Dynamics and genotypic composition of *Emiliania huxleyi* and their co-occurring viruses during a coccolithophore bloom in the North Sea

Joaquín Martínez Martínez¹,², Declan C. Schroeder² & William H. Wilson¹,²

¹Plymouth Marine Laboratory, Plymouth, UK and ²Marine Biological Association, Plymouth, UK

**Correspondence:** William H. Wilson, Bigelow Laboratory for Ocean Sciences, 60 Bigelow Drive, PO Box 380, East Boothbay, ME 04544, USA. Tel.: +1 207 747 3255 (x310); fax: +1 207 747 3258; e-mail: wwilson@bigelow.org

**Present address:** Joaquín Martínez Martínez, Present address: Bigelow Laboratory for Ocean Sciences, 60 Bigelow Drive, PO Box 380, East Boothbay, ME 04544, USA.

Received 10 October 2011; revised 19 December 2011; accepted 23 February 2012. Final version published online 29 March 2012.

**DOI:** 10.1111/j.1574-6941.2012.01349.x

**Keywords**

intraspecific succession; host-virus interaction; denaturing gradient gel electrophoresis.

**Abstract**

We studied the temporal succession of vertical profiles of *Emiliania huxleyi* and their specific viruses (EhVs) during the progression of a natural phytoplankton bloom in the North Sea in June 1999. Genotypic richness was assessed by exploiting the variations in a gene encoding a protein with calcium-binding motifs (GPA) for *E. huxleyi* and in the viral major capsid protein gene for EhVs. Using denaturing gradient gel electrophoresis and sequencing analysis, we showed at least three different *E. huxleyi* and EhV genotypic profiles during the period of study, revealing a complex, and changing assemblage at the molecular level. Our results also indicate that the dynamics of EhV genotypes reflect fluctuations in abundance of potential *E. huxleyi* host cells. The presence and concentration of specific EhVs in the area prior to the bloom, or EhVs transported into the area by different water masses, are significant factors affecting the structure and intraspecific succession of *E. huxleyi* during the phytoplankton bloom.

**Introduction**

*Emiliania huxleyi* is considered the most abundant coccolithophore in the ocean (Green & Leadbeater, 1994), has a wide distribution, forms intense blooms (Egge & Heimdal, 1994; Tyrrell & Taylor, 1996) and plays an important role in the biogeochemistry of the ocean by significantly influencing the sulphur and carbon cycles (Holligan et al., 1993; Malin et al., 1993; Simó, 2001). *Emiliania huxleyi* is a major contributor to the oceanic carbonate budget (Balch et al., 1992; Beaufort et al., 2007) which renders it a key species in ocean acidification studies, yet there exist contradictory claims on their physiological responses to increased CO₂ (Iglesias-Rodriguez et al., 2008; Beaufort et al., 2011). Vast *E. huxleyi* blooms occur during spring and summer in offshore, coastal and oceanic waters at mid-latitudes (45–55°) (Ackleson et al., 1988). Indeed, coccolithophore blooms are seasonally predictable in certain areas including the North Sea (Holligan et al., 1983). These blooms are not formed by a cosmopolitan *E. huxleyi* population with a common gene pool; instead, there is evidence of gene pools fragmentation and adaptation of local populations to their environment, where morphological and calcifying differentiation occurs (Beaufort et al., 2011). It is therefore crucial to properly differentiate morphotypes and ecotypes to avoid extrapolating findings from studies using a limited number of strains and ecosystems.

Previous studies using mesocosm systems have investigated the role viruses have in structuring different microbial components (e.g. Bratbak et al., 1993; Wilson et al., 1998; Martinez Martinez et al., 2007; Sorensen et al., 2009). It is clear from these studies that viruses are instrumental in the collapse of *E. huxleyi* blooms and allow succession of different microalgae following rapid
bacterial remineralization of organic matter (Castberg et al., 2001; Larsen et al., 2001). *Emiliania huxleyi*-specific viruses (EhVs) are known to be diverse at the genotypic level (Wilson et al., 2002b; Schroeder et al., 2003; Martínez Martínez et al., 2007). Furthermore, they have been reported to show location-specific distinctions (Rowe et al., 2011). The study of natural *E. huxleyi* blooms in different oceanic regions is indispensable for determining intraspecific diversity, clarifying the importance of viruses as mortality agents in the ocean and determining community spatial dynamics.

