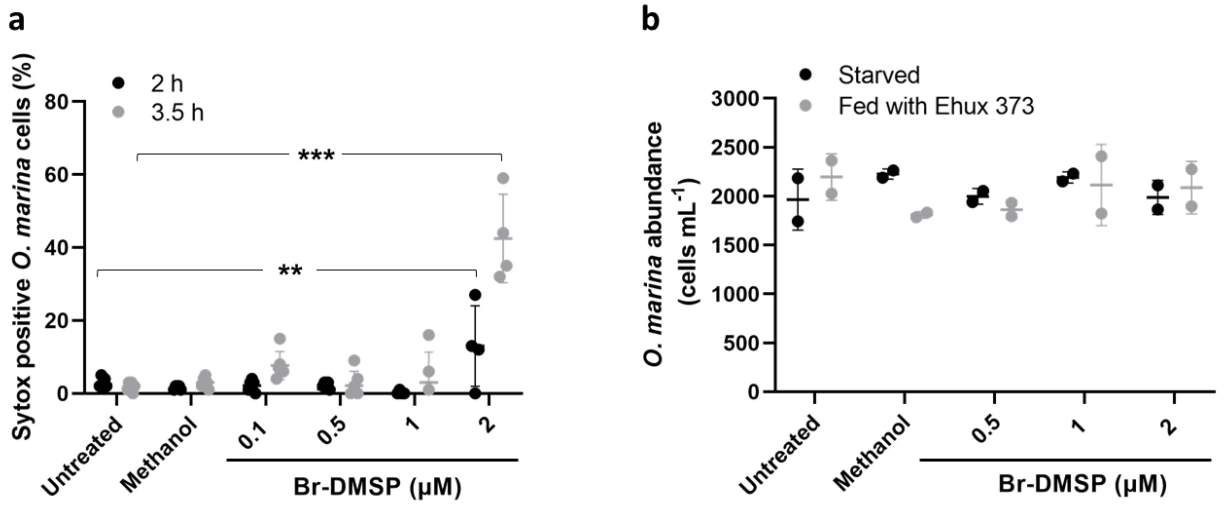
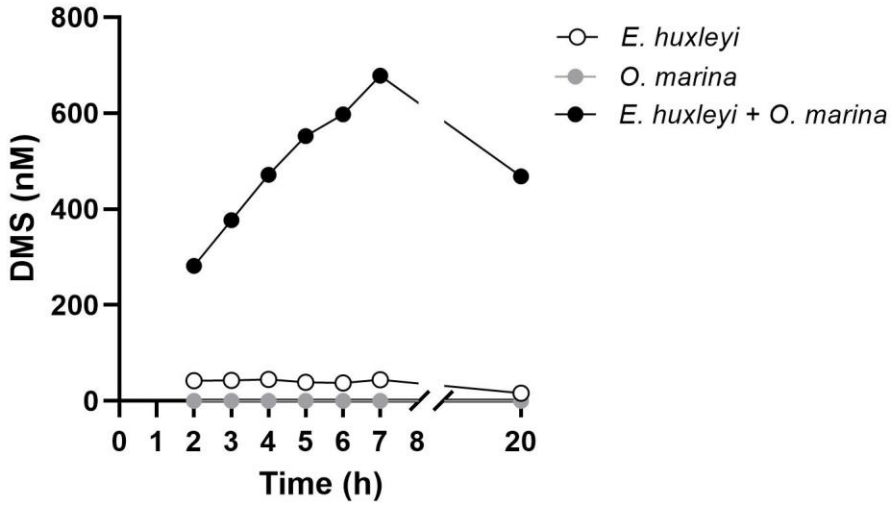
Supplemental information

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

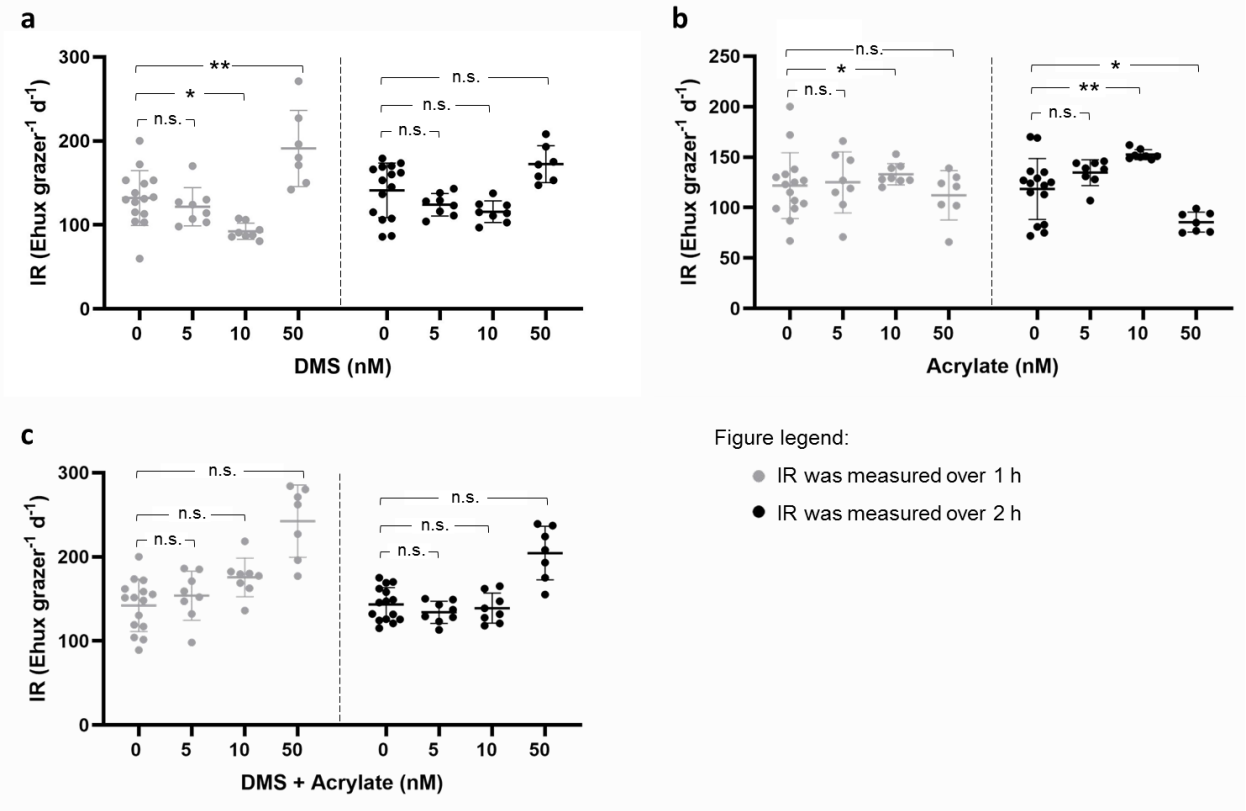
Adva Shemi, Uria Alcolombri, Daniella Schatz, Viviana Farstey, Ron Rotkopf, Shifra Ben-Dor, Miguel J. Frada, Dan S. Tawfik, Assaf Vardi



