Our data establish a pathway for DMSP biosynthesis in marine algae. This pathway has no steps in common with that in higher plants, which proceeds via SMM and 3-dimethylsulphoniopropionaldehyde[6,7]. DMSP biosynthesis must therefore have evolved independently at least twice. Our results have two other implications. The first stems from the finding that a transaminase reaction stands at the head of the DMSP pathway; this may help explain why nitrogen deficiency enhances DMSP production[12,23,30]. Depletion of cellular amino acids would favour the transamination reaction, thereby promoting DMSP synthesis when nitrogen is limiting. Second, our results suggest that DMSP may not be the only precursor of the DMS produced by living algae: DMSHB is another potential precursor *in vivo*. In support of this possibility, we have obtained preliminary evidence for extensive catabolism of supplied DMSHB to DMS in *Tetraselmis* sp. and *E. huxleyi*. □

Methods

Algae. *E. intestinalis* was collected in Florida and kept in aerated sea water at 18 °C in continuous fluorescent light (photosynthetic photon flux density, 50 μmol m^{-2} s^{-1}). *M. nummuloides* (CCMP 482) and *Tetraselmis* sp. were cultured axenically at 25 °C in the above light regime in modified Gooday's medium[24] with 1 mM NO_3^-; for *M. nummuloides*, 0.1 mM Na_2SiO_3 was added and Tris omitted. *E. huxleyi* (CCMP 373) was grown in f/2 medium[25] in daylight at 22 °C.

Radiochemicals. L-[^{35}S]SMM was synthesized enzymatically from L-[^{35}S]Met[26] or by treating L-[^{35}S]Met with 250 mM methanol in 6 M HCl at 110 °C for 4 h (ref. 27), and converted to L-[^{35}S]DMSHB with HNO_2 (ref. 28). [^{35}S]MTOB was made from L-[^{35}S]Met using L-amino acid oxidase, and converted to L-[^{35}S]MTHB using L-lactic dehydrogenase and NADH; [^{35}S]methylthiopropionate was obtained as a byproduct. Compounds were purified by ion exchange and TLE[6,7].

Labelling conditions. *E. intestinalis* fronds (50 or 100 mg) were incubated in 0.5–2.5 ml sterile sea water; ^{18}O labelling was carried out in 50-ml flasks. For phytoplankton species, labelled compounds were added to growing cultures. Incubation was at 18–21 °C for *E. intestinalis* and *E. huxleyi* and 25 °C for *Tetraselmis* and *M. nummuloides*, under fluorescent light and with gentle agitation. Uptake of ^{35}S was estimated from its disappearance from the medium.

Metabolite analysis. Most metabolites were isolated by methanol–chloroform–water extraction, ion exchange, TLE and TLC[6,7]. As MTOB broke down in these procedures, forming MTP, these compounds were isolated using 0.1 M HCl followed by ether extraction[6] (method A) or by using paired samples extracted in 0.3 ml 66 mM $NaBH_4$ (to reduce MTOB to MTHB) or in pre-neutralized $NaBH_4$ (to give the endogenous MTHB level) (method B); MTOB was estimated by difference . Method B was also used to isolate 3-dimethylsulphoniopropionaldehyde as its hydroxy derivative[7]. Metabolites were identified by their respective lability and stability in cold 2 M NaOH. ^{35}S data were corrected for recovery, determined using labelled standards, and for products formed during extraction. Protein synthesis was estimated from ^{35}S labelling of the insoluble residue; the ^{35}S was shown to be in protein-bound Met by proteolysis and TLC.

Mass spectrometry. DMSP was analysed without derivatization by FAB-MS[29]. DMSHB was derivatized as its *t*-butyldimethylsilyl ester/ether and analysed by GC-MS with SIM after on-column nucleophile-assisted *S*-demethylation[29]. Authentic DMSHB[6] was used to calibrate the SIM parameters. The diagnostic fragment ion cluster at *m/z* 321 (loss of a *t*-butyl radical) was monitored at the appropriate retention time. ^{15}N-amino acids were analysed as described[14], except that amide-^{15}N abundance was determined using *N*-ethoxycarbonyl isobutyl esters by electron impact GC-MS[30].

Received 16 January; accepted 8 May 1997.

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Acknowledgements. We thank J. Hellebust, O. Schofield and J. S. Davis for algal material. This work was supported by grants from the NSF and ONR to A.D.H. and D.A.G., from the ONR to D.R. and T.L., from the NIH to the MSU-NIH Mass Spectrometry Facility and by the C.V. Griffin Sr Foundation. (Florida Agricultural Experiment Station journal series no. R-05740.)

