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Research Paper

Role of dimethylsulfoniopropionate as an osmoprotectant following gradual salinity shifts in the sea-ice diatom *Fragilariopsis cylindrus*

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Environmental context. Dimethylsulfoniopropionate (DMSP), a small sulfur compound biosynthesised by algae, plays an important role in global climate, particularly in polar regions. We investigated salinity effects on DMSP levels, and provide the first experimental measurements of DMSP and associated physiological changes in a polar diatom across to a range of gradual salinity shifts representative of sea-ice conditions. Quantitative estimates of DMSP in polar diatoms following salinity changes will facilitate new mathematical models to predict seasonal responses and reactions to climate change.

Abstract. Although extreme environmental gradients within sea-ice have been proposed to stimulate dimethylsulfoniopropionate (DMSP) accumulation in diatoms, a taxa whose temperate counterparts show relatively low concentrations, this has yet to be experimentally validated across a range of salinities representative of sea-ice conditions. The present study examined changes in DMSP concentrations in the widespread polar diatom *Fragilariopsis cylindrus* in response to gradual salinity shifts representative of those encountered during sea-ice formation and melt. DMSP concentrations were elevated up to 127% in 70-salinity cultures. Low-salinity shifts decreased intracellular DMSP concentrations in a gradient-dependent manner that suggests DMSP recycling rather than release under milder hyposalinity shifts. Permeable membranes were detected in ~45% of 10-salinity cells; therefore, loss of membrane integrity may only partially explain DMSP release in the lowest-salinity group. Growth rates, photosynthetic efficiency of photosystem II and reactive oxygen species detection indicated only partial impairment by salinity stress in this organism. Thus, experimental evidence supports the role of DMSP as a compatible solute in the acclimation of a sea-ice diatom across large salinity gradients and measurements of associated physiological changes will improve interpretation of environmental measurements.

Additional keywords: DMSO, DMSP, F_V/F_M, membrane permeability, polar algae, reactive oxygen species, sulfur.

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Introduction

Phytoplankton dimethylsulfoniopropionate (DMSP) production and microbial conversion to the volatile gas dimethylsulfide (DMS) are believed to help regulate the Earth's radiation balance and could in part mitigate climate warming caused by greenhouse gases,[1,2] although the level of contribution is under debate.[3] Polar regions have been found to have very high DMS fluxes to the atmosphere,[4–6] with an estimated 17% of global biosulfur emissions derived from the Southern Ocean alone.[7] Concentrations of DMS, DMSP and dimethylsulfoxide (DMSO) within diatom-dominated sea-ice cores are generally one to several orders of magnitude greater than global means.[8–15] Studies have also shown DMS, DMSP and DMSO concentrations to be particularly elevated in sea-ice boundary zones.[16–20] Furthermore, large pulses in Southern Ocean atmospheric aerosols (attributed to sea-to-air DMS fluxes) were temporally correlated with spring sea-ice melt, rather than summer prymnesiophyte blooms, which were traditionally credited for the large DMS fluxes measured in the region.[21] Thus, field measurements support the hypothesis that sea-ice conditions promote accumulation of DMSP by sea-ice diatoms,[13] unlike their temperate counterparts that typically exhibit low levels per unit biomass.[22]

Critical to predicting future biogeochemical feedbacks in sensitive polar regions will be understanding how abiotic

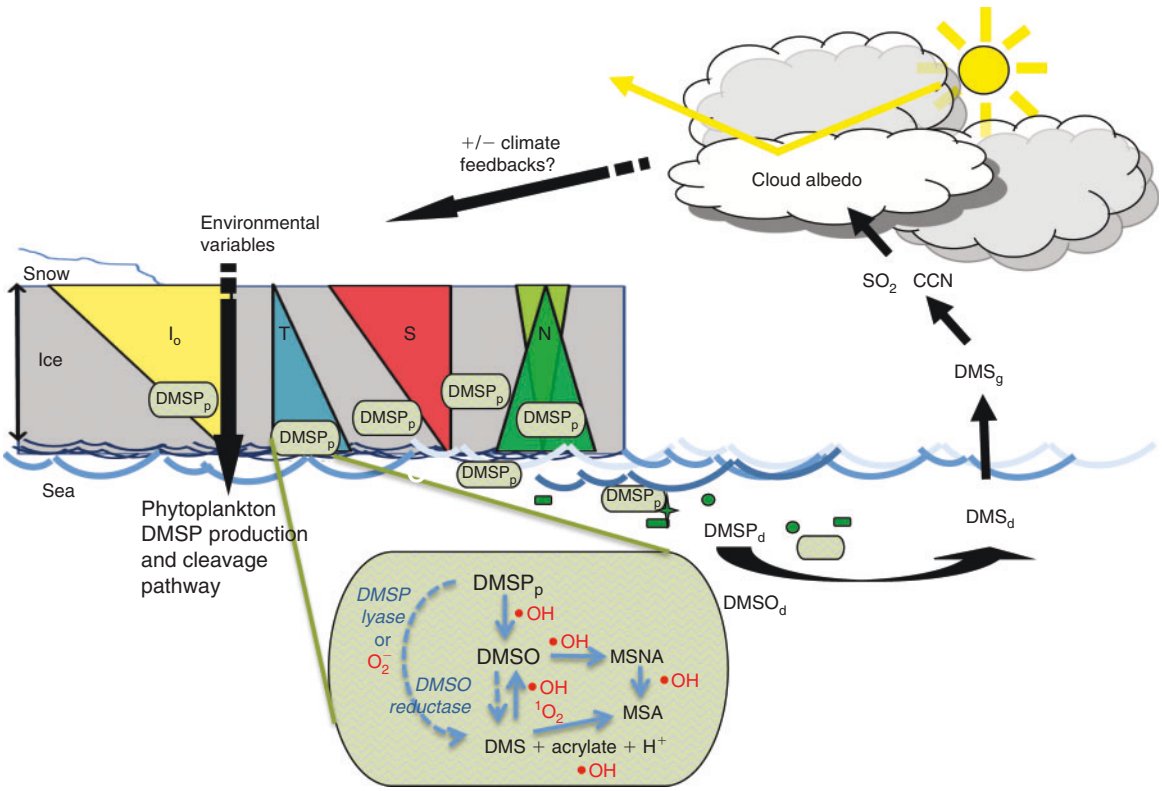

Fig. 1. Dimethylsulfoniopropionate (DMSP) production by sea-ice diatoms may be driven by the strong gradients in irradiance (Io), temperature (T), brine salinity (S) and nutrients (N) encountered within the ice column. DMSP catabolism is chemically and enzymatically mediated as part of a proposed antioxidant cascade (shown within enlarged phytoplankton cell). DMSP, dimethylsulfoxide (DMSO), dimethylsulfide (DMS), methanesulfinic acid (MSNA) and acrylate all scavenge reactive oxygen species (ROS) eventually forming methane sulfonate (MSA). DMS and DMSO readily diffuse across membranes, whereas other compounds are hydrophilic, but will be released into the dissolved fraction through lysis, changes in membrane permeability or facilitated transport. Other marine microbes can take up and convert DMSP to form the climatically active compound DMS, which is oxidised to form cloud condensation nuclei (CCN). This feedback loop between algae sulfur production and climate, the CLAW hypothesis, was first proposed several decades ago.[1]

variables affect microbial DMS, DMSP and DMSO production, particularly with regards to sea-ice (Fig. 1). Polar diatoms, the dominant algal taxa within sea-ice, encounter extreme salinity shifts during seasonal environmental cycles. For example, winter sea-ice brine salinities can exceed 200,[23] whereas salinities in summer melt ponds can drop below 10.[24] Moreover, short-term salinity fluctuations occur within the sea-ice matrix through convection and brine drainage, and may be an explanation for high spatial variability in DMSP concentrations.[14] It is well documented that salinity stress leads to ion toxicity, photosynthetic damage, decreased growth, increased respiration, disruption of enzyme activity and downstream shifts in metabolites, oxidative stress and changes in membrane permeability.[25,26] DMSP is a zwitterion believed to serve compatible solute roles (among its numerous hypothesised physiological functions).[27] Its structure closely resembles that of other well-characterised compatible solutes such as proline and glycine betaine.[28] Like other compatible solutes, DMSP has been shown to minimally inhibit enzyme activity compared with equimolar concentrations of NaCl[29] and may serve a dual role as part of a proposed DMSP antioxidant cascade (Fig. 1).[30,31] DMSP, DMSO, DMS, acrylate and methanesulfinic acid are all capable of scavenging reactive oxygen species (ROS) through a cascade of intracellular reactions cumulatively equivalent in ROS quenching power to the well-studied glutathione antioxidant system[30]. Physiologically protective biological compounds are of widespread interest to the understanding of environmental stress tolerance and may even have biotechnological applications.[32] In fact, exogenous amendment of DMSP to *Escherichia coli* cells increased salinity tolerance via cellular DMSP uptake.[33]

