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The algal osmolyte DMSP as a microzooplankton grazing deterrent in laboratory and field studies

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Using laboratory cultures, microcosm experiments in northern Puget Sound and field experiments in the coastal Gulf of Alaska, the role of dissolved DMSP as a protist grazing deterrent was examined. DMSP (20 μM) added to laboratory cultures of two ciliates (Strombidinopsis acuminatum and Favella sp.) and one dinoflagellate (Noctiluca scintillans) caused a 28–75% decrease in feeding rates; decreases were concentration-dependent with 20 nM as the lower threshold for an effect. Partial but not complete recovery of grazing rates occurred during long-term (≥24 h) exposure to dissolved DMSP, as long as concentrations remained above 12 μM. Additions of 10–30 μM dissolved DMSP to coastal planktonic communities had either no effect or mildly stimulated both phytoplankton growth and microzooplankton grazing rates, probably due to the use of DMSP as a carbon source for the growth of bacteria along with direct and indirect stimulation of phytoplankton growth. However, DMSP did strongly inhibit grazing rates in three experiments. The sensitivity of these communities to the DMSP deterrent signal could have resulted from relatively high ambient dissolved DMSP levels, or from the presence of susceptible protist grazer species. Grazer inhibition as observed in the laboratory and lack of inhibition in natural communities may arise from grazer acclimation to DMSP during 24-h field incubations. Alternatively, inhibitory effects on some grazer species could have been masked by DMSP's stimulatory effect on other portions of the food web. This study illustrates the dual role of DMSP as both a growth substrate and signaling molecule in coastal planktonic food webs.

INTRODUCTION

Contrary to the classic diatom-copepod paradigm, microzooplankton have emerged as important phytoplankton grazers in marine ecosystems, including coastal ecosystems (Murrell *et al.*, 2002; Strom *et al.*, 2007a), upwelling regions (Neuer and Cowles, 1994; Landry *et al.*, 1998), high nutrient low chlorophyll (HNLC) regions (Miller, 1993) and during oceanographic conditions such as El Niño events (Landry *et al.*, 1997). Microzooplankton, primarily protists, contribute substantially to the regulation of phytoplankton biomass levels. Worldwide, an average of 67% of phytoplankton daily growth is consumed by microzooplankton, with regional averages ranging from 59% in temperate-subpolar and polar systems to 75% in tropical-subtropical waters (Calbet and Landry, 2004).

While microzooplankton grazing can account for the loss of most or all of the daily phytoplankton production in some regions, this is not always the case. Some planktonic bacteria and phytoplankton appear to have developed mechanisms to deter grazing (Wolfe, 2000; Strom, 2002; Hamm *et al.*, 2003). Deterrence of protist grazers may result from acute toxicity, from recognition of individual prey cell characteristics, or from behavioral responses to dissolved signals. One example of the latter is the production of β-dimethylsulfoniopropionate, or DMSP.

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DMSP plays an important role in the sulfur cycle. According to the CLAW hypothesis (after Charlson *et al.*, 1987), DMSP catabolism via the DddL, DddD or DmdA pathways (Todd *et al.*, 2007; Curson *et al.*, 2008) results in the release of DMS to the atmosphere, which can be oxidized into dimethyl sulfoxide (DMSO), sulfuric acid (H_2SO_4) and sulfate aerosols. Sulfate aerosol particles can function as cloud condensation nuclei, especially over the open ocean, increasing cloud formation and impacting global climate. In phytoplankton cells, DMSP functions as a compatible solute, a cryoprotectant and, perhaps, an antioxidant (Kirst, 1989; Kirst *et al.*, 1991; Sunda *et al.*, 2002). Variation in phytoplankton intracellular DMSP concentration appears to facilitate phytoplankton survival in a fluctuating environment. For example, increased DMSP production may occur under nutrient limitation (Bucciarelli and Sunda, 2003) and when there is a decrease in temperature (van Rijssel and Gieskes, 2002).

DMSP is found in numerous species of phytoplankton, but most prominently in the Prymnesiophyceae and Dinophyceae (Keller *et al.*, 1989). The cosmopolitan coccolithophore *Emiliania huxleyi*, for example, forms extensive blooms that can result in large fluxes of DMS into the atmosphere (Malin *et al.*, 1993). Despite high intracellular levels of DMSP and/or DMSP lyase activity, some strains of *E. huxleyi* are a suitable food source for protist grazers (Wolfe *et al.*, 1994; Nejstagaard *et al.*, 1997; Strom *et al.*, 2003a). In addition to protists, copepods can consume *E. huxleyi*, though not as a preferred food source (Nejstagaard *et al.*, 1997). Although *E. huxleyi* can be a suitable food source for protist grazers, a reduction in microzooplankton grazing mortality has been shown to be a key constituent in the formation and persistence of *E. huxleyi* blooms in the Bering Sea (Olson and Strom, 2002). Like *E. huxleyi*, *Phaeocystis* is a suitable food source for protist grazers (Tang *et al.*, 2001) that has been associated with reduced microzooplankton grazing in the field (Brussard *et al.*, 1995).

The production of DMS appears to be triggered by viral lysis, physical or chemical disturbance, algal senescence and grazing (Malin and Kirst, 1997; Wolfe *et al.*, 2002). Similar processes, along with direct excretion, can result in the release of DMSP from cells into the dissolved phase without DMS production; this dissolved DMSP uptake satisfies a substantial part of the sulfur and carbon demand of marine bacteria (Kiene *et al.*, 2000; Zubkov *et al.*, 2001).

The breakdown of DMSP was originally hypothesized to act as a grazing deterrent via the accumulation of acrylate in grazer food vacuoles (Wolfe *et al.*, 1997). However, a study by Strom *et al.* (Strom *et al.*, 2003b) showed the presence of DMSP, not DMS or acrylate, acts as a chemical defense against protist grazers, causing a notable feeding rate reduction in several species. DMSP released by phytoplankton into ambient seawater appears to act as a signal molecule or indicator of inferior prey, rather than as a toxin. By acting as signaling molecules, common algal-derived compounds such as DMSP and amino acids have been shown to influence a host of planktonic community interactions, including microzooplankton herbivory (Strom *et al.*, 2007b and references therein).

In this study, we set out to explore the effect of DMSP on grazing by microzooplankton communities in northern Puget Sound and the coastal Gulf of Alaska (GoA). We also examined DMSP effects on protist feeding rates in the laboratory, using heterotrophic protist species characteristic of the aforementioned communities. We hypothesized that the addition of DMSP to ambient seawater would cause a decrease in microzooplankton grazing on both the whole community and individual level.

METHOD

Laboratory experiments

General approach

Fluorescently labeled algae (FLA) were used as tracers to determine the effect of added DMSP on protist ingestion and feeding rates. Experiments were performed using *Noctiluca scintillans*, *Favella* sp. and *Strombidinopsis acuminatum* as grazers, and *Gymnodinium simplex*, *Heterocapsa triquetra* and *Prorocentrum micans* as prey. A single prey species was fed to a single grazer species, and the number of FLA per grazer food vacuole was determined over a sampling time course to obtain an ingestion rate.

Grazers were cultured at 12°C on a 12:12 h light:dark cycle at 5–30 μmol photons $m^{-2}\,s^{-1}$. They were maintained on "ciliate medium" [autoclaved filtered seawater (FSW) that contained trace amounts of EDTA (Gifford, 1985)] and fed with non-limiting amounts of prey two times per week. At least 2 h prior to the beginning of each FLA experiment, grazers were maintained on prey concentrations equivalent to 250 μg C L^{-1}. Prey was cultured at 15°C on a 12:12 h light:dark cycle at 70–100 μmol photons $m^{-2}\,s^{-1}$ and maintained on f/2 medium without added silicate. All culturing and experimentation was under cool white fluorescent light.

FLA were prepared following the methods of Rublee and Gallegos (Rublee and Gallegos, 1989). Briefly, algal batch cultures were centrifuged at 800 $\times g$ for 5 min at

23°C, then resuspended in 7.0 mL of phosphate buffer (0.05 M Na_2HPO_4 in 3% NaCl, pH = 9), and less than 1.0 mg of 6-([4,6-dichlorotriaziny2-YL]amino)fluorescein (6-DTAF) was added to the suspension. Cells were incubated at 54°C for 2 h, then centrifuged and resuspended in phosphate buffer, repeating until the color of the suspension changed from yellow–orange to clear with final resuspension in ciliate medium. At time zero, a volume of stained cells was added to each bottle to achieve ~25 μg C L^{-1} FLA. Live and stained prey concentrations were quantified using a Sedgwick-Rafter chamber, and all grazers were observed to readily eat FLA.

Short-term effects of DMSP on ingestion

Approximately 400 grazers were suspended in 100 mL of ciliate medium in 125-mL polycarbonate bottles. Treatments (in triplicate) were: control (no chemical addition), 20 μM DMSP (purchased as DMSP-Cl from Selact BV) and 20 μM acrylate (Aldrich). Acrylate is not known to be a grazing deterrent (Strom *et al.*, 2003b) and was used as a positive control for the effect of adding a dissolved organic carbon source to the bottles (data not shown, feeding rates in the acrylate treatments were never significantly different than in the controls). Preliminary experiments indicated there was no associated change in pH with the addition of DMSP. Before chemical addition, pH values ranged from 7.43 to 7.61; with the addition of DMSP, values remained effectively unchanged (values ranged from 7.29 to 7.54).

Experiments were performed at 15°C at 5–24 μmol photons m^{-2} s^{-1}. Bottles containing grazers and unlabeled prey were randomly placed inside the incubator and allowed to acclimate at least 2 h before the experiment began. At time zero, FLA, DMSP and acrylate were added to each bottle and gently mixed by hand. Samples (20 mL) were taken after 10, 20 and 30 min for *S. acuminatum* and *Favella* sp., and 45, 90 and 135 min for *N. scintillans*. Sampling times were determined based on preliminary feeding experiments. For experiments with longer sampling times, bottles were gently inverted by hand every 15 min to ensure that unstained prey, FLA and grazers were evenly distributed.

