Supplementary material

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

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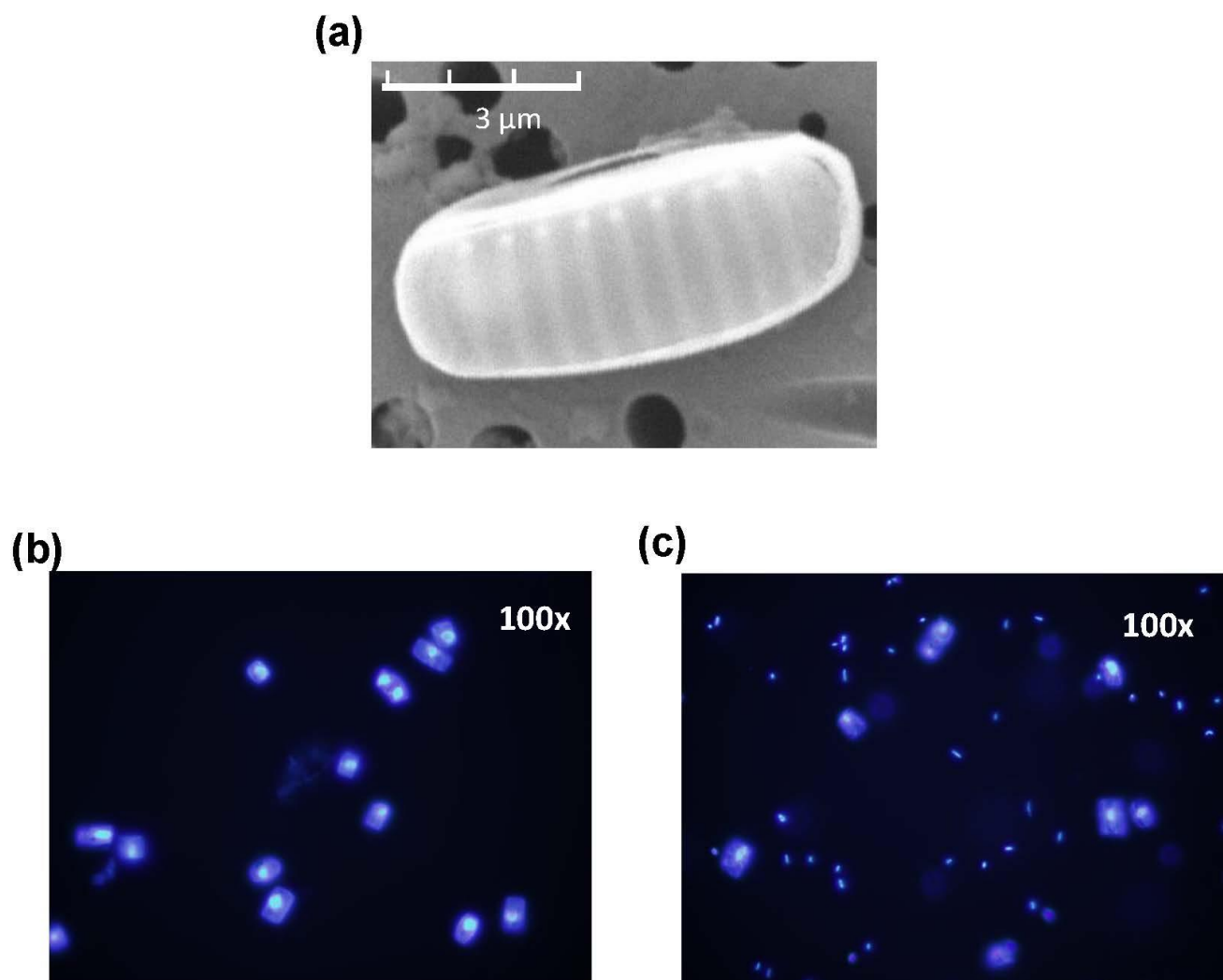6500 College Station, Brunswick, ME 04011, USA. Email: blyon@bowdoin.edu

Fig. S1. (a) SEM of acid-washed *F. cylindrus* cell. (b) DAPI stain of representative axenic *F. cylindrus* culture at end of salinity experiment. (c) DAPI stain of positive control, non-axenic stock *F. cylindrus* culture showing prominent staining of bacterial cells.
