Dimethyl sulfide acts as eat-me signal during microbial predator-prey interactions in the ocean

Adva Shemi
The Weizmann Institute of Science
Uria Alcolombri
ETH Zurich https://orcid.org/0000-0003-3561-5091
Daniella Schatz
Weizmann Institute of Science https://orcid.org/0000-0003-2740-8391
Viviana Farstey
The Interuniversity Institute for Marine Sciences
Ron Rotkopf
Weizmann Institute of Science https://orcid.org/0000-0001-9503-7348
Shifra Ben-Dor
Weizmann Institute of Science https://orcid.org/0000-0001-9604-1939
Miguel Frada
The Interuniversity Institute of Eilat https://orcid.org/0000-0001-8287-0795
Dan Tawfik
Weizmann Institute of Science https://orcid.org/0000-0002-5914-8240
Assaf Vardi (assaf.vardi@weizmann.ac.il)
Weizmann Institute of Science https://orcid.org/0000-0002-7079-0234

Research Article

Keywords: Oceanic Carbon and Sulfur Cycles, Bloom Events, Chemoattraction, Prey Cells, Emiliania huxleyi, Oxyrrhis marina

Posted Date: January 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-139243/v1

Version of Record: A version of this preprint was published at Nature Microbiology on October 25th, 2021.
See the published version at https://doi.org/10.1038/s41564-021-00971-3.

Dimethyl sulfide acts as eat-me signal during microbial predator-prey interactions in the ocean

Adva Shemi[1], Uria Alcolombri[1,2], Daniella Schatz[1], Viviana Farstey[3], Ron Rotkopf[4], Shifra Ben-Dor[4], Miguel J. Frada[3,5], Dan S. Tawfik[6], Assaf Vardi[1]*

[1]Department of Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot 7610001, Israel.

[2]Institute of Environmental Engineering, Department of Civil, Environmental and Geomatic Engineering, ETH Zurich, Zurich, Switzerland.

[3]The Interuniversity Institute for Marine Sciences, Eilat 8810302, Israel.

[4]Department of Life Sciences Core Facilities, Weizmann Institute of Science, Rehovot 7610001, Israel.

[5]Department of Ecology, Evolution and Behavior, Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, Jerusalem 91904, Israel.

[6]Department of Biomolecular Sciences, Weizmann Institute of Science, 7610001 Rehovot, Israel.

*Corresponding author. E-mail: assaf.vardi@weizmann.ac.il (A.V.)

Summary

Phytoplankton are key components of the oceanic carbon and sulfur cycles [1]. During bloom events, some species can emit massive amounts of the organosulfur volatile dimethyl sulfide (DMS) to the atmosphere, where it can modulate aerosol formation and affect climate [2,3]. In aquatic environments, DMS plays an important role as a chemical signal mediating diverse trophic-level interactions. Yet its role in microbial predator-prey interactions remains elusive with contradicting evidence for its role in algal chemical defense and in grazer's chemoattraction to prey cells [4-6]. Here, we investigated the signaling role of DMS during zooplankton-algae interactions by genetic and biochemical manipulation of the algal DMS-generating enzyme (Dimethylsulfoniopropionate lyase, DL) from the bloom-forming alga *Emiliania huxleyi* [7]. We inhibited DL activity in live *E. huxleyi* cells by the novel DL-inhibitor 2-bromo-3-(dimethylsulfonio)-propionate (Br-DMSP) [8], and overexpressed DL in the model diatom *Thalassiosira pseudonana*. We showed that algal DL activity did not serve as anti-grazing chemical defense, and paradoxically enhanced grazing by the model microzooplankton *Oxyrrhis marina* and other micro- and mesozooplankton, including ciliates and copepods. Consumption of algal prey with induced DL activity also promoted *O. marina's* growth. Overall, our results demonstrate that DMS-mediated herbivory may be ecologically important and prevalent

during prey-predator dynamics in oceanic ecosystems. The role of algal DMS acting as eat-me signal to grazers revealed here raises fundamental questions regarding the retention of its biosynthetic enzyme through the evolution of dominant bloom-forming phytoplankton in the ocean.

The ocean is the main source of atmospheric dimethyl sulfide (DMS), with an estimated flux of 13 to 37 Tg S $year^{-1}$ [1,9]. The gaseous DMS is a precursor of sulfate aerosols, mediating cloud condensation and promoting a cooling effect on Earth's climate [1,2,10]. DMS is produced by marine phytoplankton through cleavage of the zwitterionic organosulfur compound dimethylsulfoniopropionate (DMSP) by the DMSP lyase (DL) enzyme [11-13]. In phytoplankton, DMSP was proposed to act as an intracellular osmoprotectant, antioxidant and energy dissipation factor [14-16]. In addition, both DMSP and DMS were also shown to act as infochemicals (chemical cues that facilitate communication), mediating virulence or symbiosis by bacteria (for DMSP) [17,18], and promoting prey-predator interaction and parasite infection (for DMS) [19]. Notably, marine predators including invertebrates, sea birds and whales, can sense DMS and track it to find areas of high productivity [20-24]. Although DMS is highly produced via DMSP lyase (DL) in grazed algal cells [25], its signaling role during phytoplankton and zooplankton interactions is still unresolved. Two contradictory roles were proposed for DMS and its precursor DMSP, as anti-grazing defense [4,26] and pro-grazing chemoattractant [6]. We aimed to unravel this debate by combining functional genomics and physiological approaches to assess the impact of DMS during zooplankton-algae interactions. We used the bloom-forming cosmopolitan coccolithophore *Emiliania huxleyi* (Prymnesiophyceae) from which a DL enzyme was recently isolated (Alma1) [7,27-29]. The full characterization of Alma1 and its conservation among diverse taxa[7] provided a unique opportunity to unravel its ecophysiological role. We directly manipulated DL activity in prey cells in order to determine the influence of DMS on grazing dynamics by zooplankton, focusing on microzooplankton. Microzooplankton (20-200 µm) are key marine herbivores that remove 49-77% of the photosynthetic biomass daily [30-32] and in fact DMS is often produced via DL activity upon disruption of algal cells during grazing [25]. Here, we demonstrate that DMS enhances grazing efficiency by micro- and mesozooplankton, and further discussed the ecological and evolutionary implications of DMS-mediated herbivory in the ocean.

We firstly examined the effect of DMS emission on grazing dynamics between *E. huxleyi* high DL strain CCMP373 and *Oxyrrhis marina*, a heterotrophic dinoflagellate commonly used as a model microzooplankton [33]. In order to directly link between algal DMS and grazing efficiency, we applied a novel selective DL-inhibitor, 2-bromo-3-(dimethylsulfonio)-propionate (Br-DMSP) which blocked *E. huxleyi*'s DL activity *in-vitro* [8]. During short-term grazing experiments in the presence of 0.2 µM Br-DMSP, DMS release to the culture medium from grazed cells decreased by ~50%, compared to control

culture (Fig. 1a,b and Supplementary Fig. 1). Imaging of the grazer's food vacuole by florescence microscopy revealed that inhibition of DL activity led to > 50% reduction in prey content as compared to ingestion of control cells after 0.5 and 3 h of predation (Fig. 1c,d). Furthermore, the ingestion rate on Br-DMSP treated cells declined by ~74% (~35 *E. huxleyi* grazer^{-1} day^{-1}) as compared to untreated control cells (~133 *E. huxleyi* grazer^{-1} day^{-1}), based on quantification of prey clearance by flow-cytometry (Fig. 1e). Notably, even partial inhibition of DL activity led to pronounced reduction in grazing rate. As a control for a possible indirect effect of Br-DMSP, we used the green alga *Dunaliella tertiolecta* as prey since it lacks the DL enzyme. Indeed, no change was detected in the consumption of *D. tertiolecta* by *O. marina* in the presence of Br-DMSP (Supplementary Table 1).

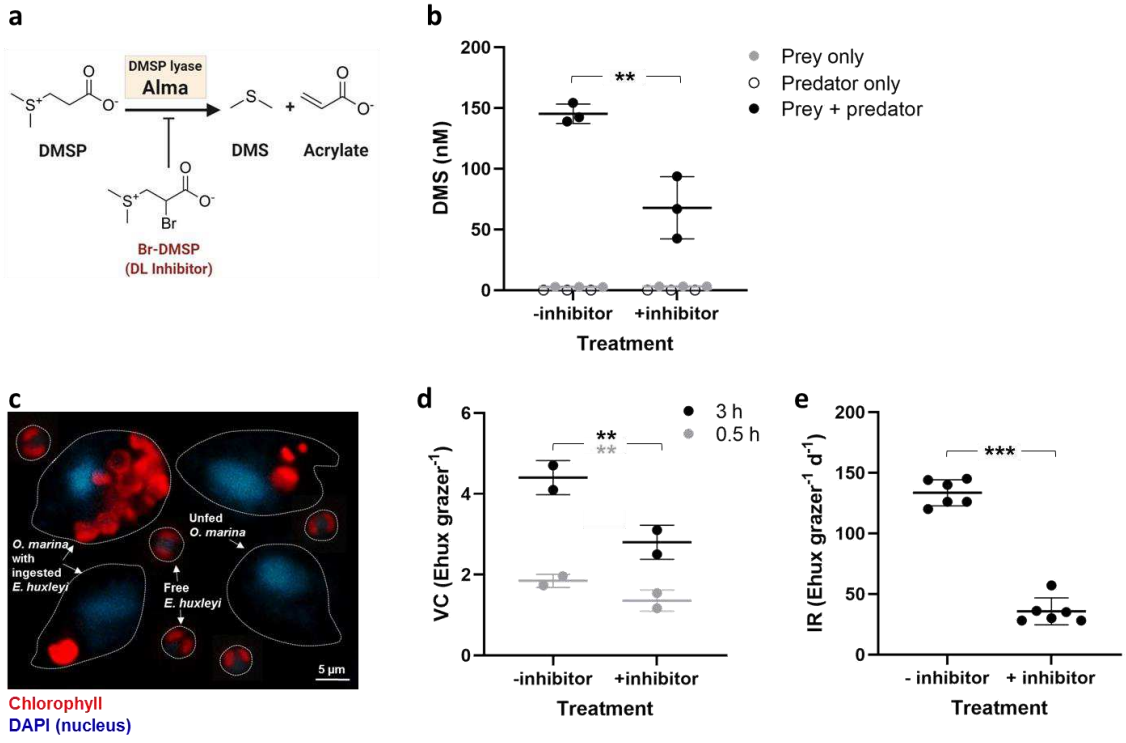

Fig 1. Reduced DMSP lyase (DL) activity of *E. huxleyi* impaired grazing by *O. marina*. **a**, The DMSP lyase Alma cleaves DMSP to DMS and acrylate. The inhibitor Br-DMSP blocks DL activity by forming an enzyme-inhibitor covalent bond at the active site, thus inhibiting the formation of DMS and acrylate [8]. **b**, The short-term (~1 h) effect of 0.2 µM Br-DMSP on DMS production *in-vivo* by *E. huxleyi* 373. Horizontal lines represent the mean ± SD; $n = 3$; $P < 0.008$ (Student's T-test). **c**, Fluorescence micrograph collage of *O. marina* with free and ingested *E. huxleyi* cells at t=30 minutes from prey addition. **d**, The effect of 0.2 µm Br-DMSP on the food vacuole content (VC, *E. huxleyi* 373 cells per grazer) of *O. marina* at t=0.5 and 3 h. A total of 800 *O. marina* cells were examined, 100 cells per biological replicate. Horizontal lines represent the mean ± SD; $n = 2$; $P < 0.003$ (generalized linear mixed model). **e**, The effect of 0.2 µm Br-DMSP on ingestion rate (IR) of *E. huxleyi* 373 cells (Ehux) by *O. marina,* quantified by flow cytometry and based on prey removal from the medium during 50 min. Approximately 2,500 cells were analyzed per sample. Horizontal lines represent the mean ± SD; $n = 6$; $P < 0.0001$ (Student's T-test).

