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Trophic uptake and transfer of DMSP in simple planktonic food chains

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ABSTRACT: Recent field studies suggest that a large portion of phytoplankton-DMSP could be lost to grazing by protozoans, but the fate of the grazed DMSP remains uncertain. In the laboratory we studied trophic uptake and transfer of phytoplankton-DMSP through simple planktonic food chains using 2 experimental approaches: (1) A direct approach measured the ingestion and retention of phytoplankton-DMSP by the heterotrophic dinoflagellate *Gyrodinium dominans*. Overall, DMSP content of *G. dominans* estimated by the direct approach was highly variable, likely because of the low *G. dominans* biomass relative to phytoplankton in the samples. (2) An indirect approach, in which the omnivorous copepod *Acartia tonsa* was allowed to prey on a mixture of phytoplankton and *G. dominans*. Using this indirect approach, *A. tonsa* retained a high concentration of *G. dominans* biomass in its guts. Combined with other feeding parameters, the copepod gut contents were used to derive the DMSP content of *G. dominans*. When fed on *Phaeocystis globosa*, *G. dominans* retained 1.64×10^{-4} nmol DMSP cell^{-1}, or 44% of the grazed DMSP. When fed on *Isochrysis galbana*, the protozoan retained 6.87×10^{-5} nmol DMSP cell^{-1}, or 32% of the grazed DMSP. *A. tonsa* selectively preyed on *G. dominans* when offered a mixture of *G. dominans* and phytoplankton, deriving 63 to 84% of their dietary DMSP from the protozoan. Our study suggests that protozoans are an important trophic linkage to transfer phytoplankton-DMSP up food chains, and that the effectiveness of this linkage is dependent on the species composition of both the phytoplankton and the higher trophic levels.

KEY WORDS: Sulfur cycle · Dimethylsulfide · Dimethylsulfoniopropionate · Microzooplankton · Mesozooplankton

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INTRODUCTION

The climatically active gas DMS is a breakdown product of DMSP, one of several osmolytes synthesized by phytoplankton (Keller & Korjeff-Bellows 1996, Kirst 1996, Welsh 2000). DMS accounts for >90% of sea-air exchange of biogenic sulfur (Lovelock et al. 1972, Nguyen et al. 1978, 1983, Andreae & Raemdonck 1983). Once in the atmosphere, DMS further reacts to form cloud condensation nuclei, thereby affecting the global radiation budget (Charlson et al. 1987, Andreae 1990). While DMSP is synthesized by phytoplankton, the conversion of DMSP to DMS is regulated by complex trophic processes in the water column (Kiene et al. 2000, Tang et al. 2000b, Simó 2001). Global measurements show poor correlations between DMS and parameters directly related to primary producers (e.g. seston-DMSP, chlorophyll, dissolved nutrients; Kettle et al. 1999), further confirming that factors other than phytoplankton play important roles in oceanic DMS and DMSP dynamics. Recent field studies show that a significant portion of phytoplankton-DMSP is consumed by grazers. For example, 91% of the phytoplankton-DMSP loss was mediated by microzooplankton grazing during an *Emiliania huxleyi* bloom in the northern North Sea (Archer et al. 2002). In the North Atlantic, microzooplankton ingested on average 44% d^{-1} of the particulate DMSP stock, accounting for 63% of the algal DMSP loss per day (Simó et al. 2002). Quantitative information on mesozooplankton grazing of phyto-

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plankton-DMSP is largely limited to laboratory studies (e.g. Kwint et al. 1996a, Tang et al. 1999). Mesocosm studies, however, suggest that when the phytoplankton community is dominated by small species, mesozooplankton control DMS and DMSP dynamics mainly by preying on microzooplankton (Kwint et al. 1996b, Levasseur et al. 1996). Although zooplankton grazing of phytoplankton-DMSP has been discussed frequently, what is less clear is the fate of the grazed DMSP. Several investigators have shown that grazing sometimes promotes DMS production (Christaki et al. 1996, Daly & DiTullio 1996, Kwint et al.1996b) due to sloppy feeding (Dacey & Wakeham 1986, but see Tang et al. 2000a), or enhanced DMSP-lyase activities in damaged phytoplankton cells (Wolfe & Steinke 1996). Through these mechanisms grazing may accelerate the conversion of DMSP to DMS and possibly the sea-air exchange of sulfur. On the other hand, if grazers accumulated ingested DMSP, they would retain DMSP within the food web (Belviso et al. 1990), transfer it up food chains (e.g. Levasseur et al. 1994) and thereby uncouple DMSP and DMS productions.

Among the planktonic grazers, copepods have been shown to accumulate DMSP in their guts or body tissues after feeding on phytoplankton (Tang et al. 1999, 2000a, Tang 2000). Whether protozoans retain ingested DMSP is less certain, and so far only indirect evidence is available: (1) Imbalances in budgeting the phytoplankton stock and production of DMSP and all measured DMSP losses in grazing studies are attributed to uptake by protozoans (Wolfe et al. 1994, Archer et al. 2001, Simó et al. 2002). (2) Field-collected omnivorous and carnivorous copepods contain DMSP in their bodies, which is likely derived from ingested protozoans (Tang et al. 1999, 2000b). Measuring protozoan-bound DMSP is difficult because most protozoans are fragile and similar in size to phytoplankton and therefore cannot be isolated easily. This is particularly problematic with field samples where protozoan biomass is generally similar to, or lower than, phytoplankton biomass. An alternative approach would be to measure DMSP in concentrated protozoan biomass in the laboratory, provided that the amount of DMSP from coexisting phytoplankton cells is known.

