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The relative significance of viral lysis and microzooplankton grazing as pathways of dimethylsulfoniopropionate (DMSP) cleavage: An *Emiliania huxleyi* culture study

Claire Evans[1], *Susanne V. Kadner, and Louise J. Darroch*
Laboratory for Global Marine and Atmospheric Chemistry, School of Environmental Sciences, University of East Anglia, Norwich, NR4 7TJ, United Kingdom

William H. Wilson[2]
The Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill, Plymouth, PL1 2PB, United Kingdom

Peter S. Liss and Gillian Malin
Laboratory for Global Marine and Atmospheric Chemistry, School of Environmental Sciences, University of East Anglia, Norwich, NR4 7TJ, United Kingdom

Abstract

Dimethylsulfoniopropionate (DMSP) cleavage was investigated during culture studies of grazing by the microzooplankter *Oxyrrhis marina* and viral lysis by *Emiliania huxleyi* virus 86 (EhV-86) on two axenic strains of *E. huxleyi*. The cleavage products of DMSP, dimethyl sulfide (DMS) and acrylic acid (AA), accumulated during viral infection of both strains, confirming that viral lysis of algae can lead directly to DMSP cleavage. AA and DMS accumulated in parallel with compromised *E. huxleyi* cells, indicating that DMSP cleavage occurred during the physical disruption of the infected cells. This is in agreement with the hypothesis that DMSP and DMSP lyase ([DL] the enzyme responsible for cleaving DMSP) are segregated in healthy or undamaged cells. During grazing, the concentrations of DMS and AA produced per eaten cell were an order of magnitude higher than the concentrations resulting from cell death caused by viral infection, suggesting that grazing is the quantitatively more significant pathway of DMS production in *E. huxleyi*. Levels of DL activity decreased in infected cultures to a minimum of 0.00065 fmol cell^{-1} min^{-1} as compared with an average of 0.09 fmol cell^{-1} min^{-1} in the control cultures, indicating that reduced DL activity in virally infected cells was responsible for the lower levels of DMSP cleavage observed during viral lysis.

Dimethyl sulfide (DMS) is a climatically active trace gas formed in seawater primarily by interactions of the microbial food web (Simo 2001). The majority of DMS originates from its precursor dimethylsulfoniopropionate (DMSP), a compound found in many taxa of algae (Keller et al. 1989). DMS and acrylic acid (AA) are formed when DMSP is cleaved by a group of isozymes known as DMSP lyases (DL), which are ubiquitous among the marine microbial community and have been found in marine bacteria (Ledyard and Dacey 1994) and DMSP-containing phytoplankton (Steinke et al. 1998). DMSP and its cleavage products generated via the action of DL have been suggested to have many functions within the cell relating to metabolic processes and maintaining homeostasis (for more details *see* Stefels 2000; Simo 2001), for example as an antioxidant cascade (Sunda et al. 2002). DMSP and its downstream products have also been implicated in cellular defense against grazing (Wolfe et al. 1997) and viral infection (Evans et al. 2006*b*).

Actively growing phytoplankton may excrete some DMS (Vairavamurthy et al. 1985) although production is enhanced during the death or injury of algal cells. Autolysis (Nguyen et al. 1988), grazing (Dacey and Wakeham 1986), viral lysis (Malin et al. 1998), and mechanical and chemical stress (Wolfe et al. 2002) have all been shown to increase DMS production. During grazing by *Oxyrrhis marina* on *Emiliania huxleyi* it was established that the concentration of DMS generated was directly related to the activity of the DL of the prey organism (Wolfe and Steinke 1996). Previously DMS production during viral lysis of phytoplankton has only been shown in the presence of bacteria (Hill et al. 1998). However, during a study of viral infection

[1] Present address: Department of Biological Oceanography, Royal Netherlands Institute for Sea Research, P.O. Box 59, NL-1790 AB Den Burg, Texel, The Netherlands.

[2] Present address: Bigelow Laboratory for Ocean Sciences, 180 McKown Point Road, POB 475, West Boothbay Harbor, Maine 04575.

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of the alga *Phaeocystis pouchetii* it was calculated that the contaminating bacteria played only a minor role in the conversion of DMSP to DMS (Malin et al. 1998).

Far less is known about the production of AA despite its ecological importance as a carbon source for some marine bacteria (e.g., Kiene 1990). Its production has been observed during in vitro tests for DL activity in *E. huxleyi* CCMP 373 and the dinoflagellate *Alexandrium tamerense* (Wolfe et al. 2002). This might suggest that both AA and DMS would be produced during grazing and viral infection.

There is some evidence to suggest that the relative importance of the different DMSP cleavage pathways may vary in the field. A Lagrangian study of a coccolithophore bloom in the North Sea revealed that microzooplankton grazing accounted for the majority of particulate DMSP degradation (Archer et al. 2001*a*), whereas viruses did not correlate with concentrations of either DMSP or DMS (Wilson et al. 2002*b*), indicating that in this case, grazing was a more significant driver of DMS biogeochemistry than viral infection. In contrast, a modeling study of seasonal patterns of DMS production in the North Sea found that leakage and lysis were far more significant DMS production processes than grazing (Archer et al. 2004), although in this case lysis caused by viral activity was not considered. Archer et al. (2004) also suggested that the reaction of DL enzymes to processes that cause DMSP release from phytoplankton is one of the keys to correctly modeling DMS production.

Viruses are numerous and ubiquitous in the world's oceans and are known to cause significant mortality of various algal species. It is pertinent to sulfur cycling that they have been implicated in the collapse of blooms of DMSP-containing algae including *E. huxleyi* (Wilson et al. 2002*a*) and *Phaeocystis* spp. (Brussaard et al. 2005). A number of viruses pathogenic to DMSP-containing phytoplankton have been isolated, and of these, the *E. huxleyi* host virus system is probably the most extensively characterized (Wilson et al. 2002*a*; Wilson et al. 2005). *E. huxleyi* is an ecologically and biogeochemically relevant species known for forming large-scale coastal and open ocean blooms at temperate latitudes that can cover 10,000 km^2 or more and are associated with high levels of DMS in the field (e.g., Malin et al. 1993).