E. huxleyi cells produce minimal amounts of DMS during exponential growth (Fig. 1b and Supplementary Fig. 2), probably because the DL and its substrate DMSP are segregated in different subcellular compartments [34]. However, algal senescence and interactions with viruses[11,35], and as we show here with grazers (Fig. 1b and Supplementary Fig. 2), trigger DL activity and significant DMS production, probably as a result of damaged cellular membranes and mixing of DL and DMSP [36-38]. Here, inhibition of the prey's DL activity suppressed grazing efficiency by *O. marina*. Hence, we hypothesize that increasing DL activity will enhance the grazing response by microzooplankton. Exogenous application of DMS or acrylate (the products of DL activity, Fig. 1a) to *E. huxleyi* strain CCMP2090, which has low DL activity and produces no detectable DMS, did not significantly alter the grazing response by *O. marina* (Supplementary Fig. 3). It is likely that bulk addition failed to mimic the natural spatiotemporal DMS gradients in seawater and in the cell's microenvironment. Thus, the cellular release of infochemicals during microbial interactions may be critical for understanding their signaling role. In order to directly address the biological context of DMS release, genetic manipulation of the DL enzyme in prey cells is critical. As *E. huxleyi* and other major DMS producing species are yet to be genetically amendable, we conducted heterologous expression of the *E. huxleyi* DL gene in the ecologically important diatom species *Thalassiosira pseudonana* [39-42]. *T. pseudonana* is similar in size to *E. huxleyi* (3-5 µm) and synthesizes DMSP (~10 mM cell^{-1}) [43], but lacks a DMSP lyase and does not produce DMS [7,43]. Thus, transgenic *T. pseudonana* can serve as a tractable model system to study the direct signaling role of DMS during grazing interactions. We overexpressed the DMSP lyase encoding gene from *E. huxleyi* 373 (*alma1*) in *T. pseudonana* and fused it to GFP at the C-terminus in order to preserve a putative target sequence at the N-terminus (Fig. 2a,b and Supplementary Fig. 4). The heterologous expression of the *E. huxleyi* DL in a transgenic *T. pseudonana* line, called Tp DL-GFP, was verified by western blot using an antibody raised against the Alma1 protein (Supplementary Fig. 4b). DL enzymatic activity in Tp DL-GFP cell lysate (8.8 ± 2.9 fmol DMS cell^{-1} min^{-1}, Fig. 2c) was comparable to *E. huxleyi* high DL strains [7,44]. Both the morphology and growth dynamic of the transgenic cells were not affected by the expression of Alma1-GFP (Supplementary Fig. 5). Live Tp DL-GFP cells produced DMS levels comparable to those found in seawater samples (~9 nM) [45,46]. We did not detect, however, a significant increase in DMS concentration during grazing by *O. marina* in Tp DL-GFP (Fig. 2d,e). The *in-vivo* DMS production in grazed Tp DL-GFP cells was predicted to be limited by the considerably lower intracellular levels of DMSP in *T. pseudonana* relative to *E. huxleyi* (Fig. 2c).

In order to investigate the direct effect of enhanced DL activity on grazing efficiency, we conducted feeding experiments with *O. marina* using Tp DL-GFP cells as prey. Grazers fed with Tp DL-GFP accumulated significantly more prey in their food vacuole over time (Fig. 2f). In accordance, the ingestion rate on Tp DL-GFP cells was ~2-4 fold higher than on wild type cells (24 ± 5 and 6 ± 4 *T. pseudonana* grazer^{-1} day^{-1} during the first 30 min,

respectively. Fig. 2g and Supplementary Fig. 6). Furthermore, ingestion by *O. marina* was also elevated during grazing *T. pseudonana* expressing only DL (Tp DL), while ingestion of *T. pseudonana* expressing only GFP (Tp-GFP) was similar to wild type (Supplementary Fig. 7c and 6b). This eliminates the possibility that the prey's GFP fluorescence modulated grazing activity, and indicates that enhanced grazing on Tp DL-GFP was solely due to the induced DMS production. Intriguingly, daily feeding on Tp DL-GFP cells significantly improved *O. marina* growth, as compared with a wild type diatom diet (Fig. 2h). Thus, DL activity in prey cells not only expedited grazing dynamics, but also enhanced the grazer's growth on a longer timescale. This may be attributed to general higher consumption of algal biomass and to specific assimilation of the diatom's DMSP as an essential nutritious sulfur [47].

In order to further examine if *O. marina* preferentially ingested DMS-producing cells, or alternatively whether the diffusible DMS may elicit a general unselective grazing activity, we conducted double-prey competition trials. The total ingestion rate of cells in a prey mixture containing Tp DL-GFP and wild type cells (1:1) was over ~2 fold higher as compared to ingestion on a mixture of wild type cells with Tp GFP (Fig. 2i). This indicated that DMS formed by DL activity in grazed cells can increase the grazing likelihood of neighboring cells with no DL activity. Therefore, DMS can potentially enhance grazing within natural algal communities where only a fraction of the cells secretes DMS. Indeed, during the double-prey competition trials there was no significant difference between the uptake of each prey type (wild type or Tp DL-GFP), and no preference by *O. marina* towards Tp DL-GFP cells (Fig. 2i). Thus, *O. marina* grazing on Tp DL-GFP was non-selective, and may be attributed to dissolved chemical signals such as DMS, which acts as an appetizing infochemical enhancing general grazing activity.

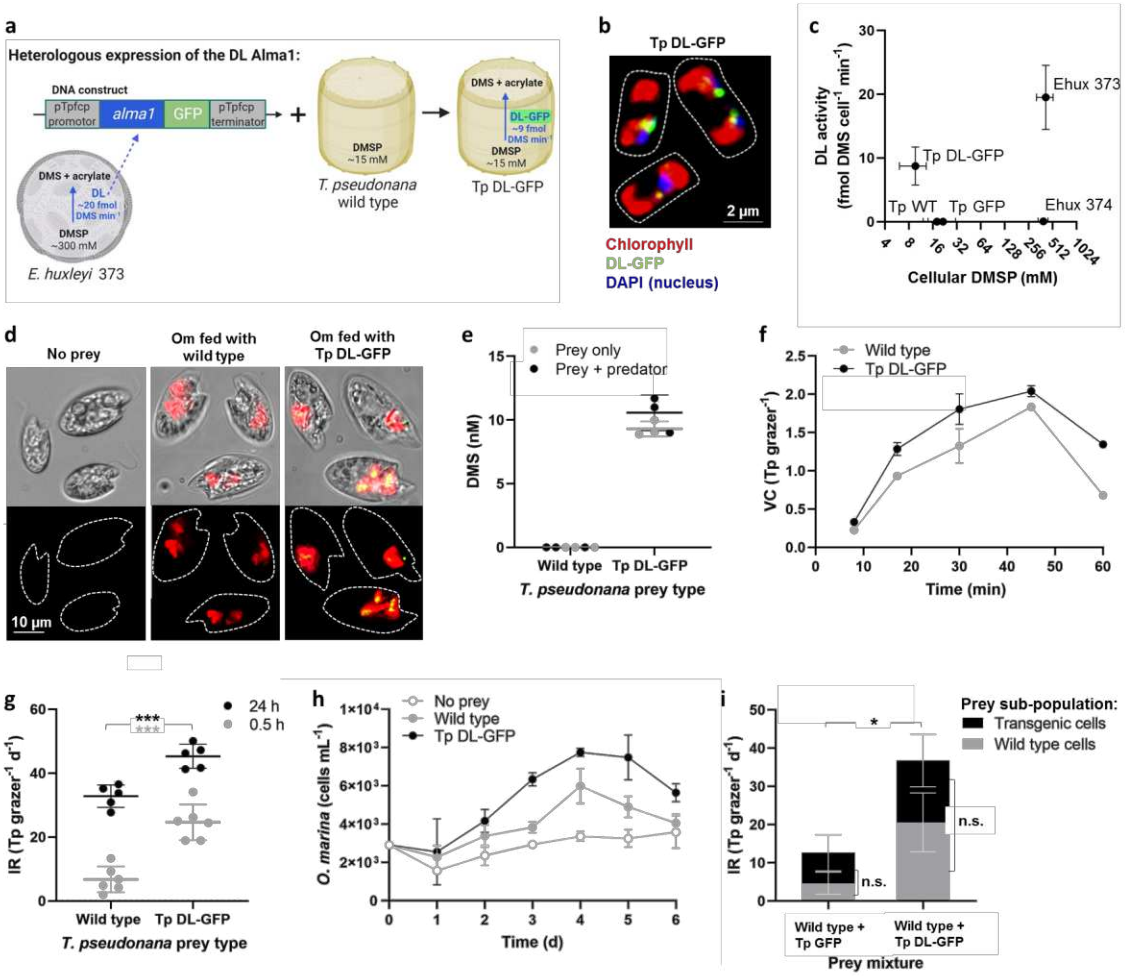

Fig. 2. Overexpression of DMSP lyase (DL) in algal prey cells enhanced grazing efficiency and growth by *O. marina*. **a**, Transgenic Tp DL-GFP cells were generated by heterologous expression of the *alma1* gene from *E. huxleyi* 373, fused to GFP, in wild type *T. pseudonana* cells. **b**, Confocal microscopy of Tp DL-GFP cells. The Alma-GFP protein can be observed as a punctate green signal in close proximity to the chloroplast. **c**, Intracellular DMSP content and DL activity in *E. huxleyi* (Ehux) strains 373 and 374, as well as in *T. pseudonana* wild type (Tp WT), Tp GFP and Tp DL-GFP. We quantified DL activity *in vitro* by measuring DMS generation in cell lysates following addition of 10 mM DMSP. Values are mean ± SD; n = 3-4. For DMSP, results from two independent measurements were averaged. **d**, Bright field and fluorescence micrographs of *O. marina* (Om) with ingested *T. pseudonana* cells. **e**, DMS emission during grazing by *O. marina* on Tp DL-GFP cells. Horizontal lines represent the mean ± SD; n = 3. **f**, Prey uptake curve based on vacuole content (VC) analysis during the first hour of grazing interaction. Values represent the mean ± SD. n = 2-4. A total of 800 grazers were analyzed for t= 8 and 17 min, and 400 grazers for t=30, 45, and 60 min. The mean VC values during 60 min were significantly different between the two prey types, $P < 0.0001$ (generalized linear mixed model). **g**, Ingestion rate (IR) analysis based on quantification of *T. pseudonana* (Tp) cell removal from the medium during 0.5 and 24 h of incubation with *O. marina*. Horizontal lines represent the mean ± SD; n = 6. $P < 0.0001$ (Student's T-test). **h**, Growth dynamics of *O. marina* under starvation conditions or daily feeding with Tp DL-GFP or wild type cells; n = 4. The growth in the Tp DL-GFP treatment was significantly faster than wild type, $P < 0.0012$ (mixed effects model). **i**, Double-prey competition trials based on IR analysis of two prey types introduced to *O. marina* in a 1:1 ratio, as measured over 2 h. Values are mean ±

SD; n = 3-4 in two independent experiments. $P < 0.001$ when comparing the total IR between the two prey mixtures. When comparing the specific IR on each prey sub-population within a mixture, $P > 0.32$, n.s.- not significant (Student's T-test).