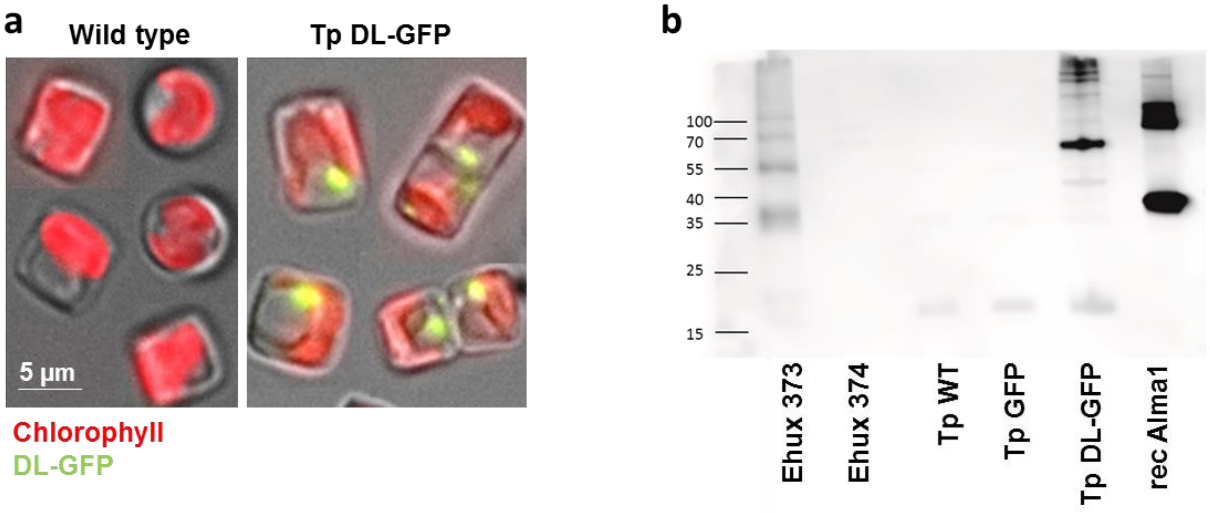
Supplementary Figure 1. Br-DMSP toxicity assays with *O. marina* cells. a, *O. marina* cells were treated with 0.1-2 µM Br-DMSP and stained with Sytox Green as an indicator of cell death. The percentage of Sytox positive cells after 2 and 3.5 hours was determined by flow cytometry. At least 250 cells were analyzed per sample. Horizontal lines represent the mean ± SD; n = 6. The percentage of Sytox positive cells in treated cells was not statistically different from the untreated control, except of 2 µm Br-DMSP at 2 h (**P < 0.002) and 3.5 h (***P < 0.001, 1-way ANOVA, followed by Dunnett's post-hoc). **b,** *O. marina* (starved or fed with Ehux 373) were treated with Br-DMSP. The inhibitor and prey were added at T_0. At T=3 h, *O. marina* cells were fixed with Lugol and quantified by light microscopy. Horizontal lines represent the mean ± SD; n = 2. A total of 622-859 cells were counted per sample. No significant changes compared to untreated control were detected, P > 0.318 (2-way ANOVA).



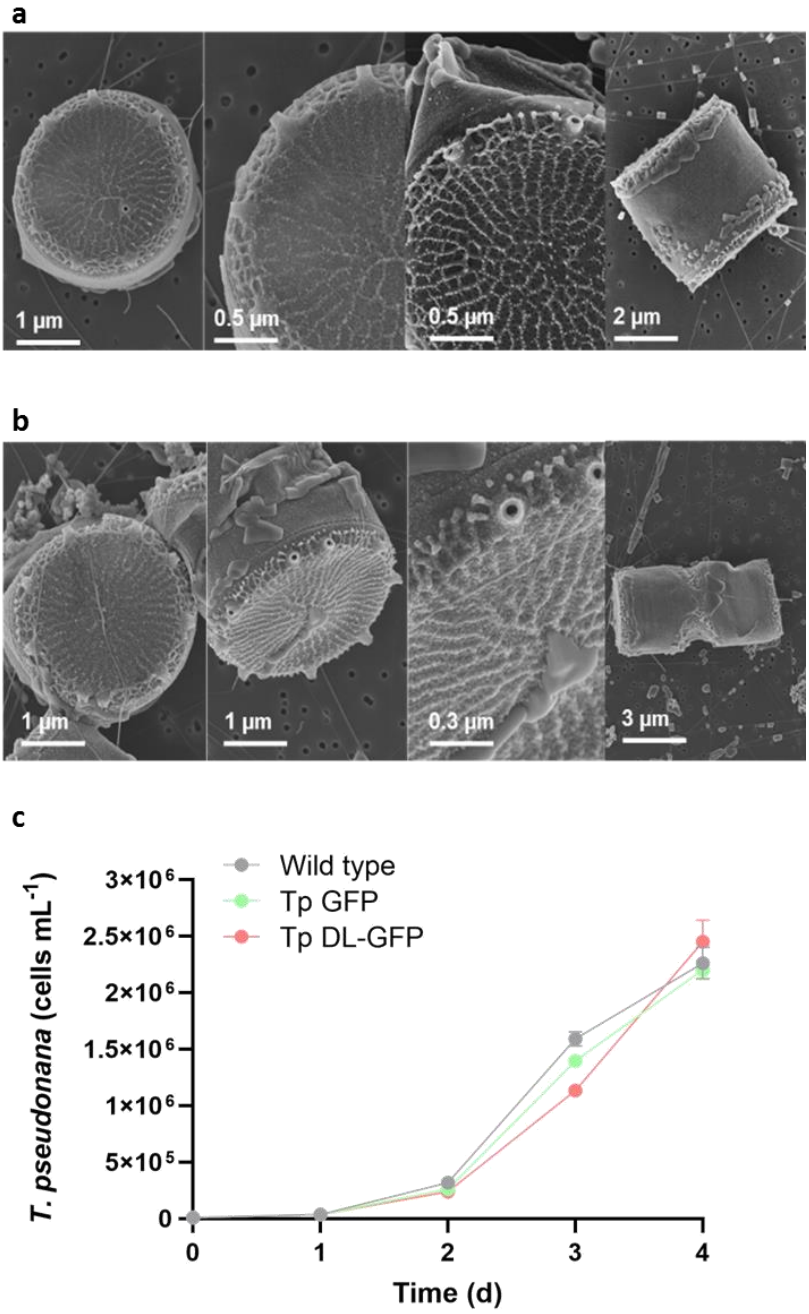
Supplementary Figure 2. DMS production during grazing by *O. marina* on *E. huxleyi*. DMS concentration in the media of *E. huxleyi* 373 was monitored in response to grazing by *O. marina*, as compared to starved predator and prey only controls. Prey: predator ratio was ~10 (initial prey concentration was 3×10^4 *E. huxleyi* cells mL^{-1}). DMS was measured directly in the sealed grazing vials. Values represent a single biological replicate (the DMS measurement itself takes ~30 min). At T_{20}, values represent the mean ± SD; *n*=3. Error bars are smaller than symbols.



