Vol. 388: 41–49, 2009
doi: 10.3354/meps08135

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published August 19

Effects of increased pCO_2 and temperature on the North Atlantic spring bloom. III. Dimethylsulfoniopropionate

Peter A. Lee[1,*], Jamie R. Rudisill[1], Aimee R. Neeley[1,7], Jennifer M. Maucher[2], David A. Hutchins[3,8], Yuanyuan Feng[3,8], Clinton E. Hare[3], Karine Leblanc[3,9,10], Julie M. Rose[3,11], Steven W. Wilhelm[4], Janet M. Rowe[4,5], Giacomo R. DiTullio[1,6]

[1]Hollings Marine Laboratory, College of Charleston, 331 Fort Johnson Road, Charleston, South Carolina 29412, USA
[2]Center for Coastal Environmental Health and Biomolecular Research, National Oceanic and Atmospheric Administration, 219 Fort Johnson Road, Charleston, South Carolina 29412, USA
[3]College of Marine and Earth Studies, University of Delaware, 700 Pilottown Road, Lewes, Delaware 19958, USA
[4]Department of Microbiology, University of Tennessee, 1414 West Cumberland Ave, Knoxville, Tennessee 37996, USA
[5]Department of Plant Pathology, The University of Nebraska, 205 Morrison Center, Lincoln, Nebraska 68583, USA
[6]Grice Marine Laboratory, College of Charleston, 205 Fort Johnson Road, Charleston, South Carolina 29412, USA
[7]*Present address:* National Aeronautics and Space Administration, Calibration and Validation Office, 1450 S. Rolling Road, Suite 4.111, Halethorpe, Maryland 21227, USA
[8]*Present address:* Department of Biological Sciences, University of Southern California, 3616 Trousdale Parkway, Los Angeles, California 90089, USA
[9]*Present address:* Aix-Marseille Université, CNRS, LOB-UMR 6535, Laboratoire d'Océanographie et de Biogéochimie, OSU/Centre d'Océanologie de Marseille, 163 Avenue de Luminy, 13288 Marseille Cedex 09, France
[10]*Present address:* CNRS (CNRS/INSU), UMR 6535, Campus de Luminy, Case 901, 163 Avenue de Luminy, 13288 Marseille Cedex 09, France
[11]*Present address:* Biology Department, MS #32, Woods Hole Oceanographic Institution, 266 Woods Hole Road, Woods Hole, Massachusetts 02543, USA

ABSTRACT: The CLAW hypothesis argues that a negative feedback mechanism involving phytoplankton-derived dimethylsulfoniopropionate (DMSP) could mitigate increasing sea surface temperatures that result from global warming. DMSP is converted to the climatically active dimethylsulfide (DMS), which is transferred to the atmosphere and photochemically oxidized to sulfate aerosols, leading to increases in planetary albedo and cooling of the Earth's atmosphere. A shipboard incubation experiment was conducted to investigate the effects of increased temperature and pCO_2 on the algal community structure of the North Atlantic spring bloom and their subsequent impact on particulate and dissolved DMSP concentrations ($DMSP_p$ and $DMSP_d$). Under 'greenhouse' conditions (elevated pCO_2; 690 ppm) and elevated temperature (ambient + 4°C), coccolithophorid and pelagophyte abundances were significantly higher than under control conditions (390 ppm CO_2 and ambient temperature). This shift in phytoplankton community structure also resulted in an increase in $DMSP_p$ concentrations and $DMSP_p$:chl *a* ratios. There were also increases in DMSP-lyase activity and biomass-normalized DMSP-lyase activity under 'greenhouse' conditions. Concentrations of $DMSP_d$ decreased in the 'greenhouse' treatment relative to the control. This decline is thought to be partly due to changes in the microzooplankton community structure and decreased grazing pressure under 'greenhouse' conditions. The increases in $DMSP_p$ in the high temperature and greenhouse treatments support the CLAW hypothesis; the declines in $DMSP_d$ do not.

KEY WORDS: Particulate DMSP · Dissolved DMSP · Climate change · Global warming · Carbon dioxide · Temperature · Biogeochemistry

INTRODUCTION

The CLAW hypothesis (Charlson et al. 1987) postulates that changes in the Earth's radiation budget and any subsequent changes in oceanic conditions will be mitigated through a feedback mechanism involving phytoplankton, the climatically active compound dimethylsulfide (DMS) and planetary albedo. In essence, the CLAW hypothesis proposes that higher sea surface temperatures (SST) will result in an enhanced DMS

*Email: leep@cofc.edu

flux to the atmosphere, presumably due to an increased production of dimethylsulfoniopropionate (DMSP), the precursor to DMS, by phytoplankton. According to the CLAW hypothesis, this DMSP increase could occur either through an increase in phytoplankton biomass or a shift in community structure towards DMSP-producing species. It is assumed that any increases in DMSP concentrations will lead to increases in the highly volatile DMS. Enhanced vertical mixing rates (i.e. stronger winds), or decreased microbial DMS consumption, however, could also increase sea-to-air DMS fluxes without a concomitant rise in DMSP production (Simó & Pedrós-Alió 1999). Once in the atmosphere, DMS would be rapidly oxidized to sulfur dioxide, with a subsequent increase in the formation of sulfate aerosols, which are a significant source of cloud condensation nuclei in remote marine environments (Ayers & Cainey 2007). Consequently, cloud coverage and ultimately planetary albedo would increase, thereby altering the Earth's radiation budget and providing a negative feedback mechanism for the regulation of planetary climate (Vallina & Simó 2007).

Global models predict that by the end of this century concentrations of atmospheric carbon dioxide (pCO_2) will have risen from current levels of 380 to >700 ppm (IPCC 2007). These changes in atmospheric CO_2 will also impact the world's oceans with anticipated increases in SST of 1 to 4°C, decreases in ocean pH of 0.3 units, and major impacts on upper-ocean stratification (Sarmiento et al. 1998, Wolf-Gladrow et al. 1999, IPCC 2007). Furthermore, such changes will have dramatic effects on marine phytoplankton communities, in terms of both the cellular physiology of individual species and the taxonomic composition of the communities (Boyd & Doney 2002, Tortell et al. 2002, Hare et al. 2007b).