In order to explore the prevalence of DL-mediated herbivory, grazing assays were conducted with microzooplankton from different taxa, size and feeding modes (i.e. direct engulfment or filter feeding. Fig. 3a and Supplementary Table 2). The fold-change between the grazing rate on Tp DL-GFP and Tp GFP cells, which differ only by their DL activity, was calculated for each grazer species (if the fold-change >1, DL-GFP cells were consumed faster than Tp GFP cells). As thecate dinoflagellates and ciliates are dominant consumers of *E. huxleyi* and are frequently observed during *E. huxleyi* blooms [48], short-term feeding experiments were conducted with the dinoflagellate *Gyrodinium dominans* and the ciliate *Strombidium* sp. as grazers. Like *O. marina*, *Strombidium* sp. consumed Tp DL-GFP prey faster than Tp GFP (~7-fold, Fig. 3a). In contrast, *G. dominans* grazed similarly on both prey types. Next, we assessed grazing by larger mesozooplankton (0.2–2 mm), on the transgenic diatoms. Cultured *Artemia salina* (brine shrimp) consumed Tp DL-GFP twice as fast as Tp GFP cells. Furthermore, wild calanoid copepods of the species *Pleuromamma indica* consumed Tp DL-GFP ~28 times more than the Tp GFP control (Fig. 3a). Notably, none of the tested predators exhibited a negative grazing response toward Tp DL-GFP prey (fold-change < 1). While DL-mediated herbivory may be species-specific, it is clearly not specific to *O. marina* alone, but shared by several important marine herbivores.

To further assess the ecological significance of DMS-mediated herbivory, we used mixed native microzooplankton assemblages from the Gulf of Aqaba in the northern Red Sea. These assemblages included a wide diversity of heterotrophic and mixotrophic species within the 5-200 µm size range, such as tintinnid ciliates, dinoflagellates, nauplii and small copepods (Supplementary Fig. 8,9). Most natural cells < 5µm were excluded during net-tow harvesting. The native grazers were subsequently fed with Tp DL-GFP or Tp GFP prey cells for 24 h. The uptake of the transgenic diatom cells by the natural grazers was confirmed by microcopy and quantified as grazing rate (g, day^{-1}) (Fig. 3b,c and Supplementary Fig. 10,11). Grazing rates in four independent experiments conducted during 2017-2020 were variable, perhaps due to changes in the taxonomic assortment of the grazer communities in each experimental flask. Nonetheless, in 3 out of 4 experiments, the native grazers removed the Tp DL-GFP prey cells significantly faster than the Tp GFP control. The highest average grazing rate was detected at February 2020, where Tp DL-GFP cells were removed on average ~10 times faster than Tp GFP control cells (0.22 ± 0.12 day^{-1}, Fig. 3b). Overall, most of the cultured and wild grazers tested exhibited a varied degree of enhanced grazing on DMS-producing prey, indicating that DMS facilitates herbivory across a wide variety of zooplankton taxa representing different size and feeding strategies. To our knowledge, this is the first study to demonstrate that DL activity in algal prey cells is coupled to enhanced grazing activity by zooplankton.

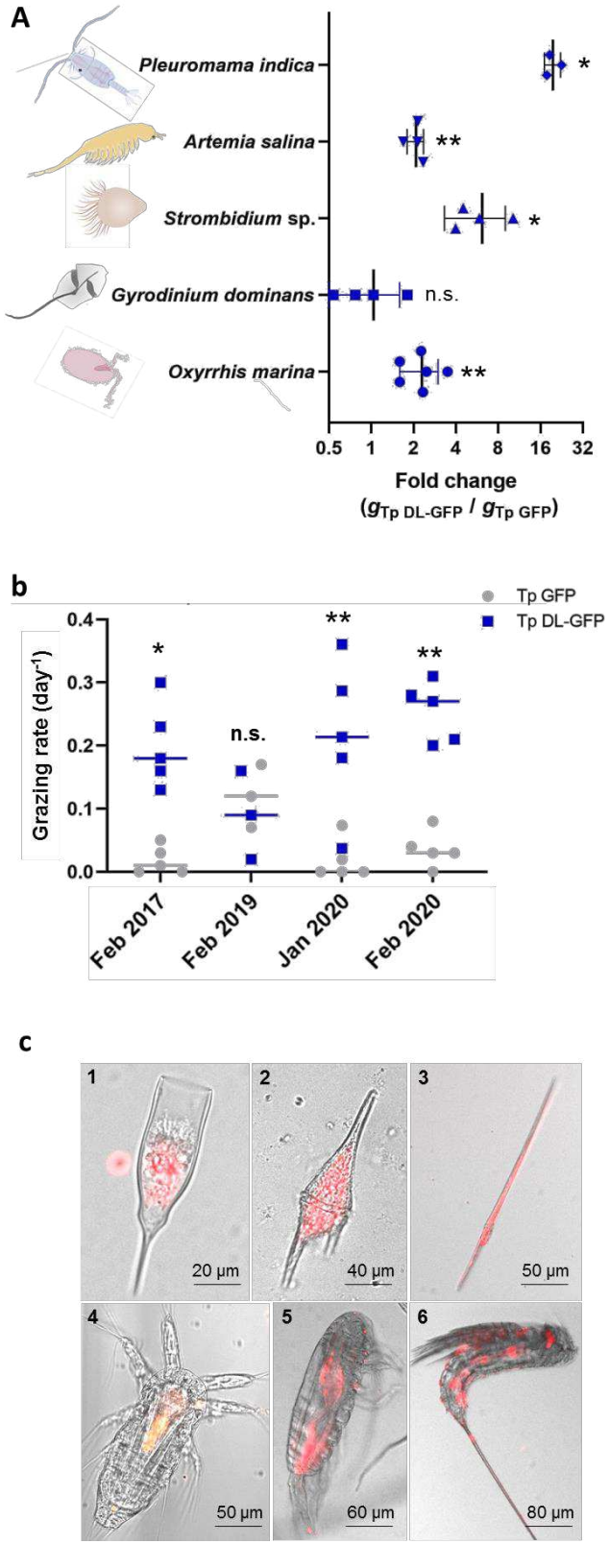

Fig. 3. Induced DMS production in algal prey enhanced grazing by diverse zooplankton. a, The grazing response of diverse zooplankton fed with Tp DL-GFP cells. Tp GFP cells were used

as control prey. The fold change in grazing rate (g, as assessed by monitoring free prey cells using flow cytometry) on each prey type was calculated, where fold change > 1 indicates faster grazing on Tp DL-GFP cells. Vertical lines represent the mean ± SD; n = 3 for *P. indica*; 4 for *A. salina*, *G. dominans* and *Strombidium* sp.; 6 for *O. marina*. n.s.- not significant, $P > 0.897$; $*P < 0.011$; $**P < 0.003$ (one-sample T-test). **b**, Grazing of Tp DL-GFP cells by natural microzooplankton from the Red Sea. Two prey types were offered to the microzooplankton assemblages: Tp GFP or Tp DL-GFP. Horizontal lines represent the mean; n=5-6. $*P < 0.017$; $**P < 0.005$ (Student's T-test). **c**, Variety of wild microzooplankton observed during grazing experiments shown in (b), including tintinnid ciliate (1), mixotrophic *Ceratium* dinoflagellates (2-3) and diverse copepods (4-6). Red represents the chlorophyll of ingested *T. pseudonana* cells added as prey (mixotrophic dinoflagellates also have endogenous chlorophyll).

Our new results corroborate previous studies showing that DMS and DMSP can act as chemoattractant signals for marine protists (including *O. marina*) and copepods [6,20,21,33]. We therefore propose a new ecological role for the DL enzyme, mediated by its DMS product (and/or potentially acrylate) which acts as a pro-grazing signal and accelerating the removal of algal cells by their immediate protozoan predators. The response of marine protists to the algal-derived DMS signal is analogous to the phagocytic activity of immune cells in the mammalians, which are attracted to damaged and apoptotic cells via secreted "eat-me" signals [49]. In light of our findings, DMS-mediated herbivory may have a significant impact on algal bloom dynamics of highly DMS-producing species like *E. huxleyi*. Since *E. huxleyi* strains can range in their DL activity over four order of magnitude [50-52], we hypothesize that grazing of high-DL strains can release DMS that acts as an appetizing signal, leading to enhanced grazing pressure imposed upon the entire blooming population (Fig. 4). Considering the constantly changing environmental conditions during bloom succession, and that late blooms are more prone to nutrient limitation and viral attack, DMS-mediated signaling may promote the removal of damaged, infected or aged algal populations by expediting a top-down grazing pressure.

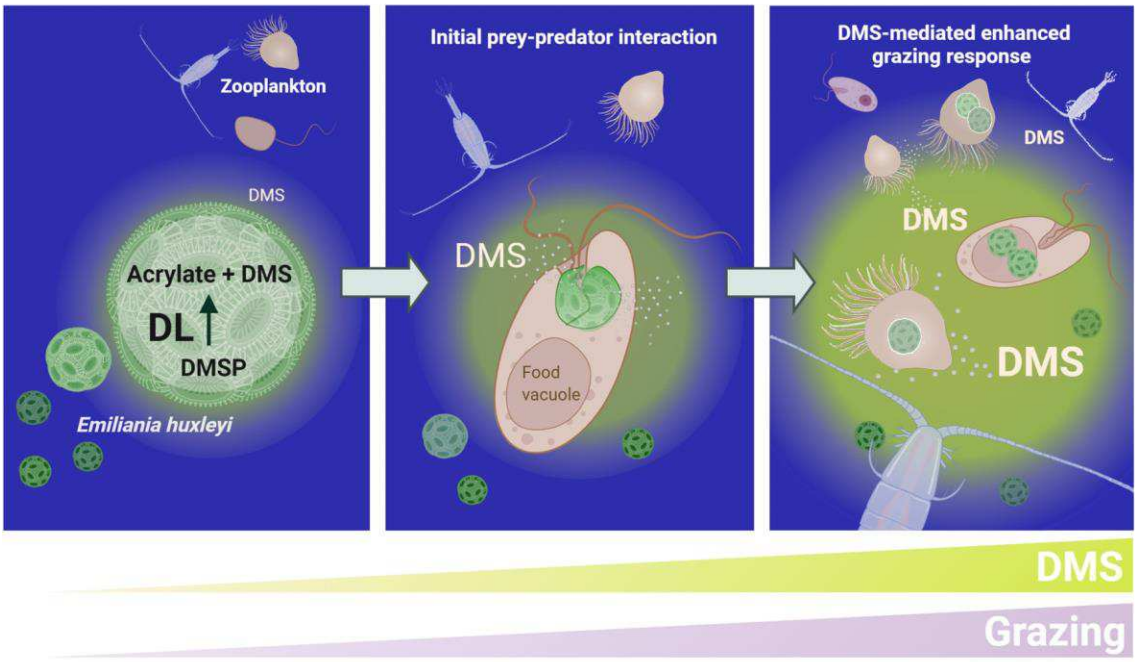

Fig. 4. **The ecological impact of algal DMS on planktonic prey-predator interactions.** A conceptual model describing DMS-mediated herbivory. Chemoattraction of zooplankton to leakage of DMS from high DMS producing algae such of *E. huxleyi* cells may facilitate initial grazing interaction (left panel). Upon dissociation of ingested cells during phagocytosis, the DL Alma1 degrades DMSP and releases more DMS into the water (middle panel). This microscale release of DMS may accumulate to high concertation, which induces an increase in the overall grazing pressure by diverse grazers on the entire phytoplankton population (right panel) and thus has wide ecological implication in the marine environment.