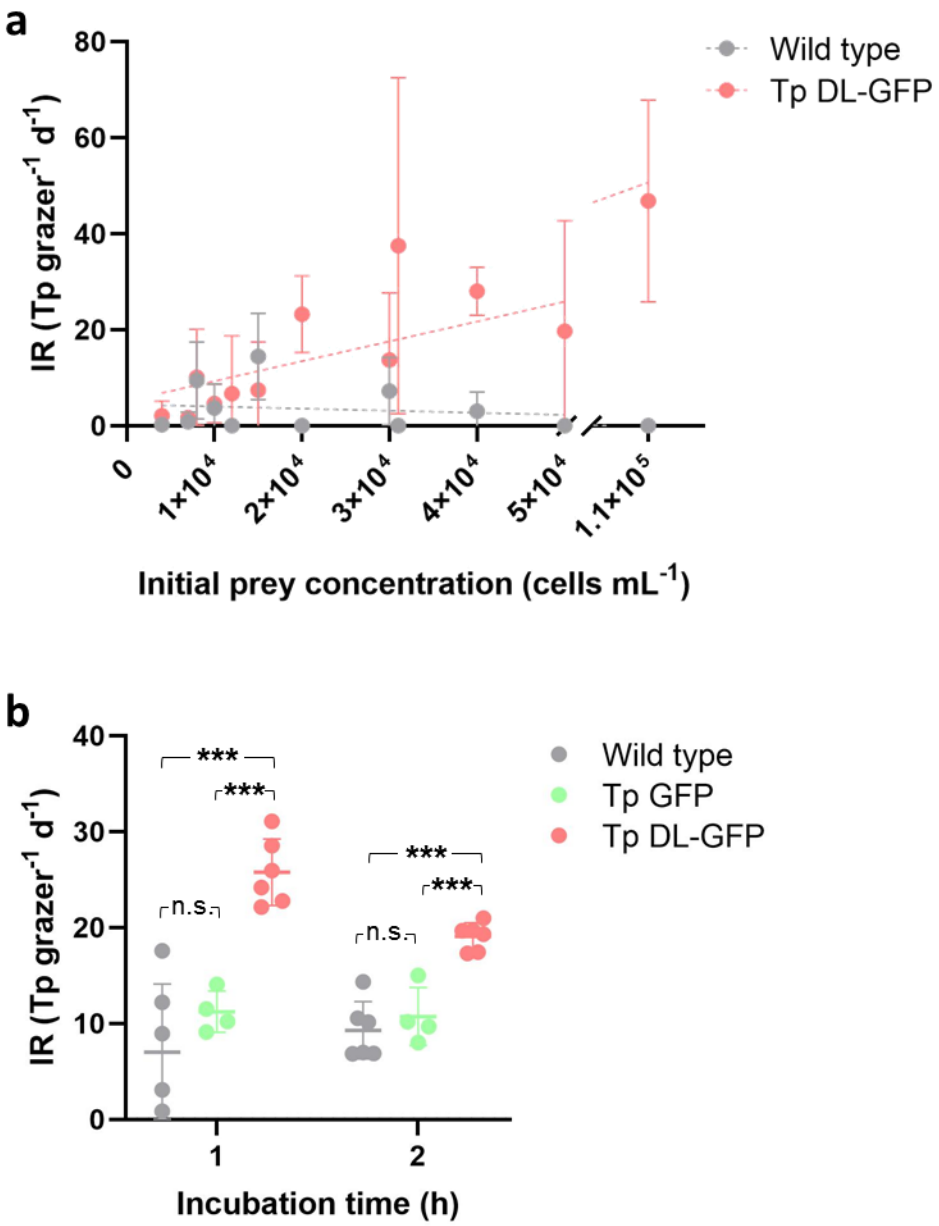
Supplementary Figure 3. Exogenous application of DL-activity products had inconsistent effect on *O. marina* grazing efficiency. DMS (**a**), acrylate (**b**), and DMS + acrylate (**c**) were added at different concentrations to *E. huxleyi-O. marina* grazing interaction. The *E. huxleyi* strain used was *Emiliania huxleyi* CCMP2090, which contain similar amount of DMSP as *Emiliania huxleyi* CCMP373, but has no detectable DMS emission, and thus considered as a low DMSP lyase strain. Ingestion rate (IR) was calculated from cell counts over 1 h and 2 h of grazing interaction by flow cytometry. Approximately 5,000 cells were analyzed per sample. Horizontal lines represent the mean ± SD; *n* = 3-4. Data from at least two independent experiments for each compound is presented; **P* < 0.049; ***P* < 0.0027 (2-way ANOVA followed by Dunnett's post-hoc).