In this study we assessed DMS and AA production during viral lysis and microzooplankton grazing on two *E. huxleyi* strains to quantitatively compare these mortality mechanisms as pathways of DMSP cleavage. DL activity was also examined in virally infected cultures to determine whether infection altered activity and, therefore, the ability of infected cells to cleave DMSP.

Methods

Culturing—Cultures of *E. huxleyi* (Lohmann) Hay et Mohler were obtained from the Provasoli-Guillard Centre for the Cultivation of Marine Phytoplankton ([CCMP] West Boothbay Harbor, Maine: strain CCMP 374) or from Brian Palenik (Scripps Institution of Oceanography, San Diego, California: strain CCMP 1516). *Dunaliella tertiolecta* CCAP 19/27 and the heterotrophic dinoflagellate *O. marina* CCAP 1133/5 were from the Culture Collection of Algae and Protozoa ([CCAP] Dunstaffnage Marine Laboratory, Scotland). All of the cultures used were axenic as judged by routine checks for bacterial contamination by staining with 4′,6-diamidino-2-phenylindole dihydrochloride and epifluorescence microscopy. Stock cultures were maintained in *f*/2 medium at 15°C under a 14-h light : 10-h dark cycle at 250 μmol photons m^{-2} s^{-1}, apart from *D. tertiolecta,* which was kept at 188 μmol photons m^{-2} s^{-1}. *O. marina* cultures were fed with *D. tertiolecta* and then transferred to the dark for several days to enable grazers to remove the prey. The viral pathogen used was *E. huxleyi* virus 86 (EhV-86) (Wilson et al. 2002*a*), and lysates were generated for each experiment on cultures of *E. huxleyi* of the same strain used in the experiment. After lysis cultures were filtered (0.22 μm) and stored in the dark at 4°C until required. Concentrated virus stocks were generated via ultrafiltration using a 30-kDa polyethersulfone membrane filter and Vivaflow 200 system (Sartorius, Goettingen, Germany). The lysate generated on *E. huxleyi* strain 1516 was dialyzed to reduce the amount of DMSP and related compounds that it contained. Concentrates (100-mL aliquots) were dialyzed twice for a minimum of 4 h against 1-liter volumes of fresh *f*/2 medium through a 10,000 nominal molecular weight cut-off membrane at 4°C in the dark.

Viral lysis experiments—Four 3-liter replicate cultures of *E. huxleyi* strain CCMP 1516 were set up in 5-liter Erlenmeyer flasks, and two 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 approximately 1. All cultures were sampled twice daily at intervals of approximately 7 h and 17 h until completion of the culture crash, which took 5 days. Samples were taken for DMSP particulate (DMSPp), DMSP dissolved (DMSPd), DMS, AA, *E. huxleyi*, and virus enumeration and *E. huxleyi* viability. Before sampling the cultures were very gently swirled to promote the resuspension of cells while minimizing the loss of DMS from the liquid phase. To investigate DL activity in *E. huxleyi* 1516, the experiment was repeated using eight 1-liter cultures in 2-liter Erlenmeyer flasks. Samples for cell and virus enumeration were taken every 24 h for 72 h. At 72 h, viral infection was established and one infected and one control culture were harvested to measure DL activity, cell enumeration, virus enumeration, and *E. huxleyi* viability. This was repeated at 78, 96, and 102 h for the remaining cultures.

A further viral lysis experiment was conducted as described above on *E. huxleyi* strain CCMP 374. Daily samples were taken for cell counts, virus particle counts, DMSPp, DMSPd, DMS, and AA for 5 days throughout the crash period.

Microzooplankton grazing experiments—Parallel grazing experiments were conducted on *E. huxleyi* strains CCMP 374 and 1516, using mid-exponential phase cultures to inoculate three 1.22-liter polycarbonate bottles. A day

later, *O. marina* was added to two bottles of each strain to serve as the grazed treatments, with the third bottle containing only *E. huxleyi* serving as the *E. huxleyi* controls. *O. marina* was also added to a further control bottle without *E. huxleyi* and served as the grazer control. Initial cell densities were *E. huxleyi* 3×10^4 cells mL^{-1} and *O. marina* 500–600 cells mL^{-1}. Before sampling, the bottles were gently rotated and then left to re-equilibrate for 10 min. Samples were taken from the controls at 0, 24, 48, 72, and 144 h and from the grazed treatments at 3 and 5 h, then approximately every 24 h before the final time point at 145 h. Treatments containing *E. huxleyi* were sampled for DMSPp, DMSPd, DMS, AA, *E. huxleyi*, and *O. marina* counts. *O. marina* numbers were also monitored in the grazing control.

Biological parameters—Phytoplankton counts were done using a Beckman Coulter Multisizer 3 with a 100-μm orifice. Autoclaved, 0.2-μm-filtered seawater was used as the electrolyte. Samples were analyzed in triplicate, and data were collected and interpreted using Coulter Multisizer3 Version 3.01a software.

The percentage of compromised *E. huxleyi* cells was measured using the cell viability stain SYTOX-Green. Cells were stained by dark incubation with 0.5 μmol L^{-1} SYTOX Green for 10 min at 15°C. Samples were analyzed on a FACSCalibur flow cytometer (Becton Dickinson Biosciences) with Milli Q as the sheath fluid. *E. huxleyi* cells were discriminated by their red autofluorescence versus the green fluorescence of the stain.

For virus enumeration, 1-mL samples were fixed in a final concentration of 0.5% glutaraldehyde for 4 h at 7°C. Samples were then snap frozen in liquid nitrogen and stored at −80°C before analysis using SYBR Green I nucleic acid gel stain (Molecular Probes Inc.) according to the method of Marie et al. (1999).