For each time point, 20 mL samples were preserved and destained by sequential addition of 250 μL alkaline Lugol's, 625 μL buffered formalin and 1.0 mL 3% sodium thiosulfate solution (modified from Sherr and Sherr, 1993). Slides were prepared by gently vacuum filtering (<20 mm Hg) the preserved samples onto 25 mm 5.0 μm pore-size polycarbonate filters and stained with a trace amount of 4',6-diamidino-2-phenylindole (DAPI) stain. Filters were mounted on slides using immersion oil and stored frozen in the dark at −20°C until enumeration. Slides were counted on a Leitz DMR epifluorescence microscope at 200–400× magnification, depending on the size of the grazer. The number of FLA per grazer food vacuole was determined for at least 100 grazers per slide; the average number of algae (FLA and unstained) consumed per grazer was calculated from the proportion of FLA in the mixture (generally 10%). Feeding rates (phytoplankton cells $grazer^{-1}$ h^{-1}) were calculated from the slope of the average number of algae consumed per grazer versus time. In cases where feeding appeared to become saturated, only the linearly increasing portion of the relationship was used.

DMSP dose-response

To determine the feeding response of grazers to varying concentrations of DMSP, two experiments were performed as above with *Favella* sp. and *H. triquetra*. DMSP target concentrations ranged from 0 to 200 μM, each in quadruplicate. For both experiments, approximately 600 grazers and unstained *H. triquetra* (387 cells mL^{-1}) were suspended in 30 mL of ciliate medium in 60-mL polycarbonate bottles. Samples (~20 mL) were obtained from each bottle 15 min after the addition of FLA and DMSP, preserved in glutaraldehyde (final concentration 0.5%) and DAPI stained. After sitting in darkness overnight at 4°C, samples were filtered and feeding rates determined from FLA ingestion as described above.

Long-term exposure to DMSP

To determine if grazer feeding rates changed during long-term exposure to DMSP, two experiments with *Favella* sp. were performed as above but sampled over a 24–28 h period after chemical addition. The first experiment was sampled every 3 h for 24 h after DMSP addition. Approximately 3600 *Favella* sp. were suspended in 450 mL of ciliate medium in each of six 500-mL polycarbonate bottles, and DMSP (20 μM) was added to three of the bottles. At each sampling time point, 30 mL from each bottle were subsampled into 40-mL polycarbonate bottles, spiked with FLA (to 10% of total prey concentration) and preserved in glutaraldehyde 15 min after FLA addition. Prior to each sampling point, the number of *H. triquetra* was determined in each 500-mL bottle, and additional live prey cells were added prior to subsampling (if necessary) to maintain a background prey concentration equivalent to 250 μg C L^{-1} (equivalent to 387 cells mL^{-1}). This experiment was conducted at 14.5°C and on a 12:12 h light:dark cycle (06:00 lights on, 18:00 lights off), beginning at approximately 11:00 h.

Sampling for the second experiment occurred 0, 6, 12, 24 and 28 h after chemical addition. FLA were not

used; instead, *Favella* sp. were starved. Phytoplankton prey was sieved out and grazers were resuspended in fresh ciliate medium 12 h before the experiment commenced. For the experiment, approximately 4200 *Favella* were suspended in 325 mL ciliate medium in each of six 500-mL polycarbonate bottles, with 20 μM DMSP added to three of these. At each sampling time point, 30 mL from each bottles were subsampled into a 60-mL polycarbonate bottle, with the addition of live *H. triquetra* concentrations equivalent to 400 μg C L^{-1}. After 5 min exposure to prey, these subsamples were preserved in glutaraldehyde. This experiment was conducted at 15°C and on a 12:12 h light:dark cycle (07:00 lights on, 19:00 lights off), beginning at approximately 09:00 h. To determine feeding rate, the total number of *H. triquetra* per *Favella* sp. food vacuole was ascertained.

Dilution experiments

General approach

A modification of the dilution technique (Landry and Hassett, 1982) was employed to measure intrinsic and net phytoplankton community growth rates, which were used to calculate losses associated with microzooplankton grazing in the presence and absence of added dissolved DMSP. The modified technique was applied to microcosms in northern Puget Sound, and to natural communities in the GoA. Instead of using net growth in series of progressively more dilute samples to estimate intrinsic growth and grazing mortality, two treatments were used (Landry *et al.*, 1984; Strom and Fredrickson, 2008). The first treatment was 100% whole seawater (WSW), which contained all organisms smaller than 202 μm and provided a measure of phytoplankton growth rate in the presence of microzooplankton grazers ($k_{100\%}$ = net growth). The second treatment consisted of 95% FSW (all particles larger than 0.2 μm removed) and 5% WSW, and provided a measure of phytoplankton growth rate in the absence of grazers. Data comparisons show that net growth rates in 5% WSW treatments are equal to intrinsic growth rates (μ) estimated from regression analyses of full dilution series (i.e. $k_{5\%} = \mu$; Strom and Fredrickson, 2008). Using these two treatments, microzooplankton grazing rates (g, day^{-1}) were calculated using the formula:

$$g = \mu - k_{100\%}$$

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Net growth rates were calculated from initial and final chlorophyll concentrations (chl) readings in each dilution treatment:

$$k = \frac{l}{t}\ \ln\left(\frac{\mathrm{chl}_{\mathrm{final}}}{\mathrm{chl}_{\mathrm{initial}}}\right)$$

where t is the incubation time in day.

Initial chlorophyll samples were obtained in triplicate or quadruplicate from the WSW and 5% WSW carboys, and final chlorophyll samples were taken from every experimental bottle. Chlorophyll sample volumes ranged from 0.138 to 1.08 L, based on the amount of chlorophyll present.

Initial and final chlorophyll samples were vacuum filtered (<150 mm Hg) onto 20 μm and 5 or 8 μm pore-size polycarbonate filters (47 mm diameter), and 0.7 μm glass fiber filters (25 mm diameter) using a three-tier cascade filtering system. The three separate size fractions (>20 μm, 5–20 or 8–20 μm, and <5 or <8 μm) were used to determine the amount of phytoplankton biomass in each size class. Total chlorophyll concentrations were obtained from the sum of the three fractions. Filters were placed in 6 mL 90% acetone and allowed to extract for 24 h at −20°C in the dark. After 24 h, filters were removed; the acetone extracts were centrifuged, and their fluorescence determined before and after acidification at room temperature in a Turner 10-AU fluorometer.

Northern Puget Sound microcosm experiments analyzed by the dilution technique

Surface water was collected south of Young Island, Washington (Table I and Fig. 1), gently poured through a 202 μm Nitex mesh into a 23-L polycarbonate carboy and transported back to Shannon Point Marine Center (SPMC) for incubation and analysis. Before incubation, and based on ambient chlorophyll readings and measurements of the cellular carbon content of cultured phytoplankton species, the carboy was enriched with 250 (high ambient chlorophyll readings) or 500 (low ambient chlorophyll reading) μg C L^{-1} of the chlorophytes *Dunaliella tertiolecta* and *Micromonas pusilla*, the prymnesiophyte *Isochrysis galbana*, and the dinoflagellate *P. micans*. The proportion of each of the three phytoplankton species added varied based on culture density, but the total amount added of all three species was equivalent to 250 or 500 μg C L^{-1}. The addition of these organisms was used to simulate phytoplankton bloom conditions, increasing the number of microzooplankton grazers in the community and the magnitude of the community grazing rate. Initial experiments indicated that, without the addition of cultured phytoplankton, grazing rates were too low to allow the detection of significant differences in grazing among treatments.

Table I: Dilution experiment location and conditions

Experiment number	Latitude °N	Longitude °W	Date	Chlorophyll (μg l^{-1}) Initial	Final (Control)	Final (DMSP)	Initial Chl >20 μm (%)	Total MZ Biomass (μg C L^{-1})
PS1	48.30	122.45	27 August 2002	3.05	2.89	3.05		
PS2	48.30	122.45	03 September 2002	3.14	3.00	3.14		
PS3	48.30	122.45	17 September 2002	7.09	7.35	7.09		
PS4[a]	48.30	122.45	16 October 2002	1.03	0.45	0.64	36	18.2
PS5[b]	48.30	122.45	22 October 2002	0.56	0.52	0.58	46	17.3
PS6[c]	48.30	122.45	13 September 2003	20.8	31.1	38.3	60	14.7
PS7	48.30	122.45	02 October 2003	2.60	4.10	4.09	86	10.1
PS8[d]	48.30	122.45	07 October 2003	23.0	39.8	40.8	95	5.4
GoA1	58.54	148.21	28 April 2003	0.30	0.22	0.22	13	6.3
GoA2	60.53	147.80	01 May 2003	0.95	1.17	1.33	66	22.5
GoA3	60.53	147.80	02 May 2003	3.34	4.25	4.31	83	15.8
GoA4	59.77	149.40	06 May 2003	3.68	4.34	4.86	74	30.4
GoA5	59.77	149.40	07 May 2003	3.28	4.51	4.69	77	23.8
GoA6	59.77	149.40	08 May 2003	5.60	9.51	9.37	77	32.7
GoA7	59.25	149.50	11 May 2003	10.7	18.0	18.0	88	13.3
GoA8	60.53	147.80	27 July 2003	0.48	0.46	0.52	17	8.6
GoA9	59.26	148.91	04 August 2003	0.33	0.28	0.29	9	13.2

Dilution experiment location and conditions, including total initial chlorophyll (μg L^{-1}), total final chlorophyll in the control and DMSP treatments, the percent of total initial chlorophyll (Chl) >20 μm and total microzooplankton (MZ) biomass (μg C L^{-1}). Chlorophyll values were calculated from the dilution method; for enriched experiments, this commenced 5 day after incubation. PS=Puget Sound, GoA=coastal Gulf of Alaska.
[a]Enriched with 500 μg C L^{-1} *Micromonas pusilla, Isochrysis galbana.*
[b]Enriched with 250 μg C L^{-1} *Isochrysis galbana, Prorocentrum micans.*
[c]Enriched with 500 μg C L^{-1} *Micromonas pusilla, Isochrysis galbana, Dunaliella tertiolecta.*
[d]Enriched with 250 μg C L^{-1} *Isochrysis galbana, Dunaliella tertiolecta.*

