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Processing of ingested dimethylsulfoniopropionate by mussels *Mytilus edulis* and scallops *Argopecten irradians*

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ABSTRACT: Dimethylsulfoniopropionate (DMSP) synthesized by marine phytoplankton is the principal source of dimethylsulfide (DMS), an important climate-affecting gas. Grazing by small zooplankton on phytoplankton often accelerates DMS production from algal DMSP. The effects of grazing by benthic suspension feeders, such as bivalve molluscs, however, have not been studied, even though their populations sometimes process a sizable fraction of local phytoplankton production. We fed *Tetraselmis* sp. Strain UW474 (27 to 42 fmol DMSP cell^{-1}) to adult mussels *Mytilus edulis* and scallops *Argopecten irradians* and studied the fate of the algal DMSP during the 24 h following ingestion. Almost none of the ingested DMSP reappeared in the environment as DMS or DMSP; the amount that appeared in the ambient water as DMS was <1% of that ingested, and the sum total that appeared either as fecal DMSP (which microbes might convert to DMS) or in the water as DMS or DMSP was ≤3 to 4% of that ingested. In the short term, therefore, thriving bivalve populations probably strongly reduce the rate of DMS formation (direct or indirect) from local algal DMSP, in contrast to zooplankton populations. Ingested DMSP is likely accumulated in the bodies of mussels and scallops. However, although we have weak evidence of partial accumulation in scallop gastrointestinal tissue, we were unable to document accumulation in mussels because of high variability and statistical nonnormality in their naturally occurring DMSP content. In total, we showed that in the 24 h following feeding, mussels and scallops do not facilitate ambient DMS formation from algal DMSP and evidently sequester most of the algal DMSP they ingest.

KEY WORDS: Dimethylsulfide · DMS · Dimethylsulfoniopropionate · DMSP · Bivalves · Mussels · Scallops · *Mytilus edulis* · *Argopecten irradians* · Phytoplanktivory

INTRODUCTION

Dimethylsulfoniopropionate (DMSP) synthesized by marine algae is the ultimate source of over half the nonanthropogenic gaseous sulfur flux to the atmosphere (Bates et al. 1992). Atmospheric sulfur flux is of interest because sulfur gases are believed to play major roles in global climate. Although DMSP itself is not volatile, dimethylsulfide (DMS) — one of its breakdown products — is a water-soluble gas which when produced in the surface waters of the oceans, can outgas to the atmosphere. Atmospheric DMS is oxidized to aerosol particulates, including cloud condensation nuclei that affect oceanic cloud cover and thus Earth's reflectivity (albedo) to incoming solar radiation (Andreae & Crutzen 1997, Malin & Kirst 1997).

The conversion of algal DMSP to DMS is mediated by biological processes, including microbial DMSP metabolism (Yoch 2002), viral lysis of phytoplankton cells (Hill et al. 1998), and grazing by animals. Dacey & Wakeham (1986) first introduced the concept that animal grazing on phytoplankton affects the dynamics of DMS production by demonstrating that feeding by copepods on dinoflagellates in laboratory cultures

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accelerated (>20-fold) the formation of DMS from algal DMSP. Subsequent investigations of grazing effects on DMS formation from algal DMSP have employed laboratory systems to study other predator–prey relations or have sought evidence in natural waters, such as correlations between DMS concentration and grazer density (e.g. Leck et al. 1990, Archer et al. 2003). Zooplankton implicated in accelerating DMS production — thereby tending to increase the DMS concentration in sea surface waters and force a greater flux of DMS to the atmosphere — include copepods (Dacey & Wakeham 1986, Lee et al. 2003), krill (Daly & DiTullio 1996, Kasamatsu et al. 2004), and microzooplankton (Archer et al. 2003). On the other hand, grazing by menhaden (Hill & Dacey 2006) or salps (Kasamatsu et al. 2004) does not accelerate DMS production, at least in the short term. The effects of feeding by benthic suspension feeders have not previously been investigated.

We report here on the processing of algal DMSP by adults of 2 species of bivalve molluscs, the blue mussel *Mytilus edulis* and the bay scallop *Argopecten irradians*. We chose these species in part because they are relatively divergent in the ways they employ the basic bivalve feeding mechanism to remove phytoplankton from the water: whereas mussels (for example) employ well-developed laterofrontal cirri, scallops lack such cirri (Jørgensen 1990).

An important reason for studying bivalves is that populations of some species can be sufficiently dense to exert a major controlling influence on local ecosystems and process a sizable fraction of local phytoplankton production (Newell 1988, Jørgensen 1990, Riisgård 1991, Heip et al. 1995, Dame 1996). These attributes are especially true of mussel and oyster populations because of the high local densities they often attain, and one reason we chose *Mytilus edulis* for study was that it is particularly well known for its dense populations. Although *Argopecten irradians* populations reach densities of 70 scallops m^{-2} (Shumway 1991), *M. edulis* populations often consist of hundreds of mussels m^{-2} (Riisgård 1991). Riisgård (1991) calculated that a population of *M. edulis* in Limfjord (Denmark) filtered 180 m^3 of ambient water m^{-2} d^{-1}, a rate equivalent to 20 times the local water column d^{-1}. Jørgensen (1990) concluded that processing of ca. 240 m^3 m^{-2} d^{-1} is typical for *M. edulis* beds. Bivalve populations dominated by *M. edulis* in parts of the Wadden Sea are able to clear all phytoplankton from the entire local volume of water in 2 to 5 d, and they harvest from 18 to >100% of local phytoplankton production (Dame 1996). Such estimates suggest that in places like the Wadden Sea, 18 to >100% of local algal DMSP production may be processed first by mussels.