O. marina samples preserved with Lugol's iodine were counted in triplicate using a Sedgwick-Rafter counting chamber. One milliliter of the preserved sample was transferred to the counting chamber and left for 10 min to enable the protists to settle. Cell number estimates were calculated on the basis of 30 randomly selected fields of view per chamber. The coefficient of variation between the three slide counts for each sample was usually ≤15%.

Chemical analysis—DMS analysis was performed on a Shimadzu GC-14B gas chromatograph with a Chromosil 330 packed column and flame photometric detector. Nitrogen was used as a carrier gas with a flow rate of 60 cm^3 min^{-1}. Column temperature was isothermal at 60°C. DMS was extracted and preconcentrated using a cryogenic purge and trap according to the method of Turner et al. (1990). The system was calibrated using DMSP standards hydrolyzed with 10 mol L^{-1} NaOH.

Five to ten milliliter samples for DMSPp analysis were passed gently through a Whatman GF/F filter in a 25-mm-diameter Swinnex filtration unit (Millipore). The filter was immediately transferred to a 21-mL glass vial, which was then filled with distilled water and 1 mL of 10 mol L^{-1} NaOH, ensuring no headspace remained. Vials were sealed gas-tight with Pharma-Fix liner crimp seals (Alltech Associates) and stored in the dark at room temperature for a minimum of 24 h before DMS analysis. DMSPd was determined from subaliquots that were used to analyze DMS or was determined from stored samples similar to that described in Turner et al. (1990). For immediate analysis, DMSPd was analyzed directly after the determination of DMS using the same method to analyze DMS, except with the addition of 1 mL 10 mol L^{-1} NaOH to the purge tube. Alternatively, GF/F filtrates that had been prepurged to remove DMS (using oxygen-free nitrogen for 20 min at 60 mL min^{-1}) were added to vials and stored as described for DMSPp. Subsamples were then removed and analyzed as DMS. Both methods yield 100% DMSP hydrolysis (Turner et al. 1990).

DL activity was determined using an adaptation of the method of Steinke et al. (2000). One liter of culture was concentrated to 50 mL by centrifugation for 10 min at 5,000 × g and 4°C. The pre-concentrated culture was then further concentrated by repeating the centrifugation step and the resultant pellet was used to perform the assay. DMSP cleavage by DL was quantified as DMS production per unit time and calculated on a per cell basis corrected for cells remaining in the supernatant.

Samples for dissolved AA were prepared by filtering 2 mL of sample through a 0.2 μm polycarbonate membrane filter (Millipore) under a gentle vacuum (5 KPa). To minimize cell damage during the filtration process the vacuum was removed after 1.5 mL of the sample had passed through the filter. The samples were filtered directly into a HPLC vial containing 20 μL 85% phosphoric acid (H_3PO_4). Samples were then analyzed on an Agilent 1100 high performance liquid chromatography (HPLC) system using an eluent of pre-mixed Milli-Q water with 0.05% H_3PO_4 (v/v) and a flow rate of 1 mL min^{-1} maintained at 40°C. The system was equipped with a temperature-controlled autosampler that was maintained at 4°C, and samples were introduced by direct injection of 100 μL. The detection wavelength was set to 205 nm with a bandwidth of 4 nm and the slit width opened to 16 nm.

Calculation of production rates—Viral lysis and grazing rates were calculated from the cell counts of the control and the infected/grazed cultures, respectively. The coefficient of growth (K) was calculated from the slope of ln cell counts plotted against time for the appropriate control culture, and the resulting value was also assumed to apply to uninfected or ungrazed *E. huxleyi* cells in all experimental treatments. Mortality coefficients were calculated from the slope of ln cell counts plotted against time for the infected and grazed populations. The slope of this line was equal to K–g, where g is the coefficient of mortality and K is the growth rate. The average number of cells (C_{av}) during a given time period was calculated according to the method of Frost (1972) and used to estimate the number of cells lysed or grazed (C_g) during the period using

$$C_g = C_{av}gt$$

where g is the coefficient of grazing or lysis, and t is time.

