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Changes in *Emiliania huxleyi* fatty acid profiles during infection with *E. huxleyi* virus 86: physiological and ecological implications

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ABSTRACT: Fatty acids profiles of *Emiliania huxleyi* strain CCMP1516 were determined in a virus-induced culture crash with *E. huxleyi* virus 86 (EhV-86). As cell numbers declined in the infected cultures due to virus lysis, a concomitant decrease in fatty acids was observed in the particulate fraction. The composition of fatty acids within infected *E. huxleyi* cells was restructured, with a shift from polyunsaturated to monounsaturated and saturated fatty acids (respective distributions changing from 70:10:20% at the start of the experiment to 44:24:32% at the final time point). In particular, decreases were seen in the major fatty acid 22:6(n-3) and in 18:5(n-3), whereas greatest increases were seen in 18:1(n-9) and 22:0. The increase in the amount and restructuring of the fatty acid pool in *E. huxleyi* was indicative of a combination of specific and non-specific effects of virus infection. Specific effects primarily included induction of metabolic pathways such as the synthesis of components involved in virus replication, the production of virions and signal transduction via sphingolipid biosynthesis. Non-specific effects due to stress were likely mediated by reactive oxygen species. Changes in the composition of virus-infected *E. huxleyi* are of significance to the food web since grazing on virus-infected blooms will decrease the amount of essential polyunsaturated fatty acids passed on to higher trophic levels. Consequently, this could decrease the overall productivity of marine ecosystems.

KEY WORDS: *Emiliania huxleyi* · Virus · Fatty acids · Lipids · Virus infection · Oxidative stress

INTRODUCTION

Within the marine environment, the primary source of fatty acids is synthesis by microalgae. Fatty acids are vital to organisms of the marine food web for energy storage, somatic growth and reproduction (Jonasdottir et al. 1995, Pond et al. 2005), and they are passed on to higher trophic levels by herbivory and the subsequent consumption of herbivores (Sargent & Whittle 1981, Fraser et al. 1989). Physiologically, their major roles are to maintain cell membrane fluidity (Pruitt 1990) and regulate hormonal processes (Bell et al. 1991).

A marine microalgal species whose lipid biochemistry is relatively well studied is the coccolithophorid *Emiliania huxleyi* (Conte et al. 1994). Within this species, there is a relatively high flow of carbon (40 to 50%) into lipids, which has been suggested as a strategy to overcome increased density due to the production of coccoliths (Fernández et al. 1994), or to obtain a storage pool to sustain protein synthesis when environmental conditions become limiting for growth (Marañón & González 1997). Unlike other microalgae, *E. huxleyi* only contains small amounts of neutral lipids in the form of triacylglycerol (TAG), and instead pro-

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duces a suite of polyunsaturated long-chain alkenes, alkenones and alkenoates (Volkman et al. 1979, Marlowe et al. 1984, Patterson et al. 1994, Fidalgo et al. 1998). Interest in the lipid composition of this species stems predominantly from the use of its long-chain (C_{37}–C_{40}) alkenones as paleo sea surface temperature proxies (Prahl & Wakeham 1987).

Characterisation of fatty acids has revealed that *Emiliania huxleyi* cells are particularly rich in (n-3) polyunsaturated fatty acids (PUFA) (Volkman et al. 1989, Conte et al. 1994). A study of 8 geographically distinct strains revealed the major fatty acids during growth to be 14:0, 16:0, 18:1(n-9), 18:4(n-3), 18:5(n-3) and 22:6(n-3), with the last being the most abundant during exponential growth and the stationary phase (Pond & Harris 1996). Within *E. huxleyi*, alkenones and alkenoates have been found to be associated with internal structures such as the endoplasmic reticulum and the coccolith-producing compartment, and it was suggested that these were membrane-unbound lipids. In contrast, fatty acids typical of membrane lipids are found in the chloroplast thylakoids and in the Golgi and plasma membranes (Sawada & Shiraiwa 2004). More recent studies indicate that *E. huxleyi* packages its neutral lipids into cytoplasmic vesicles or lipid bodies, and that a significant proportion of lipids may be associated with chloroplasts (Eltgroth et al. 2005).

The importance of virus infection in controlling phytoplankton populations and bloom dynamics is increasingly being elucidated (Brussaard 2004). *Emiliania huxleyi* is an algal species whose bloom collapses have been frequently linked to virus control in the marine environment (e.g. Wilson et al. 2002). During virus infection, the metabolism of the host cell may be taken over and driven to produce numerous virus progeny, causing the disruption of normal cellular processes and the induction of cytological, physiological and biochemical changes. In *E. huxleyi,* virus infection has been found to result in many modifications that may potentially cause, or be related to, changes in the lipid profile within the cell: these include disruption of photosynthetic processes, production of reactive oxygen species, reduction in enzyme activities, loss of membrane integrity and altered pigment composition (Evans et al. 2006, Evans et al. 2007, Llewellyn et al. 2007). Rontani et al. (2007) recently showed that the fatty acid profile of *E. huxleyi* considerably changed under conditions of oxidative damage, which is likely during virus infection due to increased production of reactive oxygen species (Evans et al. 2006). Furthermore, genome sequencing of the *E. huxleyi*-specific virus used in this study (strain EhV-86) revealed that it contains genes which encode some of the key proteins of the sphingolipid biosynthesis pathway, including the first rate-limiting enzyme, serine palmitoyl transferase (SPT) (Wilson et al. 2005). Presence of this pathway in the virus suggests that infection may induce or regulate lipid biosynthesis pathways that are not normally observed in healthy cells. In addition, electron microscopy of EhV-86 indicates that it contains an internal membrane (Schroeder et al. 2002); hence, infection by this pathogen is likely to influence host lipid biosynthesis. Despite these indications, the impact of virus infection on lipid profiles of *E. huxleyi* or any other microalgae during virus infection has, to our knowledge, not been previously investigated.