The species of mussels and other bivalves that exist today in populations dense enough to exert significant ecosystem effects occur principally in relatively shallow habitats in temperate and near-temperate parts of the globe (Dame 1996). These localized settings — which, from a global perspective, are of modest extent — are therefore the context in which bivalves could play major roles in the processing of phytoplankton DMSP; bivalves would not be significant in oceanic DMSP processing. Despite the localized nature of likely bivalve effects, the effects deserve elucidation not just because they may be locally major, as already stressed, but also because studies of ecosystem-scale DMSP processing have often been carried out in the very sorts of settings where extensive bivalve beds can occur. Important studies of DMSP and DMSP processing at ecosystem scales have been conducted, for example, in the Delaware and Chesapeake Bays (Iverson et al. 1989), Long Island Sound (Tang et al. 2000b), and the coastal Baltic (Leck et al. 1990) and North (Archer et al. 2003) Seas. In future studies of ecosystem-scale DMSP processing in locales like these, it will be desirable to have information on the possible effects of bivalve beds.

The potential of bivalves to play major roles in the processing of phytoplankton DMSP rests not only on their population densities but also on their abilities as individuals to pump water at impressively high rates through their feeding apparatus and efficiently remove wide size ranges of particulate matter from the water they pump. At temperatures near 10 to 20°C, roughly speaking (not focusing in detail on the current debate over proper measurement of pumping rates), individual *Mytilus edulis* of the size we studied (~6.5 cm shell height) pump water at 10 to 20 l h^{-1} through their feeding apparatus when feeding (Møhlenberg & Riisgård 1979, Riisgård 2001, Petersen et al. 2004), while individual *Argopecten irradians* (~5 cm) pump 4 to 10 l h^{-1} (Chipman & Hopkins 1954). *M. edulis* is one of the most effective bivalves at retaining small cells from the water it processes; it completely retains 4 µm particles, retains 90% of 3 µm particles, and 50% of 1 µm particles (Jørgensen 1990). Equally important, *M. edulis* ingests cells spanning the full natural size range of phytoplankton, including cells exceeding 100 µm (Asmus & Asmus 1993). With this breadth of diet, mussel beds seem certain to consume a great diversity of DMSP-synthesizing algal species (Keller et al. 1989). *A. irradians* fully retains 5 µm particles, retains 90% of 4 µm particles, and 50% of 2.5 µm particles (Jørgensen 1990).

Beside the potential of bivalve populations to ingest large fractions of local DMSP production, a second way in which such populations may be major players in ecosystem DMSP processing arises from the emerging paradigm that bivalves (including mussels and scallops) can be significant consumers of zooplankton

(Lehane & Davenport 2002, Wong & Levinton 2006). By depleting zooplankton populations, bivalve populations may reduce the importance of zooplankton DMSP processing (Dacey & Wakeham 1986, Archer et al. 2003, Lee et al. 2003) while simultaneously enhancing the importance of their own DMSP processing. This perspective emphasizes the importance of learning how bivalve DMSP processing resembles and differs from that of other phytoplanktivores.

Our goal was to elucidate the fate of phytoplankton DMSP in the first 24 h after adult mussels and scallops feed. In choosing the phytoplankter for our studies, we emphasized existing knowledge of the palatability and nutritional adequacy of phytoplankton for bivalves. We chose the prasinophyte *Tetraselmis* sp. because it not only synthesizes DMSP but also is (1) fully retained by feeding bivalves (Møhlenberg & Riisgård 1979), (2) known to support growth of *Mytilus edulis* (Strömgren & Cary 1984), (3) assimilated to a large extent (69 to 84%) by adult *M. edulis* (Winter 1978) and *Argopecten irradians* (Peirson 1983), and (4) commonly chosen as food for both species in aquaculture and experiments. We found that in the first 24 h following feeding, mussels and scallops do not accelerate formation of ambient DMS from DMSP they ingest, and they do not return DMSP to the environment where ambient microbes might metabolize it to form DMS.

MATERIALS AND METHODS

Mussels *Mytilus edulis*, measuring 5.5 to 8.1 cm shell height (we follow the morphological terminology of Gosling 2003), and scallops *Argopecten irradians*, 5.0 to 7.2 cm, were collected offshore from Woods Hole, Massachusetts, USA, in winter, 1 to 2 mo prior to study. They were maintained in running seawater from Vineyard Sound (31 to 33 ppt salinity, ~0.3 nmol l^{-1} particulate DMSP). Initially the water was unheated (~2°C). During the study, the water temperature was 17 to 19°C for the mussels and 14 to 15°C for the scallops. The bivalves' ambient temperature was raised to these levels gradually over 10 to 20 d. The shells of all bivalves were brushed to remove fouling organisms. During the study we monitored the exchanges of particulate DMSP (DMSPp), dissolved DMSP (DMSPd), and DMS between the bivalves and the ambient water, the elimination of DMSP in feces, and the accumulation of DMSP in tissue.