Currently, there is little direct information on the effects of climate-sensitive variables on algal community structure and the production of DMSP or DMS. While an earlier study was unable to find a link between long-term climate variations and DMS levels (Bates & Quinn 1997), more recent studies have suggested that climate-driven variations in SST and mixed layer depth may influence DMSP and DMS levels (Simó & Pedrós-Alió 1999, Wong et al. 2006). Even results obtained during a high-latitude mesocosm experiment have led to differing conclusions regarding the role of increasing pCO_2 on DMSP and DMS cycling. Wingenter et al. (2007) concluded that, although absolute DMS concentrations were not different when pCO_2 levels were doubled or tripled, time-integrated values of DMS were 26 and 18% higher, respectively. During the same experiment, Vogt et al. (2008) observed no differences in particulate DMSP ($DMSP_p$), dissolved DMSP ($DMSP_d$), total DMSP ($DMSP_t$), or DMSP-lyase activity (DLA), leading them to conclude that the system had 'a certain resilience' to changing pCO_2. These authors also reported that temporal differences in DMS levels were observed, but concluded that they were not statistically significant.

The overarching objective of the North Atlantic spring bloom (NASB) expedition in 2005 was to investigate the impact of predicted increases in SST and pCO_2 on the algal community structure and biogeochemistry of the NASB, one of the most predictable and geographically extensive oceanic phytoplankton blooms (Sverdrup 1953, Lewis 1989, Savidge & Williams 2001). The area of the North Atlantic Ocean encompassed by the NASB is known to be an important source of DMS as result of the presence of small prymnesiophytes, such as the coccolithophorid *Emiliania huxleyi* (Sieracki et al. 1993, Simó et al. 2002, Steinke et al. 2002). The presence of these calcifying phytoplankton species also makes this region a critical sink for anthropogenic CO_2 (Sabine et al. 2004). However, despite numerous intensive studies (Ducklow & Harris 1993, Sieracki et al. 1993, Savidge & Williams 2001), little is known about how this ecosystem will respond to the effects of climate change. Using a shipboard incubation system (Hutchins et al. 2003), seawater samples were manipulated to mimic SST and pCO_2 levels predicted for high-latitude oceanic regimes by the year 2100 (IPCC 2007).

This article is the third in a series of 3 companion papers that detail the findings made during the incubation experiment and focuses on the changes in DMSP concentrations and DLA that result from changes in algal community composition. The lead article (Feng et al. 2009, this volume) describes the impact of increasing temperature and pCO_2 on various biogeochemical parameters and the algal community composition, with particular emphasis on whole-community photosynthetic rates and the potential for decreased export of particulate inorganic carbon (PIC) relative to particulate organic carbon (POC) under 'greenhouse' conditions. The second article (Rose et al. 2009, this volume) describes the changes in and links between microzooplankton grazing dynamics and the algal community composition.

MATERIALS AND METHODS

The experiment was conducted aboard the RV 'Seward Johnson' between June 20 and July 4, 2005 using seawater collected at 57.58° N, 15.32° W. Additional information regarding the experimental methodology can be found elsewhere (Feng et al. 2009). However, to give the reader an overview of the incubation system, experimental design and manipulation, a summary is also presented here. The experi-

ment was carried out using acid-washed, temperature-controlled, continuous-culture incubation systems ('Ecostats'; Hutchins et al. 2003, Hare et al. 2005) that allow simultaneous manipulation of pCO_2 and temperature levels. Temperature control was achieved by the use of a thermostatically controlled heat-exchange cooling system coupled to in-line electric heaters.

A trace-metal-clean (TMC) towed-intake pumping system (Bruland et al. 2005) was used to collect surface seawater (~5 to 10 m) containing the intact bloom community, which was passed through an acid-washed 200 µm Nitex mesh (to remove mesozooplankton grazers) and collected in an acid-cleaned 50 l mixing carboy. The incubation water was then dispensed into TMC clear polycarbonate bottles (2.7 l) for incubation. At the same location, TMC surface seawater was filtered through 0.2 µm in-line filters and transferred to acid-cleaned 50 l carboys for use as dilution media during the continuous-dilution phase of the experiment. The use of TMC techniques for water collection was considered essential, as changes in ambient iron concentrations resulting from inadvertent contamination in the bulk seawater collected for the experiment, in any of the carboys used to store the seawater or in any of the incubation bottles could have significantly altered the phytoplankton community composition and intracellular DMSP concentrations and introduced random errors into the analyses (Sunda et al. 2002, Hutchins et al. 2003, Hare et al. 2007a). As a result of low *in situ* nutrient concentrations, nitrate and phosphate were added to both the initial incubation bottles and dilution media to give final concentrations of 5 and 0.31 µmol l^{-1}, respectively. These additions yielded final Si:N and Si:P ratios of 0.13 and 0.08, respectively, reflecting the silicate limitation typical of late bloom conditions during the NASB (Feng et al. 2009).

The incubation bottles were then subjected to 4 different treatments, with 6 replicates per treatment, as follows: (1) 12°C and 390 ppm CO_2 (referred to as 'ambient'); (2) 12°C and 690 ppm CO_2 ('high CO_2'), (3) 16°C and 390 ppm CO_2 ('high temperature'), and (4) 16°C and 690 ppm CO_2 ('greenhouse'). The 2 pCO_2 levels were achieved by gently pumping HEPA-filtered gas streams of either ambient air (with 390 ppm CO_2) or a commercially prepared air/CO_2 mixture containing 690 ppm CO_2. The light intensity within the ecostats was set to 30% of the surface irradiance using spectrally corrected blue plastic and neutral-density shade screen (Hutchins et al. 2003). This value corresponded to the light intensity measured at the depth where the incubation water was collected and was selected so that the light level experienced by phytoplankton in the ecostats would mimic the average light intensity experienced by cells in the 40 to 50 m mixed layer.