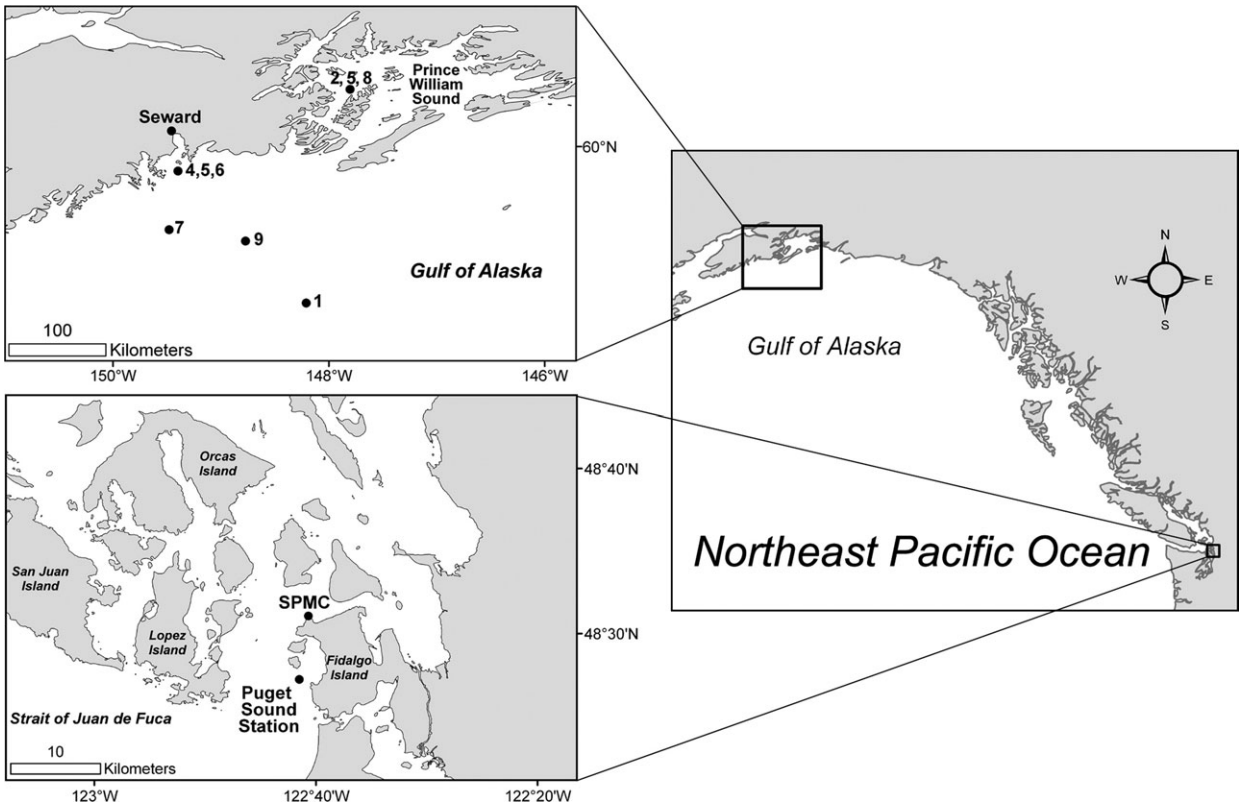

Fig. 1. Map of Pacific Ocean, showing coastal Gulf of Alaska and northern Puget Sound sampling sites. Experiment numbers for each coastal Gulf of Alaska station are indicated.

After enrichment, the water was gently siphoned into 5 4.2-L polycarbonate bottles (microcosms) and placed on a large, rotating (0.5 rpm) plankton wheel located outside the SPMC lab. Each 4.2-L bottle was filled to the top to eliminate air bubbles. The wheel was motorized and positioned inside a large Plexiglas® box with continuous flow-through seawater. Microscopic observations of the experimental water were made daily, and experiments began after approximately 5 days of incubation when the grazer community visually appeared to have increased in abundance.

To initiate experiments, water from the 5 4.2-L bottles was gently siphoned back into a 23-L carboy. All carboys, bottles, tubing and Nitex mesh were soaked in 10% HCl, rinsed in triplicate with deionized water, then rinsed with filtered or WSW prior to experiment setup. FSW was obtained by gravity filtering WSW from the carboy through a sterile 0.2 μm Gelman® pleated capsule filter. Before use, capsule filters were soaked in 10% HCl for 24 h, rinsed with deionized water and rinsed with FSW.

A separate 20-L carboy was used to make 5% WSW. A large volume of FSW was first added, then the necessary volume of WSW was gently siphoned in. Under continuous, gentle stirring, water was gently siphoned from the WSW and 5% WSW carboys into 1.2-L polycarbonate bottles (six to eight each). Half the bottles at each dilution level were then spiked with DMSP, so that each experiment consisted of three or four replicate bottles in each of the following treatments: 5% WSW with DMSP; 5% WSW without DMSP; 100% WSW with DMSP and 100% WSW without (for a total of 12–16 bottles in each experiment). DMSP treatment bottles were spiked immediately prior to incubation to achieve an enrichment of 10–30 μM DMSP. Bottles were placed back on the plankton wheel and incubated for 24 h. A Hobo Data Logger was placed inside the plankton wheel to record water temperature during incubation. Inorganic nutrient concentrations were obtained from Shannon Point Marine Center's long-term monitoring program. Nutrient concentrations averaged 16.9 μM for nitrate and 2.27 μM for phosphate. Inorganic nutrients were not added to experimental bottles because preliminary nutrient analysis indicated that, even after 5 days of incubation, nutrient concentrations were above levels likely to limit phytoplankton growth (Eppley *et al.*, 1969; Smayda, 1997).

Coastal Gulf of Alaska dilution experiments

Experiments were performed on-board the R.V. *Alpha Helix* from 24 April to 15 May, and 20 July to 12 August 2003 in the GoA (Table I and Fig. 1). Water was collected using a rosette of 5- or 10-L external spring-closing Niskin bottles, and was collected from varying depths corresponding to 50% surface irradiance (2.5–5 m except 9 m for offshore experiment GoA1). To obtain water from the Niskin bottles, one end of silicon tubing was attached to the bottle, while the other end was enclosed with a 202 μm Nitex screen (to remove copepods and other macrozooplankton) and placed in a 23-L polycarbonate carboy. To satisfy the assumptions of the dilution technique, dissolved inorganic PO_4^{-3} (0.3 μM) and NO_3^- (5.0 μM) were added to each incubation bottle.

Experiments began immediately after water collection (i.e. there was no 5-day incubation with added phytoplankton). Otherwise, methods were the same as described for northern Puget Sound experiments. All bottles were covered with layers of neutral density screen to mimic 50% surface irradiance, and then incubated for 24 h in on-deck flow-through Plexiglas® incubators.

Microzooplankton community composition

Duplicate 120 mL WSW samples were obtained directly from the WSW carboy, and preserved in acid Lugol's (10% final concentration) for subsequent estimation of microzooplankton community composition at the start of each experiment. Identification was limited to the level of genus or above. The volume of preserved sample allowed to settle for counting varied from 20 to 40 mL based on microzooplankton density; enough was settled for the enumeration of ≥200 individuals. The sample was allowed to settle for a minimum of 24 h, after which the top 10–30 mL was removed. The remaining 10 mL was transferred to a settling chamber for enumeration. Inverted microscopy combined with a computer digitizing system was used to identify and measure microzooplankton, and to convert linear dimensions to cell volumes (Roff and Hopcroft, 1986). For ciliates, a cell volume to biomass conversion factor of 0.19 pg C μm^{-3} was used (Putt and Stoecker, 1989); for dinoflagellates cell volume (V, μm^3) was converted to biomass (C, pg C) based on $\log C = -0.119 + 0.819 \log V$ (Menden-Deuer and Lessard, 2000).

Dissolved DMSP analysis

Gas chromatography (GC) was used to measure the amount of dissolved DMSP present at the beginning, end and in some cases during the 24-h incubation period for both field and laboratory experiments. For laboratory and Puget Sound microcosm experiments, DMSP samples were taken after phytoplankton addition or enrichment, respectively. During field incubations, ambient DMSP values were measured for both filtered (5% WSW) and non-FSW. From each incubation bottle,

4.5 mL of seawater was gently vacuum filtered (<5 mm Hg) through a 0.7 μm glass fiber filter (25 mm diameter) into a 5 mL polystyrene tube. Filtration was used to ensure removal of particulate DMSP. The filtrate was frozen in the dark at −20°C until time of analysis. While vacuum filtration may result in the overestimation of dissolved DMSP (Kiene and Slezak, 2006), preliminary tests showed that freezing did not reduce the amount of DMSP present in the sample. For all experiments, the expected DMSP concentrations were consistent with the concentrations measured (see Results).

DMSP was measured following the methods of White (White, 1982), except that to drive off any DMS present, samples were thawed and sparged with nitrogen gas, where sparging involved gentle bubbling of the sample on a glass fritted stripper. After sparging, 1.0 mL of ~10 N NaOH was added to a 20 mL glass vial containing 4.0 mL of sample. Vials were immediately sealed with butyl septa and crimped aluminum caps, vortexed and incubated in the dark at room temperature for 24 h.

For calibration, two sets of standards were prepared based on the estimated amount of dissolved DMSP present in (i) natural seawater and (ii) the DMSP addition treatments. Standards were processed in the same way as samples.

After 24 h, samples were analyzed on a Shimadzu GC-14A gas chromatograph equipped with a flame photometric detector. Twenty microliter of headspace sample from the DMSP addition treatments was withdrawn and directly injected into the GC using a gastight syringe. Samples from non-DMSP addition treatments were analyzed using the cryogenic purge-and-trap method described by Turner and Liss (Turner and Liss, 1985) and Wolfe *et al.* (Wolfe *et al.*, 2002). Briefly, headspace samples were passed through a Nafion drier (Permapure) and cryotrapped on a Teflon loop placed in liquid nitrogen. The loop was then placed in >80°C water and injected onto the column. Headspace samples were flushed for 0.5 min at 20 mL min^{-1}. For the GC and headspace sampler, helium was the carrier gas and column temperature was set at 90°C. When headspace analysis via the cryogenic purge-and-trap method was unavailable, non-DMSP addition treatments were analyzed by withdrawing 100 μL of headspace sample for direct injection. Detection limits for these methods were 23.3 pmol DMSP via direct injection and 4.65 pmol DMSP for headspace analysis. Data were collected and analyzed using a Shimadzu C-R8A Chromatopac integrator.

Statistical analyses

Analysis of variance repeated measures tests (ANOVAR) were used to determine differences among DMSP and control treatments in laboratory experiments, as well as in the concentration of DMSP over 24 h. In cases in which the assumption of sphericity was not met, the more conservative Greenhouse-Geisser corrected degrees of freedom (Potvin *et al.*, 1990) were used. To determine significant pairwise difference among DMSP concentrations, Student–Newman–Keuls post-hoc analyses were used. Paired *t*-tests were used to determine significant differences between DMSP samples taken at the beginning and end of each experiment, and Student's *t*-test were used to test for differences in grazing and intrinsic growth rates between control and DMSP addition treatments in Puget Sound and the GoA.