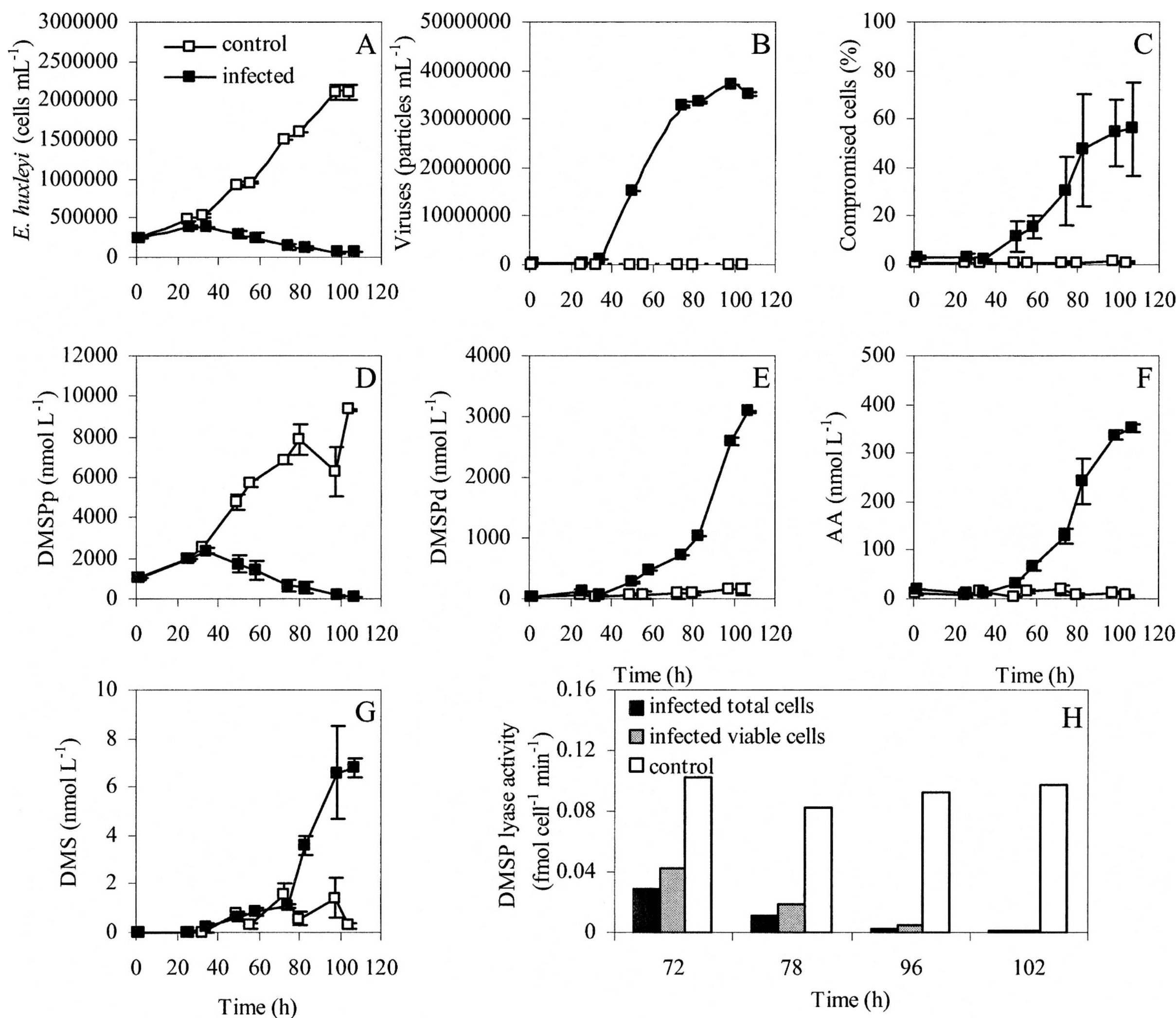

Fig. 1. The effect of viral infection in cultures of *E. huxleyi* strain CCMP 1516 on (A) *E. huxleyi* concentration, (B) virus particle concentration, (C) percentage compromised *E. huxleyi*, (D) DMSPp, (E) DMSPd, (F) AA, (G) DMS, and (H) DL activity. Other than for DL activity, data points are the mean and error bars show the range for the duplicate infected and control cultures. Where error bars are not visible they are smaller than symbol size.

DMS produced per cell lysed or grazed was calculated from the DMS produced during each diel cycle (corrected for DMS produced by live cells) and the number of cells that were lost because of viral lysis or grazing during that period. The average quantity of DMS produced per live cell was estimated from cell number, and DMS produced per diel cycle in the control cultures and the same rate of DMS production was applied to the infected and grazed cultures using the average number of uninfected or live cells during the diel cycle. Equivalent calculations were also done for AA.

Results

Viral lysis—During virus infection of *E. huxleyi* strain CCMP1516 the decline in the number of cells was noticeable after 35 h in the infected cultures, and this was concomitant with the accumulation of virus particles and compromised cells in the culture medium (Fig. 1A–C). DMSPp concentrations declined in parallel with cell density, whereas DMSPd accumulated as infection of the cultures progressed (Fig. 1D,E). DMS and AA concentrations increased in parallel during the culture crash, reflecting the increasing numbers of compromised cells (Fig. 1C,F,G). Levels of both compounds began increasing from approximately 50 h, although DMS concentrations only increased above those in the controls after 70 h. The concentration of AA was much higher than DMS with maxima at 353 nmol L^{-1} and 7 nmol L^{-1}, respectively.

In a second viral lysis experiment on strain 1516 DL activity was relatively constant in the control cultures, with average values of approximately 0.09 fmol $cell^{-1}$ min^{-1} (Fig. 1H), whereas in the infected cultures at 72 h, DL activity was lower at 0.042 fmol $cell^{-1}$ min^{-1} and continued to decrease for the rest of the experiment with a final value of 0.00065 fmol $cell^{-1}$ min^{-1}. When discounting compromised cells from the calculation of DL activity, as