Considering the importance of lipid molecules for marine food webs, the prevalence of virus infection in marine algae, and the important discovery that virus-infected *Emiliania huxleyi* are preferentially grazed by *Oxyrrhis marina* over their healthy counterparts (Evans & Wilson 2008), we investigated the fatty acid composition of *E. huxleyi* in a virus-induced culture crash. The results are examined with regards to their physiological and potential ecological significance.

MATERIALS AND METHODS

Culture method. Cultures of axenic *Emiliania huxleyi* (Lohmann) Hay et Mohler were obtained from Brian Palenik (Scripps Institution of Oceanography, San Diego, USA: strain CCMP 1516). Stock cultures were maintained in *f*/2 medium at 250 µmol m^{-2} s^{-1} under a light:dark cycle of 14:10 h at 15°C. The virus pathogen used was *E. huxleyi* virus 86 (EhV-86) (Wilson et al. 2002) and lysates were generated by the addition of 1 ml to 1 l cultures of *E. huxleyi*. After lysis, the culture lysate was filtered (0.22 µm) to remove cellular debris and stored in the dark at 4°C until required. All cultures and sampling were completed in a laminar flow hood using aseptic techniques.

Incubation experiment. Six × 6 l replicate cultures of *Emiliania huxleyi* strain CCMP 1516 were set up in 10 l polycarbonate bottles and 3 were infected with EhV-86 when they reached the mid-exponential phase. Viruses were added at a multiplicity of infection (MOI: ratio of viruses to host) of ~1. All cultures were sampled daily at ~4 h into the light period until completion of the culture crash which took 6 d. Samples were taken for fatty acid content determination of the particulate fraction, *E. huxleyi* enumeration, virus enumeration and to check for the presence of bacteria. Prior to sampling, the cultures were very gently swirled to promote the resuspension of cells.

Biological parameters. *Emiliania huxleyi* was counted with a Beckman Coulter Multisizer 3 with a 100 µm orifice, and autoclaved using 0.2 µm filtered seawater as the electrolyte. Samples were analysed in

triplicate and data were collected and interpreted using Coulter Multisizer 3 version 3.01a software.

For virus enumeration and to check for the presence of bacteria, 1 ml samples were fixed in a final concentration of 0.5% glutaraldehyde for 30 min at 7°C. The samples were then snap frozen in liquid nitrogen and stored at −80°C prior to analysis using SYBR Green I nucleic acid gel stain (Molecular Probes) and flow cytometry according to the method of Marie et al. (1999).

Fatty acid analysis. Volumes of 195 ml of culture were gently filtered onto 25 mm ashed Whatman GF/F filters. The filters were immediately transferred to 4 ml glass vials which were topped up with chloroform: methanol (2:1 v/v) and maintained at −80°C until analysis. After the addition of a 23:0 fatty acid internal standard, total lipid was extracted from cultures contained on the GF/F filters using a chloroform:methanol (2:1 v/v) solvent system (Folch et al. 1957). Total lipids were transesterified in methanol containing 1.5% sulphuric acid at 50°C for 16 h to generate fatty acid methyl esters (FAMEs, Christie 1982). FAMEs were purified by thin layer chromatography using a hexane: diethylether:acetic acid (90:10:1 v/v/v) solvent system. Purified FAMEs were dissolved in hexane to a concentration of 0.25 mg ml^{-1} and analysed by gas chromatography on a Trace 2000 GC fitted with a Restek, Stabilwax column (30 m × 0.32 mm i.d.) using hydrogen as the carrier gas.

RESULTS

During virus infection of *Emiliania huxleyi*, the decline in the number of cells was noticeable after 48 h, concomitant with the accumulation of virus particles in the culture medium (Fig. 1A,B). Control cultures grew normally until the final point at 151 h, where they decreased slightly likely due to the onset of the stationary phase. Fatty acids in the particulate fraction increased from a starting concentration of ~0.60 µg ml^{-1} in line with cell numbers during the initial phase of the experiment (Fig. 1C). In the control cultures, the levels of fatty acids reached a maximum of 1.2 µg ml^{-1} at 99 h and then declined to 0.97 µg ml^{-1} over the final 2 time points. From 51 h onwards, fatty acids declined in the particulate fraction of the infected cultures, reaching a minimum of 0.32 µg ml^{-1} at the final time point. Throughout the experiment, no bacteria were detected in any of the cultures.