Dickson et al.[34] were the first to report that cellular DMSP concentrations tend to change in the same direction as concomitant changes in salinity in the green macroalgae *Ulva lactuca*. Since then, further studies have shown DMSP concentrations change in response to instantaneous hyper- and hyposalinity changes in batch cultures of other temperate algal species,[28,35–37] whereas studies showed polar macroalgae responded to hypersaline but not hyposaline treatments.[38,39] To our knowledge, there are no reports regarding the effects of a range of gradual salinity shifts on DMSP concentrations, and associated physiological parameters including photosynthetic efficiency of photosystem II (F_V/F_M), ROS and cell membrane integrity, in a sea-ice diatom. Given their importance in polar DMSP production, a better understanding of environmental controls on sea-ice diatom DMSP concentrations is critical. The current set of experiments investigated salinity effects on DMSP concentrations and various physiological parameters in the polar diatom *Fragilariopsis cylindrus*. *F. cylindrus* is a psychrophilic pennate diatom widely distributed throughout Arctic and Antarctic regions and is a dominant species in both sea-ice and polar pelagic systems.[8,12,40–42] In order to assess environmentally relevant DMSP responses to salinity changes, salinity was gradually manipulated over a 24-h period, with

pH and carbonate alkalinity (A_c) allowed to co-vary with increasing and decreasing concentrations of sea salts, as occurs naturally.[43] We hypothesised intracellular DMSP would increase in response to increasing external salinities, and decrease in response to decreasing external salinities coinciding with increases in extracellular (dissolved) $DMSP_d$, as would be expected if it serves a compatible solute role.

Methods

Culture conditions

Axenic batch cultures of *Fragilariopsis cylindrus* (CCMP 1102 clones undergoing genome sequencing, courtesy of Dr Thomas Mock, University of East Anglia) were maintained in a low-temperature incubator system at 0 °C. Cells were grown in sterile, 2-L Teflon bottles in double-filtered (0.2-μm) 35-salinity media, bubbled with sterile air (140 mL min^{-1}) to ensure sufficient CO_2 availability. To represent natural conditions, salinity was adjusted gradually through addition of hypo-, iso- or hyperosmotic seawater media. Ultrapure water (≥18 MΩ) or sea-salts (Instant Ocean, Spectrum Brands, Inc., Cincinnati, OH) were added to sea water before filtration and nutrient addition. Cultures from different treatment groups were diluted with equal volumes of media in several stepwise salinity adjustments until final treatment salinities were reached. The individual experiments presented were separated across several years during which time our incubator system and experimental design evolved, as noted below for each experiment.

Experiment I

Cultures were maintained under continuous light (50 μE m^{-2} s^{-1} supplied by GE Plant & Aquarium fluorescent light bulbs, Cleveland, OH) to remove any diel effects. Ross Sea water amended with a 2× L1 + Si nutrient mix[44] was used for culture media and high-purity air cylinders were used to bubble air through the cultures. Five salinity treatments (10, 20, 35, 50 and 70 salinity; $n = 3$ per treatment group) were achieved through nine separate dilutions over a 23-h period, shifting cultures −2.8, −1.7, 0, +1.7 and +3.9 salinity units per dilution respectively for the five different treatment groups. At time zero (T0), a 2-L exponentially growing culture (2–2.5 × 10^6 cells mL^{-1}) was divided into five new sterile 2-L Teflon bottles and the first salinity adjustment was made. This procedure was repeated three times over a 1.5-h window, thus staggering replicates to allow for sampling time. The next salinity adjustment through media addition was made 2 h following T0 and every 3 h after that until final salinity treatments were reached at 23 h. Cultures were sampled for the various parameters listed below at T0, and on Days 1, 2, 3, 5 and 7 (T1 at 24 h, T2 at 48 h, etc.).

Experiment II

Cultures were maintained on a 16 : 8 light : dark cycle (50 μE m^{-2} s^{-1}), which is more representative of diel periodicity during the spring–autumn (melt–freeze) seasons; sampling occurred at mid-day to minimise any diurnal periodicity effects. Cultures were grown in Aquil* artificial sea water with 2× L1 + Si nutrients[45] and bubbled with 0.2-μm-filtered laboratory air. At T0, exponentially growing cultures (1.5–1.8 × 10^6 cells mL^{-1}) were divided into 10-, 35- and 70-salinity treatments ($n = 4$ per group). To minimise handling time outside the incubator, a series of four dilutions were conducted over a 22-h period starting at T0 and proceeding every 5.5 h. Each dilution adjusted culture salinity −6.25, 0 or +9 salinity units for the 10, 35 and 70 treatments respectively. Culture sampling time points were performed at days T0, T1, T2 and T7. To maintain cells at similar densities over the course of the experiment II, at T2, following sample collection, 35- and 70-salinity cultures were diluted ~50 and 30 % respectively.

Instantaneous salinity shift experiment

A small experiment in which control 35-salinity cultures were shifted instantly to 10-, 20-, 50- or 70-salinity media was also conducted in order to test the sensitivity of membrane permeability and ROS methods. Cultures were maintained as described in Experiment II. Exponentially growing cultures were divided at T0 into 500-mL culture flasks and similar volumes of hypo-, iso-, or hypertonic Aquil* media were added to achieve final 10-, 20-, 35-, 50- or 70-salinity treatments ($n = 3$ per treatment). Dilutions were staggered to allow flow cytometry analysis time. Membrane permeability and ROS were quantified 2 h following the instant salinity shift, as outlined below.

Abiotic measurements

Prior to the experiment, incubator irradiance values were measured inside media-filled Teflon bottles with a 4pi submersible sensor light meter (QSL-100, Biospherical Instruments, San Diego, CA). Samples were collected at various time points over the course of the salinity experiments for salinity, osmolality, pH and A_c measurements. Sample aliquots were filtered through 0.45-μm filters into headspace-free vials and stored at 4 °C until analysis (within 24 h of collection). Salinity was measured with a hand-held optical refractometer with automatic temperature compensation (A366ATC, Vista Series Instruments, China). A vapour-pressure osmometer (Wescor, Vapro 5520, ELITech Group, Princeton, NJ) calculated osmolality from media dew-point depression measurements. Culture pH was measured using a bench-top pH meter (Orion, Benchtop pHuture Solid-State pH System, Thermo Fisher Scientific, Waltham, MA) calibrated with pH 4.00, 7.00, 10.00 certified standards (Fisher Chemical, Pittsburgh, PA).

Experiment I

A_c was determined according to the methods of Parsons et al.[46] based on pH change following a standard acid titration. B and F constants in the Parsons' alkalinity equations are pH-, salinity- and temperature-dependent. Existing tables were used to fit the graphs of these constants to linear and polynomial equations respectively and extrapolate constants for gradients within this experiment outside the range of published tables. However, 70-salinity samples required an additional titration increment beyond Parsons' alkalinity equations, preventing calculation of A_c in this treatment in Experiment I.

Experiment II

Owing to the issue with Parsons' A_c calculations for 70-salinity cultures, a spectrophotometric method was used to measure A_c.[47]

Standard cell culture measurements

Cell counts and biovolume were measured using a Beckman Coulter Multisizer 3 (Indianapolis, IN). Cells were fixed in 2 % gluteraldehyde (Experiment I) or formalin (Experiment II). Both live and fixed aliquots of control salinity cultures were

compared to ensure fixation had no significant effect on biovolume measurements. Cell numbers and biovolume per millilitre were adjusted to account for dilutions. Growth rates (doublings per day) were calculated from cell-count data according to Eqn 1.

$$\text{Divisions per day} = (\ln N_t - \ln N_0)(T_t - T_0^{-1})(\ln 2)^{-1} \quad (1)$$

Fluorometric quantification (10-AU, Turner Designs, Sunnyvale, CA) of chlorophyll-*a* (Chl-*a*) was performed on subsamples filtered onto Whatman GF/F filters and extracted in 90 % acetone for 24–48 h in the dark at 4 °C. Subsamples from all biological replicates at the start and end of experiments were preserved with equal volumes of 4 % paraformaldehyde in phosphate-buffered saline solution (pH 7.8) and used to confirm the absence of bacterial contamination by epifluorescent microscopy[48] (optimised for *F. cylindrus* cultures). Briefly, cells were stained overnight with DAPI (4′,6-diamidino-2-phenylindole), then filtered onto 0.2-µm black polycarbonate filters. Multiple microscopic fields were assessed (more than 200 *F. cylindrus* cells observed) to confirm no bacterial contamination (Supplementary material, Fig. S1).