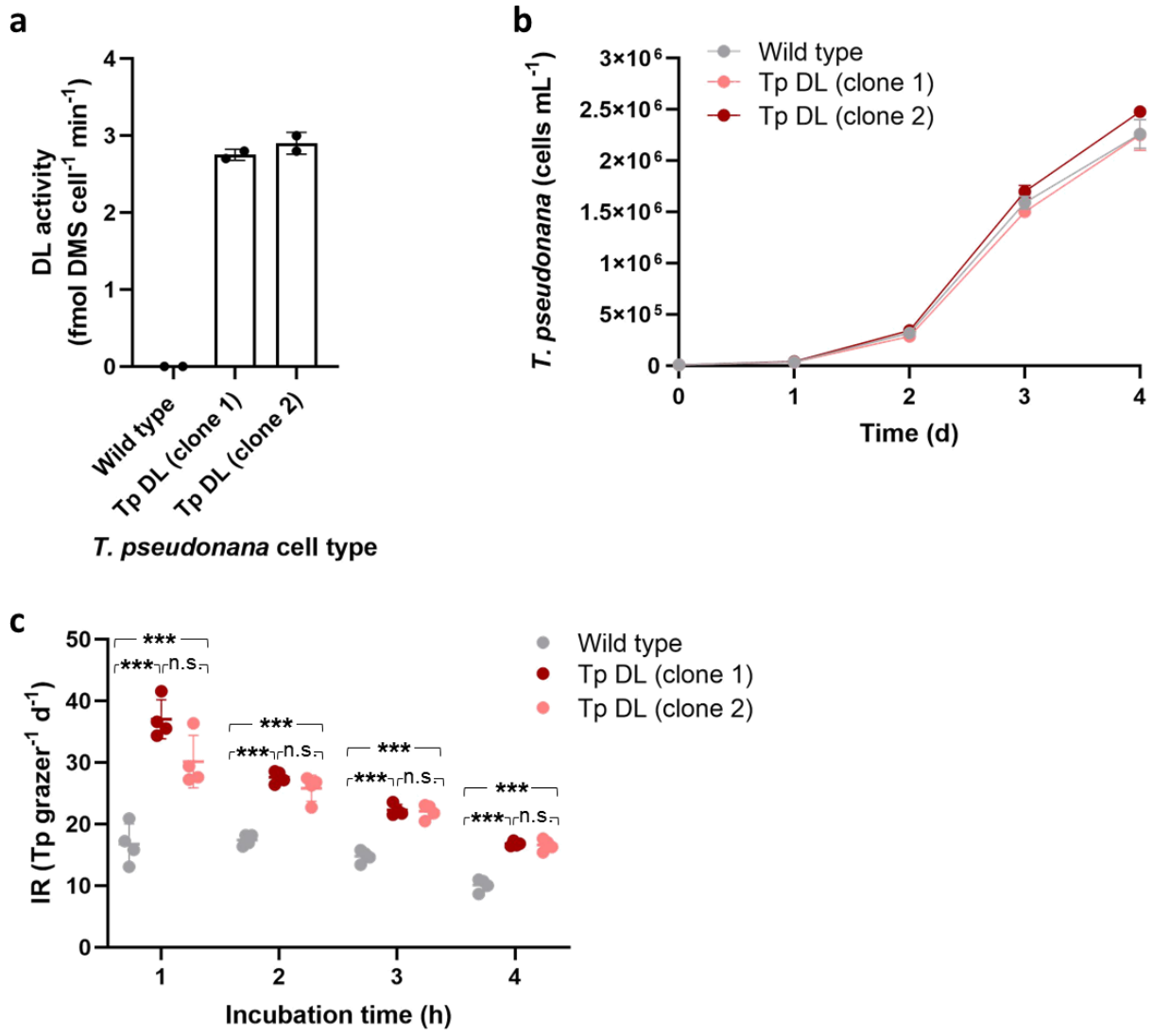
Supplementary Figure 4. Heterologous expression of the DL Alma1 from *E. huxleyi* in a *T. pseudonana* led to high DL activity. a, Merged bright field and autofluorescence micrographs of wild type and transgenic Tp DL-GFP *T. pseudonana* cells. **b,** Western-blot analysis using α-Alma polyclonal antibody raised by immunization with recombinant Alma1 [1]. Note that the antibody may have some cross reactivity. One major band is observed in Tp DL-GFP cell lysate (~65 kDa), corresponding to the size of Alma monomer (~40 kDa) plus the GFP tag. Purified Alma1 protein (recombinant Alma1, 2 ng) was used as a positive control. The purified Alma1 possesses an additional His-Tag and linker and shows the predicted monomer and dimer forms of the enzyme. Tp wild type (WT) and Tp GFP were used as negative controls. *E. huxleyi* strain CCMP373 (Ehux 373), which expresses high amounts of Alma1, was used as reference, with characteristic 4-5 major bands as previously described [1], including the monomer and dimer forms of the enzyme.