At the initial sampling point, the *Emiliania huxleyi* cells in the control cultures comprised 18% saturated, 11% monounsaturated and 71% polyunsaturated fatty acids. Results for the equivalent point in the infected cultures were approximately the same, with 20% saturated, 10% monounsaturated and 70% polyunsaturated fatty acids. The predominant saturated fatty acids detected were 14:0 and 16:0, with initial proportions to the total fatty acids of 7 and 6% respectively (Fig 2A,C). Also detected were 15:0, 18:0, 20:0 and 22:0, although these were comparatively minor components comprising 2% or less of the total (Fig. 2B,D,E,F). In the controls, the levels of saturated fatty acids did not change significantly over the course of the experiment, whereas increases were seen in all saturated fatty acids in the infected cultures after 50 h, except in 14:0 which remained at ~10% throughout (Fig. 2). At the final time point, saturated fatty acids constituted

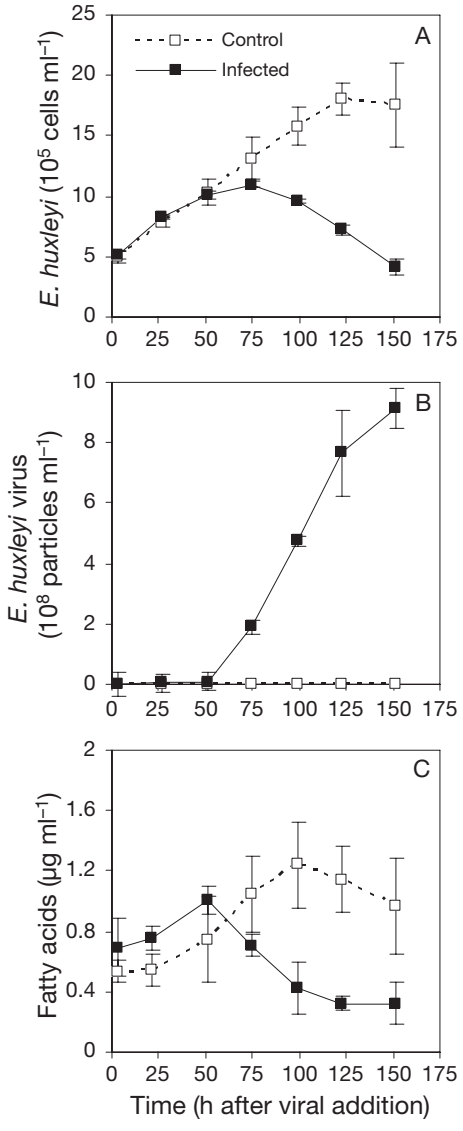

Fig. 1. EhV-86 infecting *Emiliania huxleyi*. Effect of virus infection on (A) the concentration of *E. huxleyi* cells in cultures, (B) the concentration of *E. huxleyi* virus particles, and (C) the amount of fatty acids in the particulate fraction. Data points are means ± SD of triplicate infected and control cultures