DMSP quantification

Culture subsamples were collected for total DMSP ($DMSP_t$) and $DMSP_d$ according to established methods.[49] For dissolved fractions, 25-mL aliquots were gravity-filtered through Whatman GF/F filters to remove cells; to prevent cell lysis and release of particulate DMSP ($DMSP_p$), filters were not allowed to run dry. Dissolved and total fractions were acidified with 50 % H_2SO_4 (10 µL per mL of sample) and stored at 4 °C in the dark until analysis (typically within 1 month of collection). DMSP was quantified as DMS following 2 M NaOH base hydrolysis (1 : 1 molar reaction). DMS was then collected in a cryogenic purge-and-trap system coupled to a gas chromatograph (GC), with a photometric flame detector unit (Hewlett-Packard 5890 Series II, Agilent Technologies, Santa Clara, CA). Technical replicates were always run to ensure ≥90 % precision of GC measurements; a Dixon's Q outlier test was applied to determine if values outside this range should be included in sample averages.[50] $DMSP_p$ was calculated as the difference between total and dissolved fractions. Intracellular concentrations were determined by dividing $DMSP_p$ per millilitre by cell biovolume per millilitre.

DMSO quantification

Culture subsamples were collected for total DMSO ($DMSO_t$), acidified and stored as described above for $DMSP_t$. Prior to DMSO analysis, DMS and $DMSP_t$ were removed by 2 M NaOH base hydrolysis and sparging samples with ultrahigh-purity nitrogen for 45 min. $DMSO_t$ was measured using cobalt-doped sodium borohydride tablets (Sigma–Aldrich, St Louis, MO) as described by Riseman and DiTullio[51] using the GC purge-and-trap system described above.

Physiological stress indicators

F_V/F_M was determined on dark-acclimated culture aliquots by fast-repetition-rate fluorometry using a FastTracka instrument (Chelsea Instruments, West Molesey, UK). Cultures were kept on ice in the dark for 15 to 35 min before analysis. A preliminary time-series experiment had previously established this as a sufficient dark period to empty photo-electron carriers and detect optimal F_M values in *F. cylindrus*.

Cell membrane integrity was assessed using the membrane-impermeable fluorescing dye Sytox green (Invitrogen, Carlsbad, CA). On arrival, Sytox was diluted with ultrapure water to make 50-µM working stock aliquots, which were stored at −20 °C until day of use according to manufacturer's instructions. Culture aliquots were incubated in 0.05-µM Sytox (final concentration) in the dark, on ice, for approximately 20 min before analysis by flow cytometry using a Beckman Coulter MoFlo XDP (Atlanta, GA) with standard filters. Sytox dye was excited with an argon laser at 488 nm and emission was detected with a 513-nm detector filter (26-nm bandwidth). Particle detection was triggered off forward scatter and cells were gated on a biparameteric cytogram of forward scatter and chlorophyll autofluorescence, the latter detected with a 664-nm filter (22-nm bandwidth). Relative fluorescent units (RFU) of the dye (513 nm) were measured in 20 000 cells.

Cellular ROS were detected using the fluorescent probe 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate acetyl ester (CM-H_2DCFDA; excitation 488 nm, emission 522 nm; Molecular Probes, Inc., Eugene, OR) as used by Evans and colleagues with the haptophyte *Emiliania huxleyi*.[52] CM-H_2DCFDA is membrane-permeable, and once inside the cells is hydrolysed by cellular esterases to 2′,7′-dichlorohydrofluorescein (DCFH), which in turn fluoresces on reaction with ROS. Based on optimisations in *F. cylindrus*, 4 µL of a 1 mM CM-H_2DCFDA working stock made fresh daily was added to 1-mL aliquots of cultures (4 µM final concentration) and incubated under in situ conditions for 1 h before analysis. Intracellular DCFH fluorescence was then quantified as outlined for Sytox. Cellular background fluorescence emitted in the 513–526-nm range was measured in blanks (cell aliquots with no stain added) and was subtracted from DCFH fluorescence to determine background normalised ROS fluorescence per cell. Because DCFH reactivity is dependent on cellular esterase cleavage, we also used the fluorescent esterase activity probe calcein AM (Invitrogen, Inc., Carlsbad, CA). The 1-mg mL^{-1} solution in anhydrous DMSO was diluted 1 : 10 with 0.2-µm-filtered seawater and 100 µL of this working stock was added to 1-mL culture aliquots (10 µM final concentration) and incubated and analysed as outlined for DCFH.

Statistical analysis

Data were analysed using *R* statistical software (ver. 1.11.1, R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria, see http://www.R-project.org). Because time points were not independent, treatment effects were assessed using general linear least-squares (GLS) models and specifying time as the covariate. Second-order polynomials were used to fit non-linear treatment responses over time. A recurring issue across experiments and parameters was the fact that stress conditions often resulted in increased variability in responses. Therefore, statistical models accounting for unequal variance by fitting separate variance terms for each treatment were applied and compared with models not accounting for unequal variance using log-likelihood tests. Linear contrasts were then used to compare treatments at select acute (Day 1 = T1 and Day 2 = T2) and late (Day 7 = T7) salinity acclimation time points. Weighted variance models were used for linear regressions or linear contrasts only if ANOVAs comparing unweighted and weighted variance models found this to be a statistically

Table 1. Abiotic variables associated with salinity change

Salinity, osmolality, pH and A_c (carbonate alkalinity) are listed for each treatment group. Means ± standard errors of the independent biological replicates for experiment I ($n = 3$) and experiment II ($n = 4$) were averaged from several different time points. Significant differences between treatment groups and 35-salinity control cultures are indicated for pH and A_c as: *, $P < 0.05$

Experiment	Treatment	Salinity	Osmolality (mmol kg^{-1})	pH	Carbonate alkalinity (meq L^{-1})
Experiment I	10 salinity	9.7 ± 0.2	284.8 ± 4.3	7.54 ± 0.03*	1.21 ± 0.03
	20 salinity	19.7 ± 0.3	547.4 ± 4.3	7.94 ± 0.04	1.67 ± 0.04
	35 salinity	34.8 ± 0.2	967.6 ± 4.5	8.03 ± 0.02	0.89 ± 0.09
	50 salinity	50.5 ± 0.3	1428.2 ± 16.3	8.19 ± 0.03*	2.29 ± 0.06
	70 salinity	69.5 ± 0.5	2020.9 ± 21.2	8.22 ± 0.04*	–[A]
Experiment II	10 salinity	10.9 ± 0.1	295.0 ± 1.1	7.46 ± 0.05*	1.68 ± 0.01*
	35 salinity	35.4 ± 0.2	993.0 ± 0.9	7.88 ± 0.04	3.33 ± 0.03
	70 salinity	71.5 ± 0.2	2084.4 ± 5.5	7.83 ± 0.06	4.04 ± 0.06*

[A]A_c could not be determined for the 70-salinity group in experiment I due to limitations in Parsons' method.

significant improvement ($P < 0.05$). Residuals were assessed to ensure normality and homoscedasticity assumptions were being met by the applied model.

Results

Abiotic variables

As expected, a doubling of salinity resulted in a concomitant doubling of media osmolality (Table 1). This linear relationship between salinity and osmolality was observed across all treatments in both experiments. Furthermore, as media salinities increased, A_c and pH were generally elevated and reduced respectively in a non-linear and more variable manner (Table 1). In experiment I, A_c in the 35-salinity control cultures was much lower and highly variable compared with typical seawater values; because of the high variability, the differences between treatment and control cultures were not significant. However, the A_c of the 35-salinity control cultures in experiment II was slightly higher than those typical of sea water. Nevertheless, as expected, 10- and 70-salinity treatments resulted in reduced and elevated A_c respectively compared with controls ($P < 0.0001$).