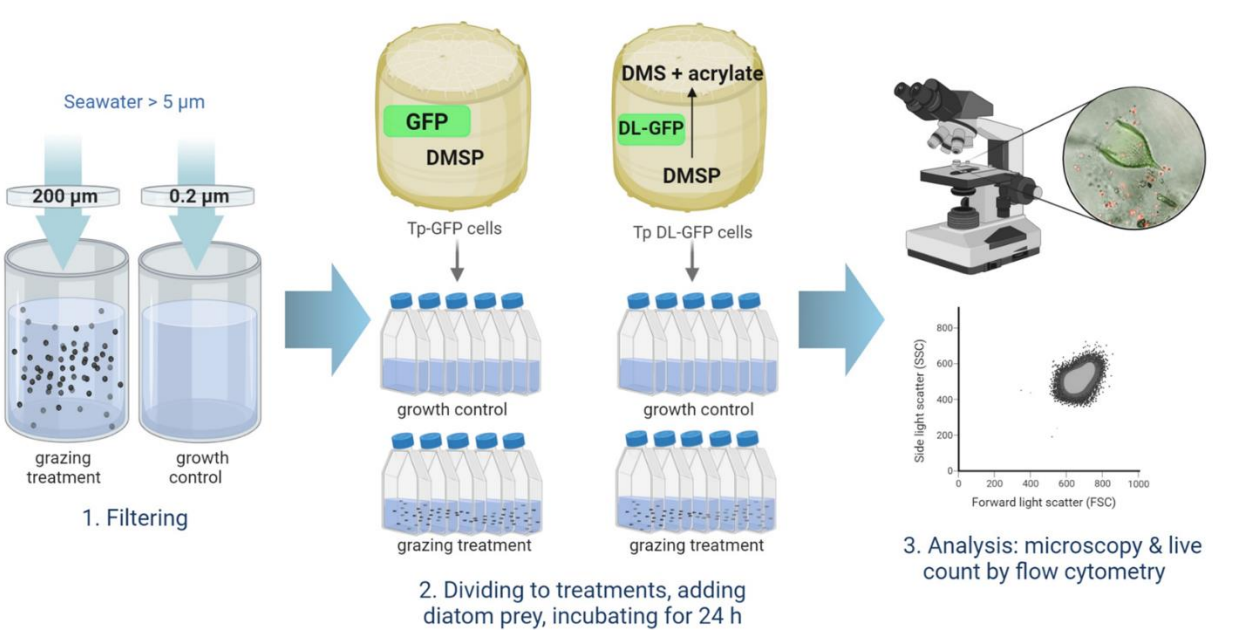
Supplementary Figure 5. Morphology and growth of Tp DL-GFP transformants are similar to wild-type cells. Scanning electron microscopy (SEM) analysis showing the delicate silica frustule of exponentially growing *T. pseudonana* cells. **a,** Wild type cells. **b,** Tp DL-GFP transformants. No differences in silica structure (thickness, structural features of the frustule such as pores and girdle bands) were observed following genetic transformation. **c,** *T. pseudonana* cell abundance during normal growth, as measured by flow cytometry. Values are mean ± SD; *n* = 6. Error bars are smaller than symbols.



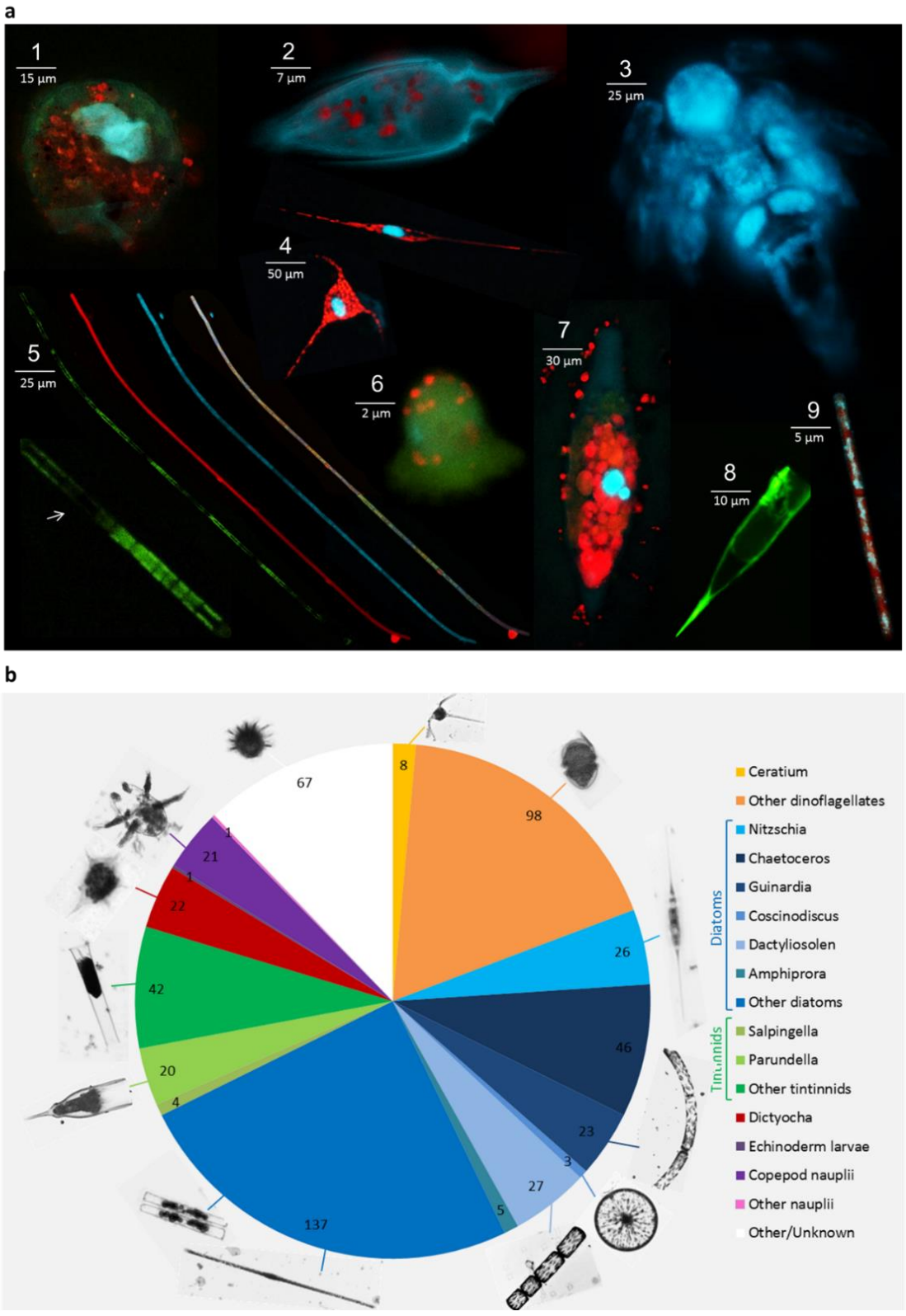
Supplementary Figure 6. A detailed examination of the short-term grazing response by *O. marina* on Tp DL-GFP transformants. a, Ingestion rate (IR) on Tp DL-GFP or wild type cells plotted as a function of prey availability. Values are mean ± SD; n = 4. The trends of the two series are significantly different, $P < 0.00001$ (ANCOVA using initial prey concentration (continuous) and genotype (categorical)). **b,** IR was calculated for 1 and 2 h time intervals. This is the continuation of the measurement presented in Figure 2g in the main text. Horizontal lines represent the mean ± SD; n = 6. ***$P < 0.0004$. No significant difference was found between wild type and Tp GFP, $P > 0.68$ (mixed effects model with random intercept for batch, followed by Tukey post-hoc).