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Grazing-activated chemical defence in a unicellular marine alga

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Marine plankton use a variety of defences against predators, some of which affect trophic structure and biogeochemistry[1]. We have previously shown[2] that, during grazing by the protozoan *Oxyrrhis marina* on the alga *Emiliania huxleyi*, dimethylsulphoniopropionate (DMSP) from the prey is converted to dimethyl sulphide (DMS) when lysis of ingested prey cells initiates mixing of algal DMSP and the enzyme DMSP lyase. Such a mechanism is similar to macrophyte defence reactions[3,4]. Here we show that this reaction deters protozoan herbivores, presumably through the

production of highly concentrated acrylate, which has antimicrobial activity[5]. Protozoan predators differ in their ability to ingest and survive on prey with high-activity DMSP lyase, but all grazers preferentially select strains with low enzyme activity when offered prey mixtures. This defence system involves investment in a chemical precursor, DMSP, which is not self-toxic and has other useful metabolic functions. We believe this is the first report of grazing-activated chemical defence in unicellular microorganisms.

We examined five axenic strains of the prymnesiophyte *E. huxleyi*, all containing 75–110 mM internal DMSP. All strains synthesized constitutive DMSP lyase, which cleaves DMSP to form DMS, acrylate and a proton[6]. However, DMS production was negligible until cells were lysed by mechanical or chemical means[2]. These strains were of similar size and growth characteristics but exhibited a bimodal distribution of *in vitro* DMSP lyase activity: strains 373 and 379 had hundreds-fold greater *in vitro* activity per cell (high activity) than strains 370, 374 and L (low activity) (Table 1).

The gross difference in enzyme activity among strains with similar DMSP titres provides a natural test for the role of this reaction as a herbivory deterrent. We selected protozoan grazers because microzooplankton are now recognized to be the dominant herbivores in marine planktonic systems[7], especially for prey $<20\,\mu m$ in diameter, such as *E. huxleyi*. Predators tested included the dinoflagellate *O. marina* and protozoa isolated from Oregon coastal waters, including a urotrichous ciliate and several flagellates. We conducted three types of grazing experiments: (1) single-prey trials with different *E. huxleyi* strains to look for gross toxicity, differences in grazing rates, or differences in predator growth rates; (2) feeding selectivity trials with prey mixtures of different *E. huxleyi* strains; and (3) feeding selectivity trials with prey mixtures of single *E. huxleyi* strains and other non-DMSP-containing prey species.

Some predators, notably *O. marina* and one flagellate, could ingest and grow on all five *E. huxleyi* strains (Fig. 1a), and DMS production during grazing reflected prey-strain DMSP lyase activity and was a reliable tracer of ingestion. DMS was not produced until *E. huxleyi* cells were actually ingested[2], suggesting that acrylate formation occurs inside predator food vacuoles. Because grazing on the high-activity strains 373 and 379 converted roughly 60% of total prey DMSP to DMS, we estimated production of ~70 mM acrylate inside predator food vacuoles per ingested cell. Such concentrated acrylate may have antimicrobial activity[5,8], and multiple prey were often observed together within a vacuole. Despite this, *O. marina* showed few negative effects. Clearance and grazing rates on high-activity strains were sometimes lower than for low-activity strains[2], but this was variable and might have been influenced by predator preconditioning.

However, grazers that tolerated high-activity strains avoided them when offered a choice of prey. This was evident in mixed-strain

Table 1 Characteristics of *E. huxleyi* strains examined

	Low activity			High activity	
Strain and origin*	370 North Sea	374 Gulf of Maine	L English Channel	373 Sargasso Sea	379 English Channel
Growth characteristics†					
Growth rate (d^{-1})	0.80	0.82	0.82	0.72	0.67
Diameter (μm)	4.4	4.9	4.7	5.3	4.8
DMSP (mM)	113	74	75	107	110
In vitro DMSP lyase‡					
Activity (fmol DMS $cell^{-1}$ min^{-1})	0.01	<0.01	0.03	12.5	6.1
NaCl requirement (mM)	1,000	–	1,000	–	–
pH optimum	>8	5	5–6	6	6

All strains were axenic as shown by DAPI staining and plating out on 1% peptone seawater–agar, and none lithified under high-nutrient culture conditions. *In vitro* enzyme preparations were similar to those described in ref. 2 but were run with saturating levels of substrate (M.S. *et al.*, in preparation).
* Strain designations are from Provasoli-Guillard National Center for the Cultivation of Marine Phytoplankton (West Boothbay Harbor, Maine, USA) except for strain L. Synonyms: 370 (451B : F451), 373 (BT-6 : CSIRO-CS-57), 374 (89E) and 379 (92A : P-92A).
† Conditions: 15 °C, 80 $\mu mol\ m^{-2}\ s^{-1}$, 18 h light : 6 h dark cycle, f/2 medium.
‡ Conditions: 20 mM DMSP, 30 °C, NaCl optimum, 160 mM citric acid/phosphate buffer, pH 6.