Growth and F_V/F_M

In experiment I, salinity shifts reduced growth rates over the 7-day experiment (T0–T7) in 10-, 50- and 70-salinity treatments – 84, 27 and 44 % respectively – relative to 35-salinity control cultures (Fig. 2a and c; $P < 0.0001$). For the first 2 days of salinity acclimation (T0–T2), growth was almost completely arrested in the 10- and 70-salinity cultures (i.e. 1 and 3 % of control growth rates respectively; $P = 0.0002$). Growth rates over the next 5 days (T2 to T7) recovered in the 70-salinity cultures, but remained reduced by 75 % in the 10-salinity treatment ($P < 0.0001$) and were also 32 % reduced in the 50-salinity treatment ($P = 0.012$) relative to the control. Experiment II showed similar effects on growth rates (after accounting for changes in cell densities from culture dilutions; Fig. 2b and d). Again, growth was almost entirely halted in the 10- and 70-salinity treatments during the first 2 days of salinity acclimation (i.e. 0 and 7 % of control growth rates respectively; $P < 0.0001$). Unlike experiment I, growth rates remained significantly reduced in the 70-salinity treatment group over the later 5 days as well (i.e. 45 % of growth rates in 35-salinity cultures; $P < 0.0001$).

F_V/F_M followed similar trends to growth (Fig. 2e and f). The most notable reduction in F_V/F_M was observed in the 10-salinity treatment group, whose F_V/F_M values were 20–35 % reduced from control cultures across all time points tested ($P < 0.0001$). However, shifts to a salinity of 70 resulted in mild F_V/F_M reductions (6–9 % reduced from controls, $P < 0.005$).

DMSP

As hypothesised, shifts to higher-salinity media led to the accumulation of intracellular $DMSP_p$; shifts to lower-salinity media resulted in reduced $DMSP_p$ (Fig. 3a and b). A GLS model fitted for unequal variances among treatments predicted $DMSP_p$ concentrations over time across all salinity groups ($P < 0.0001$; Supplementary material, Fig. S2a–d and Table S1). In experiment I, by T1 (1 h after final treatment salinities were reached), $DMSP_p$ was elevated 30 % ($P = 0.0003$) and 47 % ($P < 0.0001$) in the 50- and 70-salinity groups respectively compared with 35-salinity control cultures. By T7, $DMSP_p$ concentrations in both hypersalinity treatment groups were ~75 % elevated ($P < 0.0001$). In experiment II, the high-salinity shift showed a similar trend but a greater magnitude of change, with the 70-salinity treatment group 70 and 127 % elevated at T1 and T7 respectively relative to controls ($P < 0.0001$). Conversely, hyposalinity shifts to 20-salinity media in experiment I gradually reduced $DMSP_p$, such that by T7, intracellular concentrations were 32 % of the $DMSP_p$ concentrations in 35-salinity cultures ($P < 0.0001$). The shift to 10-salinity media, however, immediately reduced $DMSP_p$ values to 27 % of the 35-salinity controls by T1 in experiment I ($P < 0.0001$). By T2, no $DMSP_p$ was detected, and by T7, concentrations were only 8 % of control values. In experiment II, there was a similar rapid reduction to near-zero $DMSP_p$ concentrations following a shift to 10-salinity media and slight return of a small intracellular $DMSP_p$ pool by T7.

$DMSP_t$ values were also elevated and reduced with higher- and lower-salinity treatments (Fig. 3c and d). However, $DMSP_t$ concentrations were equally elevated in 50- and 70-salinity cultures at T1 (~36 % higher than controls; $P < 0.005$), and by T7, $DMSP_t$ only remained elevated in the 50-salinity group ($P < 0.005$; Fig. 3c). $DMSP_t$ concentrations in 10- and 20-salinity cultures were also comparable across the course of experiment I, with T7 values ~70 % less than control culture concentrations ($P < 0.0001$). In experiment II, by T7, $DMSP_t$ was 36 % elevated in 70-salinity cultures and 28 % reduced in the 10-salinity treatment group (Fig. 3d; $P < 0.0001$).

In both experiments, $DMSP_d$ accounted for less than 1 % of $DMSP_t$ in control and high-salinity treatments at all time points (Fig. 3e and f). Although $DMSP_d$ concentrations reached 5 % of

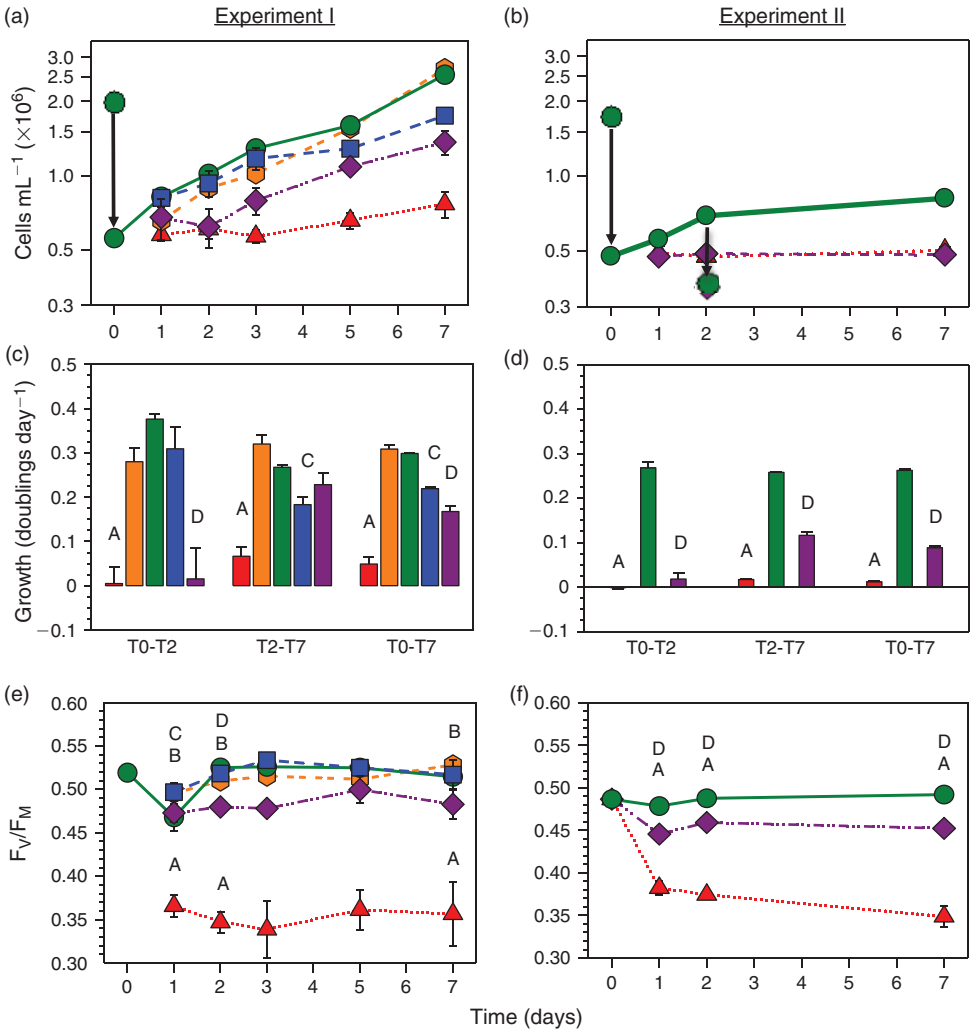

Fig. 2. Cell density, growth and photosynthetic efficiency of photosystem II (F_V/F_M) are shown for two independent salinity experiments ($n = 3$, experiment I; $n = 4$, experiment II). Means and standard errors are graphed for 10- (red triangles), 20- (orange hexagons), 35- (green circles), 50- (blue squares) and 70- (purple diamonds) salinity treatments. Significant differences between 35-salinity controls and 10-, 20-, 50-, 70-salinity treatments on Days 1 (T1), 2 (T2) or 7 (T7) are indicated by A, B, C, D respectively ($P < 0.05$). (a, b) Cells in the log scale are graphed over time. Dilutions are indicated with arrows. (c, d) Growth rates (doublings per day) after accounting for dilutions were calculated for T0–T2, T2–T7 and T0–T7. (e and f) F_VF_M over time.

the $DMSP_t$ pool at T2 in the 20-salinity treatment group, this was not significant because it was driven by one biological replicate, and returned to only 1.5 % of $DMSP_t$ in the dissolved fraction for the remainder of the experiment. However, the 10-salinity treatment group corresponded with 82, 100 and 90 % of $DMSP_t$ accounted for in the $DMSP_d$ pool at T1, T2 and T7 respectively in experiment I ($P < 0.0001$; Fig. 3e). Experiment II again showed almost the entire $DMSP_p$ pool released to the dissolved fraction 24 h following a shift to 10-salinity media ($P < 0.0001$; Fig. 3f).