Student's and paired *t*-tests and ANOVAR statistics were performed using SPSS 10.0. For all statistical analyses, $\alpha = 0.05$.

RESULTS

Laboratory experiments

Feeding by two ciliate and one dinoflagellate species was reduced by addition of 20 μM DMSP in short-term laboratory experiments (Fig. 2). Feeding by *S. acuminatum* on *G. simplex* was reduced by 50%, feeding by *Favella* sp. on *H. triquetra* by 75% and *N. scintillans* on *P. micans* by 40% (Table II). Replication of the *N. scintillans* experiment did not yield the same level of inhibition (Fig. 2D and Table II). Feeding rates in both treatments were low in the second experiment and, although DMSP addition slightly reduced feeding, the effect was not significant ($P = 0.06$).

The response of *Favella* to varying concentrations of DMSP was similar but not identical in the two dose-response experiments. In the first experiment, DMSP doses of 0.02–9.35 μM reduced ingestion to 60% of control rates, while higher doses of 50 and 189 μM reduced ingestion to 36 and 14%, respectively (Fig. 3A and C). Because control feeding rates in the second experiment were less than rates calculated with the addition of <2 μM DMSP, control rates appeared anomalously low and complicated interpretation of the response (Fig. 3B). Regardless, DMSP addition did not consistently reduce ingestion until the dose reached 2 μM. At that level, feeding rates were 49% of maximum, while a dose of 10 μM reduced ingestion to 38%, and 202 μM to 19% (Fig. 3B and D).

Long-term exposure to DMSP led to either partial or complete recovery of *Favella*. In the first 24-h experiment, DMSP addition initially reduced *Favella* grazing to 52% of control levels (Fig. 4A and C). However, grazing recovered to control levels after 9 h exposure (Fig. 4C). DMSP concentrations declined throughout

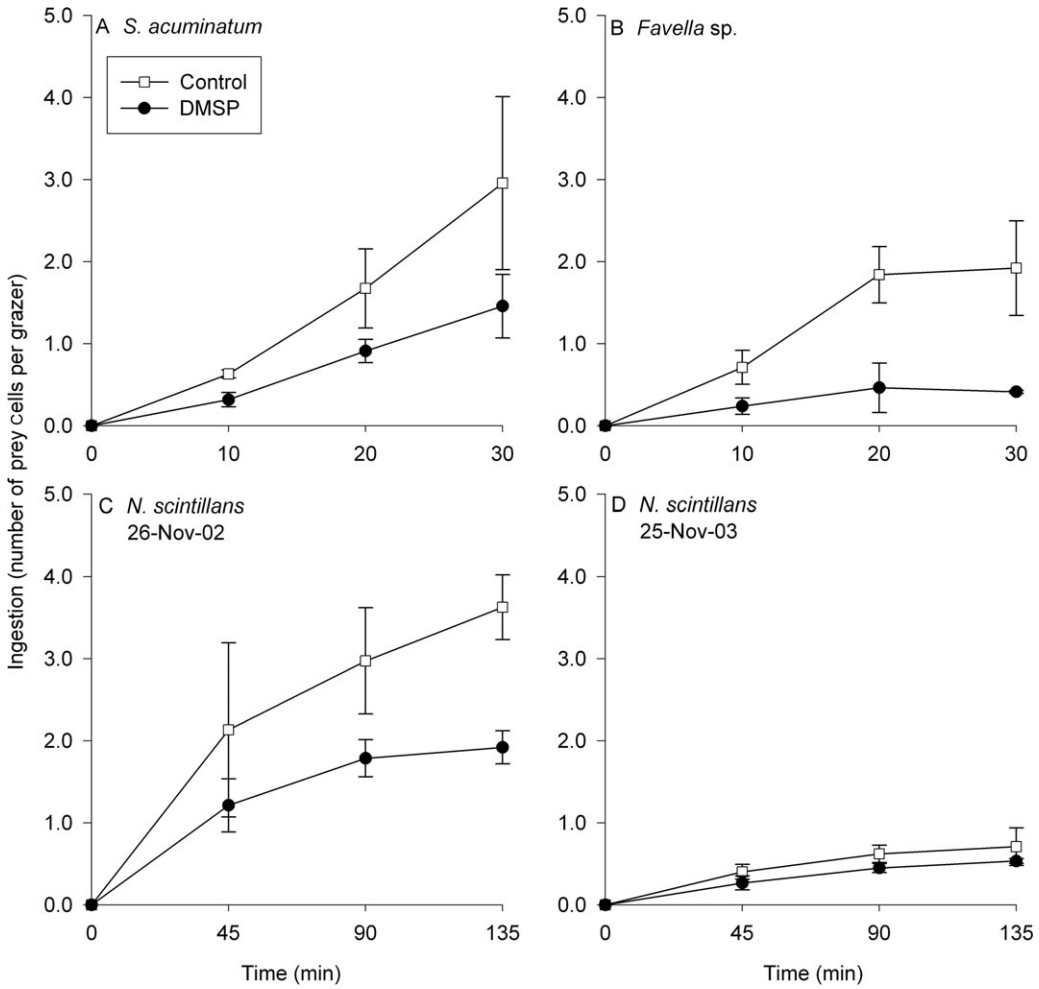

Fig. 2. Feeding time course of (**A**) *Strombidinopsis acuminatum*, (**B**) *Favella* sp., and (**C** and **D**) *Noctiluca scintillans* in the presence (filled circles) and absence (open squares) of 20 μM DMSP. The total number of ingested prey cells was calculated from ingestion of tracer quantities of fluorescently labeled algae; error bars indicate ± 1 SD, $n = 3$.

Table II: Summary of laboratory feeding experiments

Grazer	Prey	Corresponding figure	Control feeding rate	DMSP feeding rate	Percent inhibition	Feeding rate *P*-value
Strombidinopsis acuminatum	*Gymnodinium simplex*	2A	5.95 (2.16)	2.98 (0.68)	50	0.09
Favella sp.	*Heterocapsa triquetra*	2B	5.52 (1.03)	1.39 (0.91)	75	0.01*
Noctiluca scintillans	*Prorocentrum micans*	2C	1.98 (0.43)	1.19 (0.15)	40	0.04*
Noctiluca scintillans	*Prorocentrum micans*	2D	0.41 (0.07)	0.30 (0.04)	28	0.06
Favella sp.	*Heterocapsa triquetra*	3A	19.48 (4.92)	9.39 (0.69)	52	0.01*
Favella sp.	*Heterocapsa triquetra*	3B	16.58 (2.27)[a]	6.36 (1.58)	62	0.01*
Favella sp.	*Heterocapsa triquetra*	4A	9.14 (1.35)	4.77 (1.40)	48	0.02*
Favella sp.	*Heterocapsa triquetra*	4B	29.23 (4.28)	10.25 (1.04)	65	0.01*

Summary of laboratory feeding experiments including the species of grazers and prey, the figure showing the data from which rates were calculated, feeding rate (cells grazer^{-1} h^{-1}) in the control and DMSP treatments, the percent inhibition caused by the addition of 20 μM DMSP ([control rate − DMSP rate]/control rate) and the significance (*P*-value) obtained from *t*-test comparison of feeding rates in the control and DMSP treatments. Statistically significant *P*-values are noted with an asterisk; values in parentheses represent 1 SD.
[a]The 0.002 μM treatment was used as the control (see Fig. 3D).

the incubation and had reached 11 μM after 9 h (Fig. 4E); after 24 h the DMSP concentration had fallen to ~3 μM. Dose-response experiments (Fig. 3) indicated that DMSP concentrations of 3–11 μM can potentially inhibit feeding; thus, complete recovery of feeding rates suggests acclimation of the ciliates to the

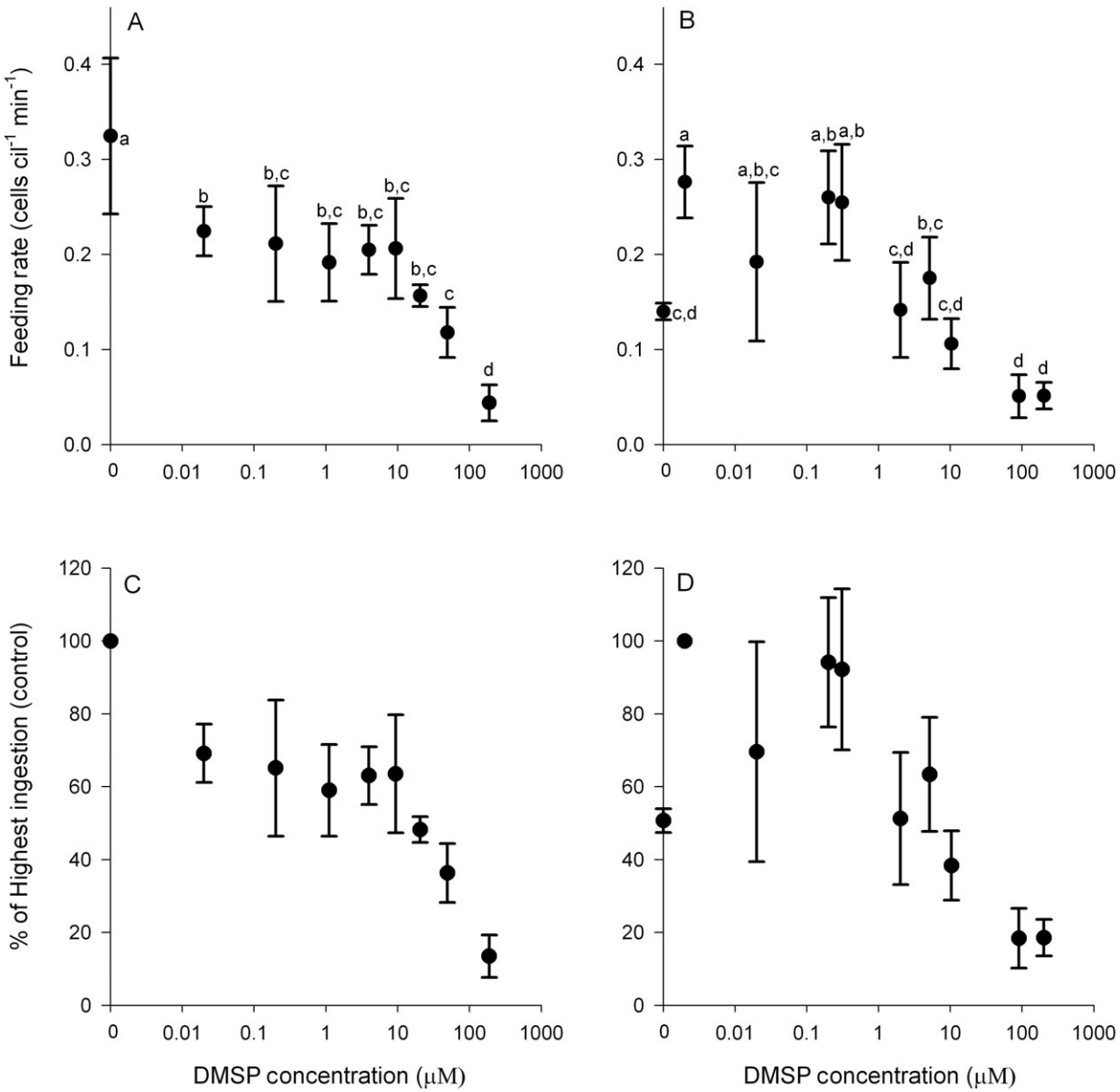

Fig. 3. Results of two dose-response experiments showing ingestion of *Heterocapsa triquetra* by *Favella* sp. over a range of added dissolved DMSP concentrations, plotted as the number of prey ingested (**A** and **B**) and as a percent of the control (**C**) or the concentration supporting the highest ingestion (0.002 μM, **D**). The total number of ingested *H. triquetra* was calculated from the ingestion of tracer quantities of fluorescently labeled *H. triquetra*. In (A) and (B), lowercase letters indicate the results of a Student–Newman–Keuls post-hoc analysis. Shared letters indicate no significant pairwise differences among the DMSP treatments. Error bars indicate ± 1 SD, $n = 4$.