The bivalve chambers employed were 3.8 l, glass, wide-mouth, screw-top jars (metal lids), each containing 2 l of seawater that had been passed through Gelman A/E glass fiber filters (nominal pore size 1 µm). To seal a jar, the glass threads were wrapped with Teflon plumbing tape prior to applying the lid, then vinyl electrical tape was wrapped around the seam between the lip of the lid and the walls of the jar. Gentle aeration of the water in the sealed jar at 60 ml min^{-1} was achieved with an external peristaltic pump (Harvard Apparatus) that drew gas from the headspace in the jar and delivered it to a submerged bubbling stone. Aeration was only occasional to minimize disturbance of the bivalves. Tubing for the aeration loop passed through 2 gas-tight ports in the lid of the jar. Two additional ports permitted access to (1) the headspace to remove gas or add O_2 and (2) the water in the jar to withdraw water samples or add food. All tubing was glass except for minimal lengths of food-grade Tygon tubing used as flexible joints and as peristaltic tubing in the pump. Jars were isolated from outside disturbances because bivalves are sensitive to environmental changes (Riisgård & Møhlenberg 1979).

The capacity of the study chambers to retain DMS was assessed in 2 preliminary tests in which chambers containing 2 l of seawater, with aeration systems operating, were injected with pure DMS (Fluka) to bring the water concentration to 300 nM. In the first test, employing filtered seawater like that used in experiments, the DMS concentration remained at 100% of the initial concentration for 3.5 h, fell to 94–99% after 19 h, and 88–96% after 26 h, demonstrating that there was little DMS leakage and/or little destruction by microbes in the water used. In the second test, employing sterile seawater, the DMS concentration remained at 100% of the original for 26 h, demonstrating that leakage was zero. Some researchers believe that absorption or adsorption of small quantities of DMS by glass can introduce errors. To test for this sort of problem, we coated jars with the syliating agent Prosil-28 (PCR) and compared them with ordinary jars, each sealed jar containing sterile seawater with 240 nM DMS. For 5 h, no change occurred in the DMS concentration in either type of jar. Accordingly, we used ordinary, untreated jars.

The *Tetraselmis* sp. used for food was a culture (not axenic) of Strain UW474, which is referable to *T. chuii* or *T. suecica* (R. A. Lewin, pers. comm.). Average DMSP content at the stage of use was 27 to 42 fmol $cell^{-1}$, and average cell dimension was 9×14 µm. The algae were grown to a density of 6 to 14×10^5 cells ml^{-1} (counted by hemacytometer) in autoclaved f/2 medium in glass carboys at 20°C with steady illumination from banks of fluorescent bulbs.

Pseudofeces production was a concern because algal cells incorporated into pseudofeces, although removed from the water, are not ingested and not available for ingestion (pseudofeces are macroscopic clumps of uneaten material assembled by and ejected from bivalves; palatable algal cells when excessively concentrated in the ambient water are sometimes included).

Our strategy to prevent pseudofeces production was to keep the algal cell density in the animals' ambient water at $\leq 1 \times 10^7$ cells l^{-1} during feeding by adding algal culture in multiple aliquots spaced at sufficiently long time intervals to allow the bivalves to clear each aliquot before the next was added. The literature on *Mytilus edulis* is unambiguous: pseudofeces production starts only at algal cell densities $>1 \times 10^7$ cells l^{-1} (Riisgård & Møhlenberg 1979, Strömgren & Cary 1984, Riisgård 1991). Because the literature on *Argopecten irradians* (Palmer & Williams 1980, Peirson 1983) is less conclusive, we repeatedly observed scallops provided with *Tetraselmis* sp. at 1×10^7 cells l^{-1}. We saw no production of pseudofeces, and no behaviors (e.g. valve clapping) ordinarily associated with pseudofeces production.

Four identical chambers were studied simultaneously in the work on each species. Three contained bivalves: 5 randomly selected individuals chamber^{-1} in the mussel study, or 2 individuals chamber^{-1} in the scallop study. The fourth chamber (the control) contained no bivalves. Food was added to, and water samples were removed from, all 4 chambers on an identical schedule.

Bivalves were placed in the chambers 5 d before the study began, and their water was changed once daily thereafter (greatly limiting access to food). On the day before the study and the day of the study itself, the water had been passed through Gelman A/E glass fiber filters. Chambers were sealed 2.5 to 3.8 h prior to addition of algal culture. The amount of algal culture initially added to each of the 4 chambers was calculated to bring the ambient cell density to 1×10^7 cells l^{-1}. A similar or smaller amount of culture was added to each chamber on 4 to 5 further occasions at intervals of 27 to 45 min. The total amount of algal DMSP added per chamber was calculated from measures of both the DMSPp in the culture added and the DMSPp concentration in the control at the end of feeding.

Water samples were withdrawn twice from each chamber during the period preceding the addition of algal culture. Water samples were then taken periodically for ca. 24 h after all the algal culture had been added. The samples were withdrawn through a glass sampling tube (tip positioned at mid-depth in the chamber water) by use of a Hamilton 50 ml gas-tight syringe.