The initial (starting) bloom community was grown in batch culture mode (i.e. no dilution) with the amended nutrients to avoid any lag-phase growth induced by sampling and to allow the biomass to increase to prevent immediate wash-out of the bloom community upon dilution. On Day 4 of the experiment, the system was switched to continuous culture mode at a dilution rate of 0.5 d^{-1}, which corresponds to the 'typical' whole-community growth rate for the area (Gaul et al. 1999). The continuous culture phase of the experiment was carried out for an additional 11 d.

During the experiment, total phytoplankton biomass (measured as chlorophyll *a* [chl *a*]) and carbonate system parameters (dissolved inorganic carbon [DIC] and pH) were monitored on a daily basis using samples collected directly from the incubation bottles using a gas-tight syringe. On a less frequent basis, samples were collected directly from the incubation bottles for cell counts (by microscopy and flow cytometry), $DMSP_t$, and $DMSP_d$. Samples for DLA, and algal pigment composition, POC, and particulate organic nitrogen (PON) were collected from the outflow. Full details of the analytical protocols used for chl *a*, DIC, pH, cell counts, PON, and POC are presented in the companion papers (Feng et al. 2009, Rose et al. 2009).

Samples for DMSP were collected and preserved using the methodology of Kiene & Slezak (2006). Small volumes of unfiltered seawater (≤20 ml) were preserved with 50% sulfuric acid (100 µl per 10 ml of sample) for the determination of $DMSP_t$. A second small volume of each sample (≤20 ml) was gravity filtered (Whatman GF/F), and the filtrate was preserved with 50% sulfuric acid for the determination of $DMSP_d$. All DMSP samples were base-hydrolyzed and measured using a cryogenic purge and trap system coupled to a Hewlett-Packard 5890 Series II gas chromatograph fitted with flame photometric detector (DiTullio & Smith 1995). $DMSP_p$ was calculated as the difference between $DMSP_t$ and $DMSP_d$. A recent study (Kiene et al. 2007) has noted that $DMSP_t$ can be underestimated when colonial *Phaeocystis* sp. is present in samples preserved using the Kiene & Slezak (2006) method. Since colonial *Phaeocystis* was not observed in the samples collected during the present study, the results presented here are unlikely to have been significantly affected by this problem.

DLA measurements were carried out following the protocol of Steinke et al. (2000). Optimal conditions for the lyase assay (25°C, pH 7, 500 mmol l^{-1} sodium chloride) were determined using a strain of *Emiliania huxleyi* (CCMP 373) that is known to possess the DMSP-lyase (Steinke et al. 1998).

Statistical analysis was conducted with the statistical software program R (www.r-project.org). A modified ANOVA test based on a percentile bootstrap

method (Wilcox 2003) was used to determine the significance of any observed differences among treatments on individual sampling days. Further details of the statistical analysis are described in Rose et al. (2009).

RESULTS

Phytoplankton pigment analyses indicated that community structure changed significantly during the course of the experiment (Fig. 1). During the first 6 d of the experiment, the abundances of pelagophytes, as indicated by the concentrations of the pigment 19′-butanoyloxyfucoxanthin (19′-But; Fig. 1A), remained unchanged, while the abundances of haptophytes (presumably coccolithophorids based on microscopic analyses) appeared to decline, as indicated by the concentration of 19′-hexanoyloxyfucoxanthin (19′-Hex; Fig. 1B). At the same time, the abundances of diatoms, as indicated by the concentrations of fucoxanthin (Fuco; Fig. 1C), increased substantially in all treatments, with the smallest increases observed under greenhouse and high temperature conditions. On Day 6, the differences in Fuco concentrations observed between the control versus high temperature and control versus greenhouse were statistically significant ($p < 0.05$; Table 1).

In the second half of the experiment, a dramatic shift in community structure was observed. Diatom abundances declined in all treatments, while increases were seen in the relative abundances of pelagophytes and coccolithophorids under both high temperature and greenhouse conditions. On Day 14, levels of Fuco in the greenhouse treatment were significantly higher than in the high CO_2 treatment, but not higher than in the ambient control (Table 1, Fig. 1C). 19′-But concentrations in the greenhouse and high temperature treatments were significantly

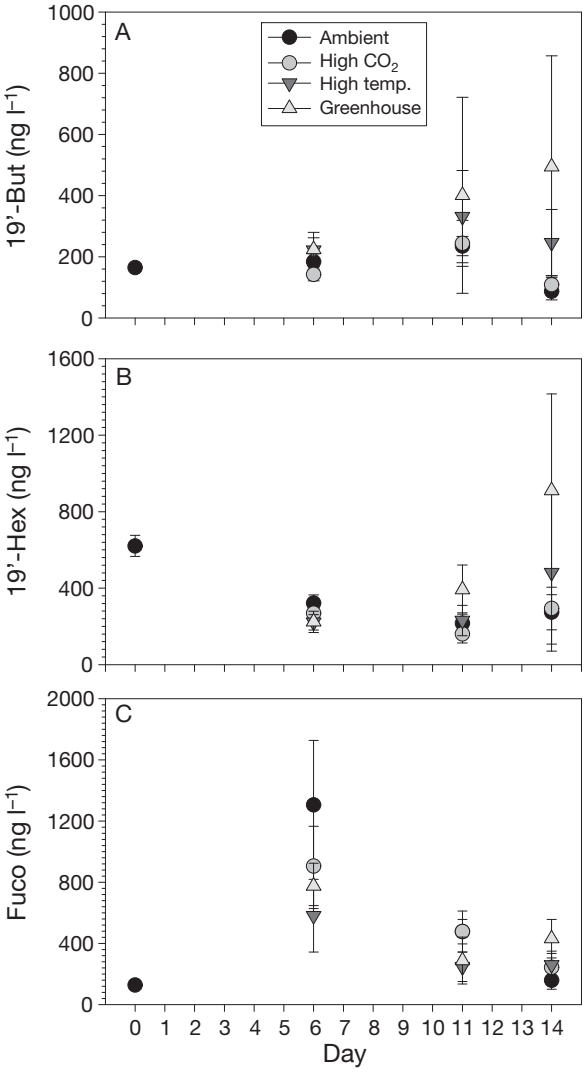

Fig. 1. Average values of (A) 19′-butanoyloxyfucoxanthin (19′-But), (B) 19′-hexanoyloxyfucoxanthin (19′-Hex) and (C) fucoxanthin (Fuco) during the course of the experiment. Error bars represent the 90% confidence interval. See 'Materials and methods' for treatment details