Our goal was to study the trophic uptake and transfer of DMSP through simple planktonic food chains. Specific objectives were to: (1) measure DMSP retention (assimilated or temporarily stored in food vacuoles) by protozoans, (2) study DMSP dynamics within simple food chains of copepod, protozoan and phytoplankton, and (3) study species-specific differences by using 2 phytoplankton species for all experiments. Our results show that the pelagic food web structure plays important roles in DMSP dynamics because of retention of ingested DMSP in protozoan biomass and selective feeding by copepods on protozoans.

MATERIALS AND METHODS

Phytoplankton and grazers. The phytoplankton and grazer species used in the present study are listed in Table 1. Non-axenic inocula of *Phaeocystis globosa* CCMP 1528 and *Isochrysis galbana* (Prymnesiophyceae) were respectively obtained from Bigelow Laboratory (Maine, USA) and University of Copenhagen (Denmark). *P. globosa* is a major DMSP producer (e.g. van Duyl et al. 1998) that frequently forms massive blooms in coastal waters (Lancelot et al. 1998). *I. galbana* is another common coastal prymnesiophyte that produces DMSP (Christaki et al. 1996, Niki et al. 2000). Phytoplankton cultures were grown in aerated L-medium at 19 ± 1°C, 60 to 100 µE m^{-2} s^{-1} with a 12:12 h dark:light cycle. The cultures were maintained in active growth by regular dilution with fresh medium. Because *P. globosa* is able to form large spherical colonies, single *P. globosa* cells were obtained by filtering the culture through an 11 µm sieve under gravity prior to experiments (Jakobsen & Tang 2002). The cosmopolitan heterotrophic dinoflagellate *Gyrodinium dominans* was originally isolated from Øresund, Denmark, and maintained in L-medium with the experimental diets for >5 generations before

Table 1. Phytoplankton and grazer species studied. Size of live phytoplankton cells was measured by Elzone particle sizer. Phytoplankton carbon content is estimated according to Strathmann (1967). Carbon content of *Gyrodinium dominans* is estimated according to Menden-Deuer & Lessard (2000)

Species	Taxonomic group	Cell size (µm)	Carbon content (pg C $cell^{-1}$)
Phaeocystis globosa	Prymnesiophyte	4.4	9.3
Isochrysis galbana	Prymnesiophyte	4.2	8.2
Gyrodinium dominans	Heterotrophic dinoflagellate	16.6 ± 2.7[a]	314
Acartia tonsa	Calanoid copepod	500[b]	

[a] *G. dominans* was fed *I. galbana* ad libitum for >5 d and observed under an inverted microscope with a B/W camera. Video images of live *G. dominans* cells were digitized by Videum 1000™ video system and analysed by SigmaScan Pro™ software for cell size (mean ± SD)
[b] Value for *A. tonsa* is approximate body length

Table 2. Experimental setup for the incubation experiments. Each type of incubation was run in triplicate. Approximate initial carbon concentrations are given in 2nd and 3rd columns. μ_p = phytoplankton specific growth rate, μ_G = *Gyrodinium dominans* specific growth rate, I_G = *G. dominans* ingestion rate, I_A = *Acartia tonsa* ingestion rate, D_p = DMSP content of phytoplankton cell, D_G = DMSP content of *G. dominans*, D_A = DMSP content of *A. tonsa*

Incubation type	Phytoplankton (ng C ml^{-1})	*Gyrodinium dominans* (ng C ml^{-1})	*Acartia tonsa* (total no.)	Sampling frequency	Parameter derived
Phaeocystis globosa					
1	230	0	0	Day 0, 1, 2, 4, 6, 8	μ_P, D_P
2	204	30	0	Day 0, 2, 4, 6, 8	μ_G, I_G, D_G
3	235	0	10	Day 0, 1	I_A, D_A
4	172	38	10	Day 0, 1	I_A, D_A
Isochrysis galbana					
1	66	0	0	Day 0, 1, 2, 4, 6, 8	μ_P, D_P
2	66	21	0	Day 0, 2, 4, 6, 8	μ_G, I_G, D_G
3	147	0	10	Day 0, 1	I_A, D_A
4	79	29	10	Day 0, 1	I_A, D_A

experiments. The size of live phytoplankton cells was measured using an Elzone particle sizer, whereas the size of live *G. dominans* was estimated from digitized video images. The cellular carbon contents of the phytoplankton and *G. dominans* were calculated from cell size according to Strathmann (1967) and Menden-Deuer & Lessard (2000). The common omnivorous calanoid copepod *Acartia tonsa* was used for the present study. Nauplii of *A. tonsa* were hatched from eggs and cohorts were raised on a food mixture of *Rhodomonas salina* (Chrysophyceae) and *Thalassiosira weissflogii* (Bacillariophyceae). Adult *A. tonsa* (within 4 wk from hatching) were collected for subsequent experiments.

For each phytoplankton species, 4 types of bottle incubation experiments were conducted simultaneously. Adult *Acartia tonsa* females were starved in filtered seawater (FSW) for 2 d to empty their gut contents prior to experiments. Phytoplankton and *Gyrodinium dominans* cultures were diluted with L-medium to the desired concentrations for the experiments. Preliminary experiments showed that *Isochrysis galbana* grew at a high rate; therefore, to avoid fouling by excessive phytoplankton concentration, the initial concentration of *I. galbana* was adjusted to lower than that of *Phaeocystis globosa* in all experiments. All incubations were done in 315 ml glass bottles (triplicate) at 19 ± 1°C, 60 to 100 µE m^{-2} s^{-1} with a 12:12 h dark:light cycle. The bottles were fastened to a rotating plankton wheel (0.4 rpm) to maintain particles in suspension. Bottle content, incubation duration, and parameters derived from each experiment are summarized in Table 2. Additional explanations for the experiments are given below.