Since the historical discovery of DMS from a seaweed about 85 years ago, the cellular function of this volatile, and of its generating enzyme, still remain elusive [53]. The unknown cellular role for the DL gene is intriguing in light of its high conservation among diverse phytoplankton species with great ecological success such as such as *E. huxleyi*, *Phaeocystis* and *Symbiodinium* [7]. While DMSP appears to serve as an osmolyte or antioxidant in several phytoplankton species [15], the physiological benefit of DMSP catabolism by DL activity is still unknown. Yet, previous studies have indicated that DL activity acts as a grazing-activated chemical defense mechanism. The proposed defense role was based on grazing experiments on *E. huxle*yi strains displaying ~4 orders of magnitude range in the DL activity [4,26,37,44]. However, since the transcriptome of these *E. huxle*yi strains differs in expression of ~10,000 genes [7,54], it is difficult to assign a specific role for a single trait as DL activity. Bulk addition of DMSP, but not of DMS or acrylate, reduced grazing rates of *E. huxle*yi cells by microzooplankton, suggesting a role in chemical defense [5]. However, it is questionable whether exogenous addition of DMS/P mimics spatiotemporal gradients in the media during prey-predator interaction. Indeed, based on our genetic and physiological approaches that enabled *in-vivo* modulation of DL activity, we show that DL activity enhanced grazing. Hence, the evolutionary retention of a DL gene comes with a marked cellular cost of prompting grazing by diverse herbivores. This conundrum indicates an essential role for DL activity in algal cells which is yet to be discovered.

At the ecological level, the response to DMS by zooplankton, and specifically microzooplankton as shown here, is akin to what has been previously described for higher predators such as fish, whales, birds and turtles. These top-predators utilize DMS as a foraging cue, to find 'hot-spots' of high biomass and high plankton concentrations [22-24,55,56]. Intriguingly, by facilitating trophic cascades, DMS may actually promote indirect defense mechanisms for the emitting phytoplankton at the population level. In the Southern Ocean, sea birds are highly sensitive to DMS emitted from *Phaeocystis* sp. blooms, track its source and feed mainly on krill, the main grazer of *Phaeocystis* sp. in this region [22]. In such tritrophic mutualism scenario, seabirds reduce grazing pressure on the phytoplankton. DMS was hypothesized to mediate pelagic tritrophic interactions in the plankton as well, where DMS-derived from phytoplankton-microzooplankton interaction triggers an indirect defense by attracting carnivorous mesozooplankton, which consume the microzooplankton and alleviate the grazing pressure imposed on the algal population [57,58]. This theory was supported by mathematical models [59,60] and evidence from coccolithophore and *Phaeocystis* blooms, where microzooplankton were found to be the main food source for mesozooplankton (copepods) rather than the coccolithophores directly [61,62]. Such copepods may be highly sensitive to DMS emitted during phytoplankton-microzooplankton grazing interaction [21]. In accord, we found that mesozooplankton (copepods and krill) fed with *O. marina* cells which were pre-treated with *E. huxleyi* or Tp DL-GFP prey, produced higher levels of fecal pellets. The mesozooplankton response was species-specific, and may

indicate enhanced copepod predation in the presence of grazing-derived DMS (Supplementary Table 3). The interlink between algal DMS and grazing at likely different trophic levels adds a new dimension to the complex trophic interactions established in oceanic ecosystems and particularly during algal blooms.

Overall, the sensing of DMS by marine biota from diverse size ranges and taxonomic groups [6,63] raises questions regarding the specificity of the DMS signal as a foraging cue, and suggests that DMS may serve as a global eat-me signal across trophic levels. The prevalence of algal-produced DMS and the ubiquity of DMS-mediated predator-prey interactions play fundamental roles in chemical communication among multiple trophic levels in the ocean.

Methods

Culture maintenance

Algal cultures (The diatom *Thalassiosira pseudonana* strain CCMP1335 and the coccolithophore *Emiliania huxleyi* CCMP373, CCMP374 and CCMP2090) were purchased from the National Center for Marine Algae and Microbiota (NCMA, East Boothbay, Maine). *Dunaliella tertiolecta* culture was kindly provided by Prof. Uri Pick, Weizmann Institute of Science. *Oxyrrhis marina* strain LB1974 was kindly provided by Dr. Matthew Johnson, Woods Hole Oceanographic Institute. *Strombidium sp.* and *Gyrodinium dominans* were isolated and kindly contributed by Dr. Albert Calbet, Institut de Ciències del Mar, CSIC. All cultures were grown at 18°C with 16 h : 8 h light:dark cycles, in light intensity of 100 µmol photons m^{-2} s^{-1} for algal cultures and 50 µmol photons m^{-2} s^{-1} for *O. marina,* supplied by cool white LED lights. Algae were diluted once weekly with autoclaved, 0.22 µm-filtered seawater (FSW) supplemented with F/2 (and silica for diatoms) [64]. *O. marina* cells were fed twice weekly with *D. tertiolecta* and were transferred every 3 weeks to a new flask containing FSW supplemented with PenStrep antibiotic solution (Sigma).

Genetic transformation of *T. pseudonana*

To overexpress the Alma1-GFP fusion protein in *T. pseudonana*, wild type cells were transformed using a biolistic particle delivery system fitted with 1350 psi rupture discs (BioRad). M10 tungsten particles (0.7 µm) were coated with 5 µg of plasmid DNA in the presence of $CaCl_2$ (2.5 M) and spermidine, according to the manufacturer's instructions. The plasmid encodes for nourseothricin resistance and Alma or Alma-GFP fusion protein. Approximately 1×10^6 cells were spread on a plate containing solid medium (1.5% Bacto agar in FSW + F/2) a few hours before bombardment. Bombarded cells were recovered in F/2 media + silica, and incubated at 18°C under constant light overnight. The culture was

then plated onto solid medium containing 100 μg mL^{-1} nourseothricin. Plates were incubated for 10 days and resistant colonies were re-streaked onto fresh solid medium containing nourseothricin. The colonies obtained were then screened for GFP fluorescence and DL activity.

Measurements of cellular DMSP, DMS emission and DMSP lyase activity

For determination of cellular DMSP concentration, cultures (~3 ml) were acidified with 1.5 % HCl and stored at 4°C for >24 hours [17]. Samples were diluted (1:100) in DDW, and DMSP was lysed to DMS by adding NaOH to a final concentration of 0.45 M. Glycine buffer (pH 3, final con. 0.8 M) was added in order to neutralize the solution (pH 8 to 9). Samples were then measured for DMS concentration in sealed glass vials, using Eclipse 4660 Purge-and-Trap Sample Concentrator system equipped with Autosampler (OI Analytical). Separation and detection were done using gas chromatography-flame photometric detector (GC-FPD, HP 5890) equipped with RT-XL sulfur column (Restek). DMS emission *in-vivo* was quantified in the culture media (filtered through GF/F filters, Whatman) or directly in the culture over time, in sealed glass vials (40 mL). The DMS measurement itself was conducted as described for the DMSP analysis. DMSP lyse activity (*in-vitro*) was estimated as DMS release and measured as previously described [7]. Cells were harvested by centrifugation. Cell pellets were resuspended in lysis buffer (Tris 100 mM pH 8.0, NaCl 100 mM, DTT 1 mM, Triton 100x 0.02%, Benzonase 250 units) and sonicated. Crude lysates were incubated with 10 mM DMSP while shaking for 10 min at 30°C. Reactions were terminated by 1000-fold dilution into sealed glass vials containing 30 ml of distilled water. The vials were kept on ice in the dark until the DMS measurement, conducted as described for the DMSP analysis. All measurements were calibrated against a standard curve of DMS, ranging from 5 to 300 nM (Sigma-Aldrich).

Western-blot analysis

For detection of the Alma1 protein expression, cells were harvested at exponential growth phase by centrifugation (10,000 × *g*, 15 min, 4°C) and plunged into liquid nitrogen. Cell pellets were then resuspended in lysis buffer and bath-sonicated (5.5 s sonication following by 10 s rest, x 5 cycles). Cell lysates and recombinant Alma1, used as a positive control [7], were separated on an Any kD SDS-PAGE (Bio-Rad). For western-blot analysis, we used a primary rabbit polyclonal antibody raised by immunization against the recombinant Alma1 protein [7] and a secondary horseradish peroxidase-conjugated anti-rabbit antibody (Sigma-Aldrich). The antibodies were diluted in Tris-buffered saline containing 0.1% Tween-20 and 5% skim-milk powder. ECL-Prime reagent (GE Healthcare) was used for detection.

Scanning electron microscopy *of T. pseudonana* cells

For morphological analysis of *T. pseudonana* cells, samples were collected during exponential growth phase. Samples (1 mL) were blotted at room temperature on Nuclepore

track-etched polycarbonate membranes (Whatman), coated with 5 nm of Iridium for improved conductance, and imaged with a Sigma 500 SEM (Zeiss) using the InLens detector.

Grazing assays with *O. marina*, *Gyrodinium dominans* and *Strombidium* sp.

Grazing assays were conducted mainly with *O. marina* as predator and *E. huxleyi or T. pseudonana* as prey. Prior to the assay, microzooplankton were starved for 3 days to clear their digestive vacuole from their routine prey, *D. tertiolecta*. Prey cells were added to the grazer culture to reach a final prey:grazer ratio of 3:1 or 10:1 (1-30 × 10^4 prey cells mL^{-1}). Grazer concentration was 3 × 10^3 cells mL^{-1}. Grazing assays with *O. marina* were conducted for 0.5-24 h, as designated in each graph. For *Gyrodinium dominans* and *Strombidium* sp., grazing assay was conducted for 3 h.

When using Br-DMSP [8], the inhibitor was added to the *E. huxleyi* culture 2 h prior to grazing interaction. The inhibitor was diluted with MeOH and added in a final concentration of 0.2 µM, which was chosen based on toxicity assays (Supplementary Fig. 1). The same volume of MeOH was used as control. The inhibitor's toxicity for *O. marina* cells was tested by using SYTOX Green (Invitrogen) staining to detect compromised cell membranes. Samples were stained with a final concentration of 1 µM SYTOX, incubated in the dark for 30 min and analyzed by an Eclipse flow cytometer, as described below (ex: 488 nm and em: 500–550 nm). An unstained sample was used to eliminate the background signal.

Determination of grazing and ingestion rates

For grazing rate (g) and ingestion rate (IR) analyses, prey and grazer were incubated in a 24 well plate. Prey cells were also added to FSW as a growth control. Each group included 4-6 biological replicates. The plate was placed in the flow cytometer autosampler, where each well was sampled every 30 min to quantify the algal cells, for a total period of 2-4 hours. The flow cytometer, Eclipse iCyt (Sony Biotechnology Inc., Champaign, IL, USA), was equipped with 488 nm solid state air cooled laser, with 25 mW on the flow cell and with standard filter set-up. Algal cells were identified by plotting the chlorophyll fluorescence (ex: 488 nm and em: 663-737 nm) against side scatter. At least 2,500 cells were collected in each sample. Grazer cells were counted manually. The algal specific growth rate (µ) was calculated for the control and grazing treatments. *g* and IR were calculated based on µ and normalized per grazer, as described by Frost [65].