An opportunity to investigate the aspects mentioned earlier, among others, was given during a multidisciplinary cruise that followed the progression of a developing *E. huxleyi*-rich phytoplankton bloom in a programme called ‘Dimethyl Sulphide biogeochemistry within a Coccolithophore bloom (DISCO)’, in the northern North Sea in June 1999. The study comprised analyses of the biological, optical and physical properties of the patch of water containing the bloom as well as studies of sulphur compounds, nutrients, halocarbons, methyamines, carbon monoxide, dissolved organic carbon and total dissolved nitrogen. In addition, the role of viruses, bacteria, phytoplankton and zooplankton, the dynamics of primary production, plankton respiration, grazing and sedimentation were investigated in relation to the biogeochemical cycling of dimethyl sulphide (DMS). The results were published elsewhere, for an overview see Burkill et al. (2002). As part of the DISCO cruise, Wilson et al. (2002a) investigated the *E. huxleyi*- and *E. huxleyi*-specific virus (EhV) dynamics by examining their concentrations through vertical profiles by analytical flow cytometry. Their aim was to obtain high-intensity sampling data of *E. huxleyi* and EhVs to gain information on their temporal and spatial dynamics in an open-water site.

In the current study, we have gone a step further in the investigation of the dynamics of *E. huxleyi* and their co-occurring virus by assessing changes in their genotypic composition during the bloom progression using specific primers. We have exploited the variations found in a gene encoding a protein with calcium-binding motifs (GPA) in *E. huxleyi* (Schroeder et al., 2005) and in the major capsid protein gene (MCP) of the *E. huxleyi*-specific viruses (Schroeder et al., 2002, 2003) to analyse samples taken during the cruise using denaturing gradient gel electrophoresis (DGGE) and sequencing analysis.

**Material and methods**

**Study site and sampling**

The samples were collected during a research cruise aboard, the RRS Discovery, between the 5 and 29 of June 1999, that followed a coccolithophore-rich phytoplankton bloom originally located at 59°N 01°E in the North Sea. The experimental design and the flow cytometry (FCM) analysis are described by Wilson et al. (2002a). Briefly, the cruise was split into three parts: (1) An initial survey to identify the bloom combining satellite imagery and measurements of *E. huxleyi* concentrations by FCM. (2) A lagrangian time series study was then conducted between the 18 and 23 of June, when the selected patch of water was traced with sulphur hexafluoride (SF₆) using methods described previously (Law et al., 1998); during this period, the water column could be divided into three layers (surface, subsurface and bottom) based on the 10.5 and 8.5 °C isotherms. (3) A final survey of the bloom (June 24–29) after the entrance of a patch of warmer, lower salinity water in the sampling area, which formed a new surface layer above the 11.5 °C isotherm. Further details of the physical structure of the study site were described by Burkill et al. (2002).

Seawater was collected twice daily from a depth profile, down to approximately 100 m, typically just after midnight and midday, using a stainless steel CTD sampler system equipped with 12 Niskin bottles (30 L). From each depth sample, 1 L of seawater was filtered onto 0.45-µm-pore size Supor-450 47-mm-diameter filters (PALL Corp). The filters were transferred to 2-mL cryotubes, snap frozen in liquid nitrogen and stored at −20 °C until further processing for total genomic DNA preparations. For virus and host enumeration using FCM, subsamples were also collected from each depth as described by Wilson et al. (2002a).

**DNA isolation**

Genomic DNA was isolated from the particulate matter retained on the Supor filters using an adapted phenol/chloroform method. The filters were cut into small easily dissolvable pieces (approximately 0.5 cm²) and placed in a 2-mL Eppendorf tube. Following the addition of 800 µL GTE buffer (50 mM glucose, 25 mM Tris-Cl pH 8.0 and 10 mM EDTA), 10 µL Protease K (5 mg mL⁻¹), 100 µL 0.5 M filter sterilized EDTA and 200 µL 10% SDS, samples were incubated at 65 °C for 1–2 h. DNA was then purified by phenol extraction as described by Schroeder et al. (2002).