Supplementary Figure 7. Different Tp DL clones exhibited similar grazing phenotypes by *O. marina*. **a,** DL activity in cell lysates of *T. pseudonana* transgenic clones. DL activity levels were measured by DMS generation following the addition of 10 mM DMSP. **b,** *T. pseudonana* growth curve. Values are mean ± SD; *n* = 3. Error bars are smaller than symbols. Growth of Tp wild type was similar to transgenic lines expressing the Alma protein. **c,** Ingestion rate (IR) values were calculated for *O. marina* feeding on the Tp DL clones as prey during 1-4 h. Horizontal lines represent the mean ± SD; *n* = 4. IR of the two Tp DL clones was similar, $P > 0.1405$, but significantly higher than on wild type, $P < 0.0001$ (generalized linear mixed model followed by Tukey post-hoc).

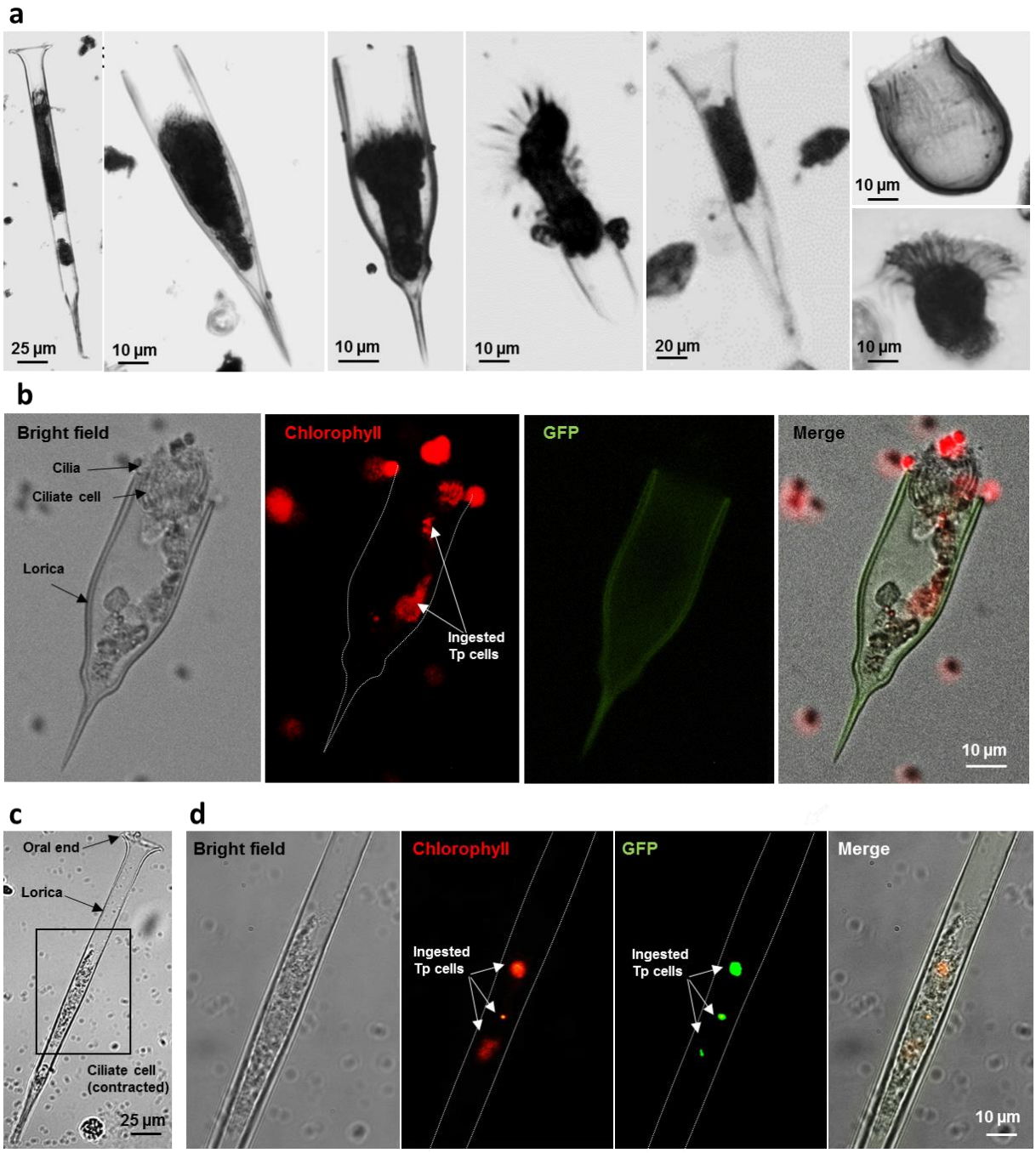


Supplementary Figure 8. Experimental design to assess grazing by natural microzooplankton assemblages on Tp DL-GFP cells. Seawater was filtered to obtain the 5-200 µm fraction and divided to two containers. One container was filtered through 0.2 µm, to remove all cells. Then, Tp DL-GFP or Tp GFP cells were added to each water faction and divided into 5-6 flasks representing biological replicates. The flasks were incubated for 24 hours on a shaded water table or a plankton wheel. Diatom cells were analyzed at t=0 and 24 h by flow cytometry and microscopy to quantify their uptake by the local grazers (as shown in Figure 3b in the main text).