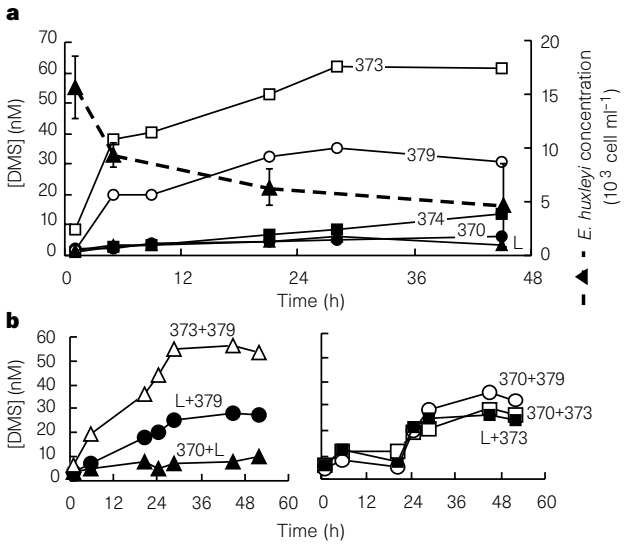

Figure 1 DMS production during grazing by *O. marina* on single (**a**) and multiple (**b**) *E. huxleyi* strains. Predators were maintained on the non-DMSP-producing chlorophyte *Dunaliella tertiolecta* and starved before the addition of *E. huxleyi* taken from exponentially growing batch cultures. Experiments were conducted in 250- or 500-ml polycarbonate bottles in 80 $\mu mol\ m^{-2}\ s^{-1}$ at 15 °C (16 h light : 8 h dark cycle). DMS was sampled and analysed by gas chromatography, and cells were counted by epifluorescence microscopy[2]. Single-strain initial prey concentrations were 15,000 ml^{-1}; grazing rates were similar on all five strains (mean and s.d. are shown). Multistrain initial prey concentrations were 20,000 ml^{-1} (10,000 each strain) and 500 predators ml^{-1}. **a**, DMS production reflects *E. huxleyi* strain DMSP lyase activity (Table 1). **b**, Left, DMS production reflects grazing on pairs of high-activity strains (373 and 379) or low-activity strains (370 and L). **b**, Right, in three of four mixtures of high- and low-activity pairs, DMS production was low for the first 24 h, indicating selective feeding on the low-activity strain, then rose rapidly as predators switched over to the high-activity strain.

feeding trials in which we could not distinguish visually between *E. huxleyi* strains, but used DMS production to detect grazing of high-activity strains (Fig. 1b, right). Feeding discrimination against high-activity strains was also demonstrated in mixed-species trials, in which we found avoidance of high-activity *E. huxleyi* strains but ingestion of low-activity strains comparable to other prey species (Fig. 2). In some instances, non-DMSP-containing prey species were consumed preferentially and completely before high-activity *E. huxleyi* prey were grazed[2].

Other predators, including the urotrichous ciliate and one gymnodinoid flagellate, grew on low-activity *E. huxleyi* strains, but could not subsist on either high-activity strain (data not shown). DMS production during grazing on high-activity strains was minimal, suggesting that there was little ingestion and lysis of prey. Short-term uptake experiments showed that, in the case of the urotrichous ciliate, all *E. huxleyi* strains were captured readily by ciliate feeding currents, but prey seemed to be ingested selectively, with strong discrimination against high-activity strains (Table 2). Active postcapture prey rejection has been observed previously for tintinnid ciliates such as *Favella*[9,10].

Taken together these results suggest that the reaction may function as a deterrent against herbivory, and imply that some DMS production may be a by-product of algal chemical defence. A defensive role for acrylate produced by unicellular phytoplankton has been suggested previously[11], but we believe this to be the first evidence that it may achieve this through a grazing-activated mechanism. Such DMS production may be widespread among marine algal taxa: the release of DMS following macroalgal tissue damage has long been known[6], and injury-activated discharge of volatiles from the DMSP-synthesizing dinoflagellate *Amphidinium carterii* that might be involved in zooplankton deterrence has been observed[12].