***O. marina* vacuole content (VC) analysis**

Manual quantification of ingested prey was adapted from previous studies [26]. Incubation of prey and predator were conducted in 50 mL culture flasks. Sub-samples were fixed with 1% PFA at two time points (0.5, 3h) for *E. huxleyi* or during a time course for *T. pseudonana* (8, 17, 30, 45 and 60 min) prey. Fixed cells were gently transferred into an

Utermoehl sedimentation system (Aquatic Research Instruments, USA) and collected onto a microscope slide for 24 hours. Ingested prey items were counted by using an IX71S1F-3-5 inverted Olympus microscope (Tokyo, Japan), equipped with an EXi Blue camera (Q Imaging, Surrey, BC, Canada). 100 grazer cells were observed per sample. The average prey content was calculated for each sample.

O. marina growth curve

To quantify the effect of DMS-producing prey on grazer growth, *O. marina* culture was divided to three dietary treatments: starvation, *T. pseudonana* wild type and Tp DL-GFP prey. Each treatment contained four 50 mL small flasks, which represent biological replicates. Prey cells were supplied daily and added to a final concentration of 3×10^4 cells mL^{-1}. The flasks were mounted onto a rotating plankton wheel and incubated with the growth condition indicated above. Daily samples for grazer cell count were fixed with 10% Lugol (Sigma-Aldrich) in a 24-well plate. *O. marina* cells were counted by using an IX71S1F-3-5 inverted Olympus microscope (described above). A total of 140-1000 cells were counted per sample.

Grazing assays with natural microzooplankton assemblages

In order to assess grazing on transgenic diatoms by natural microzooplankton, experiments were conducted during 2017-2020 at The Inter-University Institute for Marine Sciences (IUI) in Eilat, Israel. Surface water was collected from the pier area (29° 50' N, 34° 91' E) by using a concentrating plankton net (5 µm). Sampled water (4 L) was then gently sieved through a 200 µm mesh in order to obtain the 5-200 µm microzooplankton community fraction (used for the "grazing" treatment). Seawater was also filtered through 0.22 µm filter to remove all cells (used for the "growth" treatment). Each water fraction (5-200 µm and <0.22 µm) was divided to 300-830 mL polycarbonate flasks. Diatom cultures (Tp GFP or Tp DL-GFP) were added to the flasks (3×10^4 cell mL^{-1}) at T_0, including 5-6 biological replicates. Incubation flasks were placed in a shaded water-table with ambient water temperature (22-23ºC) for 24 h. In 2020, the incubation flasks were mounted onto a rotating wheel (0.3 rpm) and placed in the dark at 22ºC for 24 h. Samples for cell count were taken at T_0 and after 24 h, using FCM as describe above.

Grazing rate was calculated as previously described [66]. First, the growth rate was calculated for each flask as: $\mu = (\ln C_{24} - \ln C_0) / (T_{24} - T_0)$, where C_{24} and C_0 are the concentration of cells at T_{24} and T_0, respectively. Then, the grazing coefficient g was calculated by: $g = \mu_{growth} - \mu_{grazing}$, using the average µ for the growth treatment.

Grazing assays with *Artemia salina* and *Pleuromamma indica*

In order to assess grazing on DMS-producing prey by *Artemia salina*, grazing experiments were conducted with 15 days old females which were starved for 3 days. Four individuals were washed with FSW and carefully transferred using a pipette to 50 mL flasks containing FSW or transgenic *T. pseudonana* cultures (Tp DL-GFP or Tp GFP). Initial prey

concentration was 1×10^5 cell mL^{-1}. Algal cells were counted by FCM at T_0 and 1-3 h and *g* was calculated as in Frost [65]. Grazing experiments with *Pleuromamma indica* were conducted with wild animals collected at the northern Gulf of Aqaba, Red Sea (Station A, 29° 28' N, 34° 56' E) by using 300 µm mesh nets. Populations *P. indica* were carefully isolated from the heterogeneous assemblages. Groups of 10 individuals were transferred to petri dishes with FSW for 6-10 hours to allow evacuation of their gut content. At T_0, the animals were transferred to incubation flasks (300 mL, 10 per flask), supplemented with FSW or transgenic *T. pseudonana* cultures (Tp DL-GFP or Tp GFP, 3×10^4 cell mL^{-1}). Algal cells were counted by FCM at T_0 and 48 h and *g* was calculated as in Frost [65].

Mesozooplankton tritrophic grazing experiments

In order to assess grazing and defecation by mesozooplankton in a tritrophic setup, samples were collected during 2019-2020 at the northern Gulf of Aqaba, Red Sea (Station A, 29° 28' N, 34° 56' E) by using 300 µm mesh nets. Populations of *Euphausia diomedeae, Rhincalanus nasutus* and *Pleuromamma indica* were carefully isolated from the heterogeneous assemblages. The three species were used in separate experiments, where their gut content and FP production were quantified in response to different dietary treatments. Groups of 10-12 individuals were transferred to petri dishes with FSW for 6-10 hours to allow evacuation of their gut content. At T_0, the animals were transferred to incubation flasks (300 mL), supplemented with FSW or *O. marina* and phytoplankton cultures. *O. marina* was incubated with phytoplankton (3×10^3 cells mL^{-1} and 3×10^4 cells mL^{-1}, respectively) for a few hours prior to the addition of mesozooplankton, in order for *O. marina* to feed and allow the accumulation of grazing-derived chemical cues. Incubation flasks were mounted onto a rotating wheel (0.3 rpm) and incubated in the dark at 22ºC for 48 h. At T_{48}, the animals were collected from the incubation flasks, washed with FSW and flash-frozen in liquid nitrogen (all animals from the same flask were grouped into a single tube prior to freezing). The specific ingestion of *O. marina* cells was confirmed by detecting an *O. marina* DNA sequence within the copepod gut. DNA was extracted from whole copepods by classical phenol–chloroform extraction, and then cleaned by AMPure XP beads (Beckman Coulter). Primers for quantitative PCR were designed by Primer3 and Amplify4 (William Engels, University of Wisconsin 2015) to amplify the mitochondrial *cob-cox3* gene, which is unique to *O. marina* [67,68]. Forward primer: 5' TCATGCTTTTATCTTTCTATCCA 3'; reverse primer: 5'AGCTAAGAATAAAGTAGAAGGAGA 3'. For qPCR, Platinum SYBR Green qPCR SuperMix-UDG with ROX was used as described by the manufacturer (Invitrogen). Reactions were performed on Step-One Plus real-time PCR System (Applied Biosystems) as follows: 50 °C for 2 min, 95 °C for 2 min, 40 cycles of 95 °C for 15 s, 60 °C for 30 s.

Statistical analysis

Group comparisons were conducted with Student's t-test (for two groups) or ANOVA (for more than two groups) followed by Tukey's or Dunnett's post-hoc tests. Experiments with repeated measures per vial were tested with mixed effects models, treating vial as a random factor. A generalized linear model with Poisson distribution was used when analyzing discrete counts. All analyses were conducted in R, v. 4.0.2. GLMs were done using the package "lmerTest", v. 3.1-2.

Illustrations were created with BioRender.com.
Graphs were created with GraphPad Prism.

Acknowledgments

We thank Inbal Nussbaum and Alon Spierer for assisting in laboratory experiments; Yaara Finkel for helping in genetic transformation of *T. pseudonana*; Diana Meltzer for synthesizing Br-DMSP used in grazing experiments; Emanuel M. Avrahami for conducting scanning electron microscopy analysis for *T. pseudonana* cells; Yoav Avrahami for helping with field sampling and flow cytometry; Flora Vincent and Constanze Kuhlisch for their constructive feedback on the manuscript. We gratefully acknowledge financial support from the Israeli Science Foundation (ISF) (grant #712233) awarded to A.V. All data are available in the supplementary materials.

Author contributions: A.S. and A.V. conceptualized the research questions and hypothesis, designed the experiments and wrote the manuscript. A.S. performed all the experiments and analyzed the data. U.A., M.J.F. and D.S.T helped in establishing the experimental system, developing the experimental tools and contributed in scientific discussions. D.S. conducted genetic transformation of *T. pseudonana.* A.S. and V.F. designed and conducted mesozooplankton grazing experiments. S.B.-D. performed bioinformatics analysis. R.R. performed statistical analysis.

References

1 Simó, R. Production of atmospheric sulfur by oceanic plankton: biogeochemical, ecological and evolutionary links. *Trends. Ecol. Evol.* **16**, 287-294, doi:10.1016/S0169-5347(01)02152-8 (2001).

2 Charlson, R. J., Lovelock, J. E., Andreae, M. O. & Warren, S. G. Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. *Nature* **326**, 655-661 (1987).

3 Wang, S., Maltrud, M. E., Burrows, S. M., Elliott, S. M. & Cameron-Smith, P. Impacts of shifts in phytoplankton community on clouds and climate via the sulfur cycle. *Global Biogeochem. Cy.* **32**, 1005-1026, doi:https://doi.org/10.1029/2017GB005862 (2018).

4 Wolfe, G. V., Steinke, M. & Kirst, G. O. Grazing-activated chemical defence in a unicellular marine alga. *Nature* **387**, 894-897 (1997).

5 Strom, S., Wolfe, G., Slajer, A., Lambert, S. & Clough, J. Chemical defense in the microplankton II: Inhibition of protist feeding by β-dimethylsulfoniopropionate (DMSP). *Limnol. Oceanogr.* **48**, 230-237, doi:10.4319/lo.2003.48.1.0230 (2003).

6 Seymour, J., Simó, R., Ahmed, T. & Stocker, R. Chemoattraction to dimethylsulfoniopropionate throughout the marine microbial food web. *Science (New York, N.Y.)* **329**, 342-345 (2010).

7 Alcolombri, U. *et al.* Identification of the algal dimethyl sulfide-releasing enzyme: A missing link in the marine sulfur cycle. *Science (New York, N.Y.)* **348**, 1466-1469, doi:10.1126/science.aab1586 (2015).

8 Alcolombri, U., Lei, L., Meltzer, D., Vardi, A. & Tawfik, D. S. Assigning the algal source of dimethylsulfide using a selective lyase inhibitor. *ACS Chem. Biol.* **12**, 41-46, doi:10.1021/acschembio.6b00844 (2017).

9 Kettle, A. J. & Andreae, M. Flux of dimethylsulfide from the oceans: A comparison of updated data sets and flux models. *J. Geophys. Res.* **105**, doi:10.1029/2000JD900252 (2000).

10 Carpenter, L. J., Archer, S. D. & Beale, R. Ocean-atmosphere trace gas exchange. *Chem. Soc. Rev.* **41**, 6473-6506, doi:10.1039/C2CS35121H (2012).

11 Franklin, D. J. *et al.* Identification of senescence and death in *Emiliania huxleyi* and *Thalassiosira pseudonana*: Cell staining, chlorophyll alterations, and dimethylsulfoniopropionate (DMSP) metabolism. *Limnol. Oceanogr.* **57**, 305-317, doi:10.4319/lo.2012.57.1.0305 (2012).

12 Keller, M. D. Dimethyl sulfide production and marine phytoplankton: the importance of species composition and cell size. *Biol. Oceanogr.* **6**, 375-382, doi:10.1080/01965581.1988.10749540 (1989).