**Polymerase chain reaction (PCR) amplification and DGGE**

*Emiliania huxleyi* genotypic richness was studied by nested PCRs on the total genomic DNA preparations using three oligomers designed to the GPA gene of *E. huxleyi* strain L (Schroeder et al., 2005; Martínez Mar-
tinez et al., 2007) (Supporting Information, Table S1). Two-stage PCRs (firstly with primers GPA-F1/GPA-R1 and secondly with GPA-F2/GPA-R1) were conducted to amplify the variable region within the GPA gene that allows separation of the alleles into genotypes and coccolith morphology motif groups (CMM) (Schroeder et al., 2005). The PCRs were performed using 100 ng of total genomic DNA for the first reaction, then a 2-µL sub-sample from the first-stage PCR for the second reaction. Cycling conditions consisted of an initial denaturation at 95 °C for 5 min, 35 cycles of 95 °C for 30 s, 58/60 °C (first-/second-stage reaction) for 45 s and 72 °C for 60 s and a final extension for 5 min.

Viral diversity studies were also conducted by two-stage PCRs using oligomers designed for the MCP gene (Schroeder et al., 2002, 2003) (Table S1). PCRs using these primers coupled as MCP-F1/MCP-R1 and MCP-F2/MCP-R2 were conducted as described by Schroeder et al. (2002, 2003), respectively. Host and viral second-stage PCR products were treated with Mung Bean nuclease (Promega) according to the manufacturer’s recommendations to degrade single-stranded DNA ends. DGGE analysis of those PCR products was conducted using 30–50% linear denaturing gradient 8% polyacrylamide gels, where 100% denaturant is a mixture of 7 M urea and 40% deionized formamide. Ten microlitres of PCR products (it was estimated that all samples had approximately the same DNA concentration based on band intensity on an agarose gel) were loaded into wells with 5 µL of 2× gel loading dye [70% (v/v) glycerol, 0.05% (v/v) bromophenol blue and 0.05% (v/v) xylene cyanol]. Electrophoresis conditions and visualization of bands were as previously described by Schroeder et al. (2003).

**DNA sequencing and sequence analysis**

Selected representative single bands were excised from the DGGE gels for both *E. huxleyi* and viruses. Unfortunately, *E. huxleyi* DGGE gels for days 18–26 were lost before bands could be excised; therefore, limiting the GPA sequence database. The excised bands were incubated in 50 µL of molecular water, re-amplified and verified by DGGE. PCR products were subsequently sequenced using a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, UK) on an ABI 3100 capillary sequencer (Applied Biosystems) according to the manufacturer’s recommendations. The data for each fragment were aligned using CLUSTALW (http://www.ebi.ac.uk/clustalw/). The GPA sequences from this study (see Table 1 for GenBank accession numbers) were aligned with the GPA sequences from 15 *E. huxleyi* isolates (Schroeder et al., 2005) and from samples collected during 2 mesocosm experiments in 2000 and 2003 in a Norwegian fjord (Schroeder et al., 2003; Martinez Martinez et al., 2007) (see

### Table 1. *EhV* and *Emiliania huxleyi* genotypes found in this study and GenBank references for their sequence data

| DGGE band | GPA sequence* | CMM group | MCP sequence† | MCP genotype group | GenBank accession number |
|-----------|---------------|-----------|---------------|--------------------|-------------------------|
| EhVs      |               |           |               |                    |                         |
| a         |               |           | ehOTU3        | II                 | AY144376†               |
| b         |               |           | ehOTU1        | IV                 | AY144374†               |
| c         |               |           | ehOTU5        | V                  | AY144378†               |
| d         |               |           | ehOTU20       | XI                 | DQ084403                |
| e         |               |           | ehOTU21       | XII                | DQ084404                |
| f         |               |           | ehOTU22       | XIII               | DQ084406                |
| g         |               |           | Not available | Undetermined       | –                       |
| *E. huxleyi* |           |           |               |                    |                         |
| A         | ehOTU7        | III       |               |                    | DQ084407                |
| B         | ehOTU8        | IV        |               |                    | DQ084408                |
| C         | ehOTU9        | IV        |               |                    | DQ084409                |
| D         | ehOTU10       | III       |               |                    | DQ084410                |
| E         | ehOTU11       | IV        |               |                    | DQ084411                |
| F         | ehOTU12       | IV        |               |                    | DQ084412                |
| G         | ehOTU13       | II        |               |                    | DQ084413                |
| H         | ehOTU14       | IV        |               |                    | DQ084414                |
| I         | ehOTU15       | V         |               |                    | DQ084415                |
| J         | ehOTU17       | I         |               |                    | DQ084417                |
| K         | ehOTU18       | III       |               |                    | DQ084418                |
| L         | ehOTU16       | I         |               |                    | DQ084416                |