Phytoplankton measurements (Incubation Type 1). Phytoplankton were incubated for 8 d. Aliquots were drawn with pipettes and preserved in 4% acid Lugol's solution for cell counts (in Sedgewick-Rafter counting chambers). To measure cellular DMSP content, 10 ml aliquots were sampled with a glass syringe and slowly filtered onto GF/F filter papers. The filter papers were transferred to cryogenic vials and preserved immediately in liquid nitrogen until DMSP measurements were taken (Simó et al. 1998).

Protozoan measurements (Incubation Type 2). *Gyrodinium dominans* was incubated with phytoplankton and the change in cell concentrations was followed for 8 d. Aliquots were drawn every second day for cell counts (settling chambers for *G. dominans* and Sedgewick-Rafter counting chambers for phytoplankton cells). Additional 10 ml aliquots were drawn from each incubation bottle with a glass syringe and slowly filtered onto GF/F filter papers; the filter papers were transferred to cryogenic vials and preserved immediately in liquid nitrogen until DMSP measurements were taken. DMSP content of *G. dominans* was calculated after correcting for DMSP from phytoplankton based on cell counts and DMSP content of the phytoplankton cells.

Direct grazing experiments with copepods (Incubation Type 3). Starved *Acartia tonsa* (10 in triplicate) were removed, briefly rinsed in FSW and preserved in liquid nitrogen for background DMSP; groups of 10 *A. tonsa* were incubated with phytoplankton for 1 d. At the end of the incubation, aliquots of the bottle content were preserved for phytoplankton cell counts. The copepods were removed, rinsed and preserved for DMSP measurements.

Tri-trophic feeding experiments with protozoans and copepods (Incubation Type 4). Starved *Acartia tonsa* (10 in triplicate) were incubated with a mixture of *Gyrodinium dominans* and phytoplankton. After 1 d of incubation, aliquots were preserved for *G. dominans* and phytoplankton cell counts and the copepods were collected for DMSP measurements.

DMSP measurements. Samples were transported to Barcelona, frozen in dry ice. DMSP was quantitatively converted to DMS by alkaline hydrolysis (NaOH) of the filters and copepod individuals in air-tight vials filled with Milli-Q water, in the dark, at room temperature, for 18 to 24 h. The evolved DMS was determined following purge, cryotrapping and sulfur-specific gas chromatography procedures described elsewhere (Simó et al. 1996). The detection limit was 30 pmol.

Calculation of growth rates and ingestion rates. Growth rates of phytoplankton were derived from Days 0 to 2 of Incubation Type 1 assuming exponential growth. Growth and feeding rates of *Gyrodinium dominans* in Incubation Type 2 were derived from cell counts between Days 0 and 2 when food was not limiting: Growth rate was calculated assuming exponential growth, and ingestion rate was calculated using the iterative approach as described in Jakobsen & Hansen (1997) and Tang et al. (2001a), assuming that the phytoplankton growth rates did not differ between the grazing treatments and the controls. Ingestion rates of *Acartia tonsa* on phytoplankton in the direct grazing experiments (Incubation Type 3) were calculated according to Frost (1972). In the tri-trophic experiments (Incubation Type 4), the ingestion rate of *A. tonsa* on *G. dominans* was calculated according to Frost (1972). The phytoplankton cells were eaten by both *G. dominans* and *A. tonsa*, while *G. dominans* were eaten by *A. tonsa* at the same time; thus, the rate of ingestion of phytoplankton cells by *A. tonsa* had to be calculated by solving coupled non-linear equations as explained in Tang et al. (2001a).

Experiment with *Dunaliella tertiolecta*. To further test the hypothesis that *Gyrodinium dominans* obtain DMSP from food, we conducted an additional experiment in which *G. dominans* were incubated with the chlorophyte *D. tertiolecta*. *D. tertiolecta* produces no or little DMSP (Tang et al. 1999); therefore, *G. dominans* is expected to contain no or little DMSP when fed *D. tertiolecta*. *G. dominans* that had been growing for >5 generations on a diet of *D. tertiolecta* were incubated with *D. tertiolecta* for 1 d (970 *G. dominans* + 35 000 *D. tertiolecta* ml^{-1}, quadruplicate), and aliquots were collected for DMSP measurements. Ten ml aliquots of dense *D. tertiolecta* culture (1.45×10^5 ml^{-1}; quadruplicate) were also collected for DMSP measurements.

