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Chemical defense in the microplankton II: Inhibition of protist feeding by β-dimethylsulfoniopropionate (DMSP)

Suzanne Strom, Gordon Wolfe,[1] *Amy Slajer, Sarah Lambert, and Jennifer Clough*
Shannon Point Marine Center, Western Washington University, 1900 Shannon Point Road, Anacortes, Washington 98221

Abstract

Examples of chemical defenses and their influence on predator–prey interactions abound in the terrestrial and marine benthic ecological literature. In contrast, considerably less is known about the role of chemical defenses in marine planktonic systems. In this study, we examined the potential for the phytoplankton-produced compound dimethylsulfoniopropionate (DMSP) and its cleavage products dimethyl sulfide (DMS) and acrylate to act as chemical defenses in seawater. Although added DMS and acrylate had no effect on protist grazers, added DMSP reduced grazing on the coccolithophorid *Emiliania huxleyi* by all four tested species (dinoflagellates *Amphidinium longum, Gymnodinium* sp., and *Oxyrrhis marina* and ciliate *Coxliella* sp.). *A. longum* and *Gymnodinium* were highly sensitive to 20-μmol L^{-1} concentrations of DMSP, nearly ceasing to feed, whereas *O. marina* and *Coxliella* showed only minor reductions in grazing activity. Grazing suppression in *A. longum* was DMSP concentration–dependent over the range 0.05 to 1,000 μmol L^{-1} and was also inhibited by added glycine betaine, a structural analog of DMSP. DMSP reduced *A. longum* grazing by similar amounts on five different algal species, some of which do not produce DMSP on their own. Thus, the efficacy of DMSP as a grazing deterrent appears to depend on grazer species, but not on algal strain or species. Because DMSP does not seem to be toxic, we hypothesize that DMSP and related compounds act as signals for the presence of potentially harmful algal cells. Should such sublethal chemical defense interactions be widespread in nature, they could be important in regulating the composition and biomass of phytoplankton communities.

Chemical defenses against grazers, along with other grazing deterrence strategies, should be evolutionarily favored in marine phytoplankton. This is because most marine phytoplankton production is grazed by planktonic protists, both in coastal and in oceanic waters (Verity et al. 1993; Neuer and Cowles 1994; Landry et al. 1997). Thus, adaptations yielding even slight protection against protist grazing should confer a significant competitive advantage for a given phytoplankton strain or species (Strom in press).

Some phytoplankton species are known to be toxic to planktonic protists: contact with or ingestion of these cells results in death (Hansen 1989; Uchida et al. 1995; Kamiyama and Arima 1997). Sublethal defenses, known to be widespread in other ecosystems, have been much less studied in planktonic communities. If present, sublethal grazing deterrents could play a role in shaping phytoplankton community structure. For example, it has been hypothesized that suppression of grazing is an important element in the formation and persistence of phytoplankton blooms, including blooms of ''harmful'' taxa (Turner and Tester 1997).

Wolfe et al. (1997) have hypothesized that the cleavage of dimethylsulfoniopropionate (DMSP) into dimethyl sulfide (DMS) and acrylate constitutes a chemical defense against protist grazers. Cleavage is by the constitutive phytoplankton enzyme DMSP lyase (Steinke et al. 1998), which is activated by various physiological stressors, including pH variation and shear (Wolfe and Steinke 1996). A number of major bloom-forming phytoplankton taxa, including *Phaeocystis* spp., the coccolithophorid *Emiliania huxleyi*, and red tide dinoflagellates such as *Alexandrium* spp., contain both DMSP and DMSP lyase (Keller et al. 1989; Wolfe et al. 2002). DMSP and its cleavage products DMS and acrylate can be released directly into seawater by some phytoplankton (Keller et al. 1989; Noordkamp et al. 1998); DMS has also been identified as a product of grazing by both macro- and microzooplankton (e.g., Dacey and Wakeham 1986; Wolfe and Steinke 1996).

Grazing-activated chemical defenses analogous to the putative DMSP cleavage system are well known and widespread in terrestrial plants; they include compound classes such as the cyanogenic glycosides (Seigler 1991). In plants containing such defenses, grazing stress (mechanical, chemical, or both) results in contact between previously sequestered enzyme and substrate, with the resulting reaction yielding a chemical harmful or deterrent to the grazer. In the case of DMSP-containing phytoplankton, the harmful reaction product was hypothesized to be acrylate. At concentrations achievable in protist food vacuoles, acrylate has been shown to be toxic to some microbes (Thijsse 1964).

As part of a larger study of DMSP production and chemical defense in *E. huxleyi* (*see* Strom et al. 2003; Wolfe et al. 2002), we tested the direct effects of DMSP, acrylate, and DMS on grazing by protists. Although the DMSP defense hypothesis originally postulated that acrylate would negatively affect grazers after prey ingestion (i.e., from within the protist food vacuole), it seemed useful to explore the possibility that DMSP and its cleavage products could have

[1] Present address: Department of Biological Sciences, California State University—Chico, Chico, California 95929-0515.

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direct negative effects when present in seawater. We present evidence that DMSP acts as a generalized signal molecule promoting concentration-dependent reductions in the grazing activity of planktonic protists. If similarly active in the natural environment, DMSP release by phytoplankton has the potential to influence grazing rates and hence the net growth and accumulation of phytoplankton in the sea.

Materials and methods

General—Methods for the culture of protist grazers and algal prey are described in Strom et al. (2003). Protist grazer species employed in experiments are common in northern Puget Sound waters and, except for *Oxyrrhis marina*, are representative of species common in temperate coastal plankton communities. For all experiments described, feeding rates of protist grazers were estimated from rates of *E. huxleyi* accumulation in grazer food vacuoles. Prior to experiments, maintenance phytoplankton prey was removed from grazer stock cultures either by sieving grazers and resuspending them in ciliate medium (sterile 0.2-μm filtered seawater with dilute trace metal addition) for 12–24 h (*Coxliella* sp.) or by allowing grazers to remove prey until levels were sufficiently low that >90% of protist cells had empty food vacuoles (all other species).