*Fragments (284–287 bp) of the GPA gene encoding a protein with calcium-binding motifs.
†Fragments (99 bp) of a gene encoding the putative MCP.
‡GenBank accession numbers published previously. CMM indicates coccolith morphology motif (Schroeder et al., 2005).
Table S2 for GenBank accession numbers). The virus MCP sequences from this study (see Table 1 for GenBank accession numbers) were aligned with the MCP sequences of 10 clonal viruses isolated between 1999 and 2001 from the English Channel (Schroeder et al., 2002; Wilson et al., 2002b) and also with the MCP sequences from samples collected during the 2 mesocosm experiments in the Norwegian fjord (see Table S2 for GenBank accession numbers).

Results

EhV diversity

Here, we present key parts of gels that help illustrate and pinpoint the timing of significant changes in temporal and spatial (depth) structure of the EhV assemblage during the cruise. The full series of gels are presented in Supporting Information, Fig. S1. DGGE analysis of virus MCP fragments revealed a diverse and dynamic EhV assemblage throughout the period of study (Fig. 1, Fig. S1). DGGE MCP bands represent different genotypes (Schroeder et al., 2002, 2003; Martínez Martínez et al., 2007), and sequencing of representative selected bands corroborates that bands that migrated at the same rate (even among different gels) have the same sequence (Fig. 1). The symbols, abbreviations and GenBank accession numbers of the MCP bands and their sequences are summarized in Table 1.

DGGE gels showed a relatively stable EhV assemblage composition at all depths between June 18 and 23 (midday) (Fig. 1 and Fig. S1). For clarity, the DGGE gel images were sorted into two layers, from surface to 38 m depth (Fig. 1a) and from 38 to 100 m depth (Fig. 1b). The 38-m-depth threshold was just above the 8.5 °C isotherm (Wilson et al., 2002a). During the period of June 18–23, two more intense bands (a and b) were present in samples from all depths. Low-intensity bands during the same period had a more variable pattern and were not always easily visualized on the gels. An example of this is band (d).

From midnight of June 23, we observed a clear change in the genotypic composition of the EhV assemblage,
concurrent with the influx of a patch of warmer water into the sampling area (Wilson et al., 2002a). At this point, a new intense band, (e), became present in the upper 60 m of the water column (Fig. 1; 23 June 00:00 hours), and at some depths, this was actually the only distinguishable band by DGGE. Band (e) prevailed almost to the end of the study, but it was not detectable between 5 and 24 m on the last sampling day – 29 June (Fig. 1a). Notably, based on their relative intensity, the previously most intense bands, (a) and (b), no longer predominated in the surface 20 m from midnight of the 23 through to the 24 June, but reappeared as intense bands in the surface from the 25 June (Fig. S1).

From the 25 June, at least six bands had high intensity: (a) and (b) which were detected from surface to deep layers; (e) that was present after the 23 June; (g) (not sequenced) that was only detected in samples collected below 50 m; and (c) and (f) which followed a more irregular distribution pattern (Fig. 1 and Fig. S1).

The alignment of MCP virus sequences from DGGE bands (Fig. S2) showed that two of the six EhV genotypes detected during the bloom were identical in the amplified region to virus isolates from the English Channel [ehvOTU21 (e) was identical to EhV-84; ehvOTU22 (f) was identical to EhV-86], one more EhV genotype, ehvOTU1 (b), was the same as the virus isolate EhV-163 (isolated from a Norwegian fjord) and two other EhV genotypes, ehvOTU3 (a) and ehvOTU5 (c), were detected in this study and in the samples from the Norwegian mesocosm studies. Genotype ehvOTU20 (d) did not match with any genotype in GenBank.

**E. huxleyi diversity**

DGGE analysis of PCR products amplified with the specific primers for the GPA gene revealed a broad range of *E. huxleyi* bands (Fig. 2). Changes in presence/absence of bands were observed both temporally and spatially.
As some *E. huxleyi* strains contain a single GPA allele while other strains contain two alleles (Schroeder et al., 2005), the bands revealed in this study indicate the different alleles present instead of quantitative richness of *E. huxleyi* strains. Table 1 summarizes the symbols, abbreviations and GenBank accession numbers given to each of the GPA bands excised from the DGGE gels. The time/depth DGGE profile for *E. huxleyi* allelic richness was partial as we were not able to amplify the GPA gene from all the samples.