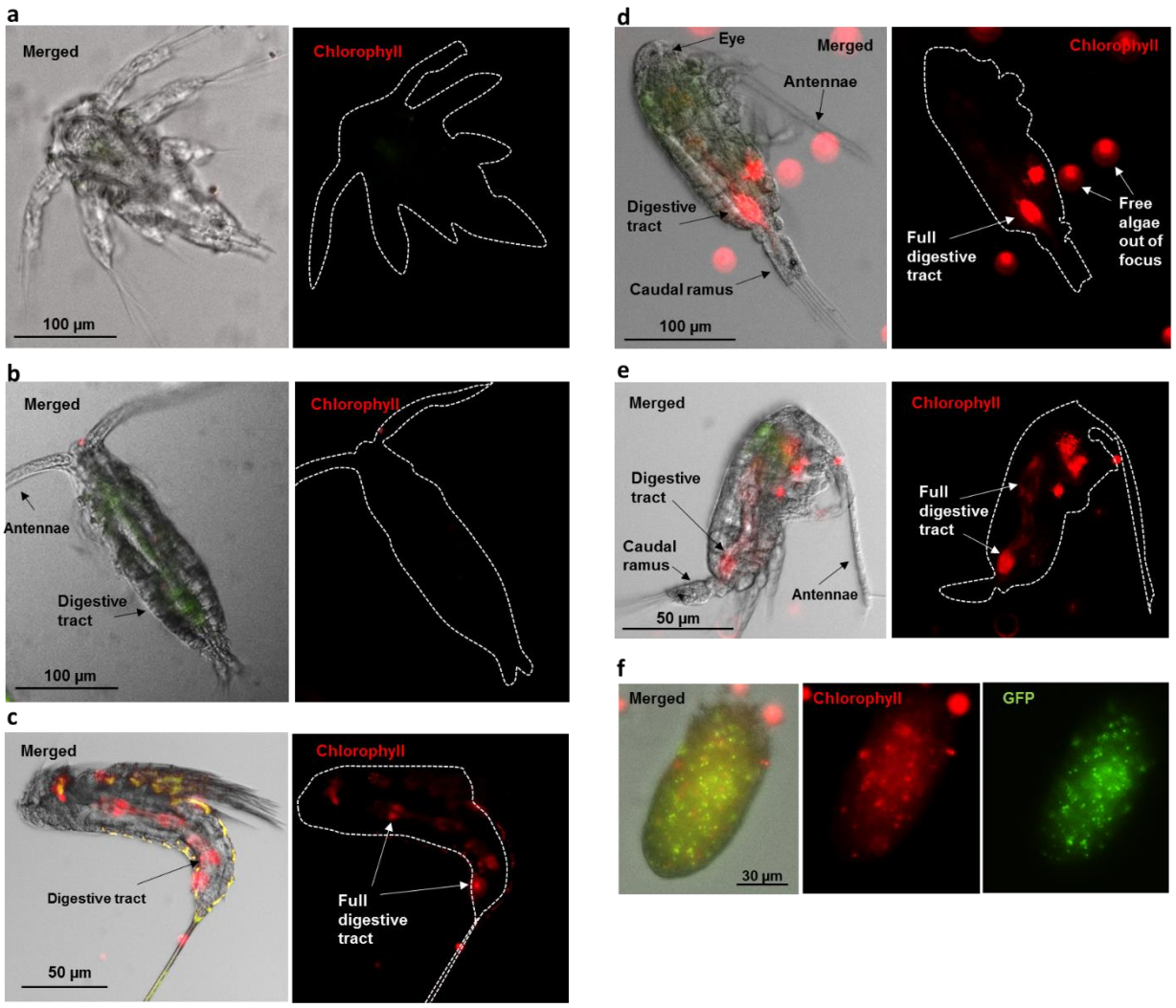
Supplementary Figure 9. Natural microplankton observed in the incubation bottles during grazing experiments. a, Samples were fixed with PFA and incubated with DAPI

nuclear stain (blue) and DiOC6, a general membrane stain (green). DAPI may have non-specific staining, facilitating the visualization of the cell's structure. Red, chlorophyll autofluorescence. (1) An unknown mixotroph. (2) The dinoflagellate *Oxytoxum* (mixotroph) or *Oxyphysis* (heterotroph). The chlorophyll may represent ingested *T. pseudonana* that was added to this sample. (3) A copepod nauplius. (4) Two *Ceratium* mixotrophic dinoflagellates. (5) *Trichodesmium*. The different fluorescence images are shown side by side. Arrow shows magnification of the segmental structure of this cyanobacteria. (6) A tintinnid ciliate, *Eutintinnus* or *Cyttarocylis*. The chlorophyll is probably ingested *T. pseudonana* that was added to this sample. (7) The ciliate *Tiarina* sp. and its symbiotic microalgae (round intracellular chlorophyll fluorescence). The smaller chlorophyll–containing cells surrounding the ciliate are *T. pseudonana* that was added to this sample. (8) A tintinnid ciliate, probably *Cyttarocylis*. (9) Unidentified. **b,** Natural microplankton were fixed with Lugol, imaged and classified to specific taxa and genus level, according to general morphological features. The number of individuals assigned to each group is indicated. A total of 551 cells were observed.



Supplementary Figure 10. Uptake of *T. pseudonana* transgenic cells by wild tintinnid ciliates. Natural microzooplankton were collected from the Red Sea and incubated with Tp DL-GFP or Tp GFP cells as prey for 24 h. **a,** A variety of tintinnids observed during grazing experiments. Samples were fixed with Lugol. **b,** A tintinnid feeding on Tp GFP cells. Some ingested diatoms are observed inside the lorica. The ciliate itself is at the oral end of the lorica, collecting prey using its cilia. The diatoms' GFP is not clearly observed in this case, since this tintinnid has natural green fluorescence. **c,**

A tintinnid fed with Tp DL-GFP cells. The ciliate cell itself is contracted inside the lorica. **d,** A magnified view of the inset in (**c**), showing the fluorescence of ingested Tp DL-GFP cells.