Table 1. Summary of the statistical analysis for 19′-butanoyloxyfucoxanthin, 19′-hexanoyloxyfucoxanthin and Fucoxanthin on Days 6 and 14 of the 14 d experiment. *$p < 0.05$ between the treatments; NS: no significant difference between the treatments. See 'Materials and methods' for treatment details

	Ambient vs.			High CO_2 vs.		High temp vs.
	High CO_2	High temp.	Greenhouse	High temp.	Greenhouse	Greenhouse
19′-butanoyloxyfucoxanthin						
Day 6	NS	NS	NS	NS	NS	NS
Day 14	NS	*	*	NS	*	NS
19′-hexanoyloxyfucoxanthin						
Day 6	NS	NS	NS	NS	NS	NS
Day 14	NS	NS	NS	NS	NS	NS
Fucoxanthin						
Day 6	NS	*	*	NS	NS	NS
Day 14	NS	NS	NS	NS	*	NS

Table 2. Summary of the averages (±1 SD; n = number of replicates) of the major pigments normalized to the sum of all fucoxanthin (Fuco) derivatives and sulfur parameters (particulate and total DSMP) normalized to the sum of the pigments for the major DMSP-producing taxa (19′-hexanoyloxyfucoxanthin [Hex] and butanoyloxyfucoxanthin [But]) as measured at the start of the experiment (initial) and on Day 14 (end of the experiment) in each of the treatments. See 'Materials and methods' for treatment details

Treatment:	Initial	Ambient	High temp.	High CO_2	Greenhouse
Pigment ratios					
But:ΣFucos	0.180 ± 0.003 (n = 3)	0.17 ± 0.05 (n = 6)	0.28 ± 0.13 (n = 6)	0.19 ± 0.07 (n = 6)	0.32 ± 0.13 (n = 6)
Fuco:ΣFucos	0.139 ± 0.001 (n = 3)	0.31 ± 0.01 (n = 6)	0.30 ± 0.11 (n = 6)	0.40 ± 0.11 (n = 6)	0.25 ± 0.04 (n = 6)
Hex:ΣFucos	0.680 ± 0.003 (n = 3)	0.52 ± 0.13 (n = 6)	0.43 ± 0.23 (n = 6)	0.42 ± 0.16 (n = 6)	0.43 ± 0.16 (n = 6)
Sulfur ratios (nmol ng^{-1})					
$DMSP_t$:Hex + But	0.29 ± 0.03 (n = 3)	0.31 ± 0.05 (n = 6)	0.25 ± 0.04 (n = 6)	0.27 ± 0.06 (n = 5)	0.24 ± 0.08 (n = 6)
$DMSP_p$:Hex + But	0.25 ± 0.03 (n = 3)	0.20 ± 0.04 (n = 6)	0.23 ± 0.03 (n = 6)	0.20 ± 0.06 (n = 5)	0.23 ± 0.07 (n = 6)

greater than those observed under ambient conditions. Similarly, 19′-But was significantly higher in the greenhouse treatment than in the high CO_2 treatment (Table 1, Fig. 1A). In the case of 19′-Hex, no significant differences were observed at the end of the experiment despite large increases in the 19′-Hex concentrations in the greenhouse treatment (Table 1, Fig. 1B).

Since it is unclear how environmental factors affect the specific pigment:chl *a* ratio in different phytoplankton groups, the relative contribution of pelagophytes, diatoms, and coccolithophorids to the total algal biomass was estimated by normalizing their diagnostic pigments to the sum of all fucoxanthin derivatives (ΣFuco), respectively (Table 2). Based on pigment analyses, algal chl *a* was dominated initially by coccolithophorids (53%), with contributions from diatoms (18%), dinoflagellates (15%), and pelagophytes (14%). However, by Day 14, dramatic differences in the community structure were evident that were apparently driven by experimental conditions in each treatment. A significant increase (55%) in the percentage of pelagophytes was observed in the 2 high temperature treatments relative to the 2 low temperature treatments. In contrast, the percentage of diatoms decreased by 27% in the high temperature treatments compared to the lower temperature treatments. Although the highest accessory pigment concentration (19′-Hex) for all treatments was observed for the coccolithophorid population, no significant change was observed in the relative percentage of coccolithophorid to the total algal community. It is worth noting that, while dinoflagellates made a small contribution to the initial phytoplankton community, there was a marked decline in dinoflagellate abundances, based on peridinin levels, in all treatments during the experiment (data not shown).