DMSP values were also normalised to Chl-*a* concentrations because these ratios are typically reported for most field sea-ice measurements. $DMSP_t$ Chl-a^{-1} values were again elevated in the high-salinity treatments, gradually reduced in the 20-salinity treatment group, but slightly elevated in 10-salinity cultures by T7, relative to 35-salinity controls (Fig. 4a and b; $P < 0.005$). The apparent elevation in Chl-*a* -normalised $DMSP_t$ in the 10-salinity group is in part due to the large pool of DMSP released from the cells to the dissolved fraction – $DMSP_d$ remained in the media unmetabolised in these axenic cultures. As expected, $DMSP_p$ Chl-a^{-1} values in the 10-salinity cultures are in fact reduced ($P < 0.0001$; Fig. 4c and d). Another factor contributing to the observed elevation in $DMSP_t$ Chl-a^{-1} concentrations in the 10-salinity group by T7 was the relative decrease in Chl-*a* cell^{-1} concentrations observed in this lowest-salinity treatment compared with 35-salinity controls ($P < 0.0005$; Fig. 4e and f). Also notable is the difference in overall cellular Chl-*a* concentrations between experiments I and II within the same treatment groups. Thus, shifts to a 70-salinity media corresponded with 86 and 139 % elevations in $DMSP_p$ Chl-a^{-1} values at T1 and T7 respectively in experiment I ($P < 0.005$; Fig. 4c) *v.* only 47 and 113 % elevations at the same time points in experiment II ($P < 0.00001$; Fig. 4d).

DMSO

Final $DMSO_t$ concentrations at T7 in experiment II showed a significant change in response to salinity (Fig. 5 and Table 2). At this time point, $DMSO_t$ concentrations were 25 % elevated in

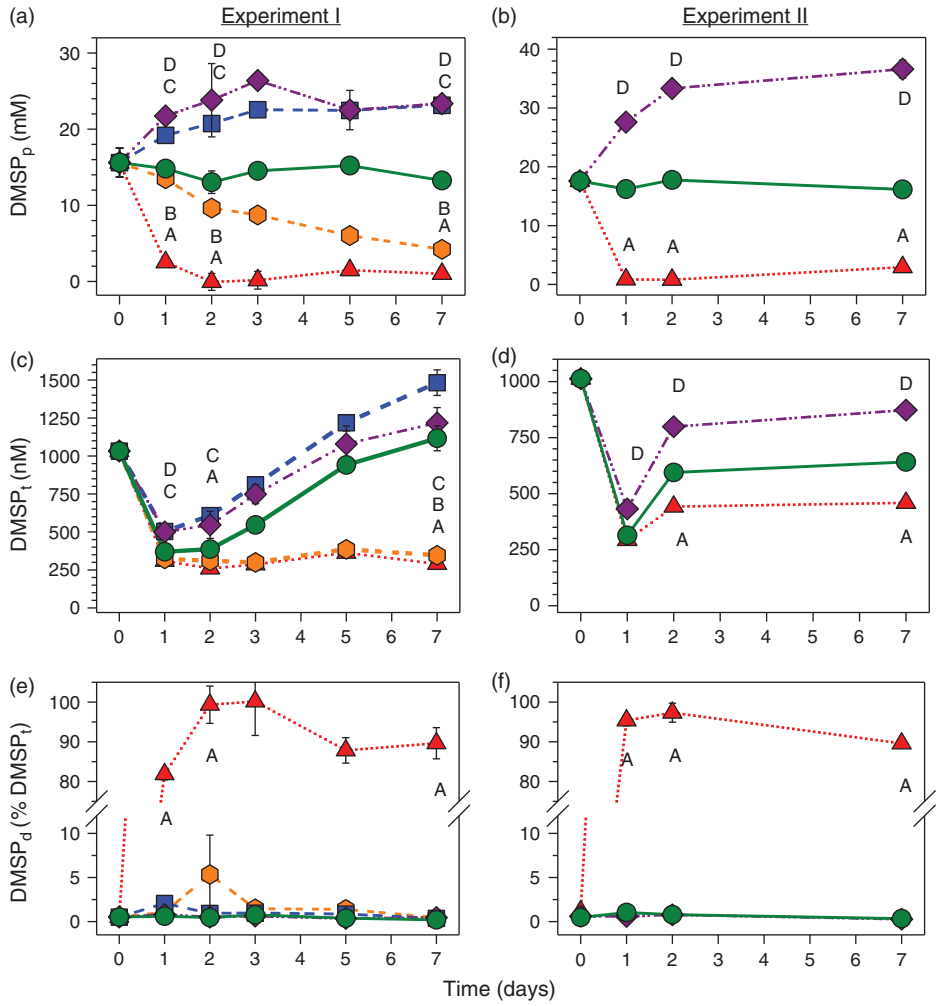

Fig. 3. Dimethylsulfoniopropionate (DMSP) changes observed over time. Means with standard errors are shown for salinity experiment I ($n = 3$) and experiment II ($n = 4$) for 10- (red triangles), 20- (orange hexagons), 35- (green circles), 50- (blue squares) and 70- (purple diamonds) salinity groups. Significant differences between treatment groups and 35-salinity controls were tested on Days 1, 2 and 7 and indicated with A, B, C or D for 10-, 20-, 50- and 70-salinity treatment groups respectively ($P < 0.05$). (a, b) Intracellular DMSP (mM) concentrations over time. (c, d) DMSP$_t$ (total DMSP) (nM) over time. (e, f) Percentage of DMSP$_t$ in the dissolved fraction.

70-salinity cultures relative to 35-salinity cultures (Table 2; $P = 0.0087$). DMSO$_t$ values normalised to cell numbers showed DMSO$_t$ concentrations per cell 73 % elevated in the 10-salinity treatment group and 110 % elevated in the 70-salinity treatment, relative to 35-salinity controls (Fig. 5; $P < 0.0001$). The percentage of DMSO$_t$ contributing to the combined DMSP$_t$ + DMSO$_t$ pool was negligible across all treatments (0.65–1.06 %; Table 2), although it was elevated in the 10-salinity group relative to controls ($P = 0.0001$).

Membrane permeability

Sytox cellular fluorescence thresholds indicative of compromised cell membranes were defined using formalin-fixed cultures as a positive control (Supplementary material, Fig. S3a, b and c). Sytox staining of *Fragilariopsis cylindrus* cells that were gradually shifted to different salinities in experiment II revealed 45–48 % of cells had permeable membranes in the 10-salinity treatment across all sampling time points, compared with less than 2 % of cells in the control and high-salinity treatments ($P < 0.0001$; Fig. 6a). Instantaneous salinity shifts performed in an optimisation experiment ($n = 3$ per treatment) also showed no increase in membrane permeability in the 35- and higher-salinity treatments (measured 2 h following salinity shift; Fig. 6b), whereas instantaneous shifts to salinities of 10 and 20 resulted in 84 and 28 % of cells respectively exhibiting permeable membranes ($P < 0.0001$).

ROS

Because low background fluorescence at 513 nm exists from small bleed-over of pigment autofluorescence, DCFH fluorescence was corrected for changes in background fluorescence at the 513-nm wavelength (Supplementary material, Fig. S4a and b). The net result was an increase in the ROS signal in the 10-salinity treatment, relative to control cultures, at T1 only ($P = 0.026$; Fig. 7a). In contrast, instantaneous salinity shifts to 70 significantly increased ROS ($P < 0.0001$), but showed decreased ROS 2 h following a shift from 35- to 10-salinity media ($P = 0.0023$; Fig. 7b). Because the ROS probe is dependent on cellular esterase activity, calcein fluorescence was used to quantify esterase activity. Calcein fluorescence was