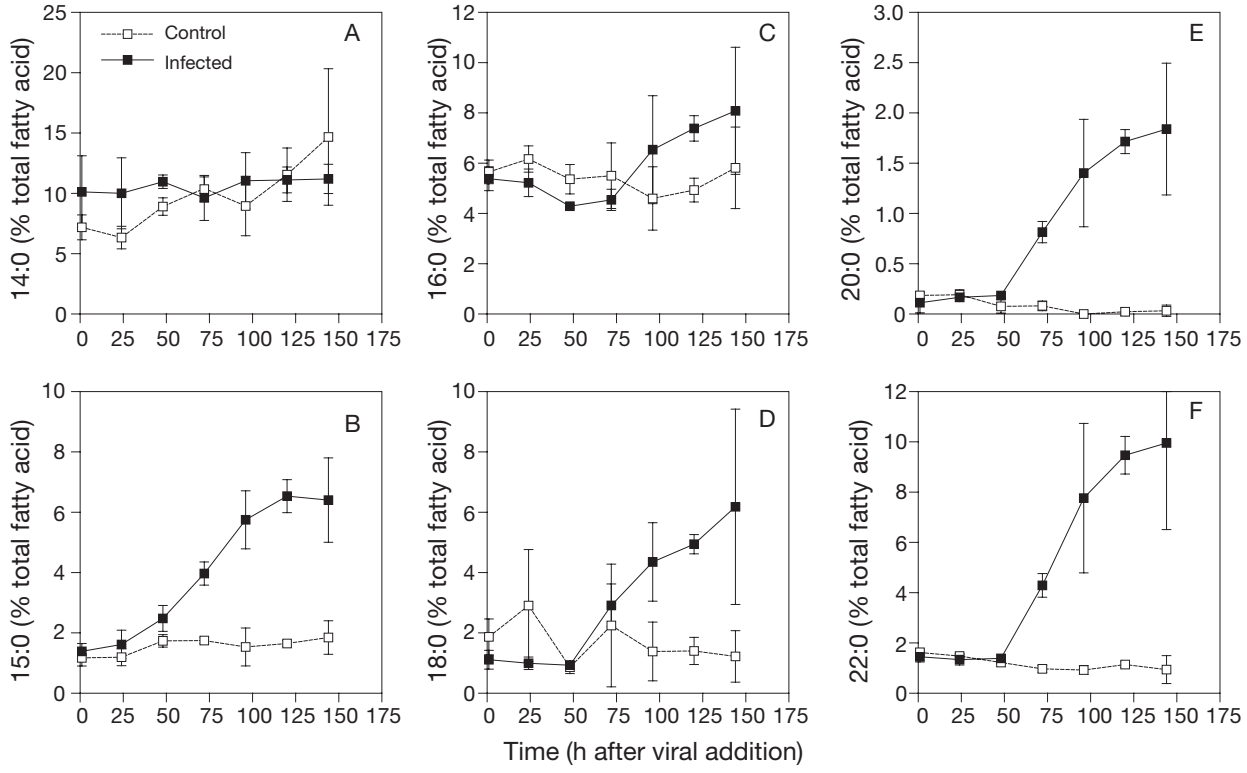

Fig. 2. EhV-86 infecting *Emiliania huxleyi*. Effect of virus infection on *E. huxleyi* saturated fatty acids (A) 14:0, (B) 15:0, (C) 16:0, (D) 18:0, (E) 20:0, and (F) 22:0 in cultures. Data points are means ± SD of triplicate infected and control cultures. Note differences in the *y*-axis scale for each of the fatty acids

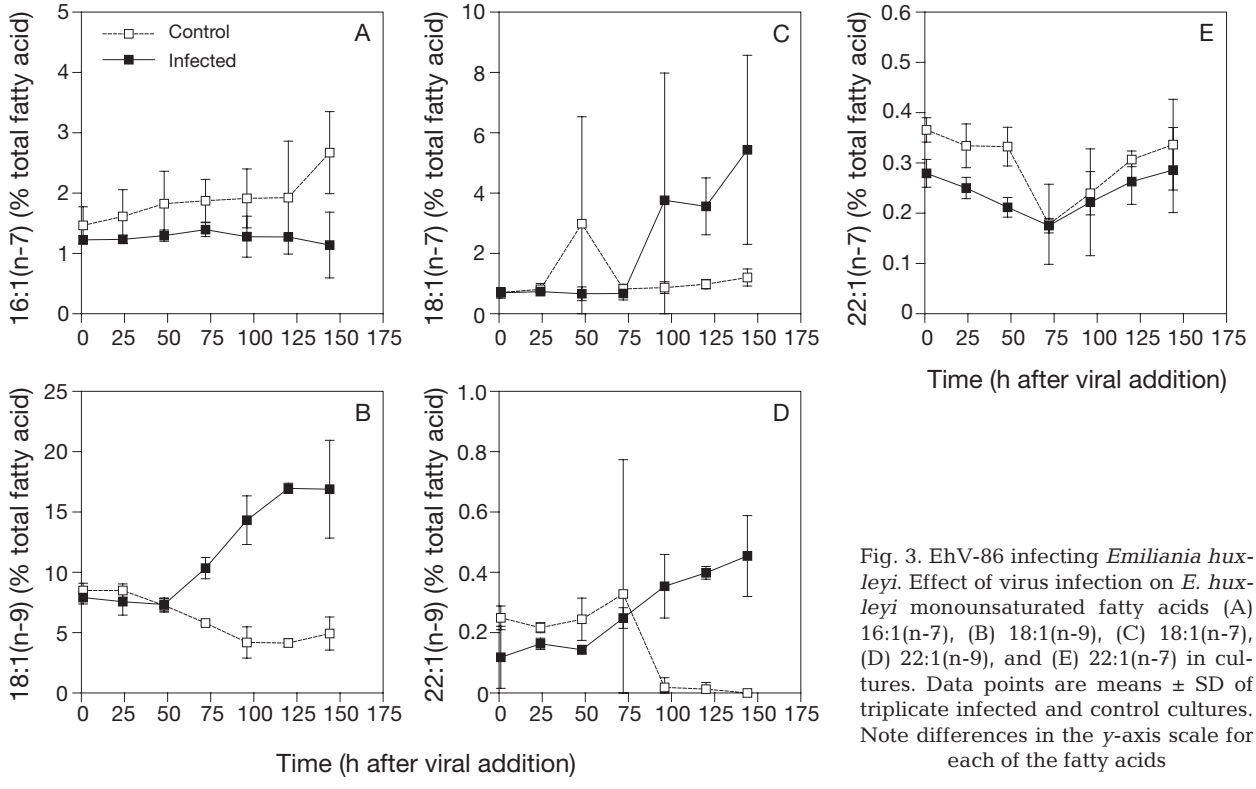

Fig. 3. EhV-86 infecting *Emiliania huxleyi*. Effect of virus infection on *E. huxleyi* monounsaturated fatty acids (A) 16:1(n-7), (B) 18:1(n-9), (C) 18:1(n-7), (D) 22:1(n-9), and (E) 22:1(n-7) in cultures. Data points are means ± SD of triplicate infected and control cultures. Note differences in the *y*-axis scale for each of the fatty acids