Experiments were initiated by combining grazers, *E. huxleyi* and ciliate medium in polycarbonate bottles (*see below*) to reach desired concentrations. *E. huxleyi* stock cultures used for experiments were in midexponential phase (densities of 3–8 $\times$ 10^5 cells ml^{-1}), and cells were added to experiment bottles to achieve initial concentrations of 5 $\times$ 10^4 cells ml^{-1}. Bottles were incubated at 15°C in darkness unless otherwise indicated. For feeding rate determinations, experimental bottles were sampled at regular intervals ranging from 5 min for voracious feeders, such as *Coxliella* sp., to 1 h for *Gymnodinium* sp. Subsamples were preserved in glutaraldehyde (final conc. 0.5%), stained with DAPI, allowed to sit overnight in darkness at 4°C, then filtered (1.0-μm pore size polycarbonate filters), slide-mounted, and frozen (−20°C) for later microscopic analysis. At least 100 individual grazers per slide were examined under blue light excitation and evaluated as to their level of feeding. Because the dinoflagellate *Amphidinium longum* feeds by myzocytosis (Calado et al. 1998), individual prey cells cannot be counted inside this grazer. Therefore individual *A. longum* were scored as feeding or nonfeeding based on presence or absence of red- or orange-fluorescing phytoplankton-derived material in food vacuoles, and feeding intensity is reported as percentage of *A. longum* population feeding. For all other protist grazers, individual prey cells in grazer food vacuoles were counted, and feeding intensity is reported as average number of cells per grazer.

Effects of added DMSP and its cleavage products DMS and acrylate on feeding rates—Trials were run using four species of protist grazers: the ciliate *Coxliella* sp. and the heterotrophic dinoflagellates *Gymnodinium* sp., *Oxyrrhis marina*, and *A. longum*. Each grazer was offered low lyase *E. huxleyi* (strain LL-2 = CCMP 374) as food. Treatments (in duplicate for *A. longum*, in triplicate for all other grazers) were: no added chemicals, added DMSP-Cl (Chemische Laboratoria, purity verified at Western Washington University by nuclear magnetic resonance spectroscopy), added DMS (Aldrich), and added acrylic acid (Aldrich). All chemicals were added to achieve concentrations of 20 μmol L^{-1}; total experiment volumes were 80 ml. Occasional checks on DMS and DMSP addition levels during this and other experiments showed that concentrations in experimental bottles were within 10% of target concentrations.

Dosage dependence of DMSP effect—Two trials were conducted in which *A. longum* was offered *E. huxleyi* LL-2 in the presence of added DMSP. In the first, DMSP was added to achieve concentrations ranging from 0.5 to 20 μmol L^{-1} (in duplicate for each of five concentrations). In the second trial, conducted approximately 3 weeks later, DMSP was added to achieve concentrations ranging from 0.05 to 0.5 μmol L^{-1} (in triplicate for each of four concentrations). For both trials, an additional replicated control treatment with no added chemicals was included. Total experiment volumes were 40 or 50 ml.

Effect of DMSP on ingestion of algal species other than E. huxleyi—Two experiments were conducted in duplicate in which *A. longum* was fed the high-quality algal diet *Rhodomonas salina*. In the first, DMSP was added to 10 μmol L^{-1}. In the second, DMSP, DMS, and acrylate were added (separately) to 1 mmol L^{-1}. In this second experiment, bottles were incubated at 12°C and pH was checked after chemical additions to ensure that acidification did not occur. Both experiments included controls with no added chemicals. A third, more extensive experiment measured the feeding rate of *A. longum* on five taxonomically diverse algal species, each offered with and without added (20 μmol L^{-1}) DMSP. Algal species tested were *R. salina*, *Mantoniella squamata*, *E. huxleyi* strain LL-2, *Dunaliella tertiolecta*, and *Isochrysis galbana*. Duplicate bottles (total volume 40 ml) were set up for each of the 10 treatments (five algal species, each with and without added DMSP). Experimental concentrations of algae (cells ml^{-1}) were 5 $\times$ 10^4 for *M. squamata*, *I. galbana*, and *E. huxleyi* (small cells, 6–10 pg C $cell^{-1}$) and 1 $\times$ 10^4 for *R. salina* and *D. tertiolecta* (larger cells, 35–49 pg C $cell^{-1}$). The experiment was conducted at 12°C in dim light (~2 μmol photons m^{-2} s^{-1}).

Relationship between inhibitory effects of DMSP and of high-lyase E. huxleyi *strains*—To investigate the possible relationship between the feeding inhibition effects of high DMSP lyase activity in *E. huxleyi* (described in Strom et al. 2003) and added DMSP, an experiment was conducted with the tintinnid *Coxliella* sp. *E. huxleyi* strains HL-1 (high lyase, CCMP 373) and LL-2 were each offered to *Coxliella* with and without added DMSP at 20 μmol L^{-1}. Triplicate bottles containing 40 ml total volume were established for each of the four treatments; the experiment was conducted at 12°C in dim light (~2 μmol photons m^{-2} s^{-1}).

Effects of different DMSP stocks and structurally related chemicals on A. longum *feeding rates*—In the first trial, two different DMSP-Cl stocks (Chemische Laboratoria and Re-