Each water sample (30 ml) was drawn under slight vacuum through a 25 mm-diameter Gelman A/E glass fiber filter supported on the fritted glass platform of a glass filter holder, and the filtrate was collected directly into a syliated (Prosil-28) glass vial (38 ml total volume) sealed with a Teflon-coated butyl rubber septum (Regis). Incubation in base converts DMSP to DMS (Dacey & Blough 1987). For measurement of DMSPp, the filter was placed in 25 ml of 1 N potassium hydroxide (KOH) in a sealed glass vial. The concentration of DMS in the vial headspace was later measured by sulfur-specific gas chromatography employing a Chromosil 330 (Supelco) column at 54°C, Sievers 350B sulfur chemiluminescence detector, and Hewlett-Packard 3390A integrator. DMS dissolved in the ambient water was measured by sparging a subsample of the filtrate with nitrogen, cryotrapping effluent DMS on cold (–15°C) Tenax-GC (Alltech), and heating the Tenax to desorb the DMS for measurement as already specified. DMSPd was measured by bringing the remaining filtrate to a concentration of 1 N KOH by addition of 8 N KOH in a sealed glass vial to convert DMSPd to DMS, measuring the DMS by headspace analysis, and subtracting preexisting (native) DMS. Standards for all assays were prepared using reagent DMS (Fluka) in background solutions that matched unknowns (e.g. DMS- and DMSP-free seawater containing 1 N KOH for standards for DMSPd measurement).

Pure O_2 was added to the headspace of each bivalve-containing chamber to replace O_2 used by the bivalves. The average rate of addition for *Mytilus edulis* was 1.6 ml O_2 g^{-1} estimated dry flesh weight h^{-1} during the first 6 h after feeding and 0.9 ml g^{-1} h^{-1} thereafter (calculated from Bayne et al. 1973, Widdows 1973). For *Argopecten irradians* (studied at a lower temperature), the average rate of addition was 0.4 ml O_2 g^{-1} h^{-1} at all times (calculated from Bricelj et al. 1987); the rise in metabolic rate during feeding in scallops has rarely been estimated, in part because some studies indicate that feeding does not increase scallop metabolic rates (Shumway 1991).

At ca. 24 h after the final addition of algae, bivalves were removed, and all fecal matter was vacuumed off the bottom of each chamber by siphoning. Siphon water (430 to 570 ml from mussel chambers, 130 to 240 ml from scallop chambers) was filtered through Gelman A/E glass fiber filters, and the filters (2 to 5 chamber^{-1}) were incubated in 1 N KOH in glass vials to convert fecal DMSP to DMS measured by headspace analysis. Tests with fish feces (Hill & Dacey 2006) indicate that the presence of fecal matter does not interfere with measurement calibration.

Each mussel from the mussel-containing chambers, plus each of 5 mussels from the same source population that had been treated identically but not fed, was then dissected into 2 parts: (1) the dark-colored digestive gland (consisting of the stomach, digestive diverticula, and associated tissue), and (2) the rest of the body, including mantle, gills, nephridia, and adductor muscles. The scallops that had been in the chambers plus 6 unfed scallops were also dissected into 2 parts: (1) the digestive gland, visceral mass, and (tiny) foot, and (2) the

rest of the body. We wanted to minimize the potential loss of DMS to the atmosphere during tissue processing. Part 1 of each species was so soft that we were able to subsample it quickly (<40 s) with scissors; we first minced the whole piece of tissue into small pieces, then mixed the pieces, before taking a subsample. Part 2 had to be processed differently because of the toughness of some of the body parts included, especially the adductor muscle. Part 2 was frozen by being dropped into liquid nitrogen, powdered with mortar and pestle while being kept frozen by additions of liquid nitrogen, and then subsampled while frozen. Each subsample was placed in 25 ml of 1 N KOH in a sealed glass vial and headspace gas later assayed for DMS.

To ensure the suitability of our procedure for analyzing tissue DMSP, we carried out 2 checks. One focused on whether the pH in the glass vials was high enough despite buffering by tissue constituents. Subsamples of mussel tissue homogenate were found to have the same DMSP concentration whether placed in 1, 2, or 3 N KOH, indicating that 1 N KOH was satisfactory. Our second check focused on whether the presence of tissue affected measurement calibration. When we added defined quantities of both reagent DMS and mussel tissue homogenate to a vial of 1 N KOH, we found that the measured DMS concentration was identical to the sum of the concentrations measured in 2 vials to which had been added, respectively, matched quantities of only reagent DMS or only homogenate, indicating that the presence of mussel tissue did not interfere with DMS quantification.

RESULTS

The total amount of algal DMSP fed was 3770 nmol chamber^{-1} in the mussel chambers and 4470 nmol chamber^{-1} in the scallop chambers. During addition of the algal culture most mussels exhibited behaviors correlated with high feeding rates: open valves, plus extended siphons and/or mantle edges (Møhlenberg & Riisgård 1979). The scallops held their valves open and had extended mantle edges. Both species visibly cleared the water between aliquots of algal culture. When we refer henceforth to the period of feeding and the end of feeding, we consider feeding to have ended when the bivalves cleared the water for the final time, nominally 30 min after addition of the last culture aliquot. The mussel and scallop studies continued for 25 and 23.5 h after feeding ended, respectively.