As in the EhV analysis, we divided the samples into surface and deep water layers to facilitate the interpretation of the DGGE gel images (Fig. 2). The 38-m-depth threshold corresponding to the 8.5 °C isotherm also marks the depth at which *E. huxleyi* numbers are just starting to reach the limit of detection by FCM analysis (Wilson et al., 2002a), indicative of very low cell concentrations in the deep layer.

In general, a similar profile of *E. huxleyi* bands was observed throughout the water column from June 18 to 26, with a few bands changing intensity in certain samples (Fig. 2). Interestingly, on 18 and 19 June, the DGGE profiles in the deep layer were most similar to those observed in the surface layer on the 24 June, specifically the noticeable common encircled bands in Fig. 2. The *E. huxleyi* assemblage composition changed significantly from 27 June. A more irregular band profile was observed during the 27–29 June period (Fig. 2), during which the combination of band migration rate in the DGGE gel (Fig. 2) and sequencing analysis (Fig. S3) of excised bands showed the presence of at least 12 different *E. huxleyi* alleles. Sequencing information alone from these short fragments was not enough to determine allelic richness as it did not allow differentiation of all the bands at the genotype level. However, the sequence data from excised bands revealed the presence of five different CMM groups of the A and B *E. huxleyi* morphotypes (Fig. S3). Four of those genotypes (CMM I to IV) were previously characterized by Schroeder et al. (2005). We were not able to determine to which morphotype CMM V belonged.

**Discussion**

In their original report on virus–host dynamics of this study site in the North Sea, Wilson et al. (2002a) suggested that large viruses (EhVs) were actively infecting hosts. However, EhV concentrations were lower than expected, grazing rates were relatively high (Archer et al., 2002) and viruses showed no evidence of influencing Dimethyl sulphide/Dimethylsulphoniopropionate (DMS/ DMSP) production (Wilson et al., 2002a). The implication was, therefore, that viruses played a minor role in the dynamics of this coccolithophore-dominated phytoplankton bloom. However, the molecular data presented here, from samples collected during the same bloom, reveal a dynamic virus–host system, concealed by what appears to be relatively uninteresting numerical population data. The sensitivity of PCR has allowed us to explore these dynamics in much greater detail, essentially revealing changing assemblages of viruses and their hosts during the course of this naturally occurring bloom in the North Sea (Figs 1 and 2, Fig. S1) beyond the limits of detection for FCM. While the FCM analysis only detected *E. huxleyi* cells up to 45 m deep, PCR and DGGE revealed the presence of different *E. huxleyi* alleles as deep as 100 m. It is worth noting that despite their wide use for describing microbial community structure based on extracted DNA (Petersen & Dahllof, 2005) PCR and DGGE only provide presence/absence data and not abundance. This is mainly owing to the qualitative nature of PCR and limitations in DGGE resolution. To our knowledge, this is the first time such a comprehensive temporal and spatial analysis of *E. huxleyi* and their corresponding viruses has been presented at the molecular level during the progression of a natural coccolithophore bloom.