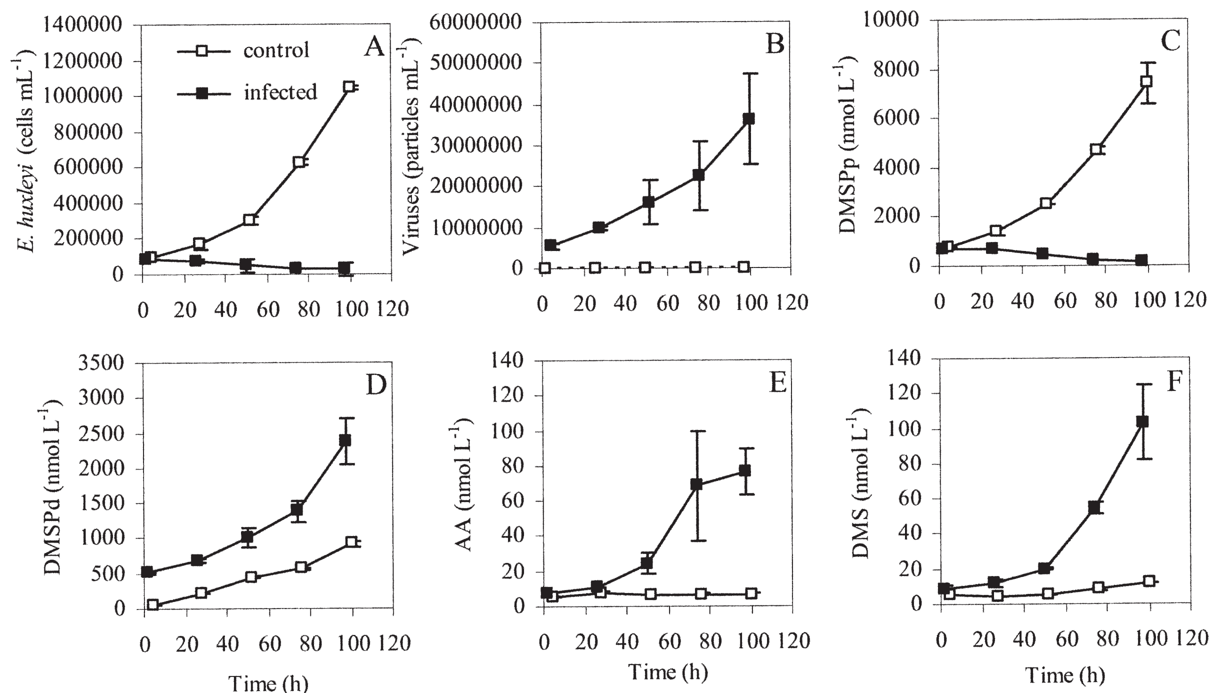

Fig. 2. The effect of viral infection in cultures of *E. huxleyi* strain CCMP 374 on (A) *E. huxleyi* concentration, (B) virus particle concentration, (C) DMSPp, (D) DMSPd, (E) AA, and (F) DMS. Data points are the mean, and error bars show the range for the duplicate infected and control cultures. Where error bars are not shown they are smaller than symbol size.

determined by SYTOX assay, it was observed that values were still much lower than observed in the control cultures with a final value of 0.00104 fmol cell^{-1} min^{-1}.

The reduction of *E. huxleyi* strain 374 densities by viral lysis was apparent from 24 h and concomitant with the production of virus particles, whereas the control cultures continued to grow normally (Fig. 2A,B). In response to changes in the cell densities, DMSPp increased in the control cultures and decreased in the infected cultures as they crashed (Fig. 2C). Initial concentrations of DMSPd were higher in the infected treatments than the controls, and this is probably because the viral lysate generated on *E. huxleyi* strain 374 was not dialyzed (Fig. 2D). Nevertheless, throughout the experiment DMSPd accumulated in all of the cultures. Viral lysis greatly enhanced the concentration of AA, reaching 77 nmol L^{-1} in the infected cultures, whereas levels remained relatively constant in the control cultures (Fig. 2E). As with viral lysis of *E. huxleyi* strain 1516, DMS production was observed in parallel with AA (Fig. 2E,F). However, in contrast with the results for strain 1516, approximately equimolar concentrations of DMS and AA were observed.

Microzooplankton grazing—During the grazing experiment on strain 1516 *E. huxleyi* densities increased in the presence and absence of *O. marina*, which was expected as the experiments were conducted in the light, but the biomass achieved was much lower in the treatments containing the grazer consistent with active grazing (Fig. 3A). Furthermore, despite *O. marina* numbers increasing in both the grazer control treatment and the grazing treatments, the increase in the latter was greater, rising from 544 mL^{-1} to 1,450 mL^{-1} as compared with 683 mL^{-1} to 1,366 mL^{-1} in the control (Fig. 3B). The concentration of DMSPp paralleled the concentration of *E. huxleyi*, with levels increasing in both treatments but exhibiting much lower concentrations in the grazed cultures as compared with the controls (Fig. 3C). DMSPd also increased in the *E. huxleyi* control and grazed cultures, although levels were higher in the control treatments, probably as a result of the higher cell density (Fig. 3D). In the grazed treatments AA accumulated rapidly and from 24 h to 144 h it increased linearly with time, reaching a maximum concentration of 77 nmol L^{-1} (Fig. 3E). During the initial phase of the experiment DMS in the grazed treatments also showed a net increase; however, after 72 h concentrations fell (Fig. 3F). As with viral lysis of *E. huxleyi* strain 1516, maximum concentrations of DMS were much lower than maximum concentrations of AA, being only 1.8 nmol L^{-1}.

During grazing on strain 374, *E. huxleyi* cell numbers increased in both treatments with the most growth seen in the *E. huxleyi* control culture (Fig. 4A). However, the difference in prey densities between the grazed and ungrazed cultures was not as pronounced as those observed during grazing on strain 1516, suggesting that *O. marina* did not graze as voraciously on strain 374. Furthermore, although *O. marina* numbers increased in the treatments containing prey, the grazer control incubation showed an even greater increase in predator concentration (Fig. 4B). Despite this, the reduction in prey density indicates that *E. huxleyi* were consumed by *O. marina* during the experiment. Again, DMSPp concentration followed the abundance of *E. huxleyi* cells increasing in all treatments