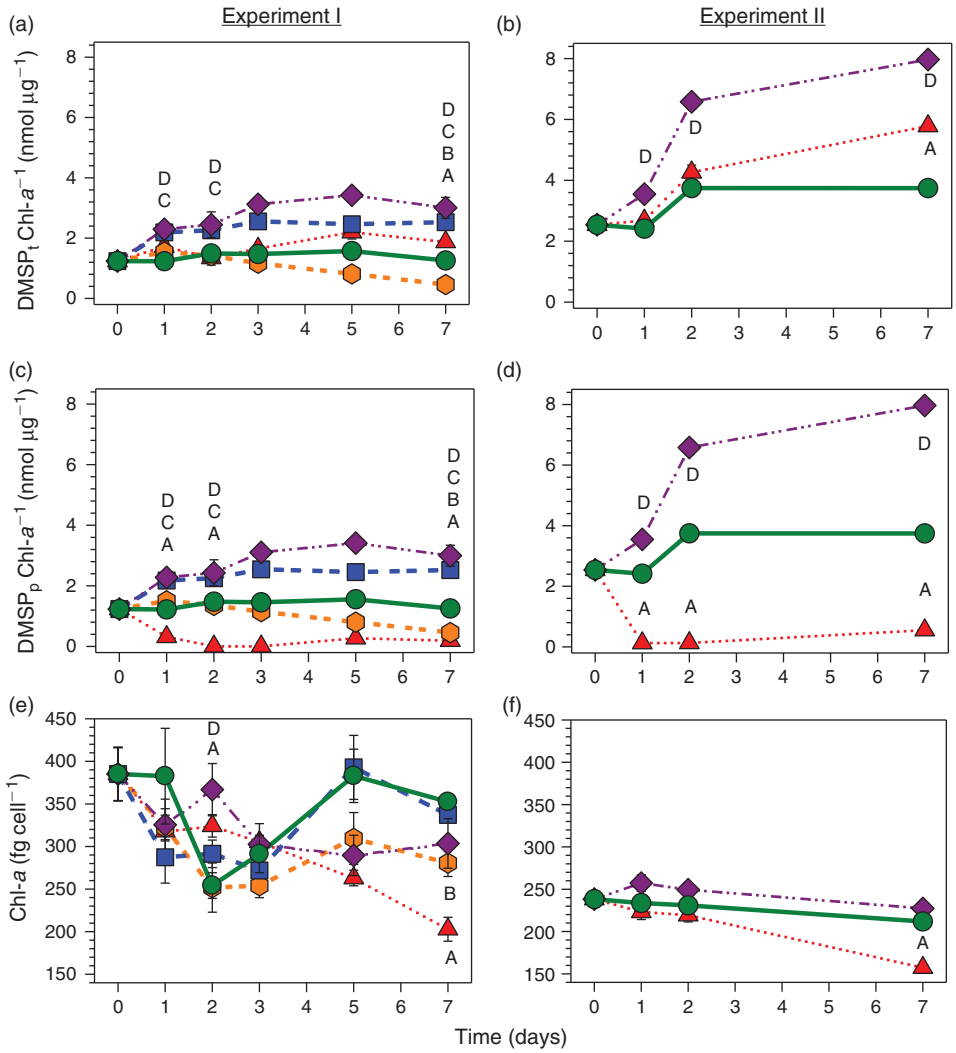

Fig. 4. Dimethylsulfoniopropionate (DMSP) changes normalised to chlorophyll-*a* (Chl-*a*) observed over time. Means with standard errors are shown for salinity experiment I ($n = 3$) and experiment II ($n = 4$) for 10- (red triangles), 20- (orange hexagons), 35- (green circles), 50- (blue squares) and 70- (purple diamonds) salinity groups. Significant differences between treatment groups and 35-salinity controls were tested on Days 1, 2 and 7 and indicated with A, B, C or D for 10-, 20-, 50- and 70-salinity treatment groups respectively ($P < 0.05$). (a, b) Total DMSP (DMSP$_t$) Chl-a^{-1} (c, d) particulate DMSP (DMSP$_p$) Chl-a^{-1} (e, f) Chl-*a* cell^{-1} concentrations are all graphed over time (days).

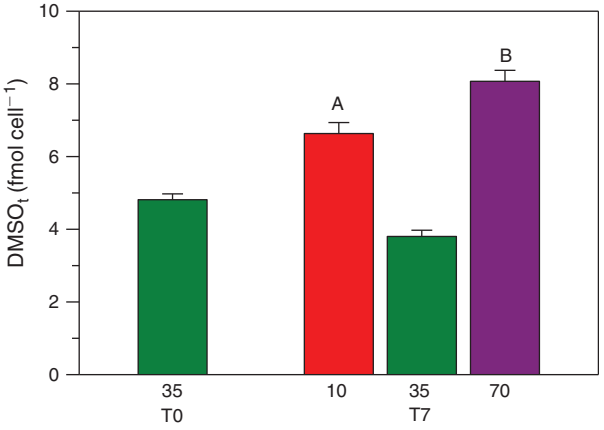

Fig. 5. Total DMSO (DMSO$_t$) changes normalised to cell density for 10- (red), 35- (green) and 70- (purple) salinity treatments. Means with standard errors are shown ($n = 4$). Significant differences at T7 between 10- or 70-salinity treatment groups and 35-salinity controls are indicated with A or B respectively ($P < 0.05$).

reduced in both 10- and 70-salinity cultures at T2 and again in 10-salinity cultures at T7 in experiment II ($P = 0.0085$, 0.044 and 0.0014 respectively; Fig. 7c). Esterase activity was also reduced 2 h following an instantaneous shift to 10-salinity media ($P = 0.0002$), but increased following an instant shift to 70-salinity media ($P = 0.0005$; Fig. 7d).

Discussion

Sea-ice diatoms experience severe shifts in environmental salinities during the natural freeze–thaw processes of ice formation and ice melt during seasonal oscillations. We hypothesised that sea-ice diatom DMSP responses to salinity changes would be consistent with a compatible solute function and could, in part, explain the quite high concentrations measured within ice cores, as well as the pulses released into the environment during ice melt. Both salinity experiments showed rapid and significant changes in DMSP$_t$, DMSP$_p$ and DMSP$_d$ pools, consistent with our hypotheses (Fig. 3).

Table 2. $DMSO_t$ and $DMSP_t$ culture concentrations

Total dimethylsulfoxide ($DMSO_t$) and total dimethylsulfoniopropionate ($DMSP_t$) concentrations and %DMSO of DMSO + DMSP pool are compared between salinity treatment groups, mean ± standard errors, $n = 4$, significant differences are indicated as: *, $P < 0.05$

	35 salinity T0	10 salinity T7	35 salinity T7	70 salinity T7
$DMSO_t$ (nM)	8.13 ± 0.01	3.39 ± 0.19	3.15 ± 0.11	3.95 ± 0.19*
$DMSP_t$ (nM)	1012.94 ± 27.56	458.94 ± 16.32*	641.28 ± 33.94	872.72 ± 8.67*
%DMSO $(DMSP + DMSO)^{-1}$	0.82 % ± 0.01	0.73 % ± 0.04 *	0.49 % ± 0.02	0.45 % ± 0.02

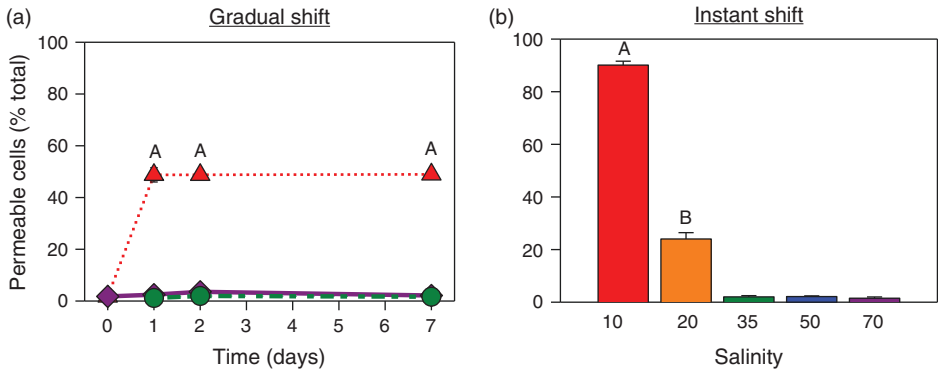

Fig. 6. Salinity treatment effects on cell membrane permeability are graphed. (a) Percentage of cells with permeable membranes after gradual salinity shifts are shown for 10- (red triangles), 35- (green circles) and 70- (purple diamonds) salinity groups ($n = 4$; experiment II). Significant differences between treatments and 35-salinity controls were tested for at T1, T2 and T7 and are indicated with the symbols A or B for 10- or 70-salinity groups respectively ($P < 0.05$). (b) Percentage of cells exhibiting permeable membranes 2 h after instantaneous salinity shifts are shown. Significant differences between treatments and 35-salinity controls are indicated by A, B, C, D for 10-, 20-, 50-, 70-salinities respectively ($P < 0.05$).