Relatively little study has been given to specific mechanisms of chemical defence in planktonic microorganisms. Many bloom-forming phytoplankton taxa, especially cyanobacteria, prymnesiophytes and 'red-tide' dinoflagellates, are acutely toxic to meso- and microzooplankton[13–15], and ingestion of a few prey cells might suppress predator activities enough to confer a selective advantage to the clonal prey population. In contrast, the products of this reaction do not seem to be acutely toxic, at least to some grazers. However, *E. huxleyi* cells need not be fully ingested and lysed to deter predators, as high-activity strains were often avoided or rejected, in some cases shortly after capture (Table 2). Influencing predator selectivity may shift grazing pressure to other prey species and reduce competition for nutrients. It is well established that phagotrophic protozoa are highly discerning feeders, using chemosensory cues as well as prey morphology and motility to select[16] or reject[10,17] their prey. Such discrimination suggests that this reaction may play a signal role, possibly by generating DMS and acrylate gradients during prey handling which are sensed by predators, but which do not produce detectable bulk DMS. Preliminary video analysis suggests that *O. marina* reacts to gradients of acrylate with an increased rate of change in direction (R.C.D.I., deg s^{-1}; 10^{-5} M acrylate R.C.D.I. = 279 ± 56, $n = 10$; control R.C.D.I. = 181 ± 15, $n = 80$; change in mean linear speed not significant; R. K. Zimmer-Faust, personal communication), consistent with a repulsion response[18].

Plants that manufacture constitutive defence toxicants are at risk of self-toxicity, and also use metabolic energy producing compounds that may not be needed. Many macrophytes avoid these risks by storing toxicants in specialized organelles[19] or by producing inducible toxins in response to predation[20]. *E. huxleyi* avoids the self-toxicity of acrylate by maintaining it as DMSP, which is not only non-toxic[21], but serves other useful cellular functions as an osmolyte[22], a cryoprotectant[23] and a methyl donor[24]. Cellular energy is therefore invested in a multi-use molecule that can be modified for instant defence whenever it is needed. Examples of marine defence compounds that serve additional ecological roles include diterpene alcohols, produced by the phaeophyte *Dictyota menstrualis*, which function as anti-fouling substances[25], and tetrodotoxin, produced by the puffer fish *Fugu niphobles*, which also serves as a sex pheromone[26]. DMSP may be an example of a multifunctional defence precursor.

In addition to their roles in this proposed defence system, micromolar levels of DMS, DMSP and acrylate may act as chemoattractants for marine bacteria[27,28] and protists[29]. These compounds may also function as chemical semaphore in trophic cascades: DMS emitted to the atmosphere seems to be a foraging cue for Antarctic procellariiform seabirds[30]. We suggest that further roles

Table 2 Short-term uptake experiment

Treatment	Prey strain	*n**	Ingested prey per predator
High-activity strains	373	22	1.8
	379	23	0.1
Negative control	fluorescent spheres	61	0.0
Low-activity strains	370	8	5.8
	374	11	3.1
	L	10	2.9
Positive control	*Chroomonas*	15	2.5

Results are for selective ingestion of low-activity *E. huxleyi* strains following capture by a urotrichous ciliate. After 1-h incubations, preserved samples were stained with DAPI and filtered and the number of ingested prey cells per predator was observed by epifluorescence microscopy. In all instances, captured prey were seen attached to the exterior of ciliates (average, 2.9 prey per predator). Fluorescent spheres (PolySciences 3.46 μm fluoresbrite carboxylate) and the chlorophyte *Chroomonas* served as negative (captured but not ingested) and positive (captured and rapidly ingested) controls. Although captured by ciliates, high-activity *E. huxleyi* strains seemed to be ingested at much lower rates than low-activity strains, consistent with low DMS production during grazing and inability of ciliates to grow on high-activity strains as the sole food source.
* Number of predators examined.