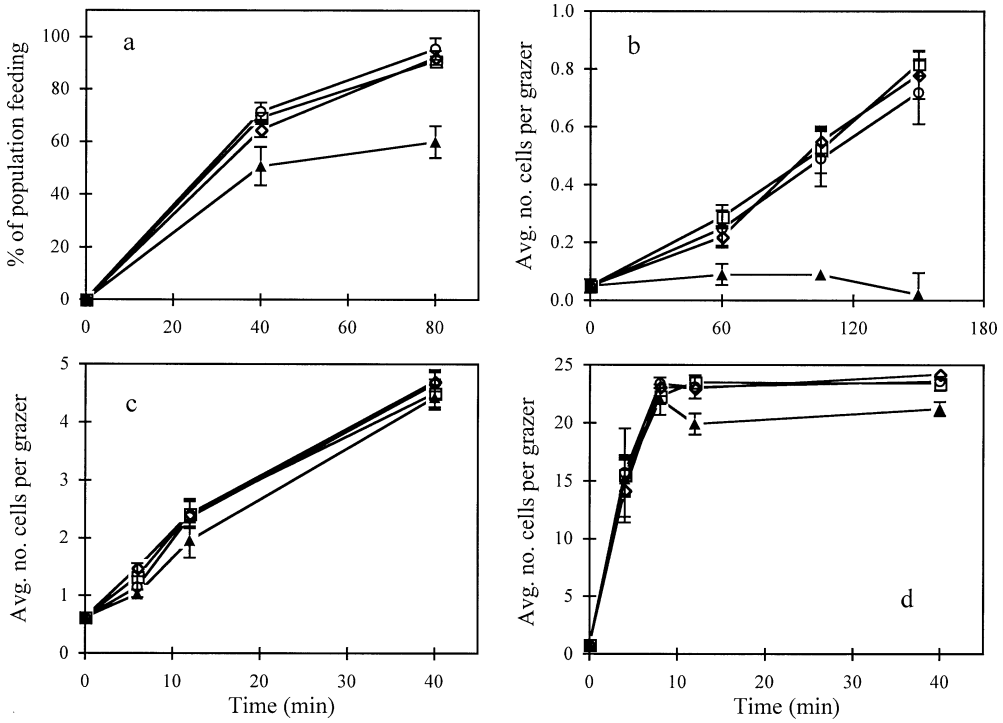

Fig. 1. Feeding responses of (a) *Amphidinium longum,* (b) *Gymnodinium* sp., (c) *Oxyrrhis marina,* and (d) *Coxliella* sp. fed low–DMSP lyase *Emiliania huxleyi* strain LL-2 in the presence of added chemicals. All chemicals added to a concentration of 20 μmol L^{-1}. Symbols show average ($n = 3$, except $n = 2$ for *A. longum*) $\pm$ 1 SD of feeding levels over time in control bottles (diamonds, no chemical addition) and bottles with added DMSP (triangles), DMS (circles), and acrylate (squares).

search Plus) were tested, each as both freshly prepared and aged (1 week at 4°C) solutions. These DMSP stocks—as well as structural analogs glycine betaine and *S*-methyl methionine (Aldrich)—were all added (20 μmol L^{-1} final conc.) to triplicate 40-ml bottles in which *A. longum* was fed *E. huxleyi* strain LL-2. Two additional triplicated control (no chemical addition) treatments were also included: one with *E. huxleyi* LL-2, the other with *E. huxleyi* strain HL-1. A second trial used *R. salina* as the diet. In this case, DMSP as well as structural analogs glycine betaine, *N*,*N*-dimethyl glycine, and glycine were added, each at 20 and 200 μmol L^{-1} (in triplicate). The control treatment consisted of *A. longum* plus *R. salina* with no added chemicals. Both trials were incubated at 12°C.

Results

All tested protist grazer species, which included both ciliates and dinoflagellates, showed some degree of feeding inhibition when offered *E. huxleyi* strain LL-2 in the presence of DMSP (Fig. 1). Feeding was not inhibited (i.e., rates were indistinguishable from control rates) for any of the four species in the presence of added DMS or acrylate, the enzyme cleavage products of DMSP (Table 1). The degree of inhibition in the presence of DMSP was variable. Feeding was reduced by a maximum of only 13% relative to control rates for the ciliate *Coxliella,* and the reduction was significant only at the 90% confidence level (Table 1). At the other extreme, feeding by the dinoflagellate *Gymnodinium* was re-

Table 1. Results (as *P* values, alpha = 0.05) of repeated measures analysis of variance, with Tukey–Kramer post hoc test, for experiments that tested the inhibitory effect of added chemicals on protist grazing activity. In all cases, prey alga was *Emiliania huxleyi* strain LL-2 (= CCMP 374); added chemicals were DMSP, acrylate, and DMS; factor 1 was chemical addition; and factor 2 was time. Post hoc test results list significant pairwise differences among chemical addition treatments; shared letters indicate no significant difference ($P \geq 0.05$). Acr, acrylate; Con, control.

	P value			Tukey–Kramer test			
Grazer	Factor 1	Factor 2	Interaction	DMSP	DMS	Acr	Con
Amphidinium longum	0.0046	0.0001	0.0291	a	b	b	b
Gymnodinium sp.	<0.0001	<0.0001	0.0002	a	b	b	b
Oxyrrhis marina	0.0614	<0.0001	0.3313	a	a,b	a,b	b
Coxliella sp.	0.0590	<0.0001	0.3412	a	a	a	a

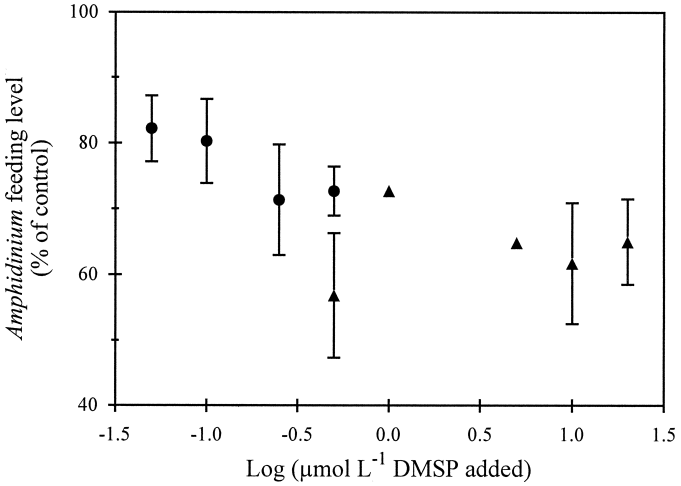

Fig. 2. Inhibition of feeding in *Amphidinium longum* over a range of DMSP addition levels. Feeding data (mean ± SD) show percentage of population with ingested *Emiliania huxleyi* LL-2 after 80 min, normalized to control feeding levels at 80 min. Circles: experiment conducted 20 Aug 99 (n = 3); triangles: experiment conducted 27 Jul 99 (n = 2).