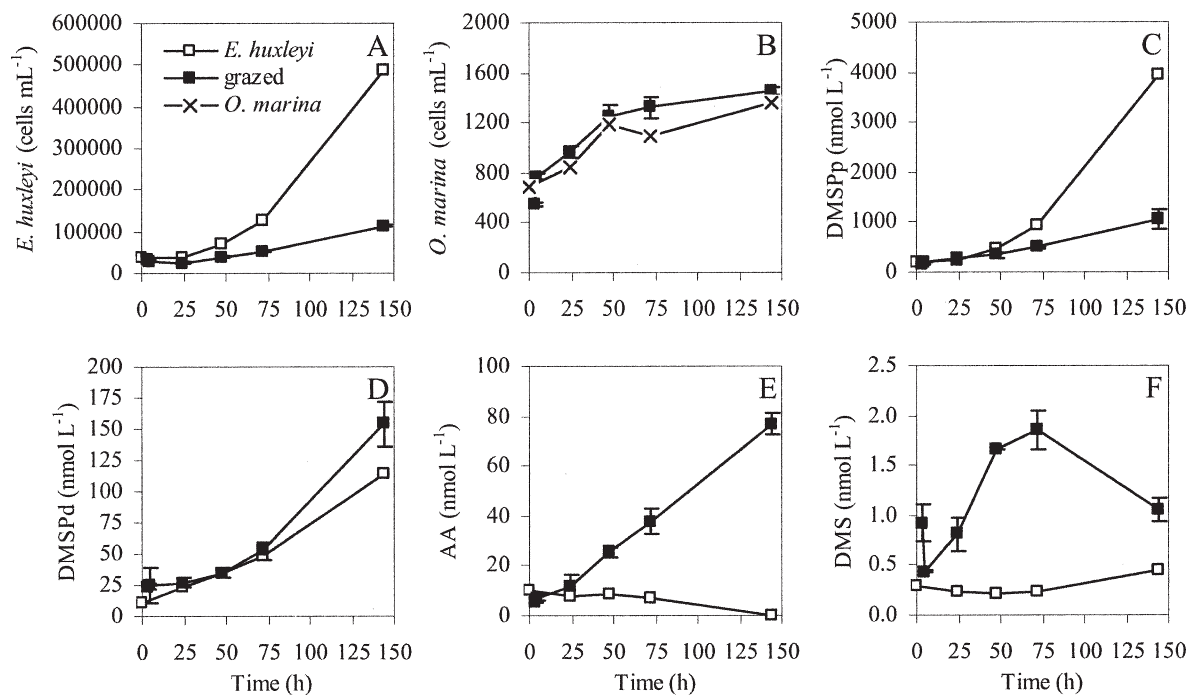

Fig. 3. The effect of microzooplankton grazing by *O. marina* in cultures of *E. huxleyi* strain CCMP 1516 on (A) *E. huxleyi* concentration, (B) *O. marina* concentration, (C) DMSPp, (D) DMSPd, (E) AA, and (F) DMS. Data points are the mean, and error bars represent the range for the duplicate grazed cultures. Where error bars are not shown they are smaller than symbol size.

containing *E. huxleyi*, but exhibiting the greatest increase in the control (Fig. 4C). DMSPd also increased over the experiment, with the highest values exhibited in the grazed cultures (Fig. 4D). AA increased significantly in response to grazing in the last 94 h with final concentrations of 76 nmol L^{-1}, whereas in the control levels dropped below detection (Fig. 4E). The levels of DMS also increased during grazing of *E. huxleyi* strain 374 to levels similar to

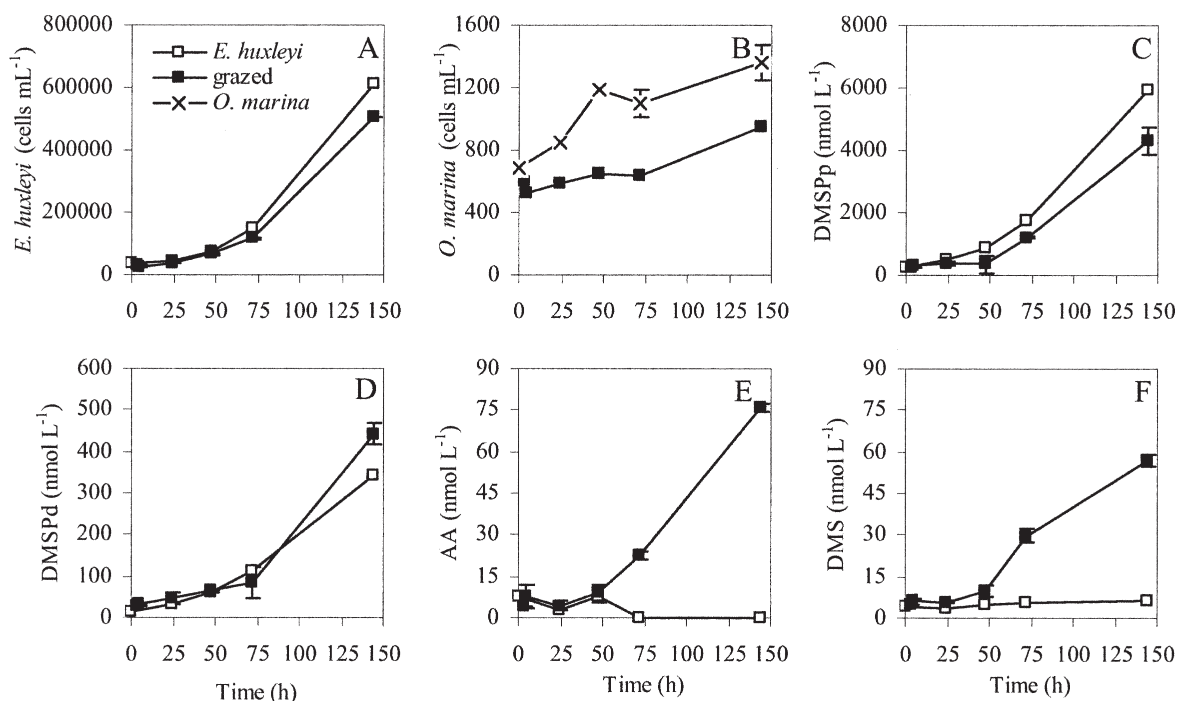

Fig. 4. The effect of microzooplankton grazing by *O. marina* in cultures of *Emiliania huxleyi* strain CCMP 374 on (A) *E. huxleyi* concentration, (B) *O. marina* concentration, (C) DMSPp, (D) DMSPd, (E) AA, and (F) DMS. Data points are the mean, and error bars represent the range for the duplicate grazed cultures. Where error bars are not shown they are smaller than symbol size.

Table 1. Dimethyl sulfide and acrylic acid production rates during viral lysis and microzooplankton grazing of *Emiliania huxleyi* strains CCMP 1516 and 374.

Emilainia huxleyi strain	Mortality source	Production rate mean (range) (fmol cell^{-1} day^{-1})	
		DMS	AA
CCMP 1516	Viral lysis	0.0105 (−0.0013 to 0.0412)	0.4508 (−0.1523 to 1.5668)
CCMP 1516	Microzooplankton grazing	0.1838 (−0.0134 to 0.5535)	3.1453 (0.4162 to 8.0800)
CCMP 374	Viral lysis	0.7875 (0.0045 to 2.3469)	0.3600 (0.0060 to 0.8734)
CCMP 374	Microzooplankton grazing	5.3170 (−0.0590 to 15.7876)	4.5957 (−0.3685 to 10.1567)

those observed for AA, with a final concentration 57 nmol L^{-1} (Fig. 4E,F).