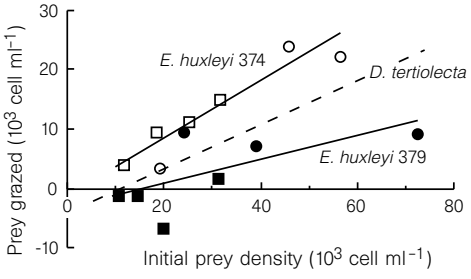

Figure 2 *O. marina* feeding selectivity on mixtures of *Dunaliella tertiolecta* and either high- or low-activity *E. huxleyi* (strains 379 or 374), with prey ratios ranging from 20% to 80% *E. huxleyi*. Combined results from two experiments with initial total prey densities of 50,000 ml^{-1} (squares) or 100,00 ml^{-1} (circles) are shown; *O. marina* densities were 250–500 ml^{-1}. Bottles were incubated in the dark at 15 °C. At time zero and after 4 or 6 h, samples were fixed with alkaline Lugol's and ungrazed prey were counted by haemocytometer; grazed prey were calculated by difference and are plotted against initial prey concentrations. High-activity strain 379 (filled symbols) was grazed at much lower rates than low-activity strain 374 (open symbols), and within each experiment, uptake rates of strain 379 did not increase with increasing prey density (filled symbols). Grazing of *Dunaliella* (broken line; data points omitted for clarity) was consistent across treatments and between experiments, suggesting that presence of high-activity *E. huxleyi* does not reduce ingestion of other prey.

for these compounds as signals in marine trophic interactions remain to be discovered. □

Methods

Response of *O. marina* to acrylate gradients. *O. marina* cell suspensions were prepared on a microscope slide without a coverslip. Neutralized acrylic acid (Aldrich) was injected by micropipette to generate spatial gradients (equilibrium concentration, 10^{-5} M), and the cell response was observed for 30 s by phase-contrast microscopy and recorded on videotape using a NEC TI-23A CCD camera[28]. A focal plane several millimetres from the surface of the slide was chosen to minimize wall effects. Cell swimming behaviour was analysed by a computer-assisted video motion analysis system (Motion Analysis model VP 110 with ExpertVision software), and rate of change in direction (R.C.D.I., deg s^{-1}), an analogue for angular velocity (turning frequency), and linear speed ($\mu m\ s^{-1}$) were calculated for 10–80 individual digitized cell paths. Control treatments were prepared with additions of seawater.

Received 17 January; accepted 10 April 1997.

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Acknowledgements. We thank B. Palenik for *E. huxleyi* strain L; L. Fessenden for protozoan isolates; R. Zimmer-Faust for the video motion analysis of *O. marina*; and E. Sherr for comments on the manuscript. This work was supported by grants from NASA and the European Community.

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Effects of sea-ice extent and krill or salp dominance on the Antarctic food web

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Krill (*Euphausia superba*) provide a direct link between primary producers and higher trophic levels in the Antarctic marine food web[1–6]. The pelagic tunicate *Salpa thompsoni* can also be important during spring and summer through the formation of extensive and dense blooms[6–9]. Although salps are not a major dietary item for Antarctic vertebrate predators[7,10], their blooms can affect adult krill reproduction and survival of krill larvae. Here we provide data from 1995 and 1996 that support hypothesized relationships between krill, salps and region-wide sea-ice conditions[11,12]. We have assessed salp consumption as a proportion of net primary production, and found correlations between herbivore densities and integrated chlorophyll-*a* that indicate that there is a degree of competition between krill and salps. Our analysis of the relationship between annual sea-ice cover and a longer time series of air temperature measurements[12,13] indicates a decreased frequency of winters with extensive sea-ice development over the last five decades. Our data suggest that decreased krill availability may affect the levels of their vertebrate predators. Regional warming and reduced krill abundance therefore affect the marine food web and krill resource management.

In the Elephant Island area near the Antarctic Peninsula (Fig. 1) both krill and salps exhibited large interannual abundance fluctuations between 1976 and 1996 (Table 1). In general, larger krill population densities were encountered during the earlier part of the data set; densities from 1984–85 until 1995–96 were on average an order of magnitude less than during previous years. Randomization tests on an analysis of variance[14] indicate that differences in abundance between these two periods are statistically significant and support lower krill abundance in recent years. In contrast, the highest salp densities occurred during three summers within the 1984–96 period.

Interannual fluctuations in krill abundance result largely from variations of year-class success: the highest population densities (for example, 1981–82) result from extremely good recruitment from the previous spawning season[11]. Relatively low densities in 1984–85, 1990–91 and 1994–95 followed two or three years of poor and intermediate krill recruitment. Good recruitment is positively correlated with early seasonal spawning (in December–February), and both are positively correlated with extensive sea-ice in the Antarctic Peninsula region the preceding winter ($n = 17$; Kendall's $T = 0.40$, $P < 0.05$; $n = 12$, $T = 0.48$, $P < 0.05$). Poor recruitment and late spawning (in March) are associated with reduced regional sea-ice formation.

In contrast to krill, salp abundance is negatively correlated with extensive sea-ice. Unlike krill, which have lifespans of more than 5 years[15], *S. thompsoni* live less than one year, and their fluctuations in abundance reflect annual variability in conditions promoting