During the early stages of the experiment, concentrations of $DMSP_d$, $DMSP_p$, and $DMSP_p$:chl *a* all declined (Fig. 2), most likely in response to the decline in

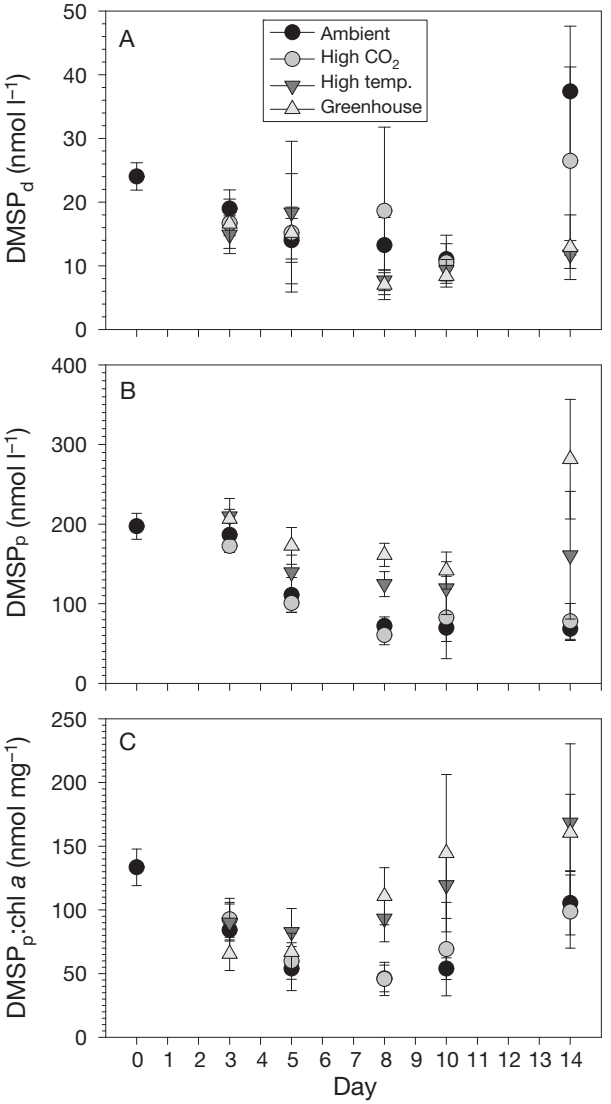

Fig. 2. Average values of (A) dissolved dimethylsulfoniopropionate ($DMSP_d$), (B) particulate DMSP ($DMSP_p$) and (C) $DMSP_p$:chl *a* ratios during the course of the experiment. Error bars represent the 90% confidence interval. See 'Materials and methods' for treatment details

Table 3. Summary of the statistical analysis for $DMSP_d$ (dissolved), $DMSP_p$ (particulate), and $DMSP_p$:chl *a* on Days 8 and 14, and DMSP-lyase activity (DLA) and DLA:chl *a* on Day 13 of the 14 d experiment. *$p < 0.05$ between treatments; NS: no significant difference between treatments. See 'Materials and methods' for treatment details

	Ambient vs.			High CO_2 vs.		High temp. vs.
	High CO_2	High temp.	Greenhouse	High temp.	Greenhouse	Greenhouse
$DMSP_d$						
Day 8	NS	NS	NS	NS	NS	NS
Day 14	NS	*	*	NS	NS	NS
$DMSP_p$						
Day 8	NS	*	*	*	*	*
Day 14	NS	*	*	NS	*	*
$DMSP_p$:chl *a*						
Day 8	NS	*	*	*	*	NS
Day 14	NS	NS	*	NS	*	NS
DLA						
Day 13	NS	NS	*	NS	*	NS
DLA:chl *a*						
Day 13	NS	NS	NS	NS	NS	NS

coccolithophorid abundances and the increase in diatom abundances. However, starting on Day 5, small increases in $DMSP_p$ and $DMSP_p$:chl *a* were observed in the high temperature and greenhouse treatments relative to the ambient control and high CO_2 treatment. On Day 8, $DMSP_p$ levels in the high temperature and greenhouse treatments were significantly higher ($p < 0.05$; Fig. 2B, Table 3) than in the high CO_2 treatment and ambient control. By the end of the experiment, $DMSP_p$ concentrations in the greenhouse treatment were significantly higher (2- to 4-fold greater; $p < 0.05$; Fig. 2B) than the final values measured in the other treatments. In the case of $DMSP_p$:chl *a* ratios, the values for the high temperature and greenhouse treatments were also significantly higher ($p < 0.05$) on Days 8 and 14, and by the end of the experiment were 50 to 60% greater than under the ambient conditions (Fig. 2C).

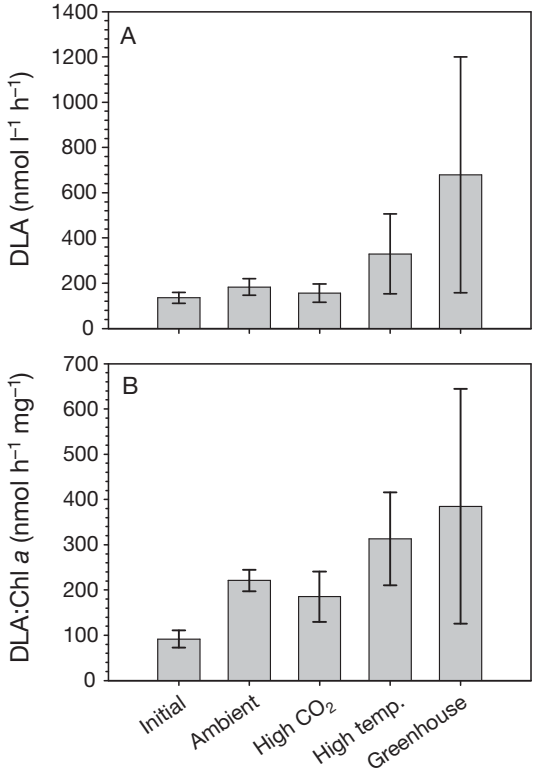

Fig. 3. Average values of (A) DMSP-lyase activity (DLA) and (B) DLA:chl *a* ratios at the start of the experiment (Initial) and in the 4 treatments on Day 13 of the 14 d experiment. Error bars represent the 90% confidence interval. See 'Materials and methods' for treatment details

Conversely, $DMSP_d$ concentrations in the high temperature and greenhouse treatments declined after Day 5 relative to the controls and high CO_2 treatments. By the end of the experiment on Day 14, $DMSP_d$ levels were significantly lower ($p < 0.05$) in both the high temperature and greenhouse treatments than under ambient conditions (Fig. 2A). The results obtained for DLA (Fig. 3A, Table 3) show a remarkably similar pattern to those obtained for $DMSP_p$, with considerably higher DLA in the greenhouse treatment. At the end of the experiment, DLA rates were ca. 2.5-fold greater in the greenhouse treatment relative to the ambient treatment ($p < 0.05$; Table 3). DLA rates were also significantly different between the high CO_2 and greenhouse treatments. Although the DLA:chl *a* ratios (Fig. 3B, Table 3) tended to follow the same pattern as DLA, the differences between the greenhouse conditions and the other treatment conditions were not as marked and none were significant.