deterrent signal. Because the 24-h experiment contained moderate densities of dinoflagellate prey as well as *Favella*, the decrease in DMSP over the course of the experiment could have been due to bacteria introduced with the algal prey culture (Kiene *et al.*, 2000 and references therein), or to DMSP uptake by phytoplankton used in the experiment (Vila-Costa *et al.*, 2006). To reduce or eliminate these possibilities, a second (28 h) experiment was performed using starved *Favella* sp. (i.e. prey cells were added only to subsamples removed at each time point, Fig. 4B). As in the unstarved experiment, *Favella* showed an initial, substantial reduction in feeding to 35% of control rates in the DMSP treatment. Rates then increased over time, reaching approximately 80% of control levels by 24 h (Fig. 4B and D). DMSP concentrations remained above 15 μM throughout the incubation (Fig. 4F). In contrast to the first experiment, complete acclimation to DMSP (i.e. recovery of feeding in DMSP-exposed ciliates to control rates) did not occur during the 28-h incubation ($P < 0.001$).

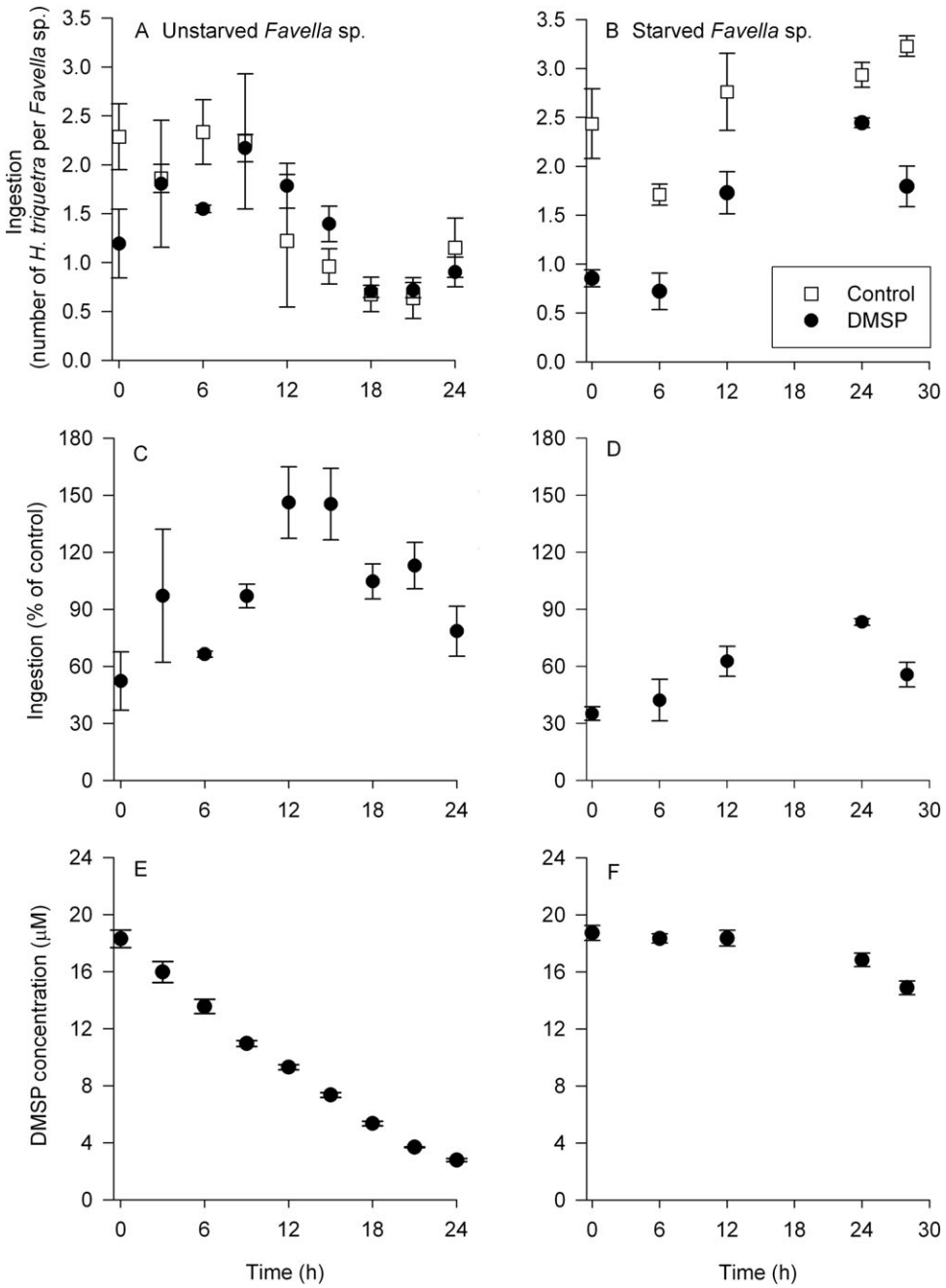

Fig. 4. Results of long-term exposure experiments showing ingestion of *Heterocapsa triquetra* over time by (**A**) unstarved *Favella* sp. (as estimated from uptake of tracer quantities of FLA) and (**B**) starved *Favella* sp. (as estimated from total prey uptake). Ingestion as percent of control for (**C**) unstarved and (**D**) starved *Favella* sp. Dissolved DMSP concentrations over time for (**E**) unstarved and (**F**) starved *Favella* sp. Error bars indicate ± 1 SD, $n = 3$.

Field experiments: environmental conditions

Water for field experiments was collected under a variety of environmental conditions (Table I). Temperatures ranged from 10.5 to 11.3°C at the northern Puget Sound station and from 6.6 to 14.9°C in the coastal GoA. Total initial chlorophyll *a* ranged from 0.3 to 10.7 $\mu g\ L^{-1}$ in the GoA and 0.6 to 23.0 $\mu g\ L^{-1}$ in northern Puget Sound, with a variable contribution (9–95%) of cells >20 μm.

Ambient dissolved DMSP concentrations were above the detection limits in 8 of the 17 dilution experiments

Table III: Ambient and experimental dissolved DMSP concentrations

	Ambient DMSP (nM)		Experimental DMSP (μM)	
Experiment	Initial	Final	Initial	Final
PS1	19.1	20.7	15.9 (1.0)	15.8 (0.8)
PS4	104	bd	17.8 (0.1)	17.2 (0.9)
PS5	17.2	bd	16.0 (2.7)	16.2 (1.0)
PS6	608	bd	ns	ns
PS7	2.6	1.7	32.6 (4.5)	30.6 (1.3)
PS8	43.8	3.6	30.2 (1.8)	24.1 (1.7)
GoA2	bd	bd	12.2 (0.6)	10.2 (0.3)
GoA3	bd	bd	11.9 (0.5)	10.2 (0.3)
GoA4	bd	bd	13.9 (1.8)	14.0 (0.8)
GoA5	bd	bd	16.4 (1.7)	12.8 (2.0)
GoA6	bd	bd	14.8 (1.0)	12.0 (0.4)
GoA7	bd	bd	15.3 (0.4)	12.5 (0.5)
GoA8	3.4	bd	ns	ns
GoA9	13.4	bd	ns	ns

Initial and final ambient (nM) and experimental (μM) dissolved DMSP concentration, with 1 SD in parentheses. bd: ambient DMSP was below detection; ns: experiment not sampled for DMSP.

Table IV: Phytoplankton growth and microzooplankton grazing rates

	Growth rate (day^{-1})		Grazing rate (day^{-1})	
Experiment	Control	DMSP	Control	DMSP
PS1	0.87 (0.16)	0.83 (0.11)	0.17 (0.05)	0.14 (0.03)
PS2	0.42 (0.20)	0.35 (0.12)	0.21 (0.09)	0.11 (0.03)
PS3	0.54 (0.09)	0.51 (0.17)	0.09 (0.06)	0.11 (0.04)
PS4	−0.07 (0.11)	−0.10 (0.07)	0.77 (0.13)	0.39 (0.11)*
PS5	0.45 (0.06)	0.50 (0.07)	0.53 (0.03)	0.47 (0.06)
PS6	0.62 (0.04)	0.64 (0.05)	0.18 (0.13)	−0.02 (0.16)*
PS7	0.44 (0.04)	0.54 (0.12)	−0.01 (0.03)*	0.09 (0.01)
PS8	0.49 (0.05)	0.51 (0.03)	−0.02 (0.03)	−0.03 (0.01)
GoA1	0.74 (0.05)	0.62 (0.05)*	0.37 (0.03)	0.30 (0.03)*
GoA2	0.30 (0.18)	0.44 (0.04)	0.08 (0.02)	0.10 (0.10)
GoA3	0.28 (0.03)	0.28 (0.07)	0.04 (0.01)	0.03 (0.06)
GoA4	0.21 (0.07)	0.29 (0.04)	0.04 (0.02)	0.01 (0.02)
GoA5	0.36 (0.03)*	0.50 (0.05)	0.07 (0.06)	0.14 (0.08)
GoA6	0.57 (0.06)	0.69 (0.16)	0.04 (0.04)*	0.18 (0.10)
GoA7	0.66 (0.05)*	0.75 (0.03)	0.15 (0.12)	0.23 (0.05)
GoA8	0.27 (0.02)	0.25 (0.03)	0.38 (0.02)	0.25 (0.03)*
GoA9	0.37 (0.09)	0.47 (0.07)	0.53 (0.07)	0.59 (0.07)

Phytoplankton growth and microzooplankton grazing rates (averages from total chlorophyll determinations, with 1 SD shown in parentheses) from Puget Sound (PS) and Gulf of Alaska (GoA) experiments. Asterisk denotes significantly lower value for control versus DMSP rate comparison (*t*-test, $P = 0.05$).