Our results showed at least three significantly different *E. huxleyi* genotypic profiles during the period of study: (1) The combination of DGGE and FCM (Wilson et al., 2002a) data showed the progression and termination of a diverse genotypic *E. huxleyi* assemblage in the surface layer between June 18 and 23 (Fig. 2). The concurrent decrease followed by an increase in EhV concentrations in the surface during the same period suggests an active infection process of *E. huxleyi* followed by the release of EhV progeny (Fig. 1). DGGE analysis showed a stable EhV assemblage (Fig. 1, Fig. S1) until the entrance of a warm patch surface water (23 June), indicating that those EhV genotypes (Fig. 1, Fig. S2) were linked to the initial *E. huxleyi* assemblage (Fig. 2) and were likely involved to a certain extent in control of the bloom through infection following ‘kill the winner’ dynamics (Thingstad, 2000). (2) The influx of warm water probably caused mixing as well as the entrance of new hosts and viruses. In turn, the new *E. huxleyi* assemblage progressively disappeared (Wilson et al., 2002a; Fig. 2). This disappearance could be partly attributed to grazing (Archer et al., 2002) and viral infection. The concomitant detection of new EhV genotypes (c and f, Fig. 1) suggests specific active infection of the decreasing *E. huxleyi* assemblage and the subsequent release of those EhVs into the water in enough numbers to allow detection by PCR and DGGE. In addition, the persistence in the water column of the EhV genotypes (a) and (b) until the last day of study (Fig. 1) hints that the remaining viruses from previous lysis events were also propagated by infection of the incoming *E. huxleyi* assemblage. (3) As the surface *E. huxleyi* assemblage...
diminished in numbers, a new assemblage formed at 30–40 m depth between 26 and 29 June (Wilson et al., 2002a; Fig. 2). It is likely that *E. huxleyi* cells that bloom in surface layers sink out with time and can be later detected in deeper water. This was especially evident between 26 and 29 June owing to the available sequence data collected for this period. For example, *E. huxleyi* alleles (L) and (C) were first detected at 30–35 m depth on the 27 and 28 June, respectively, and at 60–80 m depth on the 28 and 29 June (Fig. 2). Following this reasoning, we hypothesize that the *E. huxleyi* genotype on 18–19 June at depths below 40 m (marked by an oval in Fig. 2) revealed the presence of a remnant *E. huxleyi* genotype, from a previous bloom event, that sank to deeper water as the bloom declined. The presence of the same band at surface on 24 June (Fig. 2) can be explained by either mixing caused by the warm surface water influx or entry of similar *E. huxleyi* strains with that water patch.

Low impact of *E. huxleyi* and EhVs in DMSP production was recorded during the DISCO study (Archer et al., 2002). However, Steinke et al. (2002) measured maximum DMSP lyase activity at approximately 50 m depth on 22 June, 40 m on 23 June and 35 m on 24 June, concurrently with the lowest cell numbers recorded for the *E. huxleyi* assemblage, suggesting the production of DMSP by dying *E. huxleyi* cells. Yet, the low impact of *E. huxleyi* and EhVs in DMSP production could be explained first by the fact that coccolithophores accounted for less than 30% of the phytoplankton biomass (Widdicombe et al., 2002). Secondly, different *E. huxleyi* strains are known to have different DMSP production rates (Steinke et al., 1998). It is possible that the dominant strains during the main *E. huxleyi* bloom were not high DMS producers. It may be that the *E. huxleyi* contribution to the standing stocks of DMSP and the importance of EhVs as agents of DMS production were higher after the influx of warm surface water (24–29 June) when new *E. huxleyi* and EhV communities developed. However, DMSP and DMS measurements are lacking for this period. Further investigation would aid in establishing links between the results presented here and sulphur biogeochemical cycles in the sea. The use of well-established molecular tools, as the ones employed in this study, may be the key to answer unknown questions regarding the implications of the *E. huxleyi* virus system in local ecology, climate and biogeochemistry cycling and production of compounds such as DMS, calcite and carbon.

In summary, DGGE and sequencing analysis of *E. huxleyi* and EhV groups provided additional information about the dynamics of *E. huxleyi* blooms in open waters. Depth profiles showed ‘past, present and future’ of the progression and structuring within a natural coccolithophore-dominated bloom. The findings in this study are of great value because despite being one of the best-studied eukaryotic phytoplankton virus systems, we still know little about *E. huxleyi* natural bloom dynamics, diversity and evolution. Our results revealed highly dynamic and diverse *E. huxleyi* genotypic assemblages perhaps driven by a response to infection by equally diverse EhV genotypes seemingly following the ‘Red Queen’ effect (Van Valen, 1973), in which viral pressure leads to increased host diversity. Another possibility is a ‘Cheshire Cat’ scenario (Frada et al., 2008) in which viral infection induces the transformation or succession from diploid to virus-resistant haploid *E. huxleyi* cells, which may explain reoccurrence of certain diploid genotypes when specific viral pressure disappear. Previous studies have reported that the same limited number of *E. huxleyi* and associated EhV genotypes can reoccur over a 3-year period in a Norwegian fjord (Martinez Martinez et al., 2007) and even persist for centuries as shown in the Black Sea (Coollen, 2011). A combination of both scenarios, ‘Red Queen’ and ‘Cheshire Cat’, is more plausible.