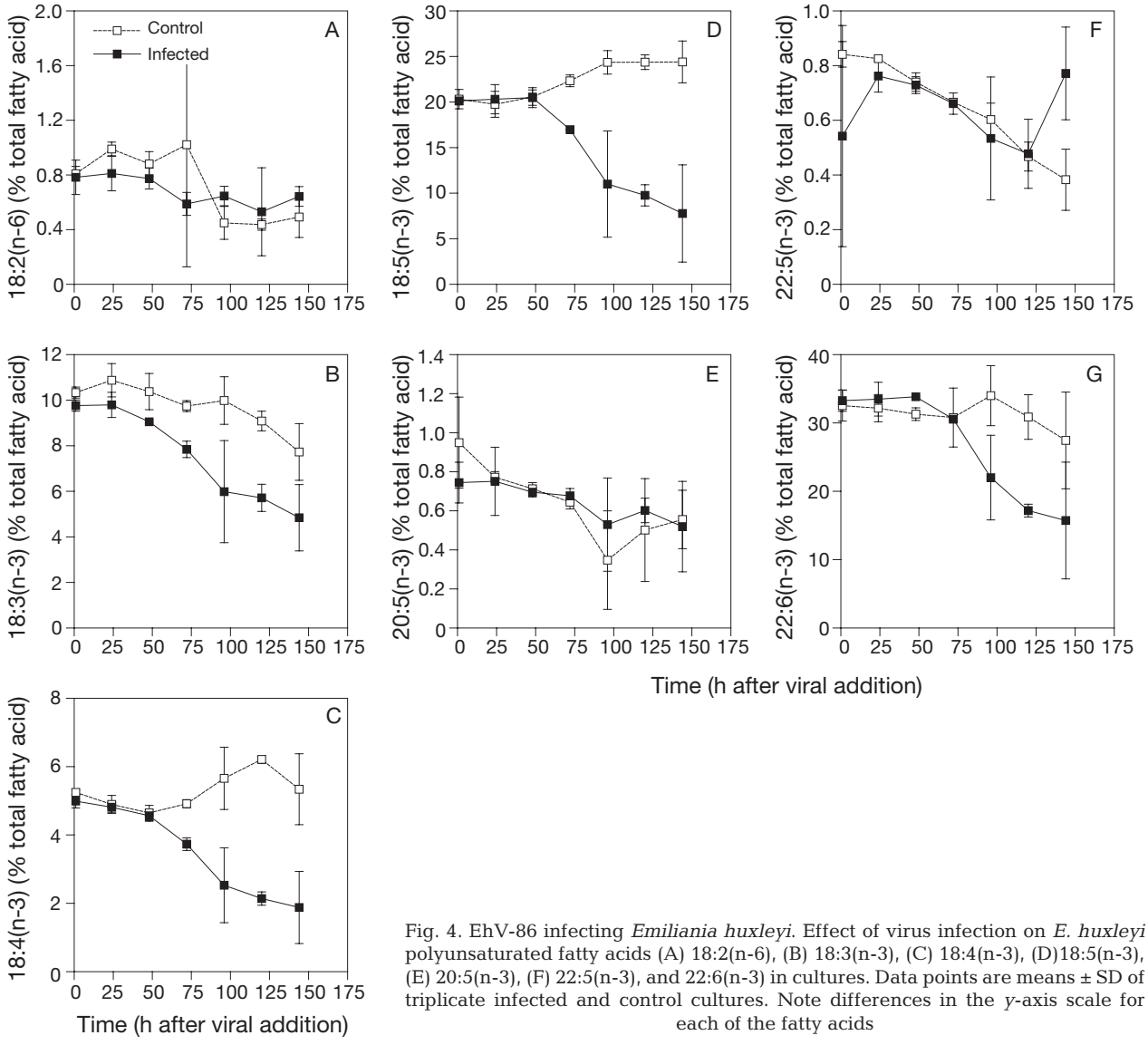

Fig. 4. EhV-86 infecting *Emiliania huxleyi*. Effect of virus infection on *E. huxleyi* polyunsaturated fatty acids (A) 18:2(n-6), (B) 18:3(n-3), (C) 18:4(n-3), (D)18:5(n-3), (E) 20:5(n-3), (F) 22:5(n-3), and 22:6(n-3) in cultures. Data points are means ± SD of triplicate infected and control cultures. Note differences in the *y*-axis scale for each of the fatty acids

44% of the total fatty acids in the particulate fraction of the virus-infected cultures. Of the monounsaturated fatty acids, 18:1(n-9) was numerically dominant, making up ~8% of the total fatty acids at the initial time point (Fig. 3B). The other monounstaurated fatty acids detected were 16:1(n-7), 22:1(n-7) and 22:1(n-9), which were present at levels of 2% or lower (Fig. 3).