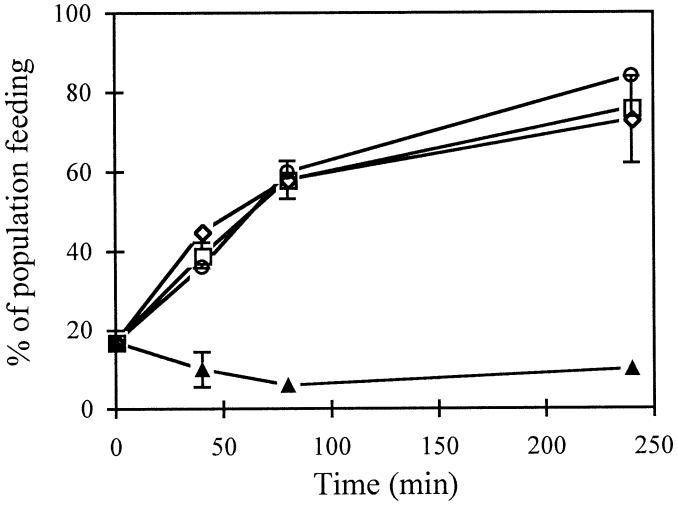

Fig. 3. Feeding (as percentage of *A. longum* population with ingested prey) on *Rhodomonas salina* over time in the presence of added DMSP, DMS, or acrylate (all at 1 mmol L^{-1}). Control treatment had no added chemicals. Symbols as in Fig. 1, showing mean ± SD (n = 2).

duced to near zero by the final time point of the experiment. Furthermore, inhibition increased over time in three out of the four grazer species in the trials (Fig. 1a,b,d).

For *A. longum*, the degree of inhibition was proportional to the concentration of added DMSP (Fig. 2). Feeding averaged 82% of control levels at the lowest DMSP addition level (0.05 μmol L^{-1}), decreasing to 61–65% at 10–20 μmol L^{-1}. Added DMSP also reduced feeding by *A. longum* on the cryptomonad *R. salina*, which otherwise sustains high feeding and growth rates in this species. An addition of 10 μmol L^{-1} DMSP reduced *A. longum* feeding to 81 and 89% of control levels at 40 and 80-min sampling time points, respectively, whereas an addition of 1 mmol L^{-1} reduced feeding to 11% of control levels (80-min time point). Additions of 1 mmol L^{-1} acrylate or DMS had no effect on feeding rates (Fig. 3). The results from the *R. salina* trials indicate that the strong dose dependence of feeding inhibition observed when *E. huxleyi* LL-2 was the diet (Fig. 2) would extend to low or zero feeding rates at higher concentrations of DMSP.

A more extensive DMSP addition experiment was conducted to determine (1) whether the inhibitory effect of DMSP extended to algal prey other than *E. huxleyi* and *R. salina* and (2) whether the strength of the inhibitory effect varied with algal species. Results (Fig. 4) showed that DMSP addition significantly reduced feeding by *A. longum* (repeated measures ANOVA, P = 0.0023). Algal species also had a significant effect on feeding rate ($P < 0.0001$). However, there was no interaction between algal species and DMSP treatment (P = 0.1704), suggesting that DMSP has a blanket inhibitory effect that does not depend on prey taxon. For reasons that are not clear, the reduction in feeding by *A. longum* on *E. huxleyi* LL-2 was considerably less in this experiment than at comparable DMSP addition levels in other experiments.

To further investigate the relationship between prey type and DMSP addition effect, *E. huxleyi* strains with differing DMSP lyase activity were each offered to *Coxliella* with and without added DMSP. High-lyase *E. huxleyi* strains were previously shown to inhibit feeding relative to low-lyase strains in this and other grazer species (Strom et al. 2003). Feeding rates were lower on high-lyase strain HL-1 (Fig. 5; two-way ANOVA, P = 0.0014), in agreement with previous findings. DMSP also reduced feeding rates on both strains by an average of 14% relative to control rates, although results were variable and the reduction was significant only at the 90% confidence level (P = 0.095). No interaction be-

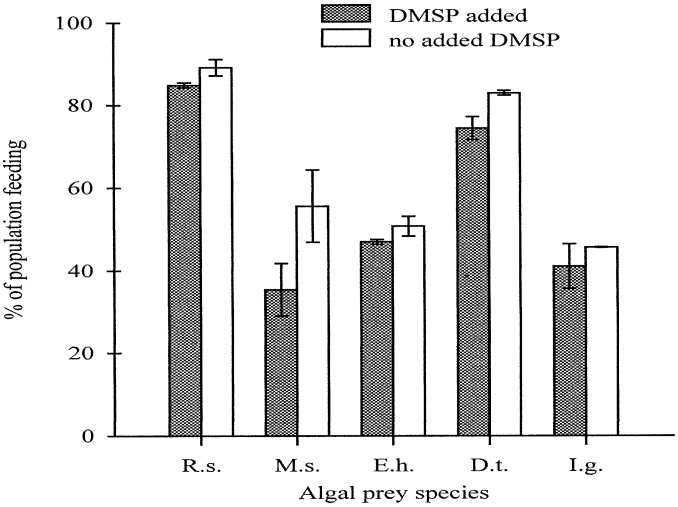

Fig. 4. Percentage of *Amphidinium longum* population with ingested prey 30 min after addition of prey cells to starved *A. longum* cultures with and without added DMSP. (Data from 60 min time point were nearly identical.) Bars show average (n = 2) and range of observations. R.s., *Rhodomonas salina*; M.s., *Mantoniella squamata*; E.h., *Emiliania huxleyi* strain LL-2; D.t., *Dunaliella tertiolecta*; I.g., *Isochrysis galbana.*

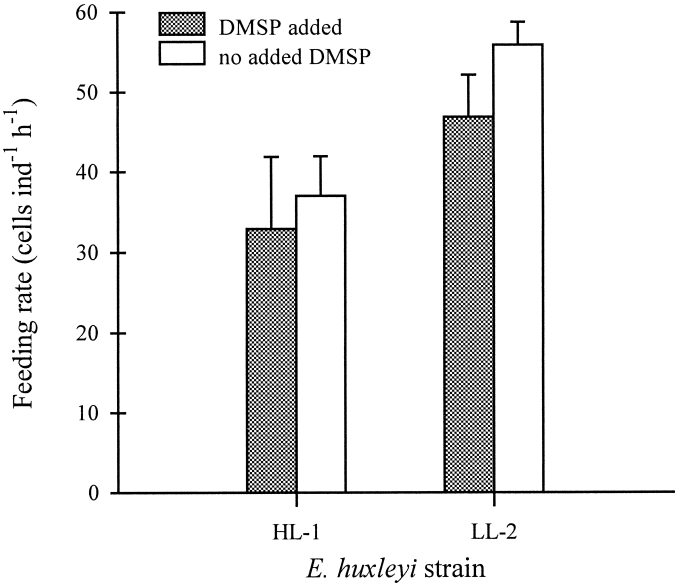

Fig. 5. Feeding rate of *Coxliella* sp. on two strains of *Emiliania huxleyi* with contrasting DMSP lyase activity (HL-1, LL-2), each with and without added DMSP. Means ± SD, $n = 3$.