(Table III). In Puget Sound, measurable DMSP concentrations ranged from 2.6 to 608 nM, with the highest concentrations (>100 nM) observed during PS4 and PS6. Ambient DMSP levels were below detection during spring in the GoA, but were low to moderate during the summer (3.4 and 13.4 nM for GoA8 and GoA9, respectively). When compared to ambient concentrations, dissolved DMSP samples from 5% WSW bottles indicated no consistent enhancement of dissolved DMSP after filtration (data not shown).

Initial dissolved DMSP concentrations in the DMSP addition treatments ranged from 11.9 to 32.6 μM over all dilution experiments (Table III). Decreases in DMSP during incubations were slight (average decrease 13%, Table III). The initial and final experimental DMSP concentrations in the GoA were lower than in Puget Sound, but for both geographic locations initial and final concentrations were >10 μM, at least 10× higher than ambient levels.

Field experiments: growth and grazing rates

Phytoplankton growth rates (total chlorophyll-based) in Puget Sound ranged from −0.10 to 0.87 day^{-1} with no consistent difference between control and DMSP treatments (two-tailed paired *t*-test, $P = 0.82$; Table IV). Over all PS experiments, treatment averages were identical (Fig. 5A, average rates = 0.47 day^{-1}). Similarly, in the GoA, rates ranged from 0.21 to 0.75 day^{-1} and treatment with DMSP yielded no consistent growth rate differences (two-tailed paired *t*-test, $P = 0.09$). However, there was a tendency for growth rates to be slightly higher in DMSP treatments in the GoA (Fig. 5A, average control rate = 0.42 day^{-1}; average +DMSP rate = 0.48 day^{-1}).

Rates of microzooplankton grazing (total chlorophyll-based) in Puget Sound ranged from −0.03 to 0.77 day^{-1}, but were usually lower than corresponding rates of phytoplankton growth (Table IV). Grazing rates averaged 0.24 day^{-1} for controls and 0.16 day^{-1} for DMSP treatments. Over the entire data set, these differences were not significant (one-tailed paired *t*-test, $P = 0.09$). However, comparisons within experiments showed two instances in which PS grazing was significantly and substantially reduced by DMSP addition (Fig. 5B). In PS4, grazing decreased from 0.77 day^{-1} in the control to 0.39 day^{-1} in the presence of added DMSP; in PS6, grazing decreased from 0.18 day^{-1} to −0.02 (effectively zero) (Table IV). These grazing rate decreases led to higher final levels of chlorophyll in DMSP treatments relative to controls: a 30% increase in PS4, and a 19% increase in PS6 (Table I). In the GoA, rates ranged from 0.01 to 0.59 day^{-1} and averages were nearly identical in the two treatments (Fig. 5B; average control rate = 0.19 day^{-1}; average +DMSP rate = 0.20 day^{-1}). However, a significant DMSP-associated grazing decrease was seen in GoA1 (0.37 to 0.30 day^{-1}, Table IV) and GoA8 (0.38 to 0.25 day^{-1}), resulting in a 0 and 13% increase in final chlorophyll concentration, respectively (Table I).

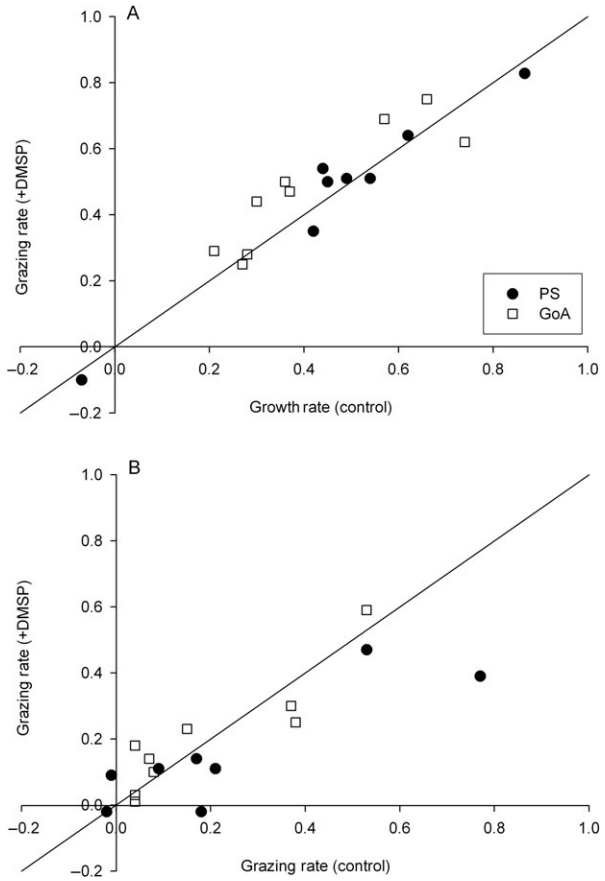

Fig. 5. (**A**) Phytoplankton growth rates (total chlorophyll-based) in control versus DMSP treatments for Puget Sound (PS) and coastal Gulf of Alaska (GoA) experiments. Solid line shows 1:1 relationship. (**B**) As in (A) but for microzooplankton grazing rates.

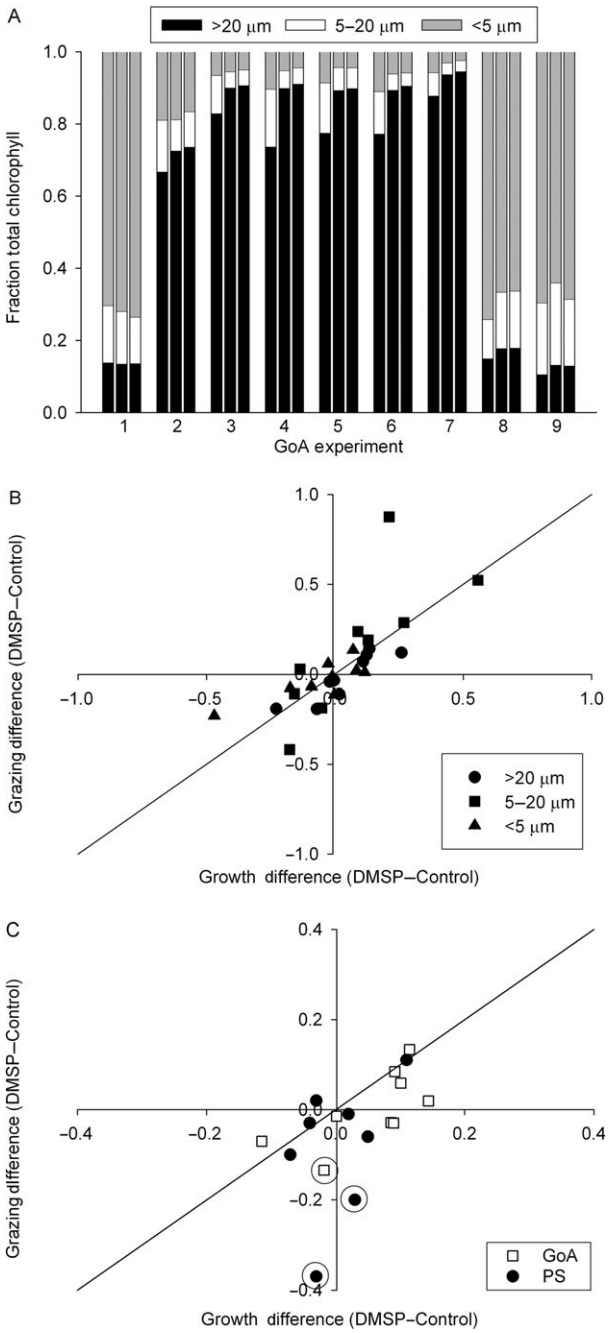

Fig. 6. (**A**) Phytoplankton size composition (based on chlorophyll size fractionation) of communities initially and after 24 h incubation in control and DMSP treatments for coastal Gulf of Alaska (GoA) experiments. In each triad, the first bar is initial chlorophyll, the second bar is final chlorophyll in the control treatment and the third bar is final chlorophyll in the DMSP treatment. (**B**) Rate differences (DMSP−control) for phytoplankton growth and microzooplankton grazing based on three chlorophyll size fractions in GoA experiments. (**C**) Rate differences as in (B) but based on total chlorophyll for GoA and Puget Sound (PS) experiments. Circled points show experiments with evidence of DMSP inhibition of grazing without a corresponding DMSP effect on phytoplankton growth.

Although DMSP effects on total chlorophyll-based rates were slight in most experiments, we considered that differential stimulation or inhibition of one chlorophyll size fraction over another might have led to changes in phytoplankton community size composition during the incubations. For example, in GoA2, grazing on the 5−20 μm size fraction decreased by 0.42 day^{-1} in the DMSP treatment, while grazing on >20 and <5 μm size fractions was nearly identical to that in the control (data not shown). However, while size composition of GoA phytoplankton communities usually changed somewhat over the time course of the incubation, there was little or no difference in the final size composition of control versus DMSP treatments (Fig. 6A). Further analysis of rate differences showed that this was due to coupled changes in phytoplankton growth and microzooplankton grazing rates within chlorophyll size fractions. For example, when grazing was decreased on a given chlorophyll size fraction, intrinsic growth of that size fraction also tended to

decrease (Fig. 6B). The same coupling was observed for rate increases. These parallel changes in growth and loss processes meant that changes in net growth were minimal, and little effect on community composition was observed. Puget Sound experiments were not analyzed in this manner because several were not size-fractionated and others had high chlorophyll concentrations in a single size class, making serial filtration data difficult to interpret.