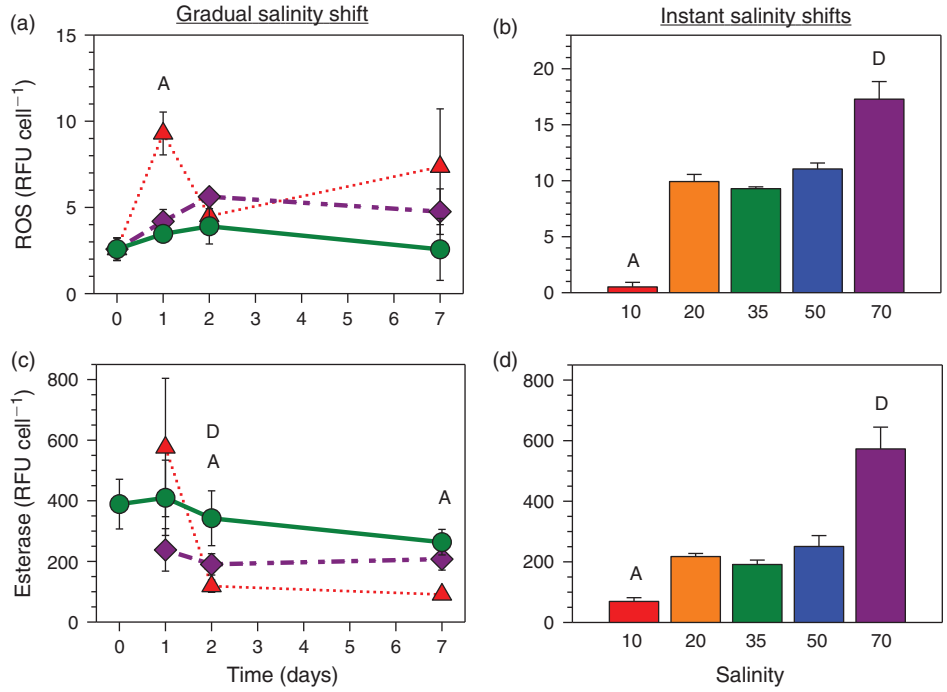

Fig. 7. Salinity treatment effects on cellular reactive oxygen species (ROS) and esterase activity are shown following gradual salinity shifts ($n = 4$; experiment II; left column) or instant salinity shifts ($n = 3$; right column). Means and standard errors are graphed for 10- (red triangles), 20- (orange), 35- (green circles), 50- (blue) and 70- (purple diamonds) salinity groups. Significant differences between treatments and 35-salinity controls are indicated by A, B, C, D for 10-, 20-, 50-, 70-salinities respectively ($P < 0.05$). (a) ROS relative fluorescence units (RFU) per cell over time. (b) ROS RFU 2 h after instant salinity shifts. (c) Calcein RFU indicative of esterase activity over time. (d) Calcein (esterase) RFU 2 h after instant salinity shifts.

DMSP as a compatible solute

F. cylindrus cultures increased $DMSP_p$ concentrations, the physiologically relevant parameter, following shifts to higher salinities and decreased $DMSP_p$ content following shifts to lower salinities (Fig. 3a and b). In the first experiment, we surveyed a range of salinities using batch cultures grown under continuous light. A salinity-dependent response pattern was observed across all five treatments and changes in $DMSP_p$ concentrations over time were predicted extremely well by second-order polynomial linear models with different curves and *y* intercepts fitted for each treatment (Fig. 3a and Supplementary material, Fig. S2a, c and Table S1). Unlike only hypersalinity DMSP responses reported in experiments with polar macroalgae,[38,39] *F. cylindrus,* and perhaps other sea-ice diatoms, show a clear and rapid response in DMSP levels to both hyper- and hyposalinity shifts, consistent with previous observations in some temperate species.[34,36,37] The present results support a compatible solute role for DMSP during sea-ice diatom salinity acclimation.

Although related, osmolyte and compatible solute functional roles are slightly different; the former depends on intracellular concentrations contributing significantly to cellular osmotic potential, whereas the latter refers to protein-stabilising characteristics of a compound (which are evident at even low concentrations). Intracellular $DMSP_p$ concentrations observed in *F. cylindrus* cultures suggest DMSP is a minor contributor to total osmotic potential when compared with other osmolytes whose intracellular concentrations are typically on the order of 300–500 mM.[35] However, Nishiguchi and Somero[53] showed fairly low in situ DMSP concentrations (100–200 mM) to be effective protein-stabilising concentrations under cold and heat shock protein-denaturing conditions. We speculate, similarly, low DMSP concentrations would also likely protect against salt-induced denaturation of proteins, as has been shown for other structurally similar, protein-stabilising solutes in the 'Hofmeister series'.[54] Furthermore, subcellular compartmentalisation can make low levels even more osmotically significant.[55] The mounting evidence for chloroplast DMSP compartmentalisation[56,57] would translate to significantly higher DMSP concentrations given that chloroplast volume has been estimated to account for 10–30 % of cell volume depending on light conditions.[58] It is an intriguing hypothesis that chloroplast-localised DMSP production could be one of the mechanisms that enable diatoms to acclimate to higher-salinity environments with minimal inhibitory effects on photosynthesis, as was observed in the current set of experiments (Fig. 2e and f).

DMSP partitioning and membrane permeability

Salinity shifts in both the 10 and 20 treatments showed a reduction in intracellular DMSP levels; however, the fate of this DMSP was different between the different downshift gradients (Fig. 3). In the 10-salinity group, 100 % of $DMSP_p$ was released to the dissolved fraction (i.e. $DMSP_d$) within 24 h of completion of this salinity shift. Some cell lysis might have occurred in the 10-salinity group, thus releasing $DMSP_p$ (along with the rest of the cell contents) into the culture media. Membrane permeability assays, however, do not support the hypothesis that compromised membrane integrity alone was responsible for the 100 % release of intracellular DMSP into the dissolved fraction by Day 2. In fact, only ~45 % of cells exhibited permeable membranes in response to a gradual shift to a salinity of 10 (Fig. 6a). However, instantaneous shifts to a salinity of 10 resulted in ~85 % of cells exhibiting compromised membranes after 2 h (Fig. 6b). It is possible that membrane integrity is a transient state and we cannot rule out the possibility that cells not showing compromised membranes at the time of measurement may have had permeable membranes at an earlier point in time. Solute leakage due to transient changes in membrane permeability following hypo-osmotic shock has previously been proposed for mannitol release in the brown macroalgae *Pilayella littoralis*, when Evans' Blue staining showed no loss of integrity following decreased salinity.[59] Alternatively, release of $DMSP_p$ and other osmolytes may be a facilitated process through cell membrane transporters, such as mechanosensitive channels that open in response to changes in membrane tension.[60] Selective release of intracellular compounds was supported by a study in *Synechocystis* of ^{14}C-labelled exudates following salinity reductions that found the osmolyte glucosylglycerol to be the major exudate, with minor amounts of amino acids, organic acids and carbohydrates released.[61] Clearly, selective release of osmolytes rather than general leakage of cellular compounds would be ecologically advantageous.

In contrast, decreases in $DMSP_p$ concentrations in the 20-salinity group occurred more gradually over the 7-day experiment (in comparison with the 10-salinity treatment; Fig. 3a) and did not correspond with any increase in the $DMSP_d$ fraction (Fig. 3c). Shifts to a salinity of 20 also had negligible effects on growth and F_V/F_M (Fig. 2c and e), indicating minimal inhibitory effects on cellular processes. Perhaps, rather than releasing an already reduced sulfur and carbon compound, cells preferably recycle this molecule into other cellular substrates when metabolically capable. However, the extreme stress of a downshift to a salinity of 10 necessitated a rapid release of osmolytes, rather than waiting for a slower recycling process to decrease $DMSP_p$ concentrations. Thus, the osmotic gradient appears to be important in determining the fate of $DMSP_p$ and this could have important implications for modelling the fate of DMSP and DMS during sea-ice melt conditions.

Possible secondary antioxidant role

Although we hypothesised salinity stress would be associated with enhanced ROS accumulation, ROS detection was only significantly elevated in the 10-salinity group at T1, although instantaneous shifts to a salinity of 70 also increased ROS (Fig. 7a and b). The latter result was consistent with previous work with an intertidal diatom in which an instant two-fold increase in media salinity induced increased DCFH cellular fluorescence (i.e. ROS) and correlated with increased glutathione and lipid peroxidation.[62] Although salinity treatment groups did show a decrease in cellular esterase activity needed to transform CM-H_2DCFDA to its ROS reactive form DCFH, esterase activity did not appear to be suppressed to the point of limiting the availability of intracellular DCFH (Fig. 7c and d). Instead, we believe the lack of elevation in ROS fluorescence indicates the gradual salinity shifts used in the current experiment allowed time for cells to up-regulate effective free radical-scavenging mechanisms, such as superoxide dismutase, carotenoids (B. Lyon, undpubl. data) and biogenic sulfur compounds.