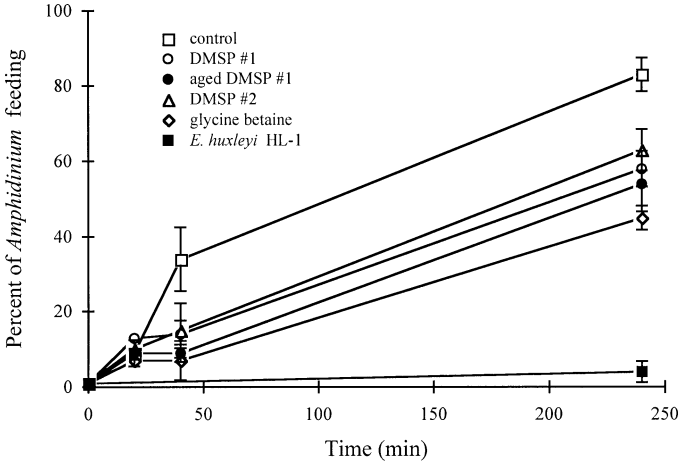

Fig. 6. Time course of feeding on *Emiliania huxleyi* strain LL-2 by *Amphidinium longum* in control treatment (no chemical addition) and treatments with added chemicals (all at 20 μmol L^{-1}): DMSP #1, DMSP stock obtained from Chemische Laboratoria; aged DMSP #1, same as DMSP #1 but solution aged 1 week at 4°C before use; DMSP #2, DMSP stock obtained from Research Plus; glycine betaine. A treatment containing no added chemicals and high-lyase *E. huxleyi* strain HL-1 was also included. Data points are means ± SD ($n = 3$).

tween strain identity and DMSP effect was seen. The approximately twofold lower feeding rate on *E. huxleyi* strain LL-2 in this experiment relative to data shown in Fig. 1 (i.e., initial slope of Fig. 1d) was attributed to the lower incubation temperature (12° vs. 15°C) and, possibly, differing physiological states of the *Coxliella* populations. Metabolic rates in *Coxliella* appear to be quite sensitive to the length of the pre-experiment starvation period.

Of the chemicals structurally similar to DMSP, only glycine betaine inhibited grazing by *A. longum* at 20 μmol L^{-1}. This inhibition was observed for both *E. huxleyi* LL-2 and *R. salina* diets (Figs. 6, 7). At 200 μmol L^{-1}, *N,N*-dimethyl glycine also inhibited feeding (Fig. 7); in most cases, the degree of feeding inhibition by these structural analogs was similar to that induced by DMSP. Glycine at 20 and 200 μmol L^{-1} (Fig. 7) and *S*-methyl methionine at 20 μmol L^{-1} (data not shown) had no effect on grazing relative to the no-addition controls. Stocks of DMSP aged 1 week showed the same inhibitory effect as fresh stocks, and stocks prepared from different chemical suppliers were equally inhibitory (Fig. 6).

Discussion

Our findings require an expanded interpretation of the DMSP/DMSP lyase defense system for microplankton. Although the reaction products acrylate and DMS, when applied externally, had no effect on grazing rates, added DMSP reduced grazing in all four protist species tested.

DMSP: A chemical defense signal—According to Berenbaum (1995, p. 3), ''A defensive chemical . . . is a substance produced in order to reduce the risk of bodily harm.'' These results demonstrate that DMSP, a chemical produced and released by numerous phytoplankton species, inhibits protist grazing on phytoplankton and thus constitutes a chemical defense. Although there are some examples in the literature of diatom-derived fatty acids and their aldehyde reaction products causing acute toxicity or reduced reproduction rates in crustacean zooplankton (Miralto et al. 1999; Pohnert 2000; Juttner 2001), there appear to be no previously published reports of phytoplankton-derived defense compounds active against protist grazing.

DMSP was originally hypothesized to function as a grazing-activated defense by means of cleavage into DMS and acrylate (Wolfe et al. 1997). Significantly, we found no negative effects of added 20 μmol L^{-1} DMS or acrylate on feeding by any of the four protist species examined. Furthermore, 1 mmol L^{-1} DMS or acrylate had no effect on

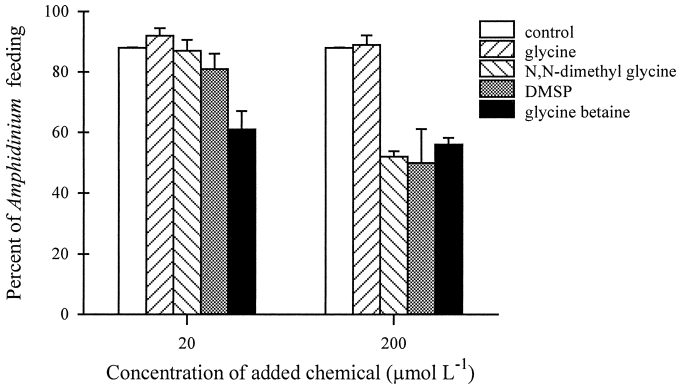

Fig. 7. Percentage of *Amphidinium longum* population feeding on *Rhodomonas salina* 240 min after experiment initiation in control treatment (no added chemicals) and treatments with added 20 and 200 μmol L^{-1} DMSP, glycine betaine, *N,N*-dimethyl glycine, and glycine. Bars show means + SD ($n = 2$).

feeding by *A. longum*, a grazer sensitive to even nanomole per liter concentrations of added DMSP. The lack of an observed acrylate effect might be due to the relatively low concentrations employed in these experiments relative to those (~70 mmol L^{-1}) hypothesized to exist within protist food vacuoles. In addition, the compound might exert a negative effect only as acrylic acid, the form that would predominate in low-pH food vacuoles. An alternative interpretation is that, at least for protists, the DMSP reaction system does not constitute an activated defense. This is supported by the results of other experiments (Strom et al. 2003) showing that protists need not ingest *E. huxleyi* cells (thus presumably activating the defense reaction) in order to exhibit reduced grazing rates.