Production rates—Mean production rates for both DMS and AA were within the same order of magnitude during mortality of *E. huxleyi* strain 374 indicating their production may have been equimolar (Table 1). However, during mortality of strain 1516 the levels of DMS produced were much lower than those of AA. When comparing experiments completed on the same strain, microzooplankton grazing was a quantitatively more significant pathway of DMS and AA production than viral lysis, with mean production rates an order of magnitude higher than during viral lysis.

Discussion

DMSP cleavage during viral infection—During virus-induced mortality of both *E. huxleyi* strains examined, DMS and AA concentrations increased, confirming the hypothesis of Malin et al. (1992) that viral lysis of phytoplankton can act as a pathway for DMSP cleavage. In previous studies of microalgae infected by viruses AA concentrations were not examined, DMS production has only been observed in cultures containing bacteria (Malin et al. 1998), and in the case of *Micromonas pusilla* it was shown to be dependent on bacteria (Hill et al. 1998). Malin et al. (1998) calculated that bacteria were negligible in DMS production during virus-induced lysis of *P. pouchetii*, and given that the cultures of *E. huxleyi* used here were free from bacterial contaminants, our results support their conclusion that most of the DMS produced could be attributed to the infected algae. These studies suggest that DMS and AA production during viral lysis could be dependent on the presence of active DL although, as far as we are aware, DL activity has not yet been assessed for *M. pusilla*. We found that levels of DMS and AA increased as compromised *E. huxleyi* cells accumulated in the cultures. It is possible that, as hypothesized for grazing (Wolfe and Steinke 1996), these compounds could be generated during viral lysis by the physical degradation of cells and the mixing of the normally segregated DL with its substrate.

DL activity was detected in infected cultures although it was significantly reduced when compared to healthy cultures. DL activity in infected cells could be reduced by a number of processes, including changes in the physiochemical conditions within the host cell to those suboptimal to the requirements of the enzyme, inactivation of the enzyme, or its loss from the host cell. Given that an in vitro assay was used, changes in intracellular conditions are unlikely to account for the differences observed between the control and infected cultures, although they may have been a factor in terms of in vivo DL activity. This would suggest that during viral infection the enzyme was inactivated or lost from the host cell. A possible explanation for the inactivation of DL is that it may be a short-lived enzyme that is constantly manufactured by the cell and viral infection terminated the production of this protein, leading to the reduction in apparent DL activity. A similar decline in the activity of phytoplankton esterase enzymes during viral infection was identified in both *M. pusilla* and *P. pouchetii*, suggesting that this phenomenon in host biochemistry is widespread (Brussaard et al. 2001).

A recent study has shown that DMS and AA at concentrations that potentially occur within virally infected cells decrease the infectivity of *E. huxleyi* viruses, and therefore DMSP cleavage may serve as an antiviral mechanism (Evans et al. 2006*b*). In this case reduction of DL activity and lower intracellular concentrations of DMS and AA during the viral infection process could serve as a counter strategy by the virus to protect the infectivity of its progeny.

Microzooplankton grazing versus viral lysis as a DMSP cleavage pathway—To our knowledge, this is the first study to directly compare viral lysis and microzooplankton grazing as mechanisms of DMSP cleavage. Results revealed that grazing is by far the most quantitatively significant process of DMS and AA production for both *E. huxleyi* strains examined. This seems surprising because during viral lysis a greater proportion of the total cell contents are probably released to the water column than during grazing, where they may be incorporated into the grazer. Indeed this has shown to be the case when copepods graze on algae that contain DMSP (Tang et al. 1999). Furthermore, during a study of *O. marina* grazing on *Isochrysis galbana* it was estimated that 67% of the ingested DMSPp was contained within the grazer (Archer et al. 2001*b*). The primary factors likely to be responsible for the differences in DMSP cleavage observed during viral lysis and grazing were their effect on the DL activity of *E. huxleyi* in combination with the physical circumstances of the mixing of DL with its substrate. During viral infection, DL activity levels were significantly reduced, which is consistent with

the lower DMS and AA levels observed when compared with grazing, assuming that the reduction in DL activity due to grazing is low or absent. Furthermore, during viral infection, DMS and AA are generated when cells start to lose integrity, and it is likely that DMSP, and possibly DL, are also lost from the cell at this point. In contrast, microzooplankton grazing facilitates the disruption of cells and mixing of DMSP with DL in a contained manner within the feeding vacuole, and digestion rates are thought to be slow enough to allow partially digested or damaged cells to continue to produce DMS (Wolfe and Steinke 1996). Overall our findings endorse the suggestion of Archer et al. (2004) that the reaction of DL to mechanisms that release DMSP from algae are a key factor in the subsequent amount of DMS produced.

A further explanation for the lower levels of DMS and AA observed as a result of viral lysis is that virally infected phytoplankton may be subject to oxidative stress. A recent study using the *E. huxleyi* host-virus system found that in virally infected cultures, cells exhibited elevated levels of intracellular reactive oxygen species (ROS) and excreted hydrogen peroxide (Evans et al. 2006*a*). DMSP has been shown to effectively scavenge hydroxyl radicals, and AA and DMS were identified as even more effective antioxidants (Sunda et al. 2002). Therefore, elevated levels of ROS during viral infection may lead to the conversion of DMSP and its cleavage products. However, it has been suggested that ROS formation may be implicated in light-aided digestion of phytoplankton by herbivorous protists (Strom 2001), indicating that during microzooplankton grazing DMSP and its downstream cleavage products may also be lost because of oxidative stress. Further studies are required to determine the link between the production of DMSP and related compounds and oxidation during the mortality of phytoplankton.

Relative production of DMS and AA by E. huxleyi strains 1516 and 374—During both mortality mechanisms it was observed that in *E. huxleyi* strain 374 the concentrations of AA and DMS produced were similar indicating production may have been equimolar, whereas in *E. huxleyi* strain 1516 DMS levels that were produced were lower than those of AA. Furthermore, DMS levels in the infected cultures took longer to increase above levels in the control when compared with AA. This suggests that in strain 1516 either AA was produced by a pathway other than DMSP cleavage or that DMS was lost by some process that did not occur or occurred at a lower rate in strain 374, indicating differences in the biochemistry of these strains. Previous genotypic characterization of *E. huxleyi* morphotypes found that the two strains used in this study could be separated based on diagnostic markers (Schroeder et al. 2005) confirming their genetic distinctness.