DISCUSSION

The results obtained for $DMSP_p$ during the experiment were consistent with the expected changes predicted by the CLAW hypothesis. There was both an increase in overall $DMSP_p$ concentrations and a shift in phytoplankton community structure toward species with a higher cellular DMSP content under greenhouse conditions. Furthermore, the pigment-normalized DMSP numbers did not vary greatly between treatments, suggesting that the intracellular DMSP content of the component of the phytoplankton community responsible for DMSP production (the coccolithophorids and pelagophytes) was not affected by the changing conditions and confirms that the observed changes in $DMSP_p$ and biomass-normalized $DMSP_p$ resulted from increases in coccolithophorid and pelagophyte abundances.

Moreover, the results show that changes in $DMSP_p$ (Fig. 2B to D) and in DLA (Fig. 3A,B) were driven primarily by changes in temperature, with increasing pCO_2 (alone) having no effect at ambient temperature. Although $DMSP_p$ and DLA levels were higher under greenhouse conditions than under elevated temperature alone by the end of the experiment, none of the observed interactive changes were statistically significant. This observation is perhaps not surprising given that increasing pCO_2 does not increase intracellular DMSP (it only rises under CO_2 limitation; Sunda et al. 2002), but can influence $DMSP_p$ levels by influencing the taxonomic composition of the phytoplankton communities (Tortell et al. 2002, Hare et al. 2007b). Despite a number of methodological differences between the present study and that of Vogt et al. (2008), the finding that increasing pCO_2 alone had little effect on $DMSP_p$ and DLA levels is remarkably consistent.

Intriguingly, the results also revealed that, under greenhouse conditions, there was a decrease in $DMSP_d$ concentrations, which contradicts the CLAW hypothesis. This decline in $DMSP_d$ was likely associated with significant changes in microzooplankton grazing pressure, species composition, and species abundance at the end of the experiment (Rose et al. 2009). These changes in the microzooplankton community may themselves be a result of the observed changes in the phytoplankton community, $DMSP_p$, and DLA. It is also worth noting that the same grazing/$DMSP_p$/DLA/$DMSP_d$ trends were observed in the high temperature treatment, which was dominated ultimately by pelagophytes.

Microzooplankton grazers have been shown to preferentially avoid prey with high DLA. The mixing of intracellular DMSP and the DMSP-lyase enzyme during prey ingestion results in the formation of DMS and acrylate, with the latter thought to render the prey unpalatable to the predator (Wolfe & Steinke 1996, Wolfe et al. 1997), or the presence of the former acting as a 'don't eat me' signal to deter grazing (Strom et al. 2003). A study has suggested that this mechanism may have been partially responsible for the development and persistence of large, anomalous coccolithophorid blooms in the Bering Sea (Olson & Strom 2002). In the current study, heavy grazing pressure in the greenhouse treatment by the initial microzooplankton community may have allowed the coccolithophores and pelagophytes to become the dominant taxa. As the phytoplankton community became less and less palatable for the grazers, less grazing took place, resulting in a decrease in the amount of DMSP released through feeding, which was reflected in the decrease in $DMSP_d$ levels.

Another potential factor that could influence DMSP release and degradation is viral infection and lysis (Malin et al. 1992). However, results obtained during subsequent studies have not provided clear-cut evidence of this possibility. In experiments using field samples, the results have been ambiguous as to the effect of viruses on DMSP or have shown no correlation between viruses and concentrations of DMSP and DMS (Bratbak et al. 1995, Wilson et al. 1998, 2002). Conversely, various studies using cultures have found that viruses can have a significant impact on the release of intracellular DMSP and can suppress the formation of DMS through the cleavage of DMSP by DMSP-lyase (Hill et al. 1998, Evans et al. 2007). In the present study, viruses were unlikely to have been a significant factor for 2 reasons. First, *in situ* water column samples collected during the cruise in the NASB region showed no discernable pattern of viral production (Rowe et al. 2008), and samples from the ecostat experiment showed no relationship between the treatments and viral production (Rowe & Wilhelm unpubl. data). Second, $DMSP_d$ was lower in all treatments at the end of the experiment rather than higher, as would be expected if viral infection resulted in cell lysis.

Unfortunately, the ecostat design precludes the accurate measurement of DMS, as active bubbling of the incubation bottles with the gas mixtures stripped the DMS from the growth media. Consequently, it is not known if the declines in $DMSP_d$ resulted from a decrease in the release of $DMSP_p$ or an increase in microbial consumption of $DMSP_d$ (or a combination of both). Thus, whether or not changes in the phytoplankton, microbial, or microzooplankton communities resulted in changes in DMS concentrations would be conjecture. A decline in microzooplankton abundances and grazing rates could result in a decline in the production of DMS. This scenario would be contrary to previous findings that suggest oceanic DMS will increase as a result of global warming, either

through stratification and temperature effects (Simó & Pedrós-Alió 1999, Wong et al. 2006) or increasing pCO_2 (Wingenter et al. 2007, Vogt et al. 2008).