Over the course of the experiment, levels of the major monounsaturate 18:1(n-9) fell by ca. 50% in the uninfected *Emiliania huxleyi* and decreases were also seen in 22:1(n-9) to levels below detection (Fig. 3B,D). Of the other monounsaturated fatty acids detected in the uninfected cells, 16:1(n-7) and 18:1(n-7) showed increases, whereas levels of 22:1(n-7) decreased initially, but were approximately equal to the starting concentrations by the final time point (Fig. 3A,C,E). As with the saturated fatty acids, increases were seen in the 18:1(n-7), 18:1(n-9) and 22:1(n-9) of virus-infected cultures, whereas no significant changes were observed in the other monounsaturates (Fig. 3). Again, the percentage of the monounsaturated fatty acids in *E. huxleyi* increased from 10 to 24% over the course of the experiment due to virus infection. Polyunsaturates represented the bulk of the fatty acids in *E. huxleyi*, with 22:6(n-3), 18:5(n-3), 18:3(n-3) and 18:4(n-3) making up 33, 20, 10 and 5% of the total respectively at the initial time point (Fig. 4B,C,D,G). The other polyunsaturated fatty acids detected were 18:2(n-6), 20:5(n-3) and 22:5(n-3), which all contributed <1% to the total respectively (Fig. 4A,E,F). As the control cultures progressed through exponential growth towards the stationary phase, decreases were observed in the fraction

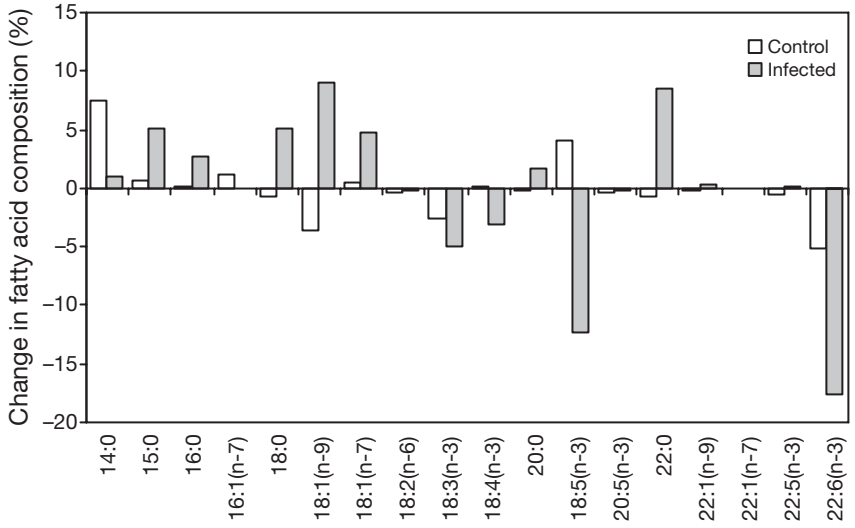

Fig. 5. EhV-86 infecting *Emiliania huxleyi*. Net changes in the fatty acid composition of control and virus-infected cultures at 151 h after the start of the experiment

of the major fatty acid 22:6(n-3) as well as in all the other polyunsaturates, with the exception of 18:4(n-3) and 18:5(n-3) which increased, the latter finally representing 24% of the total fatty acids in the control (Fig. 4). All the polyunsaturated fatty acids detected decreased in abundance during virus infection of *E. huxleyi*, with these decreases generally being more pronounced than those observed in the controls from 50 h onwards (Fig. 4). For the major fatty acids 22:6(n-3), 18:5(n-3) and 18:3(n-3), virus infection of *E. huxleyi* culminated in their final percentage of the total being reduced to 16, 8 and 5% respectively.

DISCUSSION

Over the course of the experiment, cultures of *Emiliania huxleyi* from both the uninfected controls and those containing viruses exhibited changes in the amount and composition of their fatty acids. However, changes due to virus infection were distinct and of a much greater magnitude. The decreases in the amount of fatty acids over the final 50 h of the experiment in the controls are in agreement with reports that the lipid content of algal cells decreases when cells reach the stationary phase of growth (e.g. Bell & Pond 1996). For these uninfected cells, the largest percentage increases in fatty acids were observed for 14:0 and 18:5(n-3), whereas levels of 18:1(n-9), 18:3(n-3) and 22:6(n-3) were reduced (Fig. 5). These changes are in accordance with previous reports for *E. huxleyi,* with the exception of 22:6(n-3) which has been shown to increase during the late stationary phase (Bell & Pond 1996, Pond & Harris 1996). However, as it is likely that the uninfected cultures had only just entered the stationary phase in the present study, it is possible that 22:6(n-3) increased after this point. Furthermore, different strains of *E. huxleyi* have been shown to exhibit differences not only in their fatty acid content (Pond & Harris 1996), but also in their general biochemistry; thus, it is unsurprising to find some variation when examining a previously uncharacterised strain.