Field experiments: microzooplankton community composition

Microzooplankton community composition was examined for the size-fractionated Puget Sound and all GoA experiments (Fig. 7). In Puget Sound, aloricate ciliates >20 μm consisted mostly of *Strombidium* sp. and *Strombidinopsis* sp., while *Strombidium* sp. and *Mesodinium* sp. comprised most of the ciliates <20 μm. Dinoflagellates were dominated by *Gyrodinium* sp.; *Gymnodinium* sp., *Dinophysis* sp., and *N. scintillans* were also present. Tintinnids were scarce, and consisted mostly of *Acanthostomella* sp. and *Helicostomella* sp. In PS4, 5, 6 and 7, *Strombidium* sp., *Strombidinopsis* sp. and *Mesodinium* sp. were common genera, while amorphous, unidentifiable dinoflagellates averaging 20–100 μm in size dominated PS8. Approximately half the total biomass of PS4 and 7 (55 and 47%, respectively) comprised aloricate ciliates 20–60 μm.

In the GoA, microzooplankton composition was more varied. Aloricate ciliates >20 μm consisted of different species of *Strombidium* and large *Laboea* sp., while *Strombidium* sp. and *Mesodinium* sp. comprised most of the ciliates <20 μm. *Gyrodinium* sp. and *Amphidinium* sp. were the most abundant dinoflagellates, although *Gymnodinium* sp., *Dinophysis* sp., *N. scintillans*, *Ceratium fusus* and C. *lineatum* were also present. With the exception of GoA1, tintinnids, mostly *Favella* and *Parafavella* spp., were found at each experimental location. Although most GoA experiments were highly diverse, a few experiments had dominant genera. GoA1 contained a large number of *Laboea* sp., *Gymnodinium* sp. was most

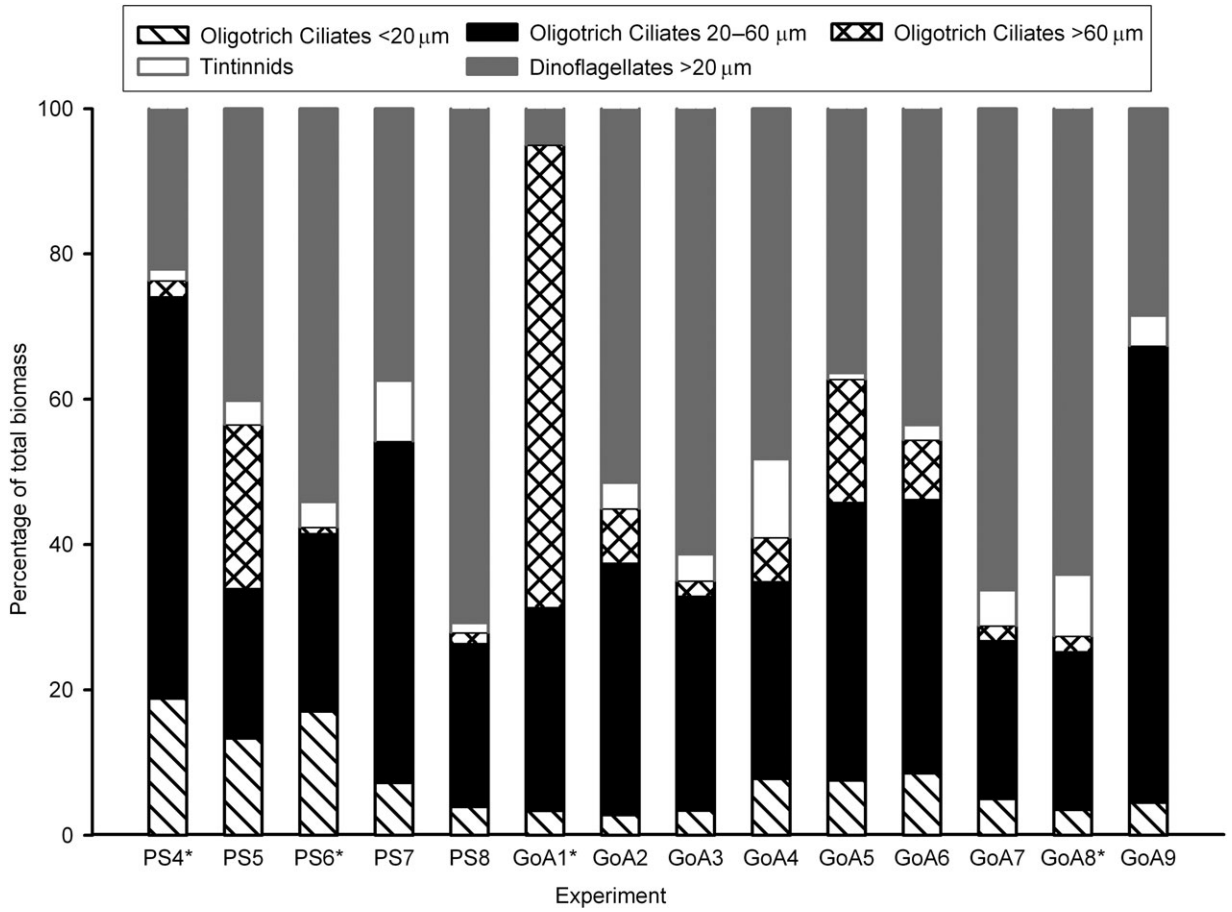

Fig. 7. Composition of microzooplankton community in size-fractionated dilution experiments. Values are from initial samples ($n = 2$). Asterisks denote experiments that experienced a significant decrease in grazing with the addition of DMSP. PS = Puget Sound, GoA = Gulf of Alaska.

abundant in GoA7 and 8, and GoA9 was dominated by *Mesodinium* sp. and small *Strombidium* sp., with aloricate ciliates making up 68% of the total biomass.

DISCUSSION

Laboratory experiments

Added DMSP consistently inhibited grazing by planktonic protists in laboratory experiments (Table II). Relative to controls, addition of DMSP decreased feeding rates by 28–75%; in agreement with previous experiments (Strom *et al.*, 2003b), the inhibitory effect varied depending on grazer species. Within a given species, additional variation in the response was apparent. For example, inhibition of *Favella* sp. by 20 μM DMSP varied from 48 to 75% depending on the experiment. This may be due to variation in feeding history and other aspects of grazer physiological condition. In conjunction with previous data, we know that six out of seven tested protist grazer species were inhibited by added DMSP, indicating that the DMSP inhibition response is widespread among phagotrophic ciliates and dinoflagellates.

Dose-response experiments suggest an inhibitory effect on *Favella* sp. at concentrations as low as 0.02 μM added DMSP (Fig. 3). However, a conservative interpretation of the pairwise comparison yields a threshold of 1–10 μM depending on the experiment. In previous research, feeding by dinoflagellate *Amphidinium longum* was reduced by DMSP concentrations as low as 0.05 μM (Strom *et al.*, 2003b), and the threshold concentration for proline inhibition of *Favella* sp. was 0.2 μM (Strom *et al.*, 2007b). Results reported here add to the emerging picture of potential protist feeding inhibition by low molecular weight, algal-derived compounds, at concentrations 10–100× higher than those typical in natural seawater.

Two experiments examining acclimation of *Favella* sp. to the inhibitory signal showed contrasting results. In the first experiment, *Favella* feeding rates returned to control levels after 9 h exposure to added DMSP; in the second experiment, complete acclimation was never observed, with feeding rates recovering gradually but remaining at or below 80% of control rates throughout the incubation (Fig. 4). There are several possible explanations for the discrepancy. Algal prey (*H. triquetra*) was continuously available in the first experiment, while it was only offered to subsamples in 5-min feeding trials during the second experiment. Continuous exposure to *H. triquetra* cells might have produced a positive cue that stimulated feeding even in the face of the inhibitory DMSP signal. Positive feeding cues associated with algal prey cell surfaces have been reported for other marine planktonic organisms including larval crabs (Hinz *et al.*, 2001). Alternatively, continuously starved *Favella* sp. might have been less able to acclimate to the inhibitory signal due to their weaker physiological condition. This hypothesis is weak, however, since a comparison of proline inhibition of well-fed versus 22-h-starved *Favella* showed no effect of starvation on the strength of the inhibition (Strom *et al.*, 2007b). Finally, the continuous decrease in dissolved DMSP concentration in the first experiment might, itself, have constituted a signal promoting resumption of control feeding levels, even though DMSP concentrations remained above inhibition thresholds as determined in shorter-term dose-response experiments.

Acclimation by *Favella* sp. to DMSP as shown here was strikingly similar to proline acclimation, in which *Favella* feeding rates slowly increased but remained at or below 80% of control rates over 24 h exposure (Strom *et al.*, 2007b). Taken together, these results show that, at least for *Favella*, long-term exposure to inhibitory compounds leads to partial and, under some conditions, complete recovery. It is possible that DMSP may act as a feeding attractant that assists microzooplankton in finding their prey. Bulk additions of DMSP would reduce, if not remove, the presence of any DMSP gradients, therefore making it difficult for predators to find or recognize prey items. However, numerous elements of these laboratory experiments are consistent with the hypothesis that DMSP acts as a signaling compound: lack of toxicity (i.e. no mortality in long-term experiments); partial acclimation upon continuous exposure; and a response threshold at concentrations 10–100× those typical of bulk natural seawater.

Field experiments

Phytoplankton growth and microzooplankton grazing rates measured in our experiments were comparable to those previously reported for the GoA and northern Puget Sound (Strom *et al.*, 2001; Liu *et al.*, 2002; Strom *et al.*, 2007a). However, addition of DMSP to field-collected or microcosm communities rarely caused a substantial decrease in microzooplankton community grazing rates (Table IV and Fig. 5B). This was the case even though DMSP concentrations in incubations remained well above potentially inhibitory levels, and remained relatively constant through the incubation (Table III). The lack of a consistent effect was surprising given that DMSP at similar concentrations in laboratory experiments strongly inhibited feeding by several representative protist species. There are at least two possible explanations for this inconsistency. The first is that

microzooplankton grazers acclimated to the signal over the 24-h incubation period of the field experiments. Even partial acclimation would lead to a reduced inhibitory effect difficult to detect using our 24-h, community-based measure of grazing intensity. Model calculations indicate that, even in cases of high feeding inhibition, inhibition must be maintained for a relatively long period of time to observe a significant decrease in microzooplankton grazing rates for communities examined during this study (Table V). For example, given a control grazing rate of 0.44 day^{-1} (s.d. 0.06), a 50% inhibition would have had to persist for at least 16 h in order to result in a statistically significant reduction in grazing rate (Table V). In our laboratory acclimation experiments, *Favella* sp. feeding rates had recovered to $\geq$60% of control levels after 12 h of DMSP exposure (Fig. 4).