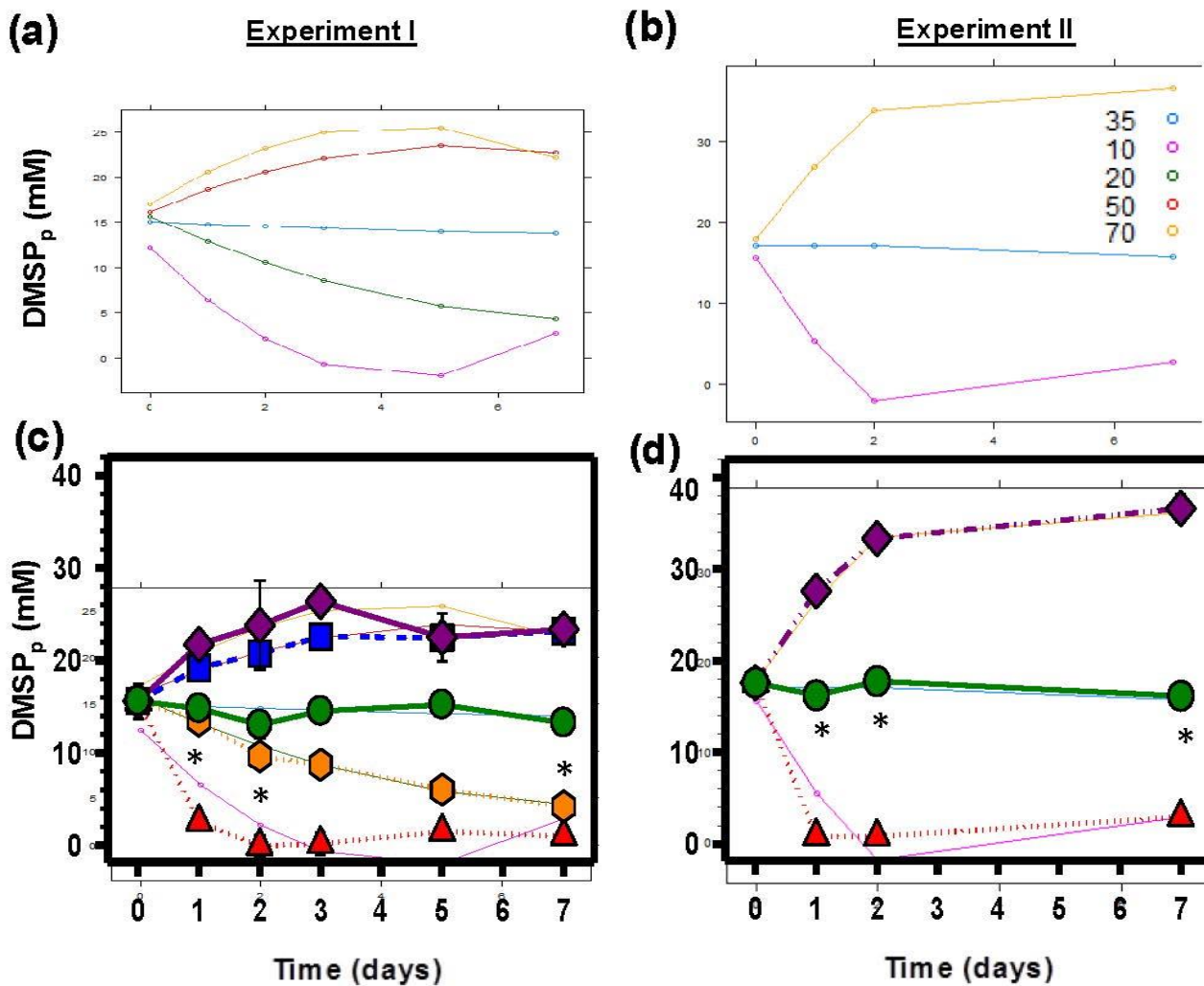
Fig. S2. Graphs of generalised linear least-squares (GLS) models for experiment I (a) and experiment II (b) showing DMSP$_p$ concentrations predicted as second-order polynomials with slopes and curves allowed to vary between treatments. (Model: DMSP ~second-order polynomial (time) × salinity treatment). (c, d) Overlays of experimental results show how well model predicts actual DMSPp concentrations over time for 10- (red triangles), 20- (orange hexagons), 35- (green circles), 50- (blue squares) and 70- (purple diamonds) salinity treatments.