Virus infection caused a decrease in the fatty acid content of the particulate phase of *Emiliania huxleyi* cultures, and total restructuring of the composition of the fatty acid pool from a dominance of polyunsaturated to monounsaturated and saturated fatty acids (Fig. 5). Virus infection is known to induce cytological, physiological and biochemical changes in *E. huxleyi*, which could be linked to the observed increases in the concentration and altered percentage composition of its fatty acids. These changes may occur as a result of specific or non-specific effects of virus infection. Specific effects would be due to the synthesis, interconversion or degradation of compounds as directed by metabolic pathways invoked by virus reproduction. This would include the synthesis of structures required to manufacture virus particles, the production of virus progeny components, or the synthesis of intracellular signalling molecules. Non-specific effects are primarily attributed to stress induced within algal cells as a consequence of virus infection.

Lipids are key molecules in the virus infection process and inhibitors of lipid biosynthesis are known to decrease replication of certain viruses, e.g. the grapevine fanleaf *Nepovirus* (Ritzenthaler et al. 2002). Virus infection can cause significant modifications to membrane systems within host cells, including the for-

mation of vesicles or spherules (Westaway et al. 1997), restructuring of the endoplasmic reticulum (Carette et al. 2000) and in particular, the production of lipids rafts (Simons & Ehehalt 2002). Lipid rafts are specific membrane microdomains involved in the localisation and concentration of virus components for the entry, assembly and budding of various types of viruses (Suzuki & Suzuki 2006). They are composed of sphingolipids and cholesterol in the outer exoplasmic leaflet, connected to phospholipids and cholesterol in the inner leaflet of the lipid bilayer, and tend to be more tightly packed than the surrounding non-raft phase. The tighter packing is facilitated by the presence of saturated hydrocarbon chains in the raft sphingolipids and phospholipids when compared with the unsaturated fatty acids of the phospholipids in the surrounding portions of the bilayer (Simons & Vaz 2004). During virus infection of *Emiliania huxleyi*, the shift in numerical dominance towards saturated fatty acids may be due to the virus-directed increases in the amount of lipid rafts present within the host's membranes as a requirement for the assembly and budding of EhV-86. Furthermore, genome sequencing of EhV-86 has revealed that it contains at least 4 genes involved in sphingolipid biosynthesis, and transcription analysis indicates that all but one of these genes are expressed during infection (Wilson et al. 2005). This supports the theory that these biomolecules are necessary for virus replication in *E. huxleyi* and could thus be implicated in rafts or other structures required for EhV-86 propagation.

The shift from a dominance of polyustaurated to saturated and monounsaturated fatty acids in the infected *Emiliania huxleyi* could also be due to the fact that these less structurally complex fatty acids are components of EhV-86 particles. Electron microscope images suggest that viruses pathogenic to *E. huxleyi* contain an internal membrane (Schroeder et al. 2002). If the presence of a membrane could be verified, it would indicate that fatty acids make up a significant proportion of the structure of EhV-86. Recent data suggests that EhV-86 is an enveloped virus that gains an external lipid membrane during a virus-budding mechanism on exit from the host cell (Mackinder et al. in press). It is likely that during the latter stages of infection, *E. huxleyi* cells would contain higher proportions of these fatty acids that would ultimately be incorporated into the virion envelope and/or become part of membranes required for virion assembly. Since the burst size of *E. huxleyi* can amount to 620 cell^{-1} (Castberg et al. 2002), the components of the *E. huxleyi* virions could represent a significant proportion of the host cell's biomass and could account for the dramatic changes seen in the fatty acid composition of infected cells. Fatty acid profiles of previously characterised virus particles have been shown to contain very low percentages of polyunsaturated fatty acids and can be markedly different from those of the host tissue (Blough & Tiffany 1968, Voiland & Bardeletti 1980, Williams & Thompson 1995). The infected cells exhibited large increases in fatty acids normally only present in small amounts in *E. huxleyi* including 15:0, 20:0, and 22:0, the last being previously undetected. However, these fatty acids have all been identified in other viruses including those from the families *Togaviridae* (Voiland & Bardeletti 1980), *Iridoviridae* (Williams & Thompson 1995) and in the case of 22:0, the *Myxoviridae*, where it was found to make up 13.7% of the PR8 influenza virus (Blough & Tiffany 1968).

Lipid molecules are not only implicated in the structure and manufacture of virus particles, but also in signalling cascades (Futerman & Hannun 2004), which may be necessary for virus replication. Wilson et al. (2005) postulated that the sphingolipid biosynthesis pathway, encoded in the genome of EhV-86, could be implicated in the regulation of apoptosis in infected *Emiliania huxleyi* cells. Sphingolipid biosynthesis leads to the formation of ceramide, an intracellular signalling molecule for apoptosis regulation, which is generated by a phospholipase-C-type reaction from its lipid precursor sphingomyelin (Merrill 2002). Indeed, viral replication in *E. huxleyi* results in the induction and active recruitment of host metacaspases, which are enzymes with a ubiquitous role in apoptosis (Bidle et al. 2007). It is possible that the induction of signalling cascades by ceramide during virus infection of *E. huxleyi* could lead to a change in lipid biosynthesis culminating in the restructuring of host cell fatty acid profiles.