A second possible explanation is that substantial grazing inhibition did occur in most or all experiments, but was masked by a concomitant stimulatory effect of DMSP on the whole community. Small net increases in growth and grazing rates in DMSP treatments were observed in many experiments (Fig. 5), presumably due to the use of DMSP as a substrate for microbial growth (see below).

Do our results mean that DMSP is rarely inhibitory in natural communities? Not necessarily. The dissolved DMSP signal in nature is likely to be patchily distributed on the microscale as well as ephemeral, due to point source release (e.g. from algal cells) and rapid uptake by bacteria. Thus, there may rarely be the opportunity for acclimation over a time course of hours to a consistently high signal, except perhaps in blooms of DMSP-producing phytoplankton. In such blooms, "bulk" concentrations of dissolved DMSP have been reported to reach hundreds of nM (Malin *et al.*, 1993; Matrai and Keller, 1993), although it can be argued that this may have been an overestimation (Kiene and Slezak, 2006). Regardless, a patchy signal distribution on the microscale is still possible. To address this, we undertook a simple modeling exercise to determine how much of an alga's intracellular DMSP would have to be transported into the surrounding phycosphere in order to reach the deterrent concentrations seen in this study. The answer is: very little. For example, transport of 0.1% of the intracellular DMSP of *Heterocapsa pygmaea* (an 8.5 μm diameter dinoflagellate) into a rather capacious phycosphere of 5 μm thickness gives a DMSP concentration of 49 μM (Fig. 8). Increasing transported quantities to 0.5% of intracellular DMSP gives rise to extremely inhibitory concentrations of 243 μM. Because intracellular DMSP concentrations are so high in some dinoflagellates and prymnesiophytes (100s of mM), an effective defense can be mounted by transferring only a minute fraction of intracellular DMSP into the volume surrounding the cell. Even though our model does not include any loss processes, at least hypothetically, this would seem to be an effective defense strategy.

Table V: Model calculations of grazing inhibition

		Percent inhibition		
Grazing rate (day^{-1})	Size fraction	25%	50%	75%
0.44 (0.06)	>20 μm	—	16 h	11 h
0.14 (0.01)	<5 μm	20 h	17 h	10 h

Estimated length of time (h) that grazing rates would have to be inhibited to achieve significantly different grazing rates (calculated as day^{-1}) in control and +DMSP treatments. Calculations include a range of grazing inhibitions experienced during lab experiments (25, 50 and 75%); for actual percent grazing inhibitions experienced during lab experiments, see Table II. Estimates are based on example microzooplankton grazing rates (day^{-1}) similar to what was seen during field experiments; for actual grazing rates experienced during field experiments, see Table IV. Standard deviation is shown in parentheses; a dashed line indicates that there would never be a significant difference in grazing rates between the two treatments.

Added DMSP did strongly inhibit microzooplankton grazing in two Puget Sound microcosm experiments (PS4 and PS6) and one GoA experiment (GoA8; Table IV); in GoA1, a significant decrease in microzooplankton grazing corresponded to a significant decrease in phytoplankton growth rates, making grazing rates difficult to interpret. During the other three experiments, there was no associated decrease in phytoplankton growth rates (Fig. 6C). This indicates a true grazing inhibition of the sort observed in the laboratory, as opposed to a general shift in whole community function (see below). Although levels of added DMSP were quite high relative to environmental concentrations, our model calculations indicate that algal DMSP release could result in these sorts of concentrations.

Possible reasons for the susceptibility to DMSP inhibition of these three particular communities include microzooplankton community composition and ambient DMSP levels. Although laboratory tests of different grazer species indicate taxon-specific sensitivity to DMSP inhibition (these data; Strom *et al.*, 2003b), there were no obviously unique taxonomic features of the affected communities in comparison with all others (Fig. 7). Protist grazers were only identified to the genus level, however, and it is possible that DMSP sensitivity is species-specific. Alternatively, elevated ambient dissolved DMSP concentrations may have played a role. Affected communities in Puget Sound microcosms had the highest ambient concentrations of any in the study: 104 and 608 nM for PS4 and PS6, respectively (Table III). DMSP levels in GoA8 were lower (3 nM) but considerably exceeded those in nearly all other

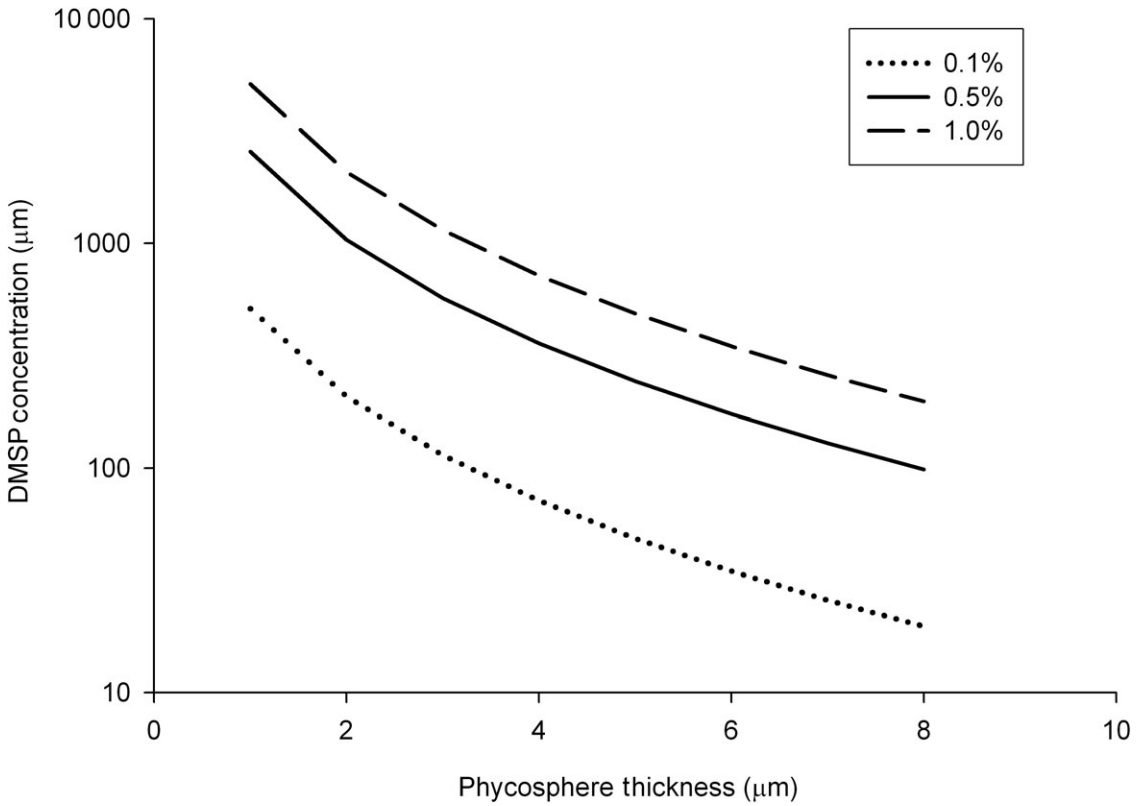

Fig. 8. DMSP concentration estimates for phycospheres of varying assumed thicknesses. Model alga: *Heterocapsa pygmaea* (diameter 8.5 μm, DMSP content 19.5 pg/cell, intracellular DMSP concentration 451 mM). DMSP and cell size data from Keller *et al.* (1989).

GoA experiments. According to our laboratory acclimation experiments (Fig. 4), long-term exposure to elevated DMSP levels would be expected to reduce, rather than increase, grazer responsiveness to added DMSP. However, we have not studied the effects of long-term exposure to submicromolar levels. Such exposure potentially sensitizes grazers so that they respond more strongly to subsequent additions; this phenomenon is often termed "potentiation". To potentiate is to augment the effect of a drug, toxin or other substance, and unfortunately little is known about this phenomenon in reference to planktonic protists.

Added DMSP often affected whole community processes. Both phytoplankton growth and microzooplankton grazing rates were frequently elevated by DMSP addition, especially in the GoA (Fig. 5). DMSP is a substrate for bacterial growth, and can constitute a substantial portion of total C and S utilization by the bacterial community (Kiene *et al.*, 2000; Zubkov *et al.*, 2001). Our DMSP additions likely elevated bacterial productivity in GoA communities. This, in turn, could have increased microzooplankton community grazing rates on phytoplankton by promoting increases in micrograzer growth and abundance, and by increasing ingestion rates of individual grazers. Some phytoplankton with osmotrophic capability may also take up DMSP (Vila-Costa *et al.*, 2006). This could directly elevate rates of phytoplankton growth; in addition, numerous photosynthetic flagellates can also feed on small particles and might have received a growth subsidy from increased availability of bacterial or algal prey (Zhang and Watanabe, 2001; Boenigk *et al.*, 2006).

The only experiment showing a significant phytoplankton growth reduction with added DMSP was GoA1 (Table IV). This was the sole experiment conducted on the outer shelf (Fig. 1), a high-nitrate, low-chlorophyll region experiencing chronic iron limitation (Martin *et al.*, 1989; Strom *et al.*, 2006). Such communities are highly sensitive to contamination by trace metals, which can inhibit phytoplankton photosynthesis and growth (Fitzwater *et al.*, 1982). It is possible that levels of trace metal contamination in DMSP stocks were inhibitory to the phytoplankton in this community.

Summary

Our laboratory experiments, in combination with those reported previously (Strom *et al.*, 2003b), show that the

inhibitory effect of μM-level DMSP is widespread among cultured protist grazers. Characteristic features of the inhibitory effect are a threshold concentration for feeding inhibition of 0.02–2 μM and partial to complete acclimation after hours of exposure to the signal. These features are similar to those exhibited by ciliates in response to dissolved amino acids, suggesting a generalized signaling property of these microbially produced, low molecular weight organic compounds. Addition of DMSP in similar concentrations to natural or phytoplankton-enriched coastal plankton communities typically did not reduce microzooplankton community grazing rates, although two clear instances of grazing inhibition were seen in Puget Sound microcosm experiments and one in the GoA. Rather, added DMSP most often had a generalized, low-level stimulatory effect on both phytoplankton growth and microzooplankton grazing rates. Wholesale DMSP enrichment does not accurately simulate the spatially patchy and ephemeral nature of dissolved organic signals as is most likely experienced in nature. These results demonstrate, however, that a common, algal-derived compound can have both inhibitory and stimulatory effects on the planktonic food web depending on scales of exposure and community condition.

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