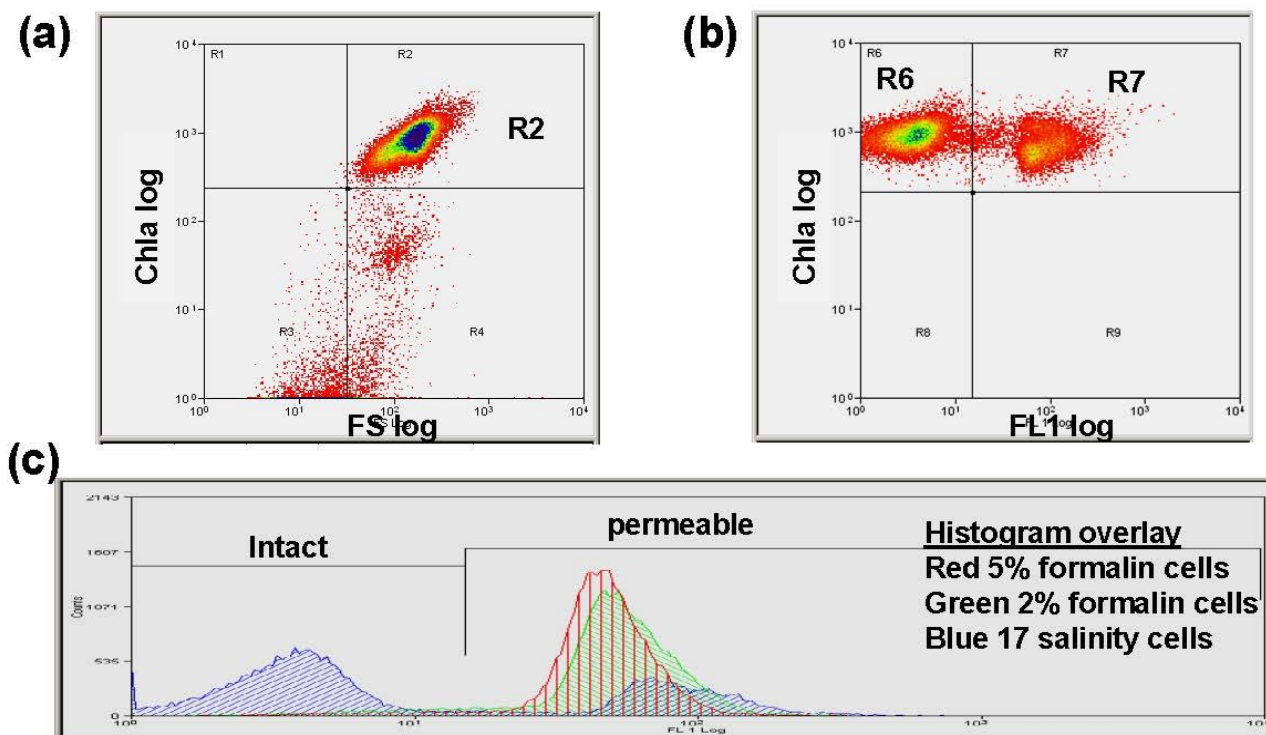
Fig. S3. Calibration of membrane permeability assay. (a) Cells were gated in R2 quadrant based on Chl-*a* fluorescence and forward scatter (FS). Percentage of particles gated as cells was consistent across all three treatments (~70–80 % of particles). (b) Cell membrane integrity was measured with Sytox, a fluorescent stain that only enters cells with compromised (permeable) membranes. Sytox fluorescence (FL1) is on the *x*-axis and subpopulations of cells with intact membranes (R6) versus compromised membranes (R7) are shown for a low-salinity shocked culture (salinity of 17). (c) Formalin-fixed cells (red and green histograms) were used to determine FL1 (*x*-axis) threshold defining compromised membranes. Histogram of a low-salinity shocked culture (blue) is also overlain.