Recently, a new method for DMSO quantification, a metabolite produced through DMSP oxidation, found high levels within sea-ice,[15] although previous work using other methods reported lower DMSO levels.[12] Our limited DMSO data set did

show a 73 and 110 % increase in $DMSO_t$ cell^{-1} at T7 for the 10- and 70-salinity treatments respectively relative to the control (Fig. 5). Whereas 10-salinity cultures released ~100 % of intracellular DMSP to the dissolved fraction by T2, $DMSP_p$ values at T7 indicate some cellular DMSP accumulation occurred following the rapid efflux of intracellular DMSP (Fig. 3a–d) and in combination with the observed increase in $DMSO_t$ cell^{-1}, lend further support for a secondary, perhaps antioxidant function. Although $DMSO_t$ represented only a small contribution to the total pool of measured sulfur species (Table 2) – compared with previously published values from temperate, non-stressed, exponentially growing cultures[63] – this could be due to higher turnover through the antioxidant cascade (Fig. 1) or high DMSO reductase activity in this polar species. We also cannot discount the possibility that significant DMSP turnover and DMS release due to bubbling could result in our underestimation of the true intracellular DMSP + DMSO pool sizes.

The fairly small difference in $DMSP_p$ between 50- and 70-salinity cultures is also in line with the DMSP antioxidant hypothesis. The shift to a salinity of 70 reduced F_V/F_M and is likely to generate more oxidative stress than a shift to a salinity of 50; DMSP synthesis may be more elevated in the 70-salinity cultures relative to 50-salinity cultures, but turnover through the antioxidant cascade may also be higher and explain the lack of significant ROS detection in the 70-salinity cultures (Fig. 7a). In experiment II, we included an 8-h dark period because previous observations by Karsten and colleagues[64] found an interactive effect of light and salinity on $DMSP_p$ accumulation and warned against looking solely under continuous light conditions. With the addition of a dark period in experiment II, $DMSP_p$ concentrations reached 37 mM in the 70-salinity group, compared with the 26 mM maximum concentration observed under continuous light in experiment I. Providing a photosynthetic recovery dark period could have mitigated some excess oxidative stress-induced free radicals caused by continuous light and thus possibly lowered DMSP turnover through the antioxidant cascade. It must be noted, however, that some experimental design changes were undertaken before experiment II in order to minimise handling time outside the incubator and costs of air cylinders for bubbling. Fewer dilutions during the salinity shift meant slightly greater salinity gradients per step. The switch to bubbling with filtered laboratory air may have also unknowingly led to slightly higher CO_2 content within the recirculated air, resulting in the moderate elevations in A_c and reductions in pH observed across all salinity groups in experiment II. As a result, we cannot rule out the possibility that this did not also influence the higher $DMSP_p$ levels observed in experiment II and emphasise the need for further examination of the effects of varying light, salinity, pH and A_c gradients on the magnitude of DMSP changes.

Implications to natural communities

Owing to logistical limitations that prevent easy separation of phytoplankton cells from the ice matrix and brine, field sea-ice DMSP values are typically derived from $DMSP_t$ measurements and normalised to Chl-*a* as a proxy for differences in biomass. The current set of experiments clearly illustrates how differences in growth rates, $DMSP_d$ contributions and Chl-*a* concentrations can significantly affect $DMSP_t$ Chl-a^{-1} values independently of actual changes in cellular $DMSP_p$ accumulation (Fig. 4). Negligible $DMSP_d$ pools for all but the most extreme hyposalinity treatment group made $DMSP_t$ and $DMSP_p$ values interchangeable for 20- through 70-salinity treatment groups. However, the large $DMSP_d$ pool in 10-salinity cultures combined with lower Chl-*a* cell^{-1} resulted in $DMSP_t$ Chl-a^{-1} concentrations greater than controls by T7 (Fig. 4a and b) – despite slower growth rates and less-dense cultures in the 10-salinity treatment group (Fig. 2a–d). Furthermore, the stand-alone effect of even minor numerical, albeit not statistically significant, decreases in Chl-*a* concentrations were demonstrated in the higher-salinity groups at T1 in experiment I (Fig. 4e). $DMSP_p$ Chl-a^{-1} elevations were more than double relative changes when normalised to cell biovolume – i.e. $DMSP_p$ Chl-a^{-1} was 78 and 86 % elevated in 50- and 70-salinity cultures respectively compared with 35-salinity cultures (Fig. 4c) *v.* $DMSP_p$ cellular concentrations being only 30 and 47 % elevated respectively (Fig. 3a). Similar effects to varying degrees were seen at other time points in both experiments. But most noticeable was the difference in both $DMSP_t$ and $DMSP_p$ values normalised to Chl-*a* between experiments I and II (Fig. 4a–d). The multiple dilutions performed in experiment II to keep cells at a similar, moderate density compared with the higher densities achieved in the batch culture design of experiment I reduced cell shading as evidenced by overall lower Chl-*a* concentrations (Fig. 4e and f). The result was 150–200 % increases in $DMSP_p$ Chl-a^{-1} values in 35- and 70-salinity cultures between experiments at T2 and T7 time points, compared with only 20–60 % increases in $DMSP_p$ biovolume values. Thus, field $DMSP_t$ Chl-a^{-1} measurements must be interpreted with care because Chl-*a* is only a proxy for biomass that is confounded by environmental conditions.

The *F. cylindrus* $DMSP_t$ Chl-a^{-1} concentrations reported here are consistent with those previously reported in field studies.[8,11,13] Although the high-salinity Chl-*a*-normalised concentrations (2–8 nmol μg^{-1}) were on the low to moderate side of average field measurements (2–25 nmol μg^{-1}), the higher field values were generally recorded from snow, ice cracks and infiltration layers. As demonstrated by the significant effects of mild shifts in self-shading in the current experiment, the large increases in solar radiation at these surface communities would reduce Chl-*a* and further elevate $DMSP_t$ Chl-a^{-1} ratios. However, the high-salinity cellular $DMSP_p$ concentrations measured in the current experiments (1000–1800 fmol cell^{-1}) were much larger than those in field samples (10–400 fmol cell^{-1}) and this is most likely an artefact of hypo-osmotic shock effects during the melting out process frequently employed to obtain sea-ice particulate fractions. Furthermore, the elevations in $DMSP_t$ values in the current experiments were the result of single salinity shifts. In polar regions, large atmospheric temperature changes occur over the course of several days and sea-ice brine salinity fluctuates with these temperature changes.[65] Salinity oscillations within brine channels could be a mechanism for cycles of ice-diatom DMSP accumulation and release that led to some of the extreme $DMSP_t$ Chl-a^{-1} values (100–300 nmol μg^{-1}) measured from sea-ice,[9] particularly in light of the reduced polar bacterial DMSP degradation rates observed by Kiene and colleagues.[16] It could also be postulated, based on our current data, that higher $DMSP_t$ concentrations are associated with more rapid and extreme salinity fluctuations, whereas $DMSP_p$ cellular recycling is more likely during more mild salinity changes (as occurred in the 20-salinity treatment group).

The current set of experiments clearly demonstrated the capabilities of *F. cylindrus*, a widespread polar diatom, for rapid

and successful acclimation to large, gradual salinity shifts, as evident by growth rates and F_V/F_M, a well-established proxy of stress in photosynthetic organisms (Fig. 2). Previous work showed instant shifts to 70-salinity media to severely impair *F. cylindrus* F_V/F_M and growth rates for several weeks.[66] This illustrates the importance of the gradient of the salinity shift when studying acclimation mechanisms. Unfortunately, owing to logistical limitations, to our knowledge, no measurements of in vivo rates of change in brine salinities during ice formation, maturation or melt have been reported, but this would be valuable information for further experiments and modelling efforts.

Conclusion

Results of these experiments confirm salinity to be a dominant abiotic variable influencing DMSP concentrations in the widespread polar diatom *Fragilariopsis cylindrus*. This supports a compatible solute function for DMSP and provides a physiological mechanism to explain the high concentrations measured in sea-ice diatom communities. Additionally, observed DMSO elevations and limited ROS detection support a secondary antioxidant function for DMSP. As has been previously suggested, organisms are likely to select for multifunctional osmolytes based on secondary benefits[60] and the many functions proposed for DMSP make it an ideal multifunctional compatible solute. Because brine salinity is dependent on ice and atmospheric temperatures, convection and drainage, further salinity studies including additional gradients and other common members of ice-diatom communities would enable modellers to better predict DMS and DMSP fluxes from sea-ice under different climate conditions, particularly if in vivo DMSP production rates[67] and turnover metabolites were simultaneously measured. Sea-ice diatom production of DMSP and release during spring ice melt have important implications on fluxes of polar atmospheric DMS; hence the potential impact of climate change on sea-ice extent and melt rates could significantly affect DMSP dynamics in polar oceans.

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