Very little is known about the role of AA within algal cells, although recent studies suggest it is an effective antioxidant (Sunda et al. 2002) and thus phytoplankton could confer some advantage by an additional metabolic route of its production. AA is a potential product of bacterial DMSP demethylation (Taylor, 1993; Kiene et al. 2000), but as far as we are aware this pathway has never been shown for marine phytoplankton. It can also be formed via the reaction of β alanine betaine, the quarternary ammonium analogue of DMSP, with OH^- (King 1988), although again there appears to be no direct evidence for this compound in algal cells.

A number of mechanisms could be responsible for the loss of DMS in cultures of strain 1516. The removal of nanomolar additions of DMS by *E. huxleyi* have been observed, with varying rates reported for different strains (Wolfe et al. 2002), so cells of strain 1516 may have been removing DMS at faster rates than those of strain 374. Although Wolfe et al. (2002) did not investigate strain 1516, strain 374 was examined and this was in the group that removed DMS at a lower rate, which would be consistent with our data. It was suggested that the mechanism of DMS removal was either cellular uptake or oxidation to dimethyl sulfoxide (DMSO). Previously, DMSO production via oxidation of DMS has been shown over diverse phytoplankton taxa, and it was hypothesized that this is because of photosensitizers produced from algal pigments (Fuse et al., 1997). It is possible that in strain 1516 consumption of DMS is implicated in a stress response pathway that is not present or occurs at a much lower rate in strain 374.

If the deficit between the AA and DMS produced during mortality of strain 1516 is as a result of DMS loss then these results call into the question the conclusion of Wolfe and Steinke (1996) that the concentration of DMS resulting from grazing on *E. huxleyi* is relative to the activity of the DL expressed by the prey (Kadner 2004), as DMS produced will also be related to the rate of its loss. It is important to emphasize that DMS and AA are not dead end products within the cell (e.g., Sunda et al. 2002), and these compounds are part of the dynamic system involved in maintaining homeostasis within phytoplankton. Further studies of the sulfur biochemistry of different *E. huxleyi* strains are warranted to determine the precise explanation for the lower rates of DMS production observed during mortality of strain 1516.

Implication for DMS production—DMS and probably AA concentrations within the oceans are the result of a complex web of production and loss processes (Simo 2001). The primary stage production of DMS can result from either direct release from phytoplankton via autolysis and exudation (Vairavamurthy et al. 1985; Nguyen et al. 1988) or via the interaction of algal cells with microzooplankton (Dacey and Wakeham 1986) or viruses (Malin et al. 1998), and it has been indicated that these production mechanisms may result in different DMS yields (Archer et al. 2004). Phytoplankton blooms are known to be terminated by both microzooplankton grazing and viral lysis or a combination of both mortality mechanisms (Evans et al. 2003).

The importance of microzooplankton grazing in the turnover of DMSPp and DMS production has been established in several in situ studies. During a coccolithophore bloom microzooplankton grazing was determined as the principal fate of DMSPp (Archer et al. 2001*a*), and during a study of microzooplankton herbivory in the North

Sea estimates of *Phaeocystis* DMSPp ingested were found to account for the DMS production rates observed (Archer et al. 2003). However, studies also indicate that even when microzooplankton are the main agents in the conversion of DMSP from the particulate to the dissolved phase they may not necessarily facilitate the direct production of significant quantities of DMS (Archer et al. 2002). These findings support the data presented here by demonstrating that DMS production is not merely a product of algal cell mortality but also of the characteristics of the DMSP-containing cells.

Although the importance of viral lysis and microzooplankton grazing as pathways of DMS and AA production should be recognized, it is important to consider that the concentrations of these compounds within the oceans are the result of a complex web of production and loss processes (Simo 2001). In addition to these processes, other biological factors, particularly the action of bacteria through the microbial loop, are central in controlling dissolved DMS and AA concentrations (Gabric et al 1993). Furthermore, physical processes such as shear stress can also combine with biological processes to influence DMS concentrations (Wolfe et al 2002).

We have observed DMS accumulation in response to apparent viral-induced decline of *E. huxleyi* blooms during two mesocosm studies, suggesting this process is significant in the marine environment (Darroch 2003; Evans 2005). Although other in situ investigations have failed to establish an unequivocal link between viral-mediated mortality and DMS production, this has been explained by other factors. During a coccolithophore bloom in the North Sea where no correlation could be identified between viruses and DMS/P (Wilson et al. 2002*b*), microzooplankton were established as the main agents of algal mortality (Archer et al. 2001*a*). Low densities of the algal host and rapid bacterial degradation of sulfur compounds have also been used to explain the inability to relate DMS production to viral termination of *E. huxleyi* blooms (Bratbak et al. 1995). However, it could also be suggested that the failure to establish this link in the field is because of the physiological consequences of viral infection on algal cells, in that this leads to a reduction in the activity of the hosts DL enzyme, a necessary component for the direct production of DMS.

This is the first report of DMSP cleavage during viral lysis of axenic phytoplankton strains. It is also the first to demonstrate that, for *E. huxleyi*, microzooplankton grazing is a quantitatively more significant pathway of DMS and AA production than viral lysis, and it is likely that this is because of decreased DL activity in virally infected cells. If these conclusions prove to be true for other phytoplankton species, these results indicate that primary DMS and AA production in the sea may vary substantially according to the dominant phytoplankton mortality mechanism. Hence, we suggest that this should be taken into consideration when attempting to model DMS production in the oceans. Our data also suggest that the mortality of closely related microalgal strains with apparently similar DMSP content and levels of DMSP lyase activity can result in different DMS yields. Resolving the significance of this finding will require the development of robust methods that enable the differentiation and sorting of different microalgal strains within natural blooms of *E. huxleyi.*

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