**Acknowledgements**

We thank scientists, officers and crew aboard RRS *Discovery* (cruise D241) for their support during the cruise. Particular thanks must also go to P. Burkill who was the PI on the cruise and to P. Nightingale, M. Liddicoat and R. Ling who provided the SF6 tracer framework for the langrangian study. This work forms part of the dimethyl sulphide biogeochemistry within a coccolithophore bloom (DISCO) project and was supported by the Natural Environmental Research Council (NERC) and UK MOD Defence Evaluation Research Agency (DERA). DCS is a Marine Biological Association of the UK Research Fellow. Research by WHW was supported through the NERC-funded core strategic research programme of the Plymouth Marine Laboratory and National Science Foundation grants EF0723730 and OCE0849363.

**References**

Ackelson S, Balch WM & Holliga PM (1988) White waters of the Gulf of Maine. *Oceanography* 1: 18–22.

Archer SD, Smith GC, Nightingale PD, Widdicombe CE, Tarran GA, Rees AP & Burkill PH (2002) Dynamics of particulate dimethylsulphoniopropionate during a langrangian experiment in the northern North Sea. *Deep Sea Res Part 2 Top Stud Oceanogr* 49: 2979–2999.

Balch WM, Holligan PM & Kilpatrick KA (1992) Calcification, photosynthesis and growth of the bloom forming coccolithophore *Emiliania huxleyi*. *Cont Shelf Res* 12: 1353–1374.
Beaufort L, Probert I & Buchet N (2007) Effects of acidification and primary production on coccolith weight: implications for carbonate transfer from the surface to the deep ocean. Geochim Geophys Geosyst 8: Q08011.

Beaufort L, Probert I, de Gariel-Thoron T et al. (2011) Sensitivity of coccolithophores to carbonate chemistry and ocean acidification. Nature 476: 80–83.

Bratbak G, Egge JK & Heldal M (1993) Viral mortality of the marine alga Emiliania huxleyi (haptophytae) and termination of algal blooms. Mar Ecol Prog Ser 93: 39–48.

Burkill PH, Archer SD, Robinson C, Nightingale PD, Groom SB, Tarran GA & Zubkov MV (2002) Dimethyl sulphide biogeochemistry within a coccolithophore bloom (DISCO): an overview. Deep Sea Res Part 2 Top Stud Oceanogr 49: 2863–2885.

Castberg T, Larsen A, Sandaa RA, Brussaard CPD, Egge JK, Heldal M, Thyrhaug R, van Hannen EJ & Bratbak G (2001) Microbial population dynamics and diversity during a bloom of the marine coccolithophorid Emiliania huxleyi (haptophyta). Mar Ecol Prog Ser 221: 39–46.

Cooen MJL (2011) 7000 years of Emiliania huxleyi viruses in the black sea. Science 333: 451–452.

Egge JK & Heimdal BR (1994) Blooms of phytoplankton including Emiliania huxleyi (haptophyta) – effects of nutrient supply in different n-p ratios. Sarsia 79: 333–348.

Frada M, Probert I, Allen MJ, Wilson WH & de Vargas C (2008) The ‘cheshire cat’ escape strategy of the coccolithophore Emiliania huxleyi in response to viral infection. P Natl Acad Sci USA 105: 15944–15949.

Green JC & Leadbeater BSC (1994) The Haptophyte Algae. Clarendon Press, Oxford.

Holligan PM, Viollier M, Harbour DS, Camus P & Champagnephilippe M (1983) Satellite and ship studies of coccolithophore production along a continental-shelf edge. Nature 304: 339–342.

Holligan PM, Fernandez E, Aiken J et al. (1993) A biogeochemical study of the coccolithophore, Emiliania huxleyi, in the North-Atlantic. Global Biogeochem Cy 7: 879–900.

Iglesias-Rodriguez MD, Halloran PR, Rickaby REM et al. (2008) Phytoplankton calcification in a high-CO2 world. Science 320: 336–340.

Larsen A, Castberg T, Sandaa RA, Brussaard CPD, Egge J, Heldal M, Paulino A, Thyrhaug R, van Hannen EJ & Bratbak G (2001) Population dynamics and diversity of phytoplankton, bacteria and viruses in a seawater enclosure. Mar Ecol Prog Ser 221: 47–57.

Law CS, Watson AJ, Liddicoat MJ & Stanton T (1998) Sulphur hexafluoride as a tracer of biogeochemical and physical processes in an open-ocean iron fertilisation experiment. Deep Sea Res Part 2 Top Stud Oceanogr 45: 977–994.