Whilst the findings of this experiment are consistent with the CLAW hypothesis in that oceanic changes associated with global warming will likely result in increased levels of $DMSP_p$, the findings do not fully support the notion that this change in $DMSP_p$ will also result in an increase in DMS levels that would lead to an increase in cloud albedo and a negative feedback mechanism on global warming. Furthermore, a number of uncertainties also remain. The responses observed during the relatively short duration of the experiment may be the result of acclimation to the experimental conditions rather than an adaptive response to 100 yr of global warming. For example, although grazing pressure was suppressed under greenhouse conditions during the experiment, will an oceanic microzooplankton community develop that is fully capable of consuming the relatively unpalatable coccolithophorids? Conversely, if no such grazing community develops, will the DMSP content of the cells still be released in the surface waters due to cell lysis when the bloom becomes senescent? A reduction in POC export due to global warming (Laws et al. 2000, Bopp et al. 2001) could result in the retention of DMSP near the surface, where it can be converted to DMS and is available for transfer to the atmosphere. Finally, how will bacterial utilization of DMSP, either through uptake or degradation to DMS and other products, be influenced by increasing temperature and changes in other climate-sensitive variables?

The results of the present study along with those of its companion studies (Feng et al. 2009, Rose et al. 2009) highlight the complex manner in which marine ecosystems may respond to global warming. Physiological (bottom-up) responses to changes in pCO_2 and temperature by the phytoplankton community (Feng et al. 2009) led indirectly to changes in the top-down pressure exerted by microzooplankton grazers (Rose et al. 2009). The impact of increasing pCO_2 and temperature on DMSP observed during this experiment is the net response of those changes and shows that further work examining these complex interactions is required.

Acknowledgements. The authors thank the captain and crew of the RV 'Seward Johnson' and fellow cruise participants for their support and many fruitful discussions. The authors also gratefully acknowledge the efforts of several anonymous reviewers whose comments greatly improved this manuscript. The present study was made possible through funding from the Biological Oceanography and Office of Polar Programs of the U.S. National Science Foundation (OCE-0423418 [0741412], OCE-0722337 and ANT-0338111 [0741411] to D.A.H., ANT-0528715 to J.M.R., OCE 0452409 to S.W.W., and OCE-0422890 to G.R.D.).

LITERATURE CITED

Ayers GP, Cainey JM (2007) The CLAW hypothesis: a review of the major developments. Environ Chem 4:366–374

Bates TS, Quinn PK (1997) Dimethylsulfide (DMS) in the equatorial Pacific Ocean (1982 to 1996): Evidence of a climate feedback? Geophys Res Lett 35:367–393

Bopp L, Monfray P, Aumont O, Dufresne JL and others (2001) Potential impacts of climate change on marine export production. Global Biogeochem Cycles 15:81–99

Boyd PW, Doney SC (2002) Modelling regional responses by marine pelagic ecosystems to global climate change. Geophys Res Lett 29,1806, doi:10.1029/2001GL014130

Bratbak G, Levasseur M, Michaud S, Cantin G, Fernandez E, Heimdal BR, Heldal M (1995) Viral activity in relation to *Emiliania huxleyi* blooms: A mechanism of DMSP release? Mar Ecol Prog Ser 128:133–142

Bruland KW, Rue EL, Smith GJ, DiTullio GR (2005) Iron, macronutrients and diatom blooms in the Peru upwelling regime: brown and blue waters of Peru. Mar Chem 93: 81–103

Charlson RJ, Lovelock JE, Andreae MO, Warren SG (1987) Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. Nature 326:655–661

DiTullio GR, Smith WO Jr (1995) Relationship between dimethylsulfide and phytoplankton pigment concentrations in the Ross Sea, Antarctica. Deep Sea Res I 42: 873–892

Ducklow HW, Harris RP (1993) Introduction to the JGOFS North Atlantic bloom experiment. Deep Sea Res II 40:1–8

Evans C, Kadner SV, Darroch LJ, Wilson WH, Liss PS, Malin G (2007) The relative significance of viral lysis and microzooplankton grazing as pathways of dimethylsulfoniopropionate (DMSP) cleavage: an *Emiliania huxleyi* culture study. Limnol Oceanogr 52:1036–1045

Feng Y, Hare CE, Leblanc K, Rose JM and others (2009) Effects of increased pCO_2 and temperature on the North Atlantic spring bloom. I. The phytoplankton community and biogeochemical response. Mar Ecol Prog Ser 388: 13–25

Gaul W, Antia AN, Koeve W (1999) Microzooplankton grazing and nitrogen supply of phytoplankton growth in the temperate and subtropical northeast Atlantic. Mar Ecol Prog Ser 189:93–104

Hare CE, DiTullio GR, Trick CG, Wilhelm SW, Bruland KW, Rue EL, Hutchins DA (2005) Phytoplankton community structure changes following simulated upwelled iron inputs in the Peru upwelling region. Aquat Microb Ecol 38:269–282

Hare CE, DiTullio GR, Riseman SF, Crossley AC, Popels LC, Sedwick PN, Hutchins DA (2007a) Effects of changing continuous iron inputs rate on a Southern Ocean algal assemblage. Deep Sea Res I 54:732–746

Hare CE, Leblanc K, DiTullio GR, Kudela RM and others (2007b) Consequences of increased temperature and CO_2 for phytoplankton community structure in the Bering Sea. Mar Ecol Prog Ser 352:9–16

Hill RW, White BA, Cottrell MT, Dacey JWH (1998) Virus-mediated total release of dimethylsulfoniopropionate from marine phytoplankton: a potential climate process. Aquat Microb Ecol 14:1–6

Hutchins DA, Pustizzi F, Hare CE, DiTullio GR (2003) A shipboard natural community continuous culture system for ecologically relevant low-level nutrient enrichment experiments. Limnol Oceanogr Methods 1:82–91

IPCC (Intergovernmental Panel on Climate Change) (2007) Climate change 2007: the physical science basis. In:

Solomon S, Qin D, Manning M, Chen Z and others (eds) Contribution of Working Group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge and New York