When the concentration of DMSPp was first measured following the end of feeding, it was 0 (i.e. below the DMSPp detection limit of 20 nmol chamber^{-1}, see legend of Table 1) in all 3 of the chambers that contained mussels, and it was only slightly above the detection limit in the scallop chambers, averaging only 37 nmol chamber^{-1} (Table 1; inexplicably, average DMSPp in the scallop chambers rose to 103 nmol chamber^{-1} at 4 h after feeding, before later falling to 0). We observed no pseudofeces production. Thus, the bivalves promptly ingested most or all of the algal DMSP provided (the DMSPp concentration would

Table 1. *Mytilus edulis* and *Argopecten irradians*. Quantities of dimethylsulfide (DMS) and dimethylsulfoniopropionate (DMSP) in the water (mean, range in parentheses, for chambers that contained bivalves). Control chambers contained no bivalves. Soon after feeding ended: values measured in bivalve-containing chambers 30 to 35 min after, and in control chambers 10 to 12 min after last aliquot of algal culture was added. Water samples were taken 5 times (mussel studies) or 6 times (scallop studies) in period following end of feeding. Max. after feeding ended: highest values observed in any of these 5 or 6 samples; all scallop-containing chambers exhibited maximum DMSPp 4 h after feeding ended. nm: DMSPp in control chambers at end of study was not meaningful because algal cells settled. Nominal detection limits were 2 nmol chamber^{-1} for DMS, and 20 nmol chamber^{-1} for DMS + dissolved DMSP (DMSPd) and for particulate DMSP (DMSPp)

	Total amount in ambient water (nmol chamber^{-1}) of: DMS	DMSPd	DMSPp
Mussels			
Chambers with mussels			
Before feeding	26 (19–33)	0	0
Soon after feeding ended	30 (9–41)	0	0
Max. after feeding ended	71 (41–111)	0	34 (19–50)
End of study: 25 h after feeding ended	47 (32–68)	0	25 (0–50)
Control chamber			
Before feeding	0	0	0
Soon after feeding ended	3	0	3770
Max. after feeding ended	12	0	3770
End of study: 25 h after feeding ended	12	0	nm
Scallops			
Chambers with scallops			
Before feeding	2 (1–2)	0	0
Soon after feeding ended	21 (20–23)	3 (0–8)	37 (15–54)
Max. after feeding ended	22 (21–23)	16 (8–22)	103 (72–156)
End of study: 23.5 h after feeding ended	17 (10–21)	6 (0–19)	0
Control chamber			
Before feeding	0	3	0
Soon after feeding ended	20	3	4470
Max. after feeding ended	23	20	4470
End of study: 23.5 h after feeding ended	23	20	nm

have been 3770 to 4470 nmol chamber^{-1} if no ingestion had occurred, as earlier noted).

Low concentrations of DMS were measured before feeding in the water of the chambers containing bivalves of both species, although the concentration of DMS in the control chambers was 0 (below the DMS detection limit of 2 nmol chamber^{-1}, see legend of Table 1) prior to the addition of algal culture, indicating that both the mussels and scallops released small quantities of DMS to the water before they were fed (Table 1). The batch of *Tetraselmis* sp. used in the mussel study contained little DMS; when the quantity fed was added to the chambers, it increased DMS by just 3 to 4 nmol chamber^{-1} on average (Table 1). During the 25 h that the mussels were studied following the end of feeding, the maximum DMS concentrations observed in the 3 mussel-containing chambers averaged 71 nmol chamber^{-1}; although DMS in the mussel chambers increased by 41 nmol chamber^{-1} (from 30 to 71 nmol chamber^{-1}) after the end of feeding, the mussel control increased by 9 nmol chamber^{-1}, indicating that the mussels added at most an average of only 32 nmol DMS chamber^{-1}, corresponding to 0.8% of the DMSP fed. The batch of *Tetraselmis* sp. used in the scallop study contained enough DMS to increase the DMS concentration by 19 to 20 nmol chamber^{-1} when the quantity fed was added to the chambers (Table 1). However, no significant further increase in DMS occurred in any of the scallop chambers, indicating that the scallops did not add DMS to the ambient water following feeding.

In the mussel study, the concentration of DMS in the alkalinized filtrates used to measure the concentration of ambient DMSPd never exceeded the native DMS concentration in the filtrates, indicating that the ambient DMSPd concentration was always 0 (Table 1). In the scallop study, small amounts of DMSPd were detected (≤0.5% of the DMSP fed), but the average amount of DMSPd in scallop-containing chambers never exceeded that in the control (Table 1), indicating that the scallops were not releasing DMSPd to the water.

Both the mussels and scallops started to release feces into the environment 2 to 3 h after feeding ended. The total amounts of released fecal DMSP accumulated during the study were similar for all 3 sets of mussels. They released 25, 31, and 50 nmol of DMSP in feces, corresponding on average to 0.9% of the DMSP fed. The 3 sets of scallops were more divergent; they released 5, 36, and 400 nmol of DMSP in feces, corresponding to 0.1, 0.81, and 8.9%, i.e. an average of 3.3% of the DMSP fed. Our vacuuming procedure for removing feces from the chambers not only collected all feces but also would have collected any pseudofeces, plus most of any *Tetraselmis* sp. that might have settled to the bottom without being ingested. Thus, our fecal DMSP measures demonstrate that if any pseudofeces production or algal settling occurred, only small quantities of DMSP could have been involved.