Malin G, Turner S, Liss P, Holligan P & Harbour D (1993) Dimethylsulphide and dimethylsulphoniopropionate in the northeast atlantic during the summer coccolithophore bloom. Deep Sea Res Part I Oceanogr Res Pap 40: 1487–1508.

Martinez Martinez J, Schroeder DC, Larsen A, Bratbak G & Wilson WH (2007) Molecular dynamics of Emiliania huxleyi and cooccurring viruses during two separate mesocosm studies. Appl Environ Microbiol 73: 554–562.

Petersen SG & Dahllöf I (2005) Improvements for comparative analysis of changes in diversity of microbial communities using internal standards in PCR-DGGE. FEMS Microbiol Ecol 53: 339–348.

Rowe JM, Fabre M-F, Gobena D, Wilson WH & Wilhelm SW (2011) Application of the major capsid protein as a marker of the phylogenetic diversity of Emiliania huxleyi viruses. FEMS Microbiol Ecol 76: 373–380.

Schroeder DC, Oke J, Malin G & Wilson WH (2002) Coccolithovirus (phycodnaviridae): characterisation of a new large dsDNA algal virus that infects Emiliania huxleyi. Arch Virol 147: 1685–1698.

Schroeder DC, Oke J, Hall M, Malin G & Wilson WH (2003) Virus succession observed during an Emiliania huxleyi bloom. Appl Environ Microbiol 69: 2484–2490.

Schroeder DC, Biggi GF, Hall M, Davy J, Martinez Martinez J, Richardson AJ, Malin G & Wilson WH (2005) A genetic marker to separate Emiliania huxleyi (prymnesiophyceae) morphotypes. J Phycol 41: 874–879.

Simó R (2001) Production of atmospheric sulfur by oceanic plankton: biogeochemical, ecological and evolutionary links. Trends Ecol Evol 16: 287–294.

Sorensen G, Baker AC, Hall MJ, Munn CB & Schroeder DC (2009) Novel virus dynamics in an Emiliania huxleyi bloom. J Plankton Res 31: 787–791.

Steinke M, Wolfe GV & Kirst GO (1998) Partial characterisation of dimethylsulfoniopropionate (DMSP) lyase isozymes in 6 strains of Emiliania huxleyi. Mar Ecol Prog Ser 175: 215–225.

Steinke M, Malin G & Liss PS (2002) Trophic interactions in the sea: an ecological role for climate relevant volatiles? J Phycol 38: 630–638.

Thingstad TF (2000) Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. Limnol Oceanogr 45: 1320–1328.

Tyrrell T & Taylor AH (1996) A modelling study of Emiliania huxleyi in the NE Atlantic. J Mar Syst 9: 83–112.

van Valen L (1973) A new evolutionary law. Evol Theor 1: 1–30.

Widdicombe CE, Archer SD, Burkill PH & Widdicombe S (2002) Diversity and structure of microplankton community during a coccolithophore bloom in the stratified northern North Sea. Deep Sea Res Part 2 Top Stud Oceanogr 49: 2887–2903.

Wilson WH, Turner S & Mann NH (1998) Population dynamics of phytoplankton and viruses in a phosphate-limited mesocosm and their effect on DMSP and DMS production. Estuar Coast Shelf Sci 46: 49–59.

Wilson WH, Tarran G & Zubkov MV (2002a) Virus dynamics in a coccolithophore-dominated bloom in the North Sea. Deep Sea Res Part 2 Top Stud Oceanogr 49: 2951–2963.
Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** DGGE gels of PCR fragments amplified with MCP primers for analysis of EhV diversity.

**Fig. S2.** Multiple sequence alignment of the EhV-MCP fragments produced in this study (ehOTUs).

**Fig. S3.** Clustal alignment of fragments within the partial *E. huxleyi* GPA-sequences obtained from excised DGGE bands from this study (‘ehuxOTUs’) (Table 1), from two mesocosm studies (‘ehuxOTUs’) (Table S2) and from isolates in culture (Schroeder *et al.*, 2005).

**Table S1.** Primers used to assess host (GPA prefix) and viral (MCP prefix) diversity.

**Table S2.** List of *E. huxleyi* and EhV genotypes from mesocosm studies in a Norwegian fjord and GenBank references for their sequence data (Martínez Martínez *et al.*, 2007).

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.