13 Curson, A. R. J. *et al.* DSYB catalyses the key step of dimethylsulfoniopropionate biosynthesis in many phytoplankton. *Nat. Microbiol.* **3**, 430-439, doi:10.1038/s41564-018-0119-5 (2018).

14 Sunda, W., Kieber, D. J., Kiene, R. P. & Huntsman, S. An antioxidant function for DMSP and DMS in marine algae. *Nature* **418**, 317-320, doi:http://www.nature.com/nature/journal/v418/n6895/suppinfo/nature00851_S1.html (2002).

15 Kirst, G. O. in *Biological and environmental chemistry of DMSP and related sulfonium compounds* (eds Ronald P. Kiene, Pieter T. Visscher, Maureen D. Keller, & Gunter O. Kirst) 121-129 (Springer US, 1996).

16 Darroch, L. *et al.* Effect of short-term light and UV stress on DMSP, DMS, and DMSP lyase activity in *Emiliania huxleyi*. *Aquat. Microb. Ecol.* **74**, 173-185, doi:10.3354/ame01735 (2015).

17 Barak-Gavish, N. *et al.* Bacterial virulence against an oceanic bloom-forming phytoplankter is mediated by algal DMSP. *Sci. Adv.* **4**, eaau5716, doi:10.1126/sciadv.aau5716 (2018).

18 Amin, S. A. *et al.* Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. *Nature* **522**, 98-101, doi:10.1038/nature14488 (2015).

19 Garcés, E., Alacid, E., Reñé, A., Petrou, K. & Simó, R. Host-released dimethylsulphide activates the dinoflagellate parasitoid *Parvilucifera sinerae*. *ISME J.* **7**, 1065-1068, doi:10.1038/ismej.2012.173 (2013).

20 Steinke, M., Stefels, J. & Stamhuis, E. Dimethyl sulfide triggers search behavior in copepods. *Limnol. Oceanogr.* **51**, 1925-1930, doi:10.4319/lo.2006.51.4.1925 (2006).

21 Breckels, M., Bode, N., Codling, E. & Steinke, M. Effect of grazing-mediated dimethyl sulfide (DMS) production on the swimming behavior of the copepod *Calanus helgolandicus*. *Mar. Drugs* **11**, 2486 (2013).

22 Savoca, M. S. & Nevitt, G. A. Evidence that dimethyl sulfide facilitates a tritrophic mutualism between marine primary producers and top predators. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 4157-4161, doi:10.1073/pnas.1317120111 (2014).

23 Bouchard, B. *et al.* Behavioural responses of humpback whales to food-related chemical stimuli. *PLOS ONE* **14**, e0212515, doi:10.1371/journal.pone.0212515 (2019).

24 Endres, C. S. & Lohmann, K. J. Perception of dimethyl sulfide (DMS) by loggerhead sea turtles: a possible mechanism for locating high-productivity oceanic regions for foraging. *J. Exp. Biol.* **215**, 3535-3538, doi:10.1242/jeb.073221 (2012).

25 Simó, R. *et al.* The quantitative role of microzooplankton grazing in dimethylsulfide (DMS) production in the NW Mediterranean. *Biogeochemistry*, doi:10.1007/s10533-018-0506-2 (2018).

26 Strom, S. *et al.* 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, doi:10.4319/lo.2003.48.1.0217 (2003).

27 Lehahn, Y. *et al.* Decoupling physical from biological processes to assess the impact of viruses on a mesoscale algal bloom. *Curr. Biol.* **24**, 2041-2046, doi:10.1016/j.cub.2014.07.046 (2014).

28 Laber, C. P. *et al.* Coccolithovirus facilitation of carbon export in the North Atlantic. *Nat. Microbiol.* **3**, 537-547, doi:10.1038/s41564-018-0128-4 (2018).

29 Burkill, P. *et al.* Dimethyl sulphide biogeochemistry within a coccolithophore bloom (DISCO): An overview. *DEEP-SEA RES PT II* **49**, 2863-2885, doi:10.1016/S0967-0645(02)00061-9 (2002).

30 Calbet, A. & Landry, M. R. Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. *Limnol. Oceanogr.* **49**, 51-57, doi:10.4319/lo.2004.49.1.0051 (2004).

31 Schmoker, C., Hernández-León, S. & Calbet, A. Microzooplankton grazing in the oceans: impacts, data variability, knowledge gaps and future directions. *J. Plankton. Res.* **35**, 691-706, doi:10.1093/plankt/fbt023 (2013).

32 Landry, M. R. & Calbet, A. Microzooplankton production in the oceans. *ICES J. Mar. Sci.* **61**, 501-507, doi:10.1016/j.icesjms.2004.03.011 (2004).

33 Breckels, M. N., Roberts, E. C., Archer, S. D., Malin, G. & Steinke, M. The role of dissolved infochemicals in mediating predator-prey interactions in the heterotrophic dinoflagellate *Oxyrrhis marina*. *J. Plankton. Res.* **33**, 629-639, doi:10.1093/plankt/fbq114 (2010).

34 Raina, J. B. *et al.* Subcellular tracking reveals the location of dimethylsulfoniopropionate in microalgae and visualises its uptake by marine bacteria. *eLife* **6**, e23008, doi:10.7554/eLife.23008 (2017).

35 Evans, C., Kadner, S. V. & Darroch, L. J. The relative significance of viral lysis and microzooplankton grazing as pathways of dimethylsulfoniopropionate (DMSP) cleavage: An *Emiliania huxleyi* culture study. *Limnol. Oceanogr.* **52**, 1036–1045 (2007).

36 van Rijssel, M. & Gieskes, W. W. C. Temperature, light, and the dimethylsulfoniopropionate (DMSP) content of *Emiliania huxleyi* (Prymnesiophyceae). *J. Sea Res.* **48**, 17-27, doi:https://doi.org/10.1016/S1385-1101(02)00134-X (2002).

37 Wolfe, G. V. & Steinke, M. Grazing-activated production of dimethyl sulfide (DMS) by two clones of *Emiliania huxleyi*. *Limnol. Oceanogr.* **41**, 1151-1160, doi:10.4319/lo.1996.41.6.1151 (1996).

38 Daly, K. L. & DiTullio, G. R. in *Biological and environmental chemistry of DMSP and related sulfonium compounds* (eds Ronald P. Kiene, Pieter T. Visscher, Maureen D. Keller, & Gunter O. Kirst) 223-238 (Springer US, 1996).

39 Poulsen, N., Chesley, P. M. & Kröger, N. Molecular genetic manipulation of the diatom *Thalassiosira pseudonana* (bacillariophyceae). *J. Phycol.* **42**, 1059-1065, doi:10.1111/j.1529-8817.2006.00269.x (2006).

40 Saade, A. & Bowler, C. Molecular tools for discovering the secrets of diatoms. *BioScience* **59**, 757-765, doi:10.1525/bio.2009.59.9.7 (2009).

41 Armbrust, E. V. *et al.* The genome of the diatom *Thalassiosira pseudonana*: ecology, evolution, and metabolism. *Science (New York, N.Y.)* **306**, 79-86, doi:10.1126/science.1101156 (2004).

42 Malviya, S. *et al.* Insights into global diatom distribution and diversity in the world's ocean. *Proc. Natl. Acad. Sci. U.S.A.* **113**, E1516-1525, doi:10.1073/pnas.1509523113 (2016).

43 Kettles, N. L., Kopriva, S. & Malin, G. Insights into the regulation of DMSP synthesis in the diatom *Thalassiosira pseudonana* through APR activity, proteomics and gene expression analyses on cells acclimating to changes in salinity, light and nitrogen. *PLOS ONE* **9**, e94795, doi:10.1371/journal.pone.0094795 (2014).

44 Steinke, M., Wolfe, G. V. & Kirst, G. O. Partial characterisation of dimethylsulfoniopropionate (DMSP) lyase isozymes in 6 strains of *Emiliania huxleyi*. *Mar. Ecol.* **175**, 215-225 (1998).

45 Lana, A. *et al.* An updated climatology of surface dimethlysulfide concentrations and emission fluxes in the global ocean. *Global Biogeochem. Cyc.* **25**, doi:10.1029/2010gb003850 (2011).

46 Royer, S. J. *et al.* A high-resolution time-depth view of dimethylsulphide cycling in the surface sea. *Sci. Rep.* **6**, 32325, doi:10.1038/srep32325 (2016).

47 Saló, V., Simó, R., Vila-Costa, M. & Calbet, A. Sulfur assimilation by *Oxyrrhis marina* feeding on a 35S-DMSP-labelled prey. *Environ. Microbiol.* **11**, 3063-3072, doi:10.1111/j.1462-2920.2009.02011.x (2009).

48 Olson, M. B. & Strom, S. L. Phytoplankton growth, microzooplankton herbivory and community structure in the southeast Bering Sea: insight into the formation and temporal persistence of an *Emiliania huxleyi* bloom. *DEEP-SEA RES PT II* **49**, 5969-5990, doi:https://doi.org/10.1016/S0967-0645(02)00329-6 (2002).

49 Li, W. Eat-me signals: keys to molecular phagocyte biology and "appetite" control. *J. Cell Physiol.* **227**, 1291-1297, doi:10.1002/jcp.22815 (2012).

50 Read, B. A. *et al.* Pan genome of the phytoplankton *Emiliania* underpins its global distribution. *Nature* **499**, 209-213, doi:10.1038/nature12221 (2013).

51 Martínez, J. M., Schroeder, D. C. & Wilson, W. H. Dynamics and genotypic composition of *Emiliania huxleyi* and their co-occurring viruses during a coccolithophore bloom in the North Sea. *FEMS Microbiol. Ecol.* **81**, 315-323, doi:10.1111/j.1574-6941.2012.01349.x (2012).

52 Krueger-Hadfield, S. A. *et al.* Genotyping an *Emiliania huxleyi* (prymnesiophyceae) bloom event in the North Sea reveals evidence of asexual reproduction. *Biogeosciences* **11**, 5215-5234, doi:10.5194/bg-11-5215-2014 (2014).

53 Haas, P. The liberation of methyl sulphide by seaweed. *Biochem. J.* **29**, 1297-1299, doi:10.1042/bj0291297 (1935).

54 von Dassow, P. *et al.* Transcriptome analysis of functional differentiation between haploid and diploid cells of *Emiliania huxleyi*, a globally significant photosynthetic calcifying cell. *Genome Biol.* **10**, R114, doi:10.1186/gb-2009-10-10-r114 (2009).

55 Foretich, M. A., Paris, C. B., Grosell, M., Stieglitz, J. D. & Benetti, D. D. Dimethyl sulfide is a chemical attractant for reef fish larvae. *Sci. Rep.* **7**, 2498, doi:10.1038/s41598-017-02675-3 (2017).

56 Savoca, M. S. Chemoattraction to dimethyl sulfide links the sulfur, iron, and carbon cycles in high-latitude oceans. *Biogeochemistry* **138**, 1-21, doi:10.1007/s10533-018-0433-2 (2018).

57 Steinke, M., Malin, G. & Liss, P. Trophic interactions in the sea: An ecological role for climate relevant volatiles? *J. Phycol.* **38**, 630-638, doi:10.1046/j.1529-8817.2002.02057.x (2002).

58 Pohnert, G., Steinke, M. & Tollrian, R. Chemical cues, defence metabolites and the shaping of pelagic interspecific interactions. *Trends Ecol. Evol.* **22**, 198-204, doi:10.1016/j.tree.2007.01.005 (2007).