Within each species, the fed and unfed bivalves dissected for tissue analysis were similar in size (Table 2). Although our assay method for tissue samples did not distinguish DMSP and DMS, we refer to the tissue material as being simply DMSP for simplicity and, more importantly, because most of the material was probably DMSP rather than DMS (Iida & Tokunaga 1986).

The gastrointestinal tissue (digestive glands) of fed mussels contained, on average, 0.72 µmol individual^{-1} more DMSP than that of unfed mussels (Table 3). Because the amount of algal DMSP supplied to the fed mussels was almost exactly the same, 0.75 µmol individual^{-1}, one might conclude that virtually all ingested DMSP remained in the gastrointestinal tissue at the end of our 25 h study. For 2 reasons, however, we are not convinced that the ingested DMSP can be localized to the gastrointestinal tissue. First, the total-tissue DMSP in the fed mussels, which averaged 2.1 µmol individual^{-1} higher than in the unfed mussels (Table 3), was far greater than could be accounted for by the algal DMSP with which the mussels were fed, suggesting that the fed mussels had higher DMSP for reasons other than feeding. Second, the statistical distribution of tissue DMSP was in some cases dramatically non-normal. Two mussels in the fed group (one each in Chambers A and B) had total-tissue DMSP levels of 25.8 and 14.2 µmol individual^{-1}, so high as to be far outside the range of most individuals. With the 2 extreme individuals present, the statistical distribution

Table 2. *Mytilus edulis* and *Argopecten irradians*. Mean (range) wet wt of all fleshy tissue, of gastrointestinal tissue, and of tissue other than the separately assayed gastrointestinal tissue in fed and unfed individuals subjected to tissue analysis. n: no. of individuals studied. No statistically significant differences existed between fed and unfed groups in any category of tissue (Mann-Whitney *U*-test, *M. edulis*, $p > 0.3$, *A. irradians*, $p > 0.3$)

Bivalves (n)	Weight of fleshy tissue (g ind.$^{-1}$)		
	All	Gastrointestinal	Other
Mussels			
Fed (15)	8.3 (4.9–14.5)	1.16 (0.81–2.32)	7.1 (4.0–12.2)
Unfed (5)	8.6 (5.9–15.0)	1.19 (0.82–2.30)	7.4 (5.1–12.7)
Scallops			
Fed (6)	10.9 (8.4–13.6)	1.81 (1.2–2.4)	9.1 (7.1–11.2)
Unfed (6)	10.0 (7.9–12.4)	1.55 (1.2–2.0)	8.4 (6.7–10.4)

Table 3. *Mytilus edulis* and *Argopecten irradians*. Mean (range) of DMSP in all fleshy tissue, in gastrointestinal tissue, and in tissue other than the separately assayed gastrointestinal tissue in individuals fed DMSP-containing *Tetraselmis* sp. and in unfed individuals. n: no. of individuals measured. Means for fed mussels are of limited interpretive value because of nonnormality

Bivalves (n)	DMSP (μmol ind.$^{-1}$)		
	All	Gastrointestinal	Other
Mussels fed			
Chamber A (5)	7.4 (1.3–25.8)	1.18 (0.2–4.5)	6.2 (1.0–21.3)
Chamber B (5)	6.3 (3.5–14.2)	1.18 (0.3–3.8)	5.1 (3.2–10.3)
Chamber C (5)	3.9 (2.2–5.5)	0.53 (0.2–0.9)	3.4 (1.5–4.9)
All (15)	5.9 (1.3–25.8)	0.96 (0.2–4.5)	4.9 (1.0–21.3)
Mussels unfed (5)	3.8 (1.5–8.4)	0.24 (0.1–0.7)	3.6 (1.4–7.8)
Scallops fed			
Chamber A (2)	4.0 (3.4–4.5)	0.99 (0.7–1.2)	3.0 (2.2–3.8)
Chamber B (2)	2.8 (0.6–5.0)	0.99 (0.2–1.7)	1.8 (0.4–3.2)
Chamber C (2)	2.2 (0.9–3.6)	0.57 (0.2–0.9)	1.7 (0.7–2.7)
All (6)	3.0 (0.6–5.0)	0.85 (0.2–1.7)	2.2 (0.4–3.8)
Scallops unfed (6)	2.9 (1.1–5.0)	0.25 (0.1–0.5)	2.6 (0.9–4.7)

of DMSP concentration in the fed mussels was dramatically non-normal (Figs. 1 & 2) whether we examined all tissues taken together (Shapiro-Wilk *W*-test, $W = 0.66$, $p = 0.00009$), the gastrointestinal tissue alone ($W = 0.61$, $p = 0.00003$), or tissues other than the separately assayed gastrointestinal tissue ($W = 0.71$, $p = 0.0004$). For carrying out mass balance calculations, the 2 extreme individuals in the fed group cannot be simply ignored as outliers. The high levels of DMSP in those 2 individuals, however, cannot be accounted for by the algal DMSP with which they were fed, even if we presume they had ingested all the algal DMSP (3.8 μmol chamber^{-1}) added to their chambers; this indicates that these individuals had unusual tissue DMSP levels before our study began. If, as a theoretical exercise, we treat the extreme individuals as outliers and remove their data, the average total-tissue DMSP in the fed mussels falls to 3.7 μmol individual^{-1}, almost identical to that of unfed mussels (Table 3). Moreover, the average DMSP in the gastrointestinal tissue of the fed group falls to 0.47 μmol individual^{-1}, and that in the other tissue to 3.2 μmol individual^{-1}, values close to those in the unfed group. In all, the data do not provide convincing evidence that the algal DMSP we fed was localized in tissue of the mussels after 25 h.