Although the reduced grazing rates that we observed in the presence of added DMSP might be due to physiological impairment, there is no evidence so far that DMSP itself is deleterious to protist grazers. Numerous protist species were able to feed and grow at high rates on *E. huxleyi* strains with low DMSP lyase activity (Strom et al. 2003). These low lyase strains have cellular DMSP contents (fg μm^{-3} cell volume) similar to those of high-lyase *E. huxleyi* strains that sometimes inhibited grazing, indicating that ingestion of DMSP itself is not harmful. However, the relatively short time course of these chemical addition experiments (Fig. 1) does not allow us to rule out the possibility that extracellular DMSP might be toxic to some protists.

Rather than directly impairing grazer function, DMSP might act as a signal molecule. Most protist grazers feed by ingesting whole phytoplankton cells through phagocytosis. This all-or-nothing feeding strategy could promote the evolution of ''early warning systems'': the ability to detect the precursors to deleterious chemicals that would be produced in an ingested prey cell. Such chemical early warning systems would be analogous to the visual recognition and avoidance of color patterns or morphologies associated with toxic prey by birds (e.g., the monarch butterfly) or fishes (e.g., many nudibranch species). An alternative possibility is that DMSP taken up by protists triggers feeding cessation not through a warning of potential toxicity, but through a signal of food vacuole fullness or other indicator of feeding satiety, perhaps related to cell turgor control.

That DMSP is a signal resulting in reduced grazing is supported by the results of other experiments presented here. The extent to which grazing was reduced in *A. longum* was directly proportional to DMSP concentration, up to the highest levels tested (Figs. 2, 3). These experiments further demonstrated that modest grazing reductions occurred even at the lowest added DMSP concentrations (50 nmol L^{-1}). DMSP concentrations of this magnitude have been measured in near-surface seawater, for example in the Iceland Basin (44 nmol L^{-1}, Archer et al. 2001) and in British coastal waters (up to 200 nmol L^{-1}, Turner et al. 1988) during dinoflagellate and prymnesiophyte blooms. It is probable that considerably higher concentrations would, at least transiently, characterize the region near a phytoplankton cell (Wolfe 2000). Thus the grazing reductions seen here could potentially be realized in natural waters. In addition, added DMSP reduced grazing on five different phytoplankton species from several different phyla (Fig. 4). The magnitude of the grazing reduction was similar for all prey species, suggesting that, at least for *A. longum*, the signaling properties of DMSP are general and do not depend on prey identity.

Experiments with structural analogs of DMSP revealed that at least one other compound, glycine betaine, consistently acted as a grazing deterrent for *A. longum*. Grazing reductions in the presence of glycine betaine were similar to, or in one instance even greater than, those in the presence of DMSP. Of the analogs we tested, glycine betaine is the most structurally similar to DMSP. A number of phytoplankton species produce glycine betaine in quantity, although as a nitrogen-containing molecule its cellular concentration is sensitive to nitrogen availability (Keller et al. 1999). It has been suggested that DMSP might substitute for glycine betaine as a compatible osmolyte under nitrogen-limiting conditions. Our data demonstrate that glycine betaine also acts as a signal molecule promoting reduced grazing by protists.

Relationships between DMSP and DMSP lyase activity in E. huxleyi—Results presented in a companion paper (Strom et al. 2003) demonstrate that protists consistently feed at higher rates on *E. huxleyi* strains with low DMSP lyase activity. Chemical analyses, however, showed that both high- and low-lyase strains have similar intracellular DMSP concentrations (15.6–18.8 fg μm^{-3}). Is the feeding reduction caused by DMSP addition related in some way to the feeding reduction seen with high-lyase *E. huxleyi* strains? Results shown in Fig. 5 indicated no interaction between strain lyase activity and DMSP addition effects. For *Coxliella* sp. at least, the presence of a high-lyase *E. huxleyi* strain does not confer increased sensitivity to DMSP. This finding is in general agreement with the observation that the inhibitory effect of DMSP does not depend on algal prey species (Fig. 4). It should be noted, however, that *Coxliella* is one of the grazer species least sensitive to both DMSP addition (Fig. 1) and high-lyase *E. huxleyi* (*see* fig. 5 in Strom et al. 2003); more sensitive grazers might give different results in such interaction experiments.

In general, protist grazers showing the greatest feeding reductions in response to high-lyase *E. huxleyi* strains (*A. longum, Gymnodinium, see* Strom et al. 2003) also showed the greatest sensitivity to DMSP addition (Fig. 1). *Coxliella* and *O. marina*, which showed little or no feeding inhibition on high-lyase *E. huxleyi*, also showed only slight feeding reductions in response to added DMSP. Although the links between DMSP and DMSP lyase activity are not completely understood (*see below*), it seems probable that some protist species are consistently more sensitive to these chemical cues than others. Thus, the extent to which a DMSP-related chemical defense could promote bloom formation in nature would depend, in part, on the composition of the protist grazer community.

Finally, preliminary data indicate that, although the studied *E. huxleyi* strains have similar intracellular DMSP concentrations, there are strain-specific differences in the amount of DMSP released. High-lyase strain 373 (HL-1) released more DMSP per cell, and low-lyase strain 370 (LL-1) released less, than other strains in batch cultures (Wolfe et al. 2002; Strom et al. unpubl. data). If DMSP signals grazers to reduce their activity, low levels of DMSP release

could lead to higher feeding rates on LL-1 than on other strains. However, low-lyase *E. huxleyi* strain LL-2 (374), which also supported high protist feeding rates, did not exhibit comparatively low DMSP release levels. Based on these preliminary results, there is not a direct relationship between DMSP release and DMSP lyase activity that would explain the consistency of protist grazing responses to these two *E. huxleyi* features. Both culture age and growth media trace metal composition influenced DMSP release in these experiments, so an in-depth study will be needed to explain the factors regulating production and release of this compound.