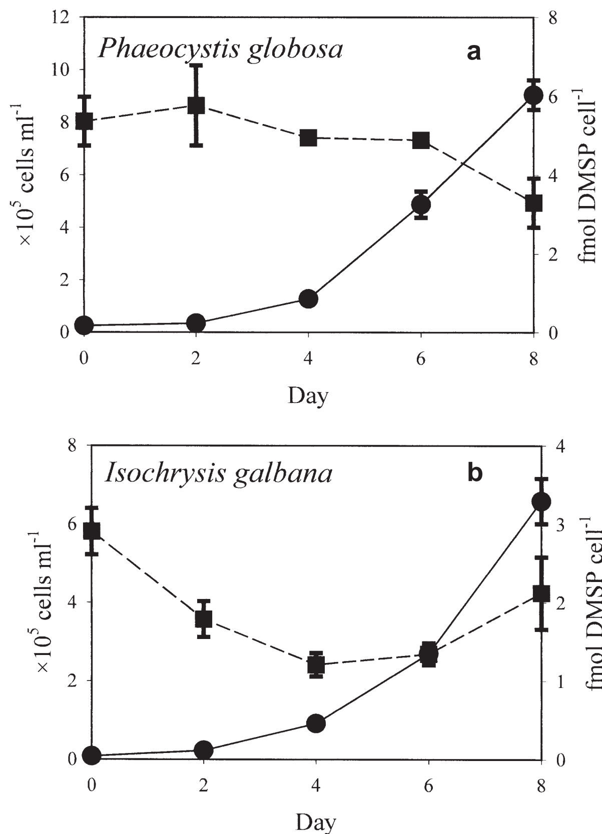

Fig. 1. Cell concentrations (●; mean ± SD of triplicates) and DMSP contents (■; mean ± SD of 2 to 3 replicates) of (a) *Phaeocystis globosa* and (b) *Isochrysis galbana*

RESULTS

Experiments with *Phaeocystis globosa*

Phaeocystis globosa grew exponentially from Days 2 to 8 during the experimental period, during which time the cellular DMSP averaged 4.86 fmol $cell^{-1}$ (SD = 0.96 fmol $cell^{-1}$) (Fig. 1). This average value was used for subsequent calculations. After 2 d starvation, *Acartia tonsa* contained a negligible amount of DMSP (below detection limit), confirming that this species accumulates no or little DMSP in the absence of DMSP-containing food (Tang 2000). When fed on *P. globosa* alone (Incubation Type 3), *A. tonsa* ingested 3.07 µg C $ind.^{-1}$ d^{-1} (mean filtration rate 15.2 ml $ind.^{-1}$ d^{-1}) or 1.60 nmol DMSP $ind.^{-1}$ d^{-1} (Fig. 2). This ingestion rate was higher than that reported in Tang et al. (2001a), likely a result of an elevated ingestion rate after starvation (Tiselius 1998). At the end of the incubation, *A. tonsa* bodies contained only 0.068 nmol DMSP $ind.^{-1}$, or 4.3% of the total ingested DMSP (Fig. 2). If the copepods contained DMSP as gut content, and assuming the gut content was at steady state, the gut passage time (T) can be expressed as a function of DMSP gut content (G) and ingestion rate (I):

$$T = \frac{G}{I} \quad (1)$$

In this experiment, the estimated gut passage time was 61 min, consistent with the general gut passage

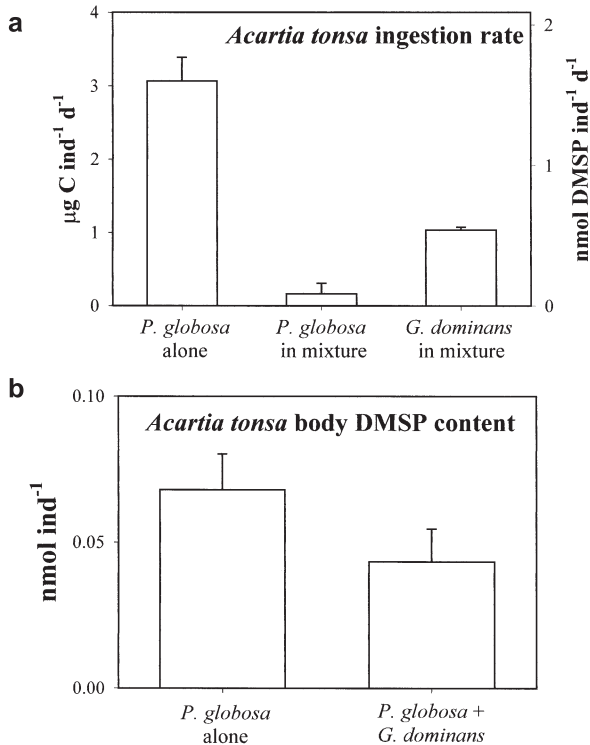

Fig. 2. *Phaeocystis globosa* experiments. (a) Carbon (left axis) and DMSP (right axis) ingestion rates of *Acartia tonsa* in single food treatment (*P. globosa*) and mixed food treatment (*P. globosa* + *Gyrodinium dominans*). See text for derivation of DMSP content of *G. dominans*. (b) DMSP content of *A. tonsa* in single food treatment and mixed food treatment. Error bars are +SD of triplicates

times for calanoid copepods (to the order of 1 h at 17 to 19°C; Mauchline 1998), further supporting the notion that *A. tonsa* contained DMSP as gut content.

When fed on a mixture of *Phaeocystis globosa* and *Gyrodinium dominans* (Incubation Type 4), *Acartia tonsa* ingested a lower amount of total carbon (1.20 µg ind.$^{-1}$ d^{-1}), similar to earlier observations (Tang et al. 2001a). Although *G. dominans* and *P. globosa* were mixed at a ratio of 1:4.5 in terms of carbon concentrations, *A. tonsa* selectively fed on *G. dominans*, deriving 86% of the total carbon intake from *G. dominans* (Fig. 2), also consistent with previous observations (Tang et al. 2001a). Mean filtration rates of *A. tonsa* in the mixed food treatment were 1.5 and 32.5 ml ind.$^{-1}$ d^{-1} on *P. globosa* and *G. dominans*, respectively. Since *A. tonsa* accumulated ingested DMSP in the gut, the observed DMSP content of *A. tonsa* could be combined with ingestion rate to deduce the cellular DMSP content of *G. dominans*. Based on Eq. (1):

$$\frac{T_M}{T_P} = \frac{G_M / I_M}{G_P / I_P}$$

where the subscript M denotes parameters for the mixed food treatment and P for the phytoplankton treatment. Rearranging this equation gives us:

$$T_M = \frac{G_M}{G_P} \times \frac{I_P}{I_M} \times T_P \quad (2)$$

Substituting body DMSP contents for parameter *G* and carbon ingestion rates for parameter *I*, T_M is estimated to be 98.7 min. Since *Acartia tonsa* accumulated 0.043 nmol DMSP ind.$^{-1}$ when fed on the food mixture (Fig. 2), this amount of DMSP would be derived from food ingested within one gut passage time, which equalled 1214 *Phaeocystis globosa* cells and 226 *Gyrodinium dominans* cells. Based on the average DMSP content of *P. globosa* from Incubation Type 1, ingested DMSP due to *P. globosa* cells equalled 5.90 × 10^{-3} nmol. The remaining 0.037 nmol DMSP must have come from ingested *G. dominans*, and the cellular DMSP content of *G. dominans* would therefore be 1.64 × 10^{-4} nmol cell^{-1}. Knowing the carbon content of *G. dominans* was 0.31 ng cell^{-1}, the carbon-specific DMSP content of *G. dominans* feeding on *P. globosa* would be 0.52 pmol DMSP ng^{-1} C. We define a trophic dilution factor (TDF) as:

$$\text{TDF} = \frac{\text{C-specific DMSP content of predator}}{\text{C-specific DMSP content of food}} \quad (3)$$

Given that the carbon-specific DMSP content of *Phaeocystis globosa* was 0.52 pmol DMSP ng^{-1} C, the TDF between *Gyrodinium dominans* and *P. globosa* was 0.52/0.52 = 1.00. From Incubation Type 2, *G. dominans* had an ingestion rate of 36.2 *P. globosa* cells ind.$^{-1}$ d^{-1} and a specific growth rate (µ) of 0.66 d^{-1}. Since *G. dominans* grew by binary fission, the generation time of 1 *G. dominans* cell can be calculated as ln2/µ = 1.05 d. Thus, before dividing, *G. dominans* ingested 38 *P. globosa* cell ind.$^{-1}$, or 1.85 × 10^{-4} nmol DMSP ind.$^{-1}$, of which 0.5 × 1.64 × 10^{-4} nmol was retained by the cell (the factor 0.5 is to correct for DMSP inherited from parent cell under steady-state condition). Therefore, the trophic uptake efficiency of DMSP by *G. dominans* feeding on *P. globosa* was 44%, and the DMSP removal efficiency was (100 – 44)% = 56%. The removal efficiency indicates the missing portion of the ingested DMSP, which could be degraded or excreted by the protozoan or released during digestion of the alga by the protozoan.

The DMSP content of *Gyrodinium dominans* can be independently calculated using data from Incubation Types 1 and 2, in which cell concentrations and particulate DMSP concentrations were measured every 2 d, for a total of 8 d. The average DMSP content of *Phaeocystis globosa* cells determined from Incubation Type 1 was 4.86 fmol cell^{-1}. Applying this value to Incubation Type 2, we calculated the particulate DMSP concentra-

Table 3. Cellular DMSP content of *Gyrodinium dominans* feeding on *Phaeocystis globosa* as derived from Incubation Type 2. Average DMSP content of *P. globosa* was obtained from Incubation Type 1 (see Fig. 1)

Day	*G. dominans* (cells ml^{-1})	*P. globosa* (cells ml^{-1})	Total particulate DMSP (nmol ml^{-1})	DMSP from *P. globosa* (nmol ml^{-1})	DMSP from *G. dominans* (nmol ml^{-1})	DMSP content of *G. dominans* (nmol cell^{-1})
0	93.7	21967	0.11	1.07×10^{-1}	1.05×10^{-3}	1.12×10^{-5}
2	349.4	14383	0.06	6.99×10^{-2}	-5.96×10^{-3}	-1.71×10^{-5}
4	582.5	9361	0.05	4.55×10^{-2}	5.62×10^{-3}	9.65×10^{-6}
6	450.3	8511	0.12	4.14×10^{-2}	7.46×10^{-2}	1.66×10^{-4}
8	170.6	46011	0.23	2.24×10^{-1}	1.06×10^{-2}	6.19×10^{-5}
						Average 6.22×10^{-5} (4.63×10^{-5})[a]

[a]Average including the negative value

tion derived from *P. globosa* cells, and the difference of that from the observed particulate DMSP concentration would be DMSP derived from *G. dominans* cells. The calculated values show considerable scatter (Table 3). The value that agrees best with the previous estimate was from Day 6 (1.66×10^{-4} nmol cell^{-1}) when the DMSP signal from *P. globosa* was relatively small. The cellular DMSP content of *G. dominans* averaged $4.63\sim6.22 \times 10^{-5}$ nmol cell^{-1} over 8 d.