In the scallops studied, no individuals were dramatically different from the others with regard to their DMSP levels, and for the most part the distributions of the values were statistically normal (Shapiro-Wilk *W*-test, $p = 0.05$ to 0.50). In all 3 fed chambers (Table 3), the amount of DMSP in the scallop gastrointestinal tissue was distinctly higher than the corresponding amount in unfed scallops, making a case for localization of ingested DMSP in the gastrointestinal tissue 23.5 h after feeding ended. The case is weak, however,

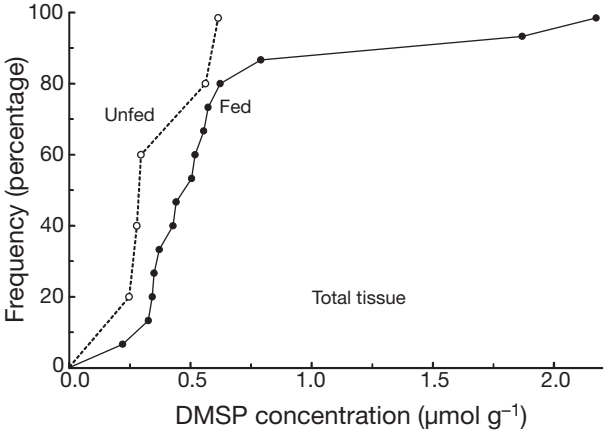

Fig. 1. *Mytilus edulis*. Cumulative frequency distribution of tissue dimethylsulfoniopropionate (DMSP) concentration (DMSP g^{-1} wet wt) in all fleshy tissue combined in (●) 15 mussels fed *Tetraselmis* sp., a DMSP-containing alga and (○) 5 unfed mussels. Each data point = DMSP concentration in 1 individual. Distributions for tissue other than the separately assayed gastrointestinal tissue in fed and unfed mussels closely resembled those shown here for total tissue. In this type of plot, normally distributed data assume a sigmoid shape, with inflexion point (frequency = 50%) at the midpoint of spread of data on *x*-axis

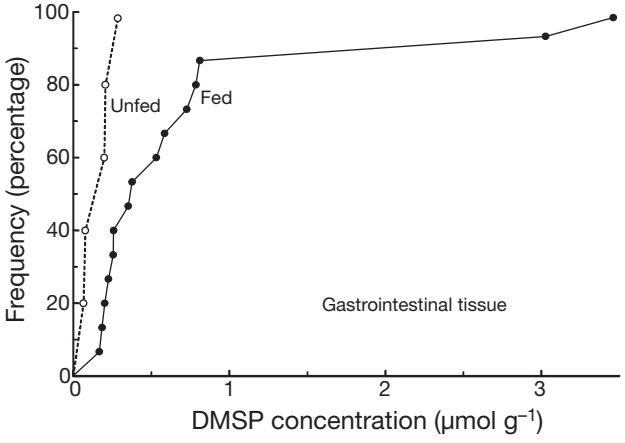

Fig. 2. *Mytilus edulis*. Cumulative frequency distribution of tissue DMSP concentration (DMSP g^{-1} wet wt) in gastrointestinal tissue (digestive gland) of the same individuals shown in Fig. 1. Each data point = DMSP concentration in 1 individual

because the 6 fed scallops taken as a group did not differ to a statistically significant extent from the 6 unfed scallops (Mann-Whitney *U*-test, p = 0.11: a parametric test could not be used because variance was higher in the fed group). Moreover, the average DMSP content of the gastrointestinal tissue in the fed scallops, being 0.6 µmol DMSP individual^{-1} higher than that in the unfed scallops, corresponded to only 27% of the DMSP fed (2.2 µmol individual^{-1}). The remainder of the ingested DMSP cannot have been localized in tissue other than the gastrointestinal tissue, because the measured DMSP in the other tissue was actually on average lower in fed than in unfed scallops (Table 3). Another troubling result for mass balance purposes was that total-tissue DMSP was almost identical in fed and unfed scallops.

To learn more about the distribution of DMSP in various subparts of bivalve non-gastrointestinal tissue, we separately analyzed the gill, mantle (including gonad), and foot in 8 mussels, and the gill, mantle, and adductor muscle in 4 scallops. DMSP was found in all types of tissue and was especially concentrated in the mussel mantle (Table 4).