Outstanding issues—

1. The DMSP addition data suggest that dissolved DMSP acts as a signal ''warning'' grazers not to feed as extensively on available prey. Chemical defense theory dictates that a warning signal will not continue to be effective in the evolutionary sense unless there is some cost associated with eating the prey that produces the signal. What is the cost? It has been hypothesized that DMSP cleavage products, in particular acrylate or acrylic acid, might be deleterious to some grazers if sufficient prey cells are ingested. Perhaps in this case DMSP signals ''here is a prey cell potentially containing a high dose of acrylate.'' Are the grazers most sensitive to DMSP also most strongly affected by ingested acrylate? Alternatively, perhaps leakage of low–molecular weight compounds such as DMSP and glycine betaine is associated with cells that are physiologically stressed. High intracellular levels of DMSP, hypothesized to act as an antioxidant, are produced by some algal species in response to chronic iron limitation, elevated CO_2, and ultraviolet exposure (Sunda et al. 2002), all sources of oxidative stress. Is there a cost to ingestion of oxidatively stressed algal prey so that protist grazers are adapted to detect and respond to chemical signals indicating that state?
2. Results presented in this and the companion paper (Strom et al. 2003) lead to a conundrum: grazing inhibition covaried with DMSP lyase activity, yet the products of the DMSP cleavage reaction did not inhibit feeding when added exogenously. Is higher DMSP release the reason that *E. huxleyi* strains with high DMSP lyase activity are eaten at reduced rates by protists? Our data demonstrate that DMSP release could potentially lead to grazing suppression of the magnitude observed for some protists in the presence of high-lyase *E. huxleyi* cells (e.g., *compare* Fig. 1b in this paper with fig. 3b in Strom et al. 2003). Furthermore, other dissolved signals might also differ among strains. On the other hand, the presence of high-lyase *E. huxleyi* cells, even in high concentrations, did not inhibit feeding on a preferred prey type by *A. longum* (*see* fig. 6 in Strom et al. 2003). This observation points to cell surface–associated cues as important in prey recognition; although our lectin results (Strom et al. 2003) did not show gross differences in cell surface sugars among *E. huxleyi* strains, numerous other compound types might be important in cell–cell recognition. In terms of interspecific competition among phytoplankton, release of a compound whose presence reduces grazing on all nearby phytoplankton cells would seem less effective than production of deterrent compounds bound to the prey cell surface. In general, we do not believe that a single chemical cue is responsible for the range of behavioral interactions we have observed between protist grazers and phytoplankton prey.
3. What is the metabolic cost to the alga of the DMSP-based chemical defense system, and what are the relationships between this chemical defense and other cellular functions of DMSP? DMSP is known to act as both an osmolyte and a cryoprotectant in certain algal species (Vairavamurthy et al. 1985; Karsten et al. 1996; Stefels 2000). Most recently, it has been proposed that DMSP, acrylate, and DMS are all active as cellular antioxidants (Sunda et al. 2002). The observations that DMSP lyase is a constitutive phytoplankton enzyme, that lyase activity levels are stable for hundreds of generations or more in laboratory culture (Steinke et al. 1998), and that the presence and activity of grazers does not exert a strong influence on lyase activity (Wolfe and Steinke 1996) all indicate that this enzyme system has important functions other than grazer defense. How do these multiple functions influence the evolution of different DMSP release and DMSP lyase activity levels in various algal strains and species? An obvious hypothesis is that the metabolic cost of maintaining a DMSP defense system leads to acquisition of this system by only a subset of taxa. Experiments to date, however, do not show any difference in the maximum growth rates attainable by high- versus low-lyase *E. huxleyi* strains (Wolfe et al. 2002). Metabolic costs of the DMSP system might not be apparent under the resource-replete conditions that we typically employ in the laboratory. Experiments under conditions of nutrient and light limitation, which take into account the multiple functions of DMSP and DMSP lyase, will be necessary to understand the taxonomic and geographic distributions of this chemical defense.

Results of this study demonstrate that DMSP, a compound produced and released by some marine phytoplankton species, is a chemical defense against protist grazers. Because DMSP is not known to be toxic—whereas one of its cleavage products, acrylate, might have physiological effects at sufficient concentrations within protist food vacuoles—we hypothesize that DMSP acts as a signal molecule rather than directly as a toxin. Grazing inhibition by glycine betaine, a close structural analog of DMSP, was also consistently observed, demonstrating that at least two low–molecular weight, phytoplankton-produced compounds can signal protists to reduce grazing activity. The prevalence of phagotrophy among protists might result in evolutionary pressure to detect and avoid prey that are potentially toxic or otherwise suboptimal. Although grazing reductions were proportional to DMSP concentrations and added DMSP reduced grazing similarly on a range of algal species, some protist grazers exhibited much higher sensitivity to given levels of DMSP than others. Thus, the efficacy of DMSP as a chemical defense appears to depend on grazer species but not on algal strain or species. Important unresolved issues include the

relationship between DMSP deterrence and feeding deterrence associated with high DMSP lyase activity, as reported in a companion paper (Strom et al. 2003). In addition, the relationships between chemical defense and other known functions of the DMSP system are certain to influence the evolution, distribution, and metabolic costs of this defense.

References

ARCHER, S. D., C. E. STELFOX-WIDDECOMBE, P. H. BURKILL, AND G. MALIN. 2001. A dilution approach to quantify the production of dissolved dimethylsulphoniopropionate and dimethyl sulphide due to microzooplankton herbivory. Aquat. Microb. Ecol. **23:** 131–145.

BERENBAUM, M. R. 1995. The chemistry of defense: Theory and practice, p. 1–16. *In* T. Eisner and J. Meinwald [eds.], Chemical ecology: The chemistry of biotic interaction. National Academy Press.

CALADO, A. J., S. C. CRAVEIRO, AND O. MOESTRUP. 1998. Taxonomy and ultrastructure of a freshwater, heterotrophic *Amphidinium* (Dinophyceae) that feeds on unicellular protists. J. Phycol. **34:** 536–554.