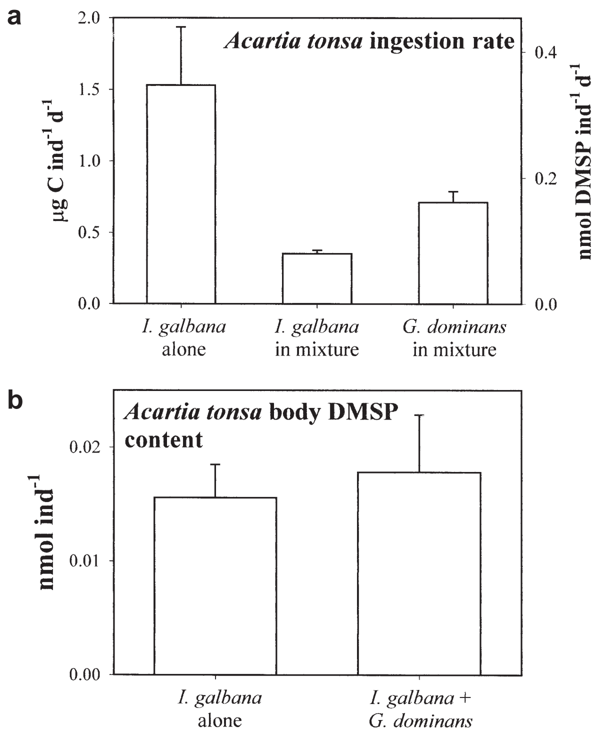

Fig. 3. *Isochrysis galbana* experiments. (a) Carbon (left axis) and DMSP (right axis) ingestion rates of *Acartia tonsa* in single food treatment (*I. galbana*) and mixed food treatment (*I. galbana* + *Gyrodinium dominans*). See text for derivation of DMSP content of *G. dominans*. (b) DMSP content of *A. tonsa* in single food treatment and mixed food treatment. Error bars are +SD of triplicates

Experiments with *Isochrysis galbana*

The cellular DMSP content of *Isochrysis galbana* varied during exponential growth and averaged 1.86 fmol cell^{-1} (SD = 0.66 fmol cell^{-1}) over 8 d (Fig. 1). When fed only on *I. galbana*, *Acartia tonsa* ingested 1.57 µg C ind.$^{-1}$ d^{-1} (mean filtration rate 9.4 ml ind.$^{-1}$ d^{-1}) (Fig. 3) or 0.35 nmol DMSP ind.$^{-1}$ d^{-1}, and retained 0.016 nmol DMSP ind.$^{-1}$ (Fig. 3). Thus, the estimated gut passage time was 65.8 min. When fed a mixture of *I. galbana* and *Gyrodinium dominans*, *A. tonsa* ingested 1.06 µg C ind.$^{-1}$ d^{-1} (Fig. 3) and retained 0.018 nmol DMSP ind.$^{-1}$ (Fig. 3). Although *G. dominans* made up only 27 % of the carbon concentration in the mixture, *A. tonsa* derived 67 % of their carbon intake by ingesting *G. dominans* (Fig. 3). Mean filtrate rates of *A. tonsa* in the mixed food treatment were 5.5 ml ind.$^{-1}$ d^{-1} on *I. galbana* and 28.7 ml ind.$^{-1}$ d^{-1} on *G. dominans*. The lower ingestion rate in the mixed food treatment corresponds to a longer gut passage time (Mauchline 1998). From Eq. (2), the estimated gut passage time was 110 min in the mixed food treatment, during which time *A. tonsa* would have ingested 3280 *I. galbana* cells, or 6.10×10^{-3} nmol DMSP from *I. galbana*. In the same duration *A. tonsa* would have ingested 173 *G. dominans* cells, which would account for the remaining 1.19×10^{-2} nmol DMSP in the gut. This gives a cellular DMSP content of *G. dominans* of 6.87×10^{-5} nmol cell^{-1}. Carbon-specific DMSP content of *G. dominans* feeding on *I. galbana* was 0.22 pmol DMSP ng C^{-1}, lower than that for the *Phaeocystis globosa* experiments. This indicates that retention of DMSP in *G. dominans* biomass is dependent on food type. The carbon-specific DMSP content of *I. galbana* was 0.35 pmol DMSP ng C^{-1}, yielding a TDF of 0.63 between *G. dominans* and *I. galbana*. The generation time of a *G. dominans* cell feeding on *I. galbana* was 1.17 d, during which the cell would have ingested 58.9 *I. galbana* cells, or 1.09×10^{-4} nmol DMSP. Accordingly, the uptake efficiency of DMSP was 32 %, and the removal efficiency 68 %.

Table 4. Cellular DMSP content of *Gyrodinium dominans* feeding on *Isochrysis galbana* as derived from Incubation Type 2. Average DMSP content of *I. galbana* was obtained from Incubation Type 1 (see Fig. 1)

Day	*Gyrodinium dominans* (cells ml^{-1})	*Isochrysis galbana* (cells ml^{-1})	Total particulate DMSP (nmol ml^{-1})	DMSP from *I. galbana* (nmol ml^{-1})	DMSP from *G. dominans* (nmol ml^{-1})	DMSP content of *G. dominans* (nmol cell^{-1})
0	64	8100	0.015	1.51×10^{-2}	-9.30×10^{-5}	-1.45×10^{-6}
2	207	2467	0.007	4.60×10^{-3}	2.10×10^{-3}	1.02×10^{-5}
4	289	1247	0.005	2.32×10^{-3}	2.93×10^{-3}	1.02×10^{-5}
6	80	817	0.004	1.52×10^{-3}	2.88×10^{-3}	3.60×10^{-5}
8	9	977	0.005	1.82×10^{-3}	2.98×10^{-3}	3.44×10^{-4}
						Average 1.00×10^{-4} (7.98×10^{-5})[a]

[a]Average including the negative value

Table 5. Estimates of DMSP content of *Gyrodinium dominans* feeding on *Dunaliella tertiolecta*. *D. tertiolecta* contributed negligible DMSP to the samples

Replicate	*Gyrodinium dominans* (cells ml^{-1})	*Dunaliella tertiolecta* (cells ml^{-1})	Total particulate DMSP (nmol ml^{-1})	DMSP content of *Gyrodinium dominans* (nmol cell^{-1})
1	2570	3530	4.7×10^{-3}	1.83×10^{-6}
2	2270	2800	6.4×10^{-3}	2.82×10^{-6}
3	2450	1980	6.3×10^{-3}	2.57×10^{-6}
4	2630	2560	6.0×10^{-3}	2.28×10^{-6}
				Average 2.38×10^{-6}

The cellular DMSP content of *Gyrodinium dominans* was independently estimated from Incubation Type 2 (Table 4). Similar to the *Phaeocystis globosa* experiments, the data show considerable scatter. The closest agreement with the previous estimate was on Day 6 when the *Isochrysis galbana* concentration was the lowest. The cellular DMSP content of *G. dominans* feeding on *I. galbana* averaged $0.79\sim1.00 \times 10^{-4}$ nmol cell^{-1} over 8 d.