Kiene RP, Slezak D (2006) Low dissolved DMSP concentrations in seawater revealed by small-volume gravity filtration and dialysis sampling. Limnol Oceanogr Methods 4: 80–95

Kiene RP, Kieber DJ, Slezak D, Toole DA and others (2007) Distribution of cycling of dimethylsulfide, dimethylsulfoniopropionate, and dimethylsulfoxide during spring and early summer in the Southern Ocean south of New Zealand. Aquat Sci 69:305–319

Laws EA, Falkowski PG, Smith WO, Ducklow H, McCarthy JJ (2000) Temperature effects on export production in the open ocean. Global Biogeochem Cycles 14:1231–1246

Lewis MR (1989) The variegated ocean: a view from space. New Sci 1685:37–40

Malin G, Turner SM, Liss PS (1992) Sulphur: the plankton/climate connection. J Phycol 28:590–597

Olson MB, Strom SL (2002) Phytoplankton growth, microzooplankton herbivory and community structure in the southeast Bering Sea: insight into the formation and temporal persistence of an *Emiliania huxleyi* bloom. Deep Sea Res II 49:5969–5990

Rose JM, Feng Y, Gobler CJ, Gutierrez R, Hare CE, Leblanc K, Hutchins DA (2009) Effects of increased pCO_2 and temperature on the North Atlantic spring bloom. II. Microzooplankton abundance and grazing. Mar Ecol Prog Ser 388: 27–40

Rowe JM, Saxton MA, Cottrell MT, DeBruyn JM and others (2008) Constraints on viral production in the Sargasso Sea and North Atlantic. Aquat Microb Ecol 52:233–244

Sabine CL, Felly R, Grube N, Key R and others (2004) The oceanic sink for anthropogenic CO_2. Science 305:367–371

Sarmiento JL, Hughes TMC, Stouffer RJ, Manabe S (1998) Simulated response of the ocean carbon cycle to anthropogenic climate warming. Nature 393:245–249

Savidge G, Williams PJleB (2001) The PRIME 1996 cruise: an overview. Deep Sea Res II 48:687–704

Sieracki ME, Verity PG, Stoecker DK (1993) Plankton community response to sequential silicate and nitrate depletion during the 1989 North Atlantic spring bloom. Deep Sea Res II 40:213–225

Simó R, Pedrós-Alió C (1999) Role of vertical mixing in controlling the oceanic production of dimethyl sulphide. Nature 402:396–399

Simó R, Archer SD, Pedrós-Alió C, Gilpin L, Stelfox-Widdicombe CE (2002) Coupled dynamics of dimethylsulfoniopropionate and dimethylsulfide cycling and the microbial food web in surface waters of the North Atlantic. Limnol Oceanogr 47:53–61

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, Archer SD, Turner SM, Liss PS (2000) Determinations of dimethylsulphoniopropionate (DMSP) lyase activity using headspace analysis of dimethylsulphide (DMS). J Sea Res 43:233–244

Steinke M, Malin G, Archer SD, Burkill PH, Liss PS (2002) DMS production in a coccolithophorid bloom: evidence for the importance of dinoflagellate DMSP lyases. Aquat Microb Ecol 26:259–270

Strom S, Wolfe G, Holmes J, Stecher H, Shimeneck C, Lambert S, Moreno E (2003) Chemical defense in the microplankton. I. Feeding and growth rates of heterotrophic protists on the DMS-producing phytoplankter *Emiliania huxleyi.* Limnol Oceanogr 48:217–229

Sunda W, Kieber DJ, Kiene RP, Huntsman S (2002) An antioxidant function for DMSP and DMS in marine algae. Nature 418:317–320

Sverdrup HU (1953) On the conditions for the vernal blooming of phytoplankton. J Cons Int Explor Mer 18:287–295

Tortell PD, DiTullio GR, Sigman DM, Morel FMM (2002) CO_2 effects on taxonomic composition and nutrient utilization in an Equatorial Pacific phytoplankton assemblage. Mar Ecol Prog Ser 236:37–43

Vallina SM, Simó R (2007) Strong relationship between DMS and the solar radiation dose over the global surface ocean. Science 315:506–508

Vogt M, Steinke M, Turner S, Paulino A and others (2008) Dynamics of dimethylsulphoniopropionate and dimethylsulphide under different CO_2 concentrations during a mesocosm experiment. Biogeosciences 5:407–419

Wilcox RR (2003) Applying contemporary statistical techniques. Academic Press, New York

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(Suppl A):49–59

Wilson WH, Tarran GA, Zubkov MV (2002) Virus dynamics in a coccolithophore-dominated bloom in the North Sea. Deep Sea Res II 49:2951–2963

Wingenter OW, Haase KB, Zeigler M, Blake DR and others (2007) Unexpected consequences of increasing CO_2 and ocean acidity on marine production of DMS and CH_2CII: potential climate impacts. Geophys Res Lett 34, L05710, doi: 10.1029/2006GL028139

Wolf-Gladrow DA, Riebesell U, Burkhart S, Bijma J (1999) Direct effects of CO_2 concentration on growth and isotopic composition of marine plankton. Tellus B Chem Phys Meterol 51:461–476

Wolfe GV, Steinke M (1996) Grazing-activated production of dimethyl sulfide (DMS) by two clones of *Emiliania huxleyi.* Limnol Oceanogr 41:1151–1160

Wolfe GV, Steinke M, Kirst GO (1997) Grazing-activated chemical defense in a unicellular marine alga. Nature 387: 894–897

Wong CS, Wong SKE, Peña A, Levasseur M (2006) Climatic effect on DMS producers in the NE sub-Arctic Pacific: ENSO in the upper ocean. Tellus B Chem Phys Meterol 58: 319–326

Editorial responsibility: Matthias Seaman, Oldendorf/Luhe, Germany

Submitted: August 8, 2008; Accepted: May 28, 2009
Proofs received from author(s): July 30, 2009