59 Lewis, N. *et al.* Grazing-induced production of DMS can stabilize food-web dynamics and promote the formation of phytoplankton blooms in a multitrophic plankton model. *Biogeochemistry* **110**, 303-313, doi:10.1007/s10533-011-9649-0 (2012).

60 Lewis, N. D. Role of infochemical mediated zooplankton grazing in a phytoplankton competition model. *Ecol. complex.* **16**, pp. 41-50-2013 v.2016, doi:10.1016/j.ecocom.2012.10.003 (2013).

61 Hansen, F. C., Reckermann, M., Breteler, W. C. M. K. & Riegman, R. Phaeocystis blooming enhanced by copepod predation on protozoa: evidence from incubation experiments. *Mar. Ecol. Prog. Ser.* **102**, 51-57 (1993).

62 Levasseur, M. *et al.* Production of DMSP and DMS during a mesocosm study of an *Emiliania huxleyi* bloom: Influence of bacteria and *Calanus finmarchicus* grazing. *Mar. Biol.* **126**, 609-618, doi:10.1007/BF00351328 (1996).

63 Buchan, A., LeCleir, G. R., Gulvik, C. A. & González, J. M. Master recyclers: features and functions of bacteria associated with phytoplankton blooms. *Nat. Rev. Microbiol.* **12**, 686, doi:10.1038/nrmicro3326 (2014).

64 Guillard, R. R. L. & Ryther, J. H. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (cleve) Gran. *Can. J. Microbiol.* **8**, 229-239, doi:10.1139/m62-029 (1962).

65 Frost, B. W. Effects of size and consentration of food particles on the feeding behavior of the marine planktonic copeopd *Calanur Pacificus. Limnol. Oceanogr.* **17**, 805-815, doi:10.4319/lo.1972.17.6.0805 (1972).

66 Johnson, M. D., Michelle, R. & Stoecker, D. K. Microzooplankton grazing on *Prorocentrum minimum* and *Karlodinium micrum* in Chesapeake Bay. *Limnol. Oceanogr.* **48**, 238-248, doi:10.4319/lo.2003.48.1.0238 (2003).

67 Slamovits, C. H., Saldarriaga, J. F., Larocque, A. & Keeling, P. J. The highly reduced and fragmented mitochondrial genome of the early-branching dinoflagellate *Oxyrrhis marina* shares characteristics with both apicomplexan and dinoflagellate mitochondrial genomes. *J. Mol. Biol.* **372**, doi:10.1016/j.jmb.2007.06.085 (2007).

68 Untergasser, A. *et al.* Primer3Plus, an enhanced web interface to Primer3. *Nucleic acids Res.* **35**, W71-74, doi:10.1093/nar/gkm306 (2007).

Figures

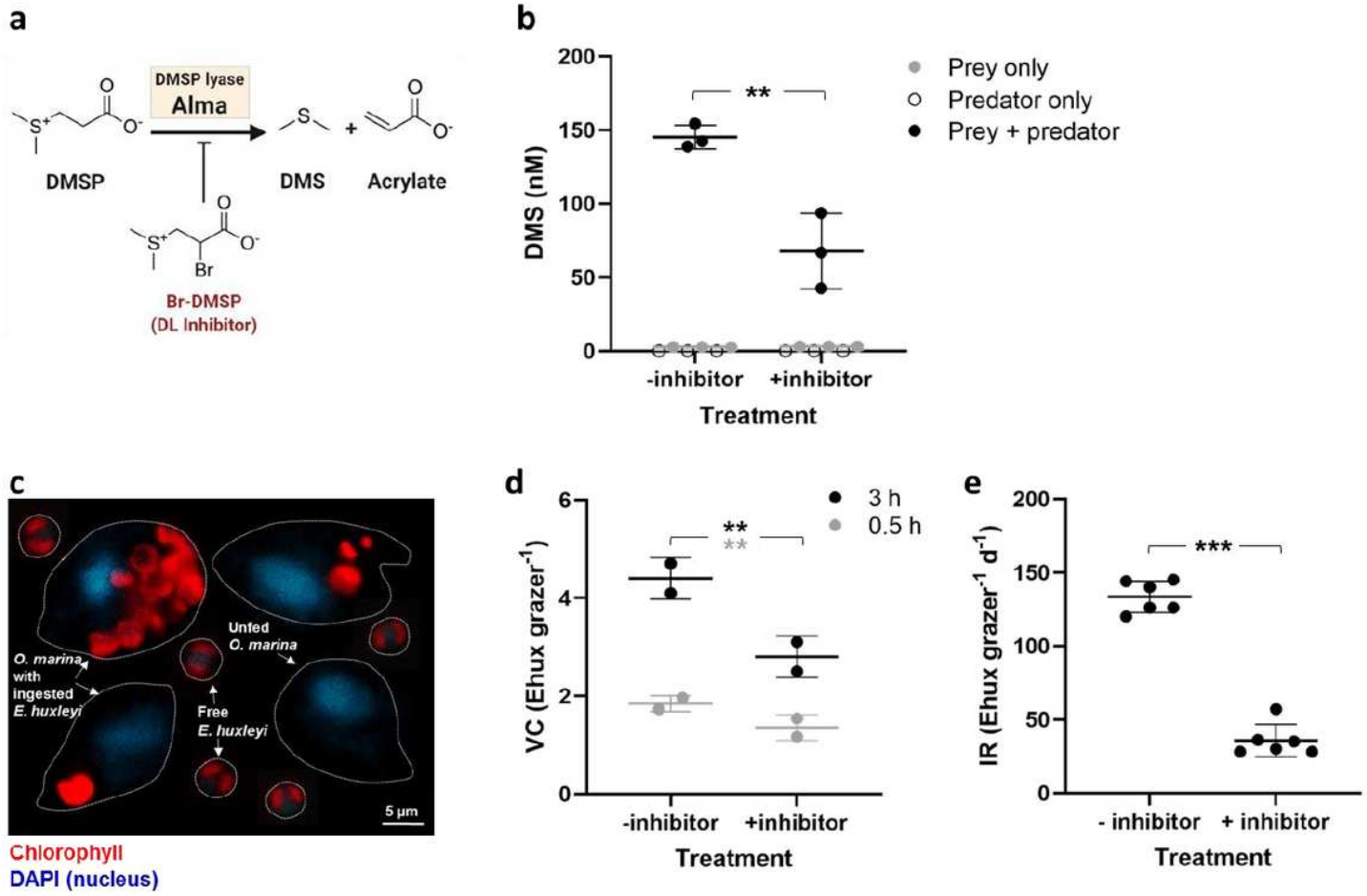

Figure 1

Reduced DMSP lyase (DL) activity of E. huxleyi impaired grazing by O. marina. a, The DMSP lyase Alma cleaves DMSP to DMS and acrylate. The inhibitor Br-DMSP blocks DL activity by forming an enzyme-inhibitor covalent bond at the active site, thus inhibiting the formation of DMS and acrylate 8. b, The short-term (~1 h) effect of 0.2 µM Br-DMSP on DMS production in-vivo by E. huxleyi 373. Horizontal lines represent the mean ± SD; n = 3; P < 0.008 (Student's T-test). c, Fluorescence micrograph collage of O. marina with free and ingested E. huxleyi cells at t=30 minutes from prey addition. d, The effect of 0.2 µm Br-DMSP on the food vacuole content (VC, E. huxleyi 373 cells per grazer) of O. marina at t=0.5 and 3 h. A total of 800 O. marina cells were examined, 100 cells per biological replicate. Horizontal lines represent the mean ± SD; n = 2; P < 0.003 (generalized linear mixed model). e, The effect of 0.2 µm Br-DMSP on ingestion rate (IR) of E. huxleyi 373 cells (Ehux) by O. marina, quantified by flow cytometry and based on prey removal from the medium during 50 min. Approximately 2,500 cells were analyzed per sample. Horizontal lines represent the mean ± SD; n = 6; P < 0.0001 (Student's T-test).

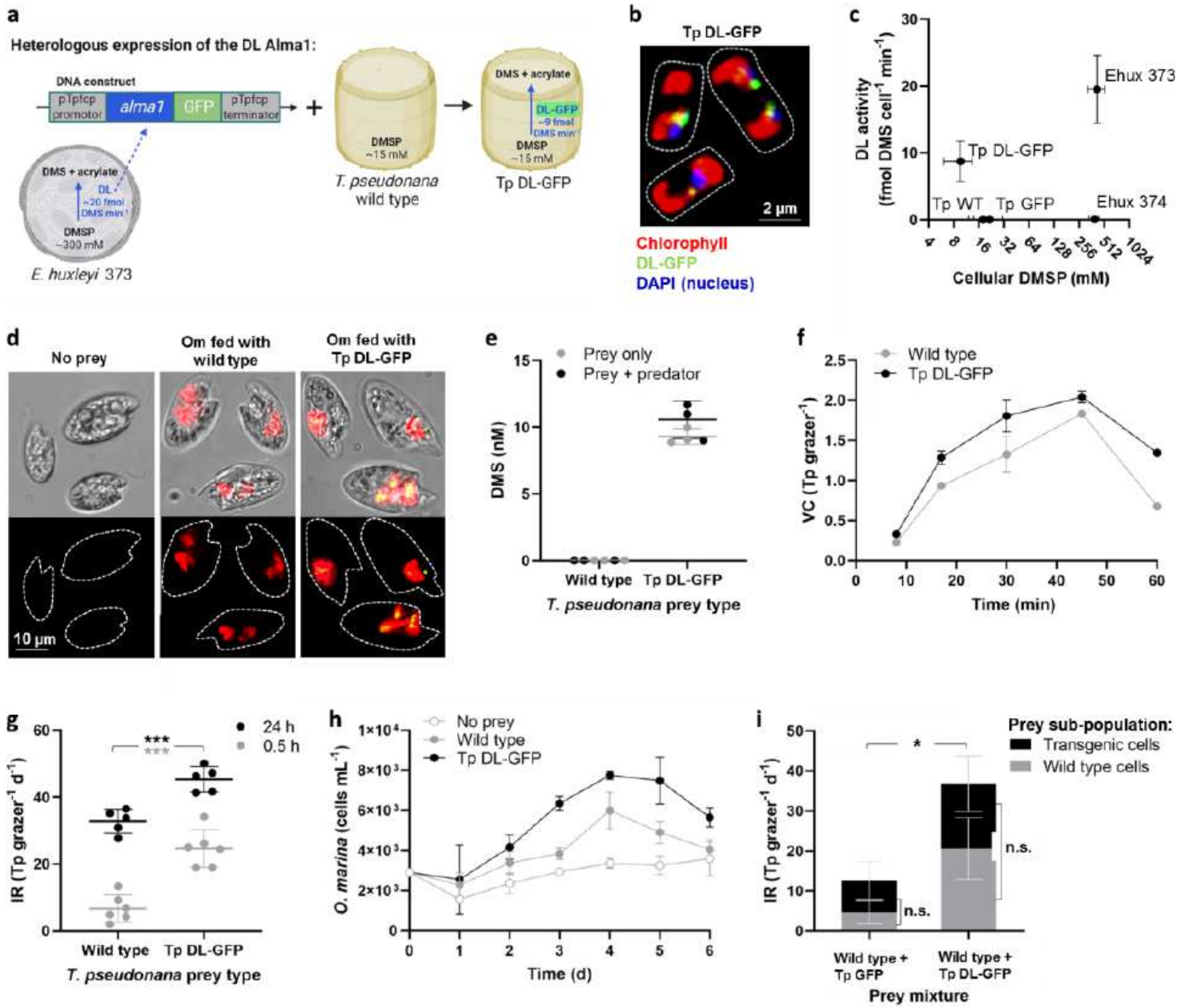

Figure 2

Overexpression of DMSP lyase (DL) in algal prey cells enhanced grazing efficiency and growth by O. marina. a, Transgenic Tp DL-GFP cells were generated by heterologous expression of the alma1 gene from E. huxleyi 373, fused to GFP, in wild type T. pseudonana cells. b, Confocal microscopy of Tp DL-GFP cells. The Alma-GFP protein can be observed as a punctate green signal in close proximity to the chloroplast. c, Intracellular DMSP content and DL activity in E. huxleyi (Ehux) strains 373 and 374, as well as in T. pseudonana wild type (Tp WT), Tp GFP and Tp DL-GFP. We quantified DL activity in vitro by measuring DMS generation in cell lysates following addition of 10 mM DMSP. Values are mean ± SD; n = 3-4. For DMSP, results from two independent measurements were averaged. d, Bright field and fluorescence micrographs of O. marina (Om) with ingested T. pseudonana cells. e, DMS emission during grazing by O. marina on Tp DL-GFP cells. Horizontal lines represent the mean ± SD; n = 3. f, Prey uptake curve based on vacuole content (VC) analysis during the first hour of grazing interaction. Values