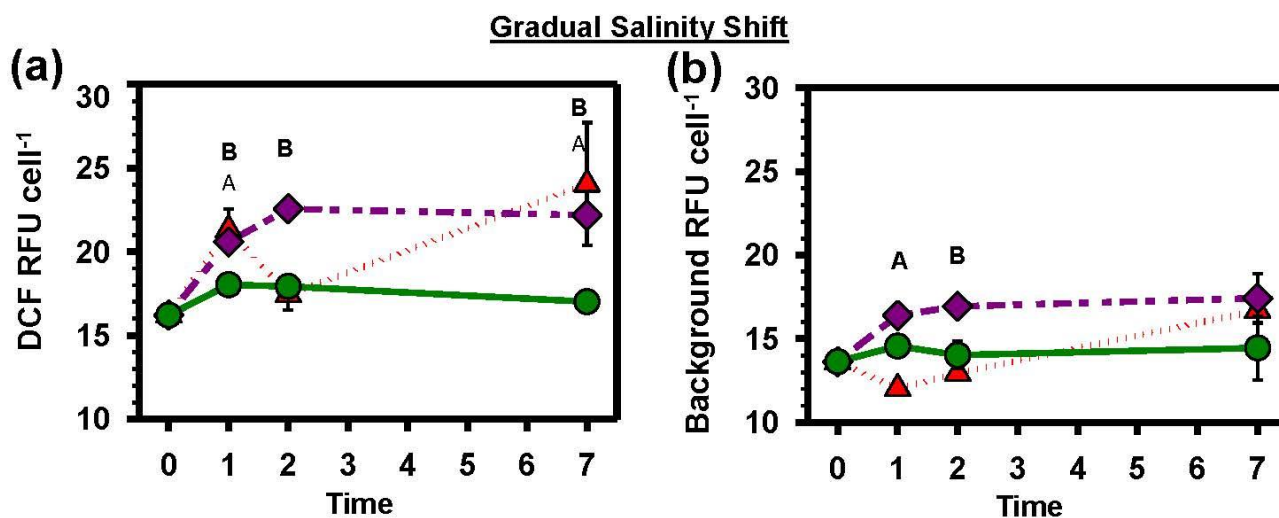
Fig. S4. Non-normalised DCFH cellular fluorescence and background fluorescence. Means and standard errors for 10- (red triangles), 35- (green circles) and 70- (purple diamonds) salinity cultures (*n* = 4) are graphed. Significant differences between treatments and 35-salinity controls were tested for at T1, T2 and T7 and are indicated with the symbols A or B for 10- or 70-salinity groups respectively (*P* < 0.05). (a) DCFH relative fluorescent units (RFU) per cell are graphed. (b) Cellular background fluorescence in the absence of dye is shown.

Table S1.   DMSP$_p$ linear model statistical results

A generalised linear least-squares (GLS) model, with unequal variances accounted for by fitting separate variance terms for each treatment, was fitted by maximising the restricted log-likelihood (REML) using *R* statistical software. The data were fitted to a second-order polynomial with slopes and curves allowed to vary between treatments and all treatments were compared with 35-salinity control treatment (*P* value for 35-salinity represents how different this treatment was from zero). Model: DMSP$_p$ ~second-order polynomial (time) × salinity treatment

Experiment	Parameter	*y* intercept	s.e.	*t* value	*P* value
I	35-salinity treatment	14.40872	0.44408	32.44622	<0.0001
	Slope	−3.96822	4.212914	−0.94192	0.3493
	Curve	0.73294	4.212914	0.17397	0.8624
I	10-salinity treatment	−10.95467	0.929111	−11.79048	<0.0001
	Slope	−25.80637	8.814323	−2.92778	0.0045
	Curve	32.90555	8.814323	3.73319	0.0004
I	20-salinity treatment	−4.79078	0.564779	−8.48256	<0.0001
	Slope	−32.24856	5.357969	−6.0188	<0.0001
	Curve	7.90937	5.357969	1.47619	0.1441
I	50-salinity treatment	6.20506	0.603142	10.28789	<0.0001
	Slope	24.92142	5.721905	4.35544	<0.0001
	Curve	−12.63457	5.721905	−2.2081	0.0303
I	70-salinity treatment	7.82272	1.081557	7.23284	<0.0001
	Slope	20.17586	10.260546	1.96635	0.053
	Curve	−22.64056	10.260546	−2.20656	0.0304
II	35-salinity treatment	16.85262	0.39744	42.40296	<0.0001
	Slope	−3.94092	2.858328	−1.37875	0.176
	Curve	−1.12483	2.672866	−0.42083	0.6762
II	10-salinity treatment	−11.31289	0.912949	−12.3916	<0.0001
	Slope	−17.63447	6.215745	−2.83706	0.0073
	Curve	40.00393	6.274956	6.37517	<0.0001
II	70-salinity treatment	11.76833	0.604142	19.47943	<0.0001
	Slope	43.56277	4.184129	10.41143	<0.0001
	Curve	−28.25643	4.125792	−6.84873	<0.0001