002) revealed that pigment-specific grazing rates on prymnesiophytes was actually low and dinoflagellates and cryptophytes formed a more important food source for the microzooplankton grazers in their parallel study. This further supports the prediction made by Wilson et al. (in press) who discussed that viruses would play a much more important role towards the end of a bloom when virus concentrations would reach a threshold level required to cause termination of that bloom. Indeed, numerous mesocosm studies have been conducted where high concentrations of LVLPs have been observed following the demise of the *E. huxleyi* population (Bratbak et al., 1993; Wilson et al., 1998; Castberg et al., 2001). It is difficult to measure such termination at sea since it is not often logistically possible to be on a ship throughout the progression of a bloom. The transect conducted on 30 July 1999 was probably the equivalent of the latter stages (Station 1) and termination (Stations 2–4) of an *E. huxleyi* bloom. The bloom had been tracked in the South West approaches of the English Channel by the Plymouth Marine Laboratory Remote Sensing Data Analysis Service (RSDAS http://www.npm.ac.uk/rsdas/) from the end of June 1999 and both the high reflectance and high chlorophyll areas had disappeared by 14 August (data not shown).

Since the concentration of LVLPs was significantly higher in Stations 2–4 (Figure 2B), it was assumed the majority of them were derived from lysing *E. huxleyi* cells. If the burst size of infected *E. huxleyi* is >300, as Figure 5B suggests, then this is not an unreasonable assumption to make. Indeed, other algal viruses have been shown to have very large burst sizes of up to 4100 (Sandaa et al., 2001). Unfortunately, one of the drawbacks of AFC is that it is difficult to prove that separate clusters identified on AFC scatter plots can be attributed to specific virus groups, therefore we can only make assumptions on the likely hosts from viruses detected in natural samples.

Surface chlorophyll concentrations inferred from satellite imagery (ranging from 1.4 g l^{-1} at Station 1 to 2–7.5 g l^{-1} at Stations 2–4: data not shown), taxonomy data (Fileman et al., 2002) and AFC analysis revealed that a bloom was still occurring in the high reflectance area. It had switched from *E. huxleyi* domination (Station 1) to a mixture of autotrophic dinoflagellates (<50 µm), nanophytoplankton, cryptophytes and picoeukaryotes, whose concentrations were all higher inside the high reflectance area suggesting that virus-induced *E. huxleyi* lysis had triggered a rapid succession of the phytoplankton community. Castberg et al. (2001) reached a similar conclusion using

molecular methods (denaturing gradient gel electrophoresis) where they demonstrated that a new eukaryote community developed following the virus-induced demise of an *E. huxleyi* bloom during a seawater mesocosm experiment. It is worth noting that viruses that infect all these different phytoplankton strains would probably have a similar AFC signature to *E. huxleyi*-specific viruses, though this has not been rigorously examined.

Following *E. huxleyi* cell lysis, high concentrations of organic matter would be released, hence contributing to an increase in bacteria concentrations inside the high reflectance bloom (Figure 3B). Concurrently, the virus to bacteria ratio (VBR) decreased from an average of 33.0 (±5.2) outside the bloom to an average of 13.9 (±5.9) at Stations 2–4 (Figure 3A). Despite these VBRs being higher than usual they are still within the range expected for productive open water systems (Wommack & Colwell, 2000). Wilson et al. (in press) demonstrated that a drop in VBR between different thermal layers in an *E. huxleyi* bloom in the North Sea, correlated with an increase in bacteria production. Hence, it is likely that the increase in bacteria concentrations observed in the current study (Figure 3B) is indicative of increased bacteria production responding to a flux of organic matter caused by *E. huxleyi* cell lysis. A similar increase in bacteria concentration was observed by Castberg et al. (2001) following the demise of an *E. huxleyi* bloom during a recent mesocosm study. In addition, these workers observed a dramatic change in bacteria community composition where there was an increase in bacteria diversity following the collapse of the *E. huxleyi* bloom. Similarly, Bratbak et al. (1998) demonstrated that bacteria production increased following algal virus lysis of *Phaeocystis pouchetii* cultures.

Virus isolation

The use of AFC and TEM can be criticized for not providing foolproof evidence for the presence of viruses. Usually Koch's postulates must be met before the presence of an infectious agent can be proved. Briefly, to fulfil Koch's postulates the infectious virus must be filterable, transferable and cause the same disease symptoms, following propagation, in a healthy host. The two virus strains isolated in this study, *Eh*V84 and *Eh*V86 (Figure 7) met these criteria. *Eh*V84 was isolated from a water sample collected from a routine sampling trip four days before the main transect. Satellite imagery showed that *Eh*V84 was also collected from a high reflectance area on 26 July 1999 (data not shown), unfortunately we do not have any further relevant data available for this day. The isolation of a second virus strain, *Eh*V86, from water collected inside the high reflectance area of the bloom during the transect, confirmed that viruses must have been involved in the demise of the *E. huxleyi* bloom, further supporting data collected during the transect.

Basic characterization revealed *Eh*V84 and *Eh*V86 were lytic viruses (Figure 6) approximately 170 nm–190 nm in diameter with an icosahedral symmetry (Figure 7), similar to LVLPs observed in fixed samples collected from the bloom (Figure 5). The sulphurous odour observed following host cell lysis is likely to be caused by dimethyl sulphide, a potent climatically active biogenic gas. *Emiliania huxleyi* has

high intracellular DMSP concentrations (Keller et al., 1989) and elevated DMS levels are found in blooms of this species (Malin et al., 1993; Matrai & Keller, 1993). Previously, laboratory studies have also revealed that viral lysis of phytoplankton does significantly increase DMS/ DMSP release (Hill et al., 1998; Malin et al., 1998).

Characterization of *Eh*V84 and *Eh*V86 showed they are similar to other viruses that belong to the family *Phycodnaviridae*, a group of large double-stranded DNA viruses that infect aquatic algae (van Etten et al., 1991; van Etten & Meints 1999). Indeed, *Eh*V84 and *Eh*V86 are further characterized by Schroeder et al. (in press) who demonstrated that they belong to the family *Phycodnaviridae*. These workers performed a phylogenetic analysis of the DNA polymerase gene, revealing that *Eh*V84 and *Eh*V86 actually belong to a new virus genus they propose to call the coccolithovirus. Similar sized *E. huxleyi*-specific viruses have also been isolated from coastal seawater off the coast of Bergen, Norway (Tonje Castberg, personal communication), suggesting that these viruses are widespread.

Taken together, transect and isolation data suggest that viruses were the major contributor to the demise of the *E. huxleyi* population in the high reflectance area off the coast of Plymouth in July 1999. It is likely there was close coupling between microalgae, bacteria and viruses that contributed to a large organic carbon input. Consequent cycling influenced the succession of an *E. huxleyi*-dominated population to a more characteristic mixed summer phytoplankton community. The isolation of *E. huxleyi*-specific viruses confirmed the involvement of viruses in this succession. Further characterization of these viruses may help to further understand their ecological role, mechanism of infection and role in biogeochemical cycling.

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