Previous studies of virus-infected *Emiliania huxleyi* indicate that it is subjected to stress when its physiology and biochemistry are disrupted (Evans et al. 2006, Evans et al. 2007, Llewellyn et al. 2007). Importantly, virus-infected *E. huxleyi* cells have been shown to exhibit increased intracellular production of reactive oxygen species (ROS) and excretion of hydrogen peroxide, indicating that cells have been subjected to elevated levels of oxidative stress (Evans et al. 2006).

Lipids, particularly polyunsaturated fatty acids, are prone to breakdown by reactive oxygen species, with the products of lipid peroxidation possibly initiating further oxidation reactions that cause a chain reaction within biological systems (Halliwell & Gutteridge 1989). Peroxidation of lipids can cause disruption of the plasma membrane, and loss of *Emiliania huxleyi* membrane integrity has been observed in cultures subjected to virus infection (Evans et al. 2007). Furthermore, the change in the composition of pigment profiles in *E. huxleyi* during infection indicates that

they are subject to oxidative stress, with largest decreases having been reported for β-carotene, possibly because of its role in scavenging ROS or due to ROS-induced degradation of the photosystems where this pigment is located (Llewellyn et al. 2007). Indeed, during virus infection, decreases were seen in the fraction of 18:5(n-3), which is a fatty acid that has been postulated to be a component of the thylakoid membranes and intimately linked with photosynthetic processes in *E. huxleyi* (Pond & Harris 1996). The photosynthetic capacity of *E. huxleyi,* as represented by the F_v/F_m (a measurement of photosystem II efficiency), is reduced during virus infection (Evans et al. 2006). This supports the conclusion that 18:5(n-3) and other fatty acids, particularly the polyunsaturates, are lost during virus infection as a consequence of the reaction of ROS with components of the chloroplasts, which are associated with significant proportions of lipids (Eltgroth et al. 2005), particularly unsaturated fatty acids (Sawada & Shiraiwa 2004). Oxidative stress induced by growing *E. huxleyi* in the presence of increased CO_2 was shown to result in strongly altered fatty acid profiles and a lack of appreciable amounts of the polyunsaturated fatty acids 18:5, 18:3 and 22:6 (Rontani et al. 2007). During virus infection of *E. huxleyi,* the fatty acids most reduced in their percentage contribution were 18:3(n-3), 18:5(n-3) and 22:6(n-3), supporting the hypothesis that virus-induced oxidative stress might have caused restructuring of the fatty acid pool by peroxidation of the polyunsaturated fatty acids.

It is probable that a combination of these specific and non-specific effects of virus infection leads to the changes observed in *Emiliania huxleyi* fatty acid content, with the net result being cells that contain more fatty acids, but with a shift in dominance from polyunsaturates towards saturates. Considering the recent finding that virus-infected *E. huxleyi* cells are preferentially and more rapidly grazed by *Oxyrrhis marina* than their non-infected counterparts (Evans & Wilson 2008), these virus-induced changes could have implications for food web productivity. Stress treatments that affect algal cell composition have a major impact on their food value (e.g. Pinto et al. 2003), as the primary determinant of the quality of food passed on to higher trophic levels is its biochemical composition (Brown & Miller 1992). In marine environments, polyunsaturated fatty acids with 20 carbon atoms or more, including 20:5(n-3) and 22:6(n-3), are essential compounds that can, for example, limit zooplankton productivity (Jonasdottir et al. 1995, Bell et al. 2007). Pond & Harris (1996) suggested that *E. huxleyi* is of exceptionally high nutritional quality in terms of lipids, and may be a major source of (n-3) polyunsaturated fatty acids to the marine ecosystem for some periods of the year. However, if grazing occurs on *E. huxleyi* blooms which are virus infected (as observed in other algal species, Evans et al. 2003), the concentration of polyunsaturated fatty acids available to higher trophic levels would be lower per unit of biomass than if the bloom was not subjected to infection. Furthermore, the fact that infected cells stimulate increased rates of grazing and are preferentially consumed exacerbates this effect (Evans & Wilson 2008). Further work is required to determine the implications of altered *E. huxleyi* fatty acid content on their palatability to grazers and the consequences for the rest of the food web.

Understanding the lipid biochemistry of *Emiliania huxleyi* is crucial in understanding the functioning of the ecosystems in which this alga occurs due to its importance as a prolific lipid producer (Fernández et al. 1994) and the high nutritional value of its polyunsaturated fatty acids (Pond & Harris 1996). Therefore, processes that significantly affect the fatty acid composition of this alga warrant further study in order to fully assess their biogeochemical impact. Furthermore, there is scope to use biochemical changes, such as the occurrence of rare fatty acids, as biomarkers of virus-induced mortality, which has the potential to greatly increase our understanding of the frequency, prevalence and potential implications of virus infection in marine phytoplankton.

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