represent the mean ± SD. n = 2-4. A total of 800 grazers were analyzed for t= 8 and 17 min, and 400 grazers for t=30, 45, and 60 min. The mean VC values during 60 min were significantly different between the two prey types, $P < 0.0001$ (generalized linear mixed model). g, Ingestion rate (IR) analysis based on quantification of T. pseudonana (Tp) cell removal from the medium during 0.5 and 24 h of incubation with O. marina. Horizontal lines represent the mean ± SD; n = 6. $P < 0.0001$ (Student's T-test). h, Growth dynamics of O. marina under starvation conditions or daily feeding with Tp DL-GFP or wild type cells; n = 4. The growth in the Tp DL-GFP treatment was significantly faster than wild type, $P < 0.0012$ (mixed effects model). i, Double-prey competition trials based on IR analysis of two prey types introduced to O. marina in a 1:1 ratio, as measured over 2 h. Values are mean ±SD; n = 3-4 in two independent experiments. $P < 0.001$ when comparing the total IR between the two prey mixtures. When comparing the specific IR on each prey sub-population within a mixture, $P > 0.32$, n.s.- not significant (Student's T-test).

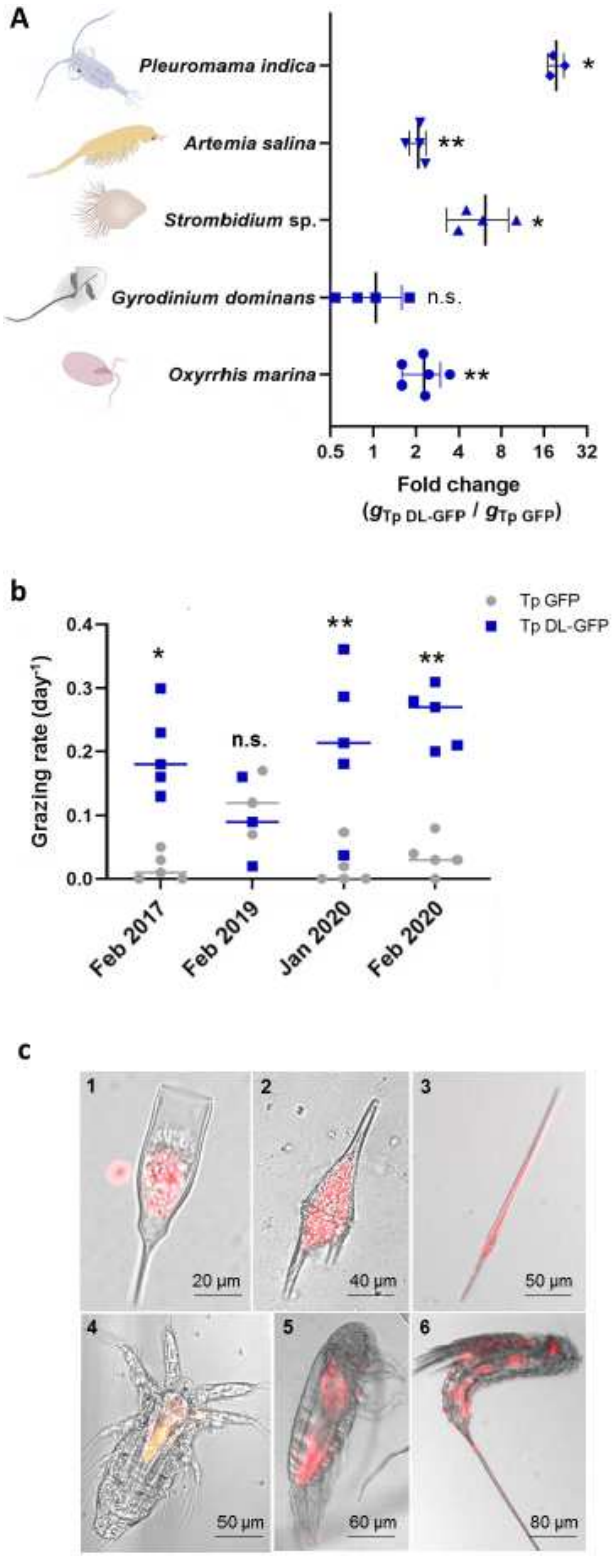

Figure 3

Induced DMS production in algal prey enhanced grazing by diverse zooplankton. a, The grazing response of diverse zooplankton fed with Tp DL-GFP cells. Tp GFP cells were used as control prey. The fold change in grazing rate (g, as assessed by monitoring free prey cells using flow cytometry) on each prey type was calculated, where fold change > 1 indicates faster grazing on Tp DL-GFP cells. Vertical lines represent the mean ± SD; n = 3 for P. indica; 4 for A. salina, G. dominans and Strombidium sp.; 6 for O. marina. n.s.- not

significant, P > 0.897; *P < 0.011; **P < 0.003 (one-sample T-test). b, Grazing of Tp DL-GFP cells by natural microzooplankton from the Red Sea. Two prey types were offered to the microzooplankton assemblages: Tp GFP or Tp DL-GFP. Horizontal lines represent the mean; n=5-6. *P < 0.017; **P < 0.005 (Student's T-test). c, Variety of wild microzooplankton observed during grazing experiments shown in (b), including tintinnid ciliate (1), mixotrophic Ceratium dinoflagellates (2-3) and diverse copepods (4-6). Red represents the chlorophyll of ingested T. pseudonana cells added as prey (mixotrophic dinoflagellates also have endogenous chlorophyll).

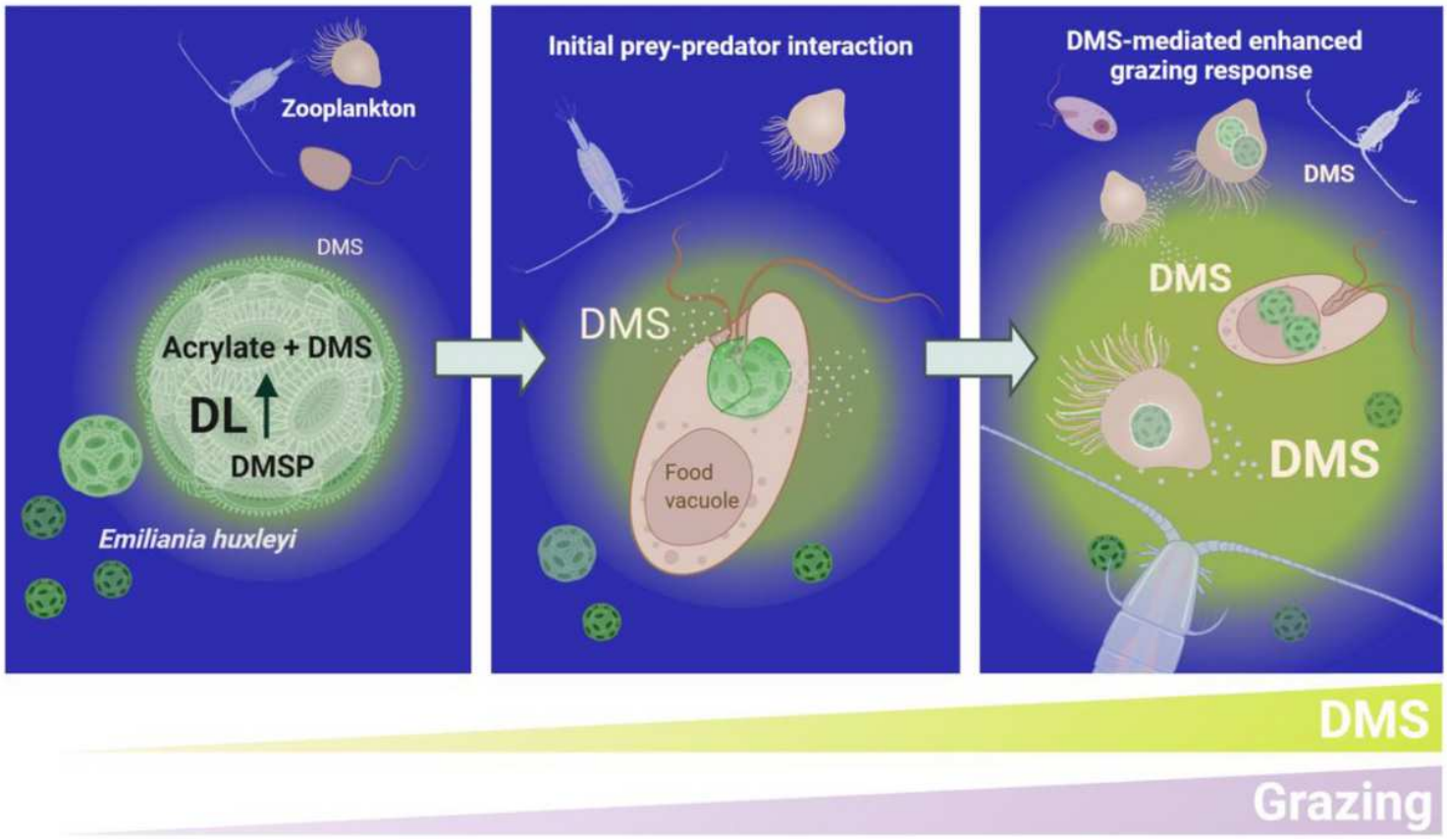

Figure 4

The ecological impact of algal DMS on planktonic prey-predator interactions. A conceptual model describing DMS-mediated herbivory. Chemoattraction of zooplankton to leakage of DMS from high DMS producing algae such of E. huxleyi cells may facilitate initial grazing interaction (left panel). Upon dissociation of ingested cells during phagocytosis, the DL Alma1 degrades DMSP and releases more DMS into the water (middle panel). This microscale release of DMS may accumulate to high concertation, which induces an increase in the overall grazing pressure by diverse grazers on the entire phytoplankton population (right panel) and thus has wide ecological implication in the marine environment.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ShemietalSuppMaterials.pdf