Experiment with *Dunaliella tertiolecta*

This additional experiment was designed to further test the hypothesis that *Gyrodinium dominans* obtains DMSP from its prey. Measurements on a dense *Dunaliella tertiolecta* culture ($\geq 7 \times 10^5$ cells sample^{-1}) yielded no detectable DMSP. During the experiment, *G. dominans* grew at an optimal specific growth rate of 0.94 d^{-1} and had an average DMSP content of 2.38×10^{-6} nmol cell^{-1} (Table 5). Thus, when fed on *D. tertiolecta*, *G. dominans* contained 1 to 2 orders of magnitude less DMSP than when fed on *Phaeocystis globosa* and *Isochrysis galbana*. These observations support the argument that *G. dominans* ingestion results in DMSP uptake. Nevertheless, one may ask: Does the small amount of DMSP indicate de novo DMSP synthesis by *G. dominans*?

To measure the DMSP content of *Dunaliella tertiolecta*, we injected an equivalent of 7×10^5 cells into the reaction-analysis system. The detection limit of the gas chromatograph was 0.03 nmol injection^{-1}; thus, the DMSP content of *D. tertiolecta* would be $\leq 4.3 \times 10^{-8}$ nmol cell^{-1}. The doubling time for *Gyrodinium dominans* in this experiment was 0.74 d. If we assume that all DMSP came from *D. tertiolecta*, the ingestion rate of *G. dominans* would have been $(2.38 \times 10^{-6}) \div (4.3 \times 10^{-8}) \div 0.74 = 74.8$ cells ind.$^{-1}$ d^{-1}, or 1.3×10^4 µm^3 ind.$^{-1}$ d^{-1}, consistent with the theoretical maximum ingestion rate for heterotrophic dinoflagellates of this size (Hansen et al. 1997). This analysis shows that the small amount of DMSP in *G. dominans* feeding on *D. tertiolecta* does not necessarily indicate de novo DMSP synthesis by *G. dominans*. Instead, undetectable amounts of DMSP in *D. tertiolecta* could be bioaccumulated to a detectable level in *G. dominans*. Wolfe et al. (1994) also reported that the heterotrophic dinoflagellate *Oxyrrhis marina* accumulated trace amounts of DMSP when fed on *D. tertiolecta*.

DISCUSSION

Since Charlson et al. (1987) hypothesized that oceanic production of DMSP and DMS could significantly affect the global climate (known as the CLAW hypothesis), interest in DMSP dynamics has increased rapidly (e.g. Kiene et al. 1996, Stefels et al. 2000). Most DMSP studies have focussed on phytoplankton and bacteria, whereas little is known of the roles of grazers and higher consumers. Protozoans and other microzooplankton are major grazers in the ocean, especially in areas dominated by small phytoplankton such as solitary *Phaeocystis* sp. cells (Weisse & Scheffel-Möser

1990, Archer et al. 2000) and *Emiliania huxleyi* (Archer et al. 2001, 2002). Recent field studies reported that microzooplankton ingested 43 to 57% of the algal DMSP stock per day, and channelled 63 to 91% of phytoplankton-DMSP losses (Archer et al. 2002, Burkill et al. 2002, Simó et al. 2002); thus, the fate of the ingested DMSP will be a key factor in DMSP dynamics. If the micrograzer only acts as a releaser of phytoplankton-DMSP into the dissolved phase, where it is made available to bacteria, then microzooplankton grazing will accelerate the coupling between phytoplankton-DMSP and its transformation products such as DMS. If DMS is produced and released during digestion of the alga by the micrograzer, the coupling between algal DMSP and DMS is still strengthened. Conversely, if DMSP accumulates in the grazer's biomass, it can be further transferred to higher trophic levels and the coupling between DMSP and DMS productions will be weakened. Similar considerations would apply to mesozooplankton.

Retention of ingested DMSP in the protozoan cells is usually deduced indirectly based on the disappearance of algal DMSP and production of known DMSP breakdown products in grazing experiments. For example, Wolfe et al. (1994) observed that when *Oxyrrhis marina* (heterotrophic dinoflagellate) grazed on *Emiliania huxleyi*, 30 to 76% of the ingested DMSP was released as dissolved DMSP, whereas the missing 24 to 70% was assumed to be metabolized or retained by the grazers. In another laboratory study, Belviso et al. (1990) estimated that ciliates decomposed 40 to 50% of ingested DMSP and assumed that the remaining 50 to 60% was retained temporarily in the cells. Christaki et al. (1996) estimated that >65% of the prey's DMSP was lost to dissolved form when *Isochrysis galbana* was grazed by the ciliate *Strombidium sulcatum*, whereas the remaining portion was thought to be taken up by bacteria or the ciliate. A field grazing experiment in a North Atlantic *E. huxleyi* bloom showed that 67% of the ingested DMSP was released by microzooplankton as dissolved DMSP and DMS, and the other 33% was assumed to be retained in the microzooplankton biomass (Simó et al. 2002). In a similar bloom in the North Sea, Burkill et al. (2002), assuming an uptake efficiency of 30% for microzooplankton, estimated that 70% of the phytoplankton-DMSP ingested by microzooplankton was released as dissolved DMSP, only a small fraction (<7%) was converted to DMS, and the fate of the remaining fraction (>23%) was uncertain.