DISCUSSION

Our most salient result is that phytoplanktivory by mussels *Mytilus edulis* and scallops *Argopecten irradians* does not accelerate the conversion of algal DMSP to ambient DMS during the 24 h following feeding (Table 1), at least when the bivalves are feeding on *Tetraselmis* sp. Strain UW474 (a strain of unknown DMSP lyase status). The near-zero *accumulation* of DMS in the study chambers indicates that there was little DMS *production*, because we know from our tests with reagent DMS that DMS persisted when added to the water in the chambers. The failure of bivalve phytoplanktivory to accelerate the formation of ambient DMS is consistent with the 'sloppy feeding' hypothesis for acceleration of DMS production by copepod and krill grazing. As argued by Kasamatsu et al. (2004) and others, if break-up and incomplete ingestion of phytoplankton cells during feeding are instrumental in the prompt acceleration of DMS production by grazing, phytoplanktivores that ingest algal cells whole (such as bivalves) would not be expected to accelerate DMS production, except possibly in ways long-delayed after feeding.

Table 4. *Mytilus edulis* and *Argopecten irradians*. Mean (range) concentration of DMSP relative to wet wt in 3 tissues. Muscle analyzed was foot in mussels and adductor muscle in scallops. n: no. of individuals studied

Bivalves (n)	DMSP (nmol g^{-1})		
	Gill	Mantle	Muscle
Mussels (8)	33 (5–140)	900 (47–2900)	67 (8–190)
Scallops (4)	72 (29–100)	31 (11–57)	110 (62–170)

As argued in the 'Introduction', mussels or other bivalves may ingest large fractions of local algal DMSP production in ecological settings in which their populations are dense (Newell 1988, Jørgensen 1990, Riisgård 1991, Heip et al. 1995, Dame 1996). Our results show that in these types of settings, bivalve populations probably strongly reduce rates of production of ambient DMS in the short term. The bivalves themselves essentially produce no ambient DMS from the algal DMSP they ingest during the first 24 h following feeding. Simultaneously, they remove phytoplankton that otherwise might have been subjected to processes that accelerate ambient DMS formation from algal DMSP, such as grazing by small zooplankton (Dacey & Wakeham 1986, Archer et al. 2003, Lee et al. 2003). The bivalves also seem likely to slow short-term DMS production by reducing populations of small zooplankton, based on the emerging paradigm that bivalves are significant zooplankton consumers (Lehane & Davenport 2002, Wong & Levinton 2006).

Based on assays of DMSP and DMS, an apt description of the fate of algal DMSP ingested by bivalves is that, during the 24 h following feeding, it disappears from their environment: almost none can be found as DMS, DMSPd, or DMSPp in the ambient water, or in feces. The mussels in our study added some DMS and DMSPp to the ambient water in the hours following feeding (Table 1) and eliminated some DMSP in feces, but the total of all outputs represented just 2 to 3% of the algal DMSP ingested. The scallops, although they added nothing in net fashion to the ambient water (Table 1), eliminated more DMSP in feces than the mussels on average, but the total of their outputs was only about 3% of the DMSP ingested. In these respects, mussels and scallops are unique among the animal phytoplanktivores thus far investigated. Menhaden, although they produce almost no ambient DMS, release considerable DMSPd and fecal DMSP in the 24 h after feeding (Hill & Dacey 2006). Salps, while apparently not releasing DMS or DMSPd, excrete unbroken algal cells back into the environment in feces within 24 h (Kasamatsu et al. 2004). Only the bivalves essentially fail to release any DMSP or DMS back into their environments after DMSP ingestion (although in principle they might release DMSP derivatives [e.g. demethylated products; Tang et al. 2000a] not measured in the present study and not typically measured in other studies of animal phytoplanktivory).

The DMSP ingested by the bivalves in our study seems most likely to have been in their bodies 24 h

after the end of feeding. We obtained weak evidence of accumulation of ca. 30% of ingested DMSP in the gastrointestinal tissue of the scallops. We were unsuccessful, however, in documenting accumulation in the mussels because of the high and non-normal variation among individuals in their naturally occurring content of tissue DMSP. Similar variation has been observed in other populations of *Mytilus edulis* (Hill et al. 1995). *A priori,* a simple expedient for studies like the present one would seem to be to allow mussels to void tissue DMSP prior to investigation; however, we have found that some individuals exhibit high tissue DMSP levels even after 5 wk of dietary DMSP deprivation (Hill et al. 1995). The fact that we were unable to document the accumulation of most ingested DMSP in the tissue of the mussels leaves open the possibility that considerable DMSP might have been transformed to non-DMSP/non-DMS products. The unusual statistical distributions provide a more parsimonious explanation in our opinion, however.

Tang et al. (2000a) discussed the chemical forms in which DMSP accumulated in animal tissue might ultimately be lost; they demonstrated that in the copepod *Temora longicornis*, tissue DMSP primarily returns to the environment as DMSPd. From evidence we have (authors' pers. obs.), populations of *Mytilus edulis* on average lose about half their tissue DMSP over the course of 35 d of dietary DMSP deprivation (some individuals lose none, but others lose most). If the lost tissue DMSP enters the ambient water as DMSPd (available for microbial breakdown to DMS) or as DMS, it might contribute to DMS outgassing to the atmosphere. An important local role of bivalve populations might, therefore, be to smooth the rate at which DMS outgases: during a bloom of DMSP-containing phytoplankton, bivalves might take up the DMSP promptly (preventing access to it by zooplankton [or viruses, etc.] which would cause quick conversion to DMS), but thereafter they might mediate slow production of ambient DMS from the DMSP.

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