Supplementary Figure 11. Uptake of photosynthetic prey by wild microzooplankton copepods. Micrograph images of small herbivorous copepods captured during grazing experiments. (**a-b**) Copepods from incubation bottles with no added diatom prey. Note that there is no chlorophyll signal in these animals. **a,** Copepod nauplius. **b,** A female *Mecynocera*. (**c-e**) Copepods from incubation bottles with added diatom prey (Tp GFP or Tp DL-GFP). It is highly probable that the copepods were feeding on the added transgenic diatoms (note that their digestive system is full with chlorophyll). Yet we cannot rule out that these copepods were feeding on large diatoms (~50-200 µm) which were also present in the natural assemblages. (**c**) *Microsetella*. (**d-e**) Two copepods from the family of Clausocalanidae. **f,** A fecal pellet, probably produced by a small copepod that consumed transgenic diatoms expressing GFP.

Supplementary Table 1. The effect of Br-DMSP on the consumption of high, low and no-DMS producing prey by *O. marina*.

Prey	DMS producer*	n	$g_{control}$ (day^{-1})	$g_{Br\text{-}DMSP**}$ (day^{-1})	*P*
D. tertiolecta	No	5	3.2 ± 1.3	3.5 ± 1.3	>0.700
E. huxleyi 374	Yes, low	5	9.3 ± 0.5	6.7 ± 0.3	<0.001
E. huxleyi 373	Yes, high	6	10.2 ± 1.0	8.3 ± 0.6	<0.002

*DMS producer definition is based on *in-vitro* activity assay.
**Br-DMSP was added to the co-culture in a final concentration of 200 nM.
$g_{control}$ = grazing rate measured over 3 hours, MeOH was added at t=0.
$g_{Br\text{-}DMSP}$= grazing rate measured over 3 hours, Br-DMSP was added at t=0.

Grazing assays were conducted as described in the Methods section.

Supplementary Table 2. Grazing rates measured for different grazers fed with *T. pseudonana* cells. Values are mean ± SD.

Grazer	*n*	$g_{\text{Tp GFP}}$ (day^{-1})	$g_{\text{Tp DL-GFP}}$ (day^{-1})	*P*
Pleuromamma indica (copepod)	3	0.1 ± 0.3	3.1 ± 0.4	< 0.0005
Artemia salina (brine shrimp)	4	2.2 ± 1.0	4.6 ± 0.6	< 0.006
Strombidium sp. (ciliate)	4	0.3 ± 0.4	1.8 ± 0.8	< 0.018
Gyrodinium dominans (dinoflagellate)	4	0.9 ± 1.9	2.7 ± 1.4	> 0.20
Oxyrrhis marina (dinoflagellate)	6	2.3 ± 1.6	5.3 ± 1.6	< 0.022

Supplementary Table 3. Fecal pellet production by mesozooplankton following tritrophic interaction with *O. marina* and DMS-producing phytoplankton.

Mesozooplankton (# per flask)	Prey	n	*Om ingested	FP production (#FP animal^{-1} d^{-1})	FP biovolume (10^4 mm^3 animal $^{-1}$ d^{-1})	**FP carbon (ng C animal $^{-1}$ d^{-1})	*P*	FP ratio (diet X : no prey)
Euphausia diomedeae (12)	No prey	3	-	2.2 ± 1.8	2.7 ± 2.4	15.2 ± 13.3	-	1.0 ± 0.8
	Om only	3	+	5.8 ± 0.9	10.3 ± 4.8	56.6 ± 26.1	0.397	3.7 ± 1.7
	Om + Ehux 374	3	+	14.8 ± 4.9	20.9 ± 3.5	115.2 ± 19.4	0.005**	7.6 ± 1.3
	Om + Ehux 373	3	+	29.6 ± 4.4	37.5 + 6.8	206.1 ± 37.2	<0.001 ***	13.6 ± 2.5
	Om + Tp GFP	3	+	7.7 ± 1.5	12.8 ± 1.6	70.4 ± 8.7	0.091	4.6 ± 0.6
	Om + Tp DL-GFP	2	++	5.8 ± 1.2	8.2 ± 2.6	45.3 ± 14.2	0.772	2.3 ± 0.1
Pleuromamma indica (10)	No prey	3	-	1.9 ± 1.3	3.5 ± 3.1	19.4 ± 17.1	-	19.4 ± 0.9
	Om only	3	+	4.6 ± 2.2	15.9 ± 9.2	87.7 ± 50.4	0.515	4.6 ± 2.6
	Om + Ehux 374	3	+	6.9 ± 1.3	20.9 ± 3.6	114.9 ± 19.8	0.221	5.9 ± 1.0
	Om + Ehux 373	2	+	7.7 ± 4.4	21.9 ± 6.0	120.3 ± 33.2	0.240	6.2 ± 1.7
	Om + Tp GFP	3	+	6.2 ± 4.7	18.1 ± 10.2	99.8 ± 56.3	0.362	5.1 ± 2.9
	Om + Tp DL-GFP	3	+	9.6 ± 3.7	42.1 ± 12.0	231.4 ± 66.2	0.002**	11.9 ± 3.4
Rhincalanus nasutus (10)	No prey	2	-	1.9 ± 0.3	5.6 ± 2.7	31.3 ± 15.0	-	1 ± 0.5
	Om + Tp GFP	2	+	4.5 ± 0.1	7.0 ± 0.4	38.5 ± 2.1	0.571	1.2 ± 0.1
	Om + Tp DL-GFP	2	++	10.7 ± 4.6	26.2 ± 9.8	144.3 ± 54.0	0.075	4.6 ± 1.7

* The crustaceans gut content was measured by qPCR in order to estimate their direct feeding response on *O. marina* (Om). For technical reasons, gut content analysis based on prey-DNA content yielded very low values and could not be quantified accurately. Thus only approximate evaluation of *O. marina* ingestion by the crustaceans is presented.
**Assuming a carbon : volume ratio of 55 µg C mm^{-3} [2].

Statistical significance (*P*) is related to FP biovolume and carbon, and was calculated by 1-way ANOVA with Dunnett's post-hoc test, for compering each diet to the no-prey treatment.

Supplementary references

1 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).

2 Dagg, M. J., Jackson, G. A. & Checkley, D. M. The distribution and vertical flux of fecal pellets from large zooplankton in Monterey bay and coastal California. *DEEP-SEA RES PT I* **94**, 72-86, doi:https://doi.org/10.1016/j.dsr.2014.09.001 (2014).