Direct measurement of DMSP in protozoan biomass is difficult due to the fact that protozoans cannot be easily separated from co-existing phytoplankton. In the present study, we allowed *Gyrodinium dominans* to graze down co-existing phytoplankton and grow to high densities in the laboratory, then concentrated the biomass for measurements. Using this approach we were able to detect the small amount of DMSP in *G. dominans* cells. However, there was still considerable scatter in the data, and the values tended to be low at the beginning of the incubation when phytoplankton concentrations were relatively high (Tables 3 & 4). On average, *G. dominans* retained 0.6 to 1 × 10^{-4} nmol DMSP $cell^{-1}$. To our knowledge, only one strict heterotroph, the osmotrophic dinoflagellate *Crypthecodinium cohnii*, has been reported to contain DMSP in culture (Ishida & Kadota 1967, Keller et al. 1989). The general belief is that heterotrophs are not able to synthesize DMSP de novo, as photosynthesis is a requisite for the biosynthesis chain from sulfate reduction to DMSP formation (Malin & Kirst 1997). Simó et al. (2002) found a good match between primary production and DMSP biosynthesis over a 2 wk period, as well as over a whole day-night cycle, which indicated that DMSP production was light-dependent and coupled to photosynthesis. Our additional experiment with the dinoflagellate feeding on *Dunaliella tertiolecta* also supported the argument that *G. dominans* relies on food for DMSP uptake. We also used the copepod *Acartia tonsa* as a natural agent to concentrate *G. dominans* biomass in their guts to yield measurable DMSP signals. Using this indirect approach we estimated that *G. dominans* contained 0.7 to 1.6 × 10^{-4} nmol DMSP $cell^{-1}$. Thus the 2 approaches yield similar ranges of DMSP content for *G. dominans*. The indirect approach can be further developed for field applications, especially when direct measurements on protozoan biomass are not feasible. This approach has the advantage that it does not require manipulation of protozoan cells, and that copepods are easy to collect and handle. It does require that one knows the contribution of gut DMSP content from phytoplankton cells, which could be estimated from gut pigment measurements (Harris et al. 2000) and ambient particulate DMSP data. A limitation of the indirect approach is that it does not apply to copepod species that assimilate a substantial amount of ingested DMSP into body tissues (e.g. Tang et al. 1999).

The processing of ingested DMSP by *Gyrodinium dominans* differed between food types. For example, *G. dominans* retained ingested DMSP more efficiently when fed on *Phaeocystis globosa* (44%) than when fed on *Isochrysis galbana* (32%). Regardless of the dietary difference, these values are comparable to other researchers' estimates based on disappearance of food DMSP (24 to 70% of ingested DMSP, see earlier discussion). It therefore appears that retention in biomass and release in dissolved forms are the 2 main mechanisms by which protozoans process ingested DMSP. Interestingly, TDF was also higher with *P. globosa* (1.00) than with *I. galbana* (0.63). Thus, with *G. dominans* as the grazer, the carbon dynamics and DMSP

dynamics were more tightly coupled in a *P. globosa*-based food chain than in an *I. galbana*-based food chain. By retaining ingested DMSP in their biomass, protozoans can transfer DMSP further up the food chain. In the present study, the omnivorous copepod *Acartia tonsa* fed on both phytoplankton and *G. dominans*. However, in the mixed food treatments, the copepods selectively fed on *G. dominans*, deriving most of their carbon ration by ingesting the protozoan. In terms of DMSP, *A. tonsa* obtained 63 to 84% of dietary DMSP by feeding on *G. dominans* in the food mixture. Thus, the predatory food chain appeared to be more important than the direct grazing food chain for transferring DMSP to omnivorous copepods.

Based on this and other studies, protozoans retain ~30% of ingested DMSP in the cells. In systems where protozoans exert strong top-down control on phytoplankton (e.g. Weisse & Scheffel-Möser 1990, Archer et al. 2002), one-third of the phytoplankton-DMSP could be channelled through protozoans to higher trophic levels such as copepods. A related question is: What may happen to DMSP within the copepod bodies? In species such as *Acartia tonsa*, ingested DMSP remains mainly as gut content (Tang 2000, this study) for a relatively short time (e.g. 1 gut passage time) before being channelled to the microbial and detrital food chains via defecation (Tang 2001, Tang et al. 2001b). The copepod species *Temora longicornis* assimilates part of the ingested DMSP into its body tissues, making it available for higher trophic levels (Tang et al. 1999). The assimilation is a function of ambient salinity and food concentration, and reaches a maximum after which further ingested DMSP is likely disposed of as faecal material or excreta (Tang et al. 1999, 2000a). In a field study, Tang et al. (2000b) found that most mesozooplankton contained very little DMSP; the copepod *T. longicornis* was the only species with a considerable amount of DMSP. During the months when the zooplankton community was dominated by *T. longicornis*, the zooplankton pool accounted for >70% of the particulate DMSP in the water column, whereas at other times, the zooplankton pool of DMSP was negligible (Tang et al. 2000b). Thus, the importance of mesozooplankton as a trophic linkage for transferring DMSP up the food chain highly depends on their species composition.

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