DACEY, J. W., AND S. G. WAKEHAM. 1986. Oceanic dimethylsulfide: Production during zooplankton grazing on phytoplankton. Science **233:** 1314–1316.

HANSEN, P. J. 1989. The red tide dinoflagellate *Alexandrium tamarense*: Effects on behaviour and growth of a tintinnid ciliate. Mar. Ecol. Prog. Ser. **53:** 105–116.

JUTTNER, F. 2001. Liberation of 5,8,11,14,17-eicosapentaenoic acid and other polyunsaturated fatty acids from lipids as a grazer defense reaction in epilithic diatom biofilms. J. Phycol. **37:** 744–755.

KAMIYAMA, T., AND S. ARIMA. 1997. Lethal effect of the dinoflagellate *Heterocapsa circularisquama* upon the tintinnid ciliate *Favella taraikaensis.* Mar. Ecol. Prog. Ser. **160:** 27–33.

KARSTEN, U., K. KÜCK, C. VOGT, AND G. O. KIRST. 1996. Dimethylsulfoniopropionate production in phototrophic organisms and its physiological function as a cryoprotectant, p. 143–153. *In* R. P. Kiene, P. Visscher, G. Kirst, and M. Keller [eds.], Biological and environmental chemistry of DMSP and related sulfonium compounds. Plenum Press.

KELLER, M. D., W. K. BELLOWS, AND R. R. L. GUILLARD. 1989. Dimethyl sulfide production in marine phytoplankton, p. 167–183. *In* E. S. Saltzman and W. J. Cooper [eds.], Biogenic sulfur in the environment. American Chemical Society.

———, R. P. KIENE, P. A. MATRAI, AND W. K. BELLOWS. 1999. Production of glycine betaine and dimethylsulfoniopropionate in marine phytoplankton. II. N-limited chemostat cultures. Mar. Biol. **135:** 249–257.

LANDRY, M. R., AND OTHERS. 1997. Iron and grazing constraints on primary production in the central equatorial Pacific: An EqPac synthesis. Limnol. Oceanogr. **42:** 405–418.

MIRALTO, A., AND OTHERS. 1999. The insidious effect of diatoms on copepod reproduction. Nature **402:** 173–176.

NEUER, S., AND T. J. COWLES. 1994. Protist herbivory in the Oregon upwelling system. Mar. Ecol. Prog. Ser. **113:** 147–162.

NOORDKAMP, D. J. B., M. SCHOOTEN, W. W. C. GIESKES, L. J. FORNE, J. C. GOTTSCHAL, AND M. VAN RIJSSEL. 1998. High acrylate concentrations in the mucus of *Phaeocystis globosa* colonies. Aquat. Microb. Ecol. **16:** 45–52.

POHNERT, G. 2000. Wound-activated chemical defense in unicellular planktonic algae. Agnew. Chem. Int. Ed. **39:** 4352–4354.

SEIGLER, D. S. 1991. Cyanide and cyanogenic glycosides, p. 35–77. *In* G. A. Rosenthal and M. R. Berenbaum [eds.], Herbivores: Their interactions with secondary plant metabolites. Academic Press.

STEFELS, J. 2000. Physiological aspects of the production and conversion of DMSP in marine algae and higher plants. J. Sea Res. **43:** 183–197.

STEINKE, M., G. V. WOLFE, AND G. O. KIRST. 1998. Partial characterization of dimethylsulfoniopropionate (DMSP) lyase isozymes in 6 strains of *Emiliania huxleyi.* Mar. Ecol. Prog. Ser. **175:** 215–225.

STROM, S. L. In press. Novel interactions between phytoplankton and microzooplankton: Their influence on the coupling between growth and grazing rates in the sea. Hydrobiologia.

———, G. WOLFE, J. HOLMES, H. STECHER, C. SHIMENECK, S. LAMBERT, AND E. MORENO. 2003. Chemical defense in the microplankton I: Feeding and growth rates of heterotrophic protists on the DMS-producing phytoplankter *Emiliania huxleyi.* Limnol. Oceanogr. **48:** 217–229.

SUNDA, W. G., D. J. KIEBER, R. P. KIENE, AND S. A. HUNTSMAN. 2002. An antioxidant function for DMSP and DMS in marine algae. Nature **418:** 317–320.

THIJSSE, G. J. E. 1964. Fatty-acid accumulation by acrylate inhibition of beta-oxidation in an alkane-oxidizing *Pseudomonas.* Biochim. Biophys. Acta **84:** 195–197.

TURNER, J. T., AND P. A. TESTER. 1997. Toxic marine phytoplankton, zooplankton grazers, and pelagic food webs. Limnol. Oceanogr. **42:** 1203–1214.

TURNER, S. M., G. MALIN, P. S. LISS, D. S. HARBOUR, AND P. M. HOLLINGAN. 1988. The seasonal variation of dimethyl sulfide and dimethylsulfoniopropionate concentrations in nearshore waters. Limnol. Oceanogr. **33:** 364–375.

UCHIDA, T., M. YAMAGUCHI, Y. MATSUYAMA, AND T. HONJO. 1995. The red-tide dinoflagellate *Heterocapsa* sp. kills *Gyrodinium instriatum* by cell contact. Mar. Ecol. Prog. Ser. **118:** 301–303.

VAIRAVAMURTHY, A., M. O. ANDREAE, AND R. L. IVERSON. 1985. Biosynthesis of dimethylsulfide and dimethylpropiothetin by *Hymenomonas carterae.* Limnol. Oceanogr. **30:** 59–70.

VERITY, P. G., AND OTHERS. 1993. Composition, productivity and nutrient chemistry of a coastal ocean planktonic food web. Cont. Shelf Res. **13:** 741–776.

WOLFE, G. V. 2000. The chemical defense ecology of marine unicellular plankton: Constraints, mechanisms, and impacts. Biol. Bull. **198:** 225–244.

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

———, ———, AND G. O. KIRST. 1997. Grazing-activated chemical defense in a unicellular marine alga. Nature **387:** 894–897.

———, S. L. STROM, J. L. HOLMES, AND M. B. OLSON. 2002. DMSP cleavage by marine phytoplankton in response to physical, chemical, or dark stress. J. Phycol. **38:** 948–960.

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