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RESEARCH ARTICLE

Spatial variation in dimethylsulfoniopropionate (DMSP) production in *Ulva lactuca* (Chlorophyta) from the Northeast Pacific

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Abstract Although dimethylsulfoniopropionate (DMSP) has a variety of functions in marine macroalgae including that of a cryoprotectant, an osmolyte, a way to remove excess sulfur and energy, an antioxidant, and an allelopathic precursor, the latter two functions are believed to be the most important in *Ulva lactuca* L. (=*U. fenestrata*) in intertidal populations on the coast of Washington state, USA. The present study found significant variation in DMSP concentrations among *U. lactuca* collected in May 2005 from six sites ranging from 47°54.45′N (Possession Point, Whidbey Island, WA, USA) to 48°30.55′N (Shannon Point Beach, Anacortes, WA, USA), and also among individuals within sites, and among tissues (basal tissues near the holdfast, middle of the blades, and tips). Concentrations ranged from 37 to 224 μmol g^{-1} fresh mass (FM). In several 10-day experiments between July 2001 and August 2004 with *U. lactuca* collected from several places on the coast of Washington, the effects of nutrient level (DIN), light intensity and wavelength, and grazing by the herbivorous gastropod *Lacuna vincta*, were examined. None of these manipulations resulted in DMSP concentrations that differed significantly from controls, and variance in DMSP concentrations within each experiment was very low. Although DMSP concentrations in *U. lactuca* may be affected by factors not tested in these experiments, it is also possible that the observed spatial differences reflect constitutive genotypic or phenotypic differences among geographically separated *U. lactuca* populations or among cryptic *Ulva* species.

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Introduction

Many species of northeastern Pacific ulvoid algae (Phylum Chlorophyta, Order Ulvales) produce the tertiary sulphonium compound dimethylsulfoniopropionate (DMSP) (Van Alstyne et al. 2001), which can be cleaved by the enzyme DMSP lyase to form dimethyl sulfide (DMS) and acrylic acid (Cantoni and Anderson 1956). DMSP has been hypothesized to function as a cryoprotectant (Kirst et al. 1991; Karsten et al. 1992), a compatible solute (Reed 1983; Edwards et al. 1987, 1988; Kirst 1989; Kirst et al. 1991; Karsten et al. 1992), an antioxidant (Sunda et al. 2002), an allelopathic precursor, (Sieburth 1960), and a way to expel excess sulfur and energy (Stefels 2000). In Washington state USA, osmoregulation and cryoprotection do not seem to be important functions of DMSP in the ulvoid macroalga *Ulva lactuc*a (=*U. fenestrata*). DMSP concentrations in *U. lactuca* vary little in response to salinity changes (Van Alstyne et al. 2003b) and the alga rarely experiences freezing conditions. Instead, the primary function appears to be herbivore deterrence.

Sea urchins (*Strongylocentrotus droebachiensis)* avoid *U. lactuca* in laboratory preference experiments and are deterred from feeding by the products of DMSP cleavage, acrylic acid and DMS (Van Alstyne et al. 2001; Van Alstyne and Houser 2003).

Environmental nutrient concentrations, irradiance, and grazing rates can affect resource allocation within plants, particularly the division of resources between growth and defense (e.g., Bryant et al. 1983). One mechanism generating this division is the differential allocation of resources to herbivore-deterrent natural products, which, in marine algae, can be correlated with environmental conditions (reviewed by Cronin 2003; Van Alstyne et al. 2003a). Differences in the allocation of resources to defensive natural products within species can arise as a result of genetic differences between individuals, phenotypic plasticity, or some combination of the two.

There are numerous examples of phenotypic plasticity in marine algal natural products as well as examples of algal compounds that do not respond to environmental change. For example, brown algal phlorotannin concentrations tend to be lower when seawater nitrogen concentrations are high (Ilvessalo and Tuomi 1987; Yates and Peckol 1993; Arnold et al. 1995), whereas terpenes show little response to changes in nitrogen concentrations (Cronin and Hay 1996b; Puglisi and Paul 1997). The brown alga *Sargassum filipendula* contained lower concentrations of phlorotannins in shaded than better-lit habitats, but phlorotannin concentrations did not differ between shaded and unshaded individuals following field manipulations (Cronin and Hay 1996b). Green algal natural products also can respond to environmental change. When grown at higher irradiances, DMSP concentrations increased in the polar chlorophytes *Acrosiphonia arcta*, *Enteromorpha bulbosa*, *Ulothrix implexa*, and *U. subflaccida*, as well as in the temperate alga *Blidingia minima* (Karsten et al. 1991, 1992). DMSP concentrations also increased with increasing salinity in several of these species (Karsten et al. 1992).

One consequence of phenotypic variation in the production of these compounds is that their concentrations can vary both spatially and temporally. However, spatial and temporal variability also can result from genetic control, even when environmental conditions are correlated with compound concentrations. Spatial variation that is genetically determined can arise from localized selection, particularly when gene flow is limited. For example, a defensive natural product could occur at higher concentrations at sites with higher densities of herbivores because the herbivores preferentially consume algae that contain lower concentrations of the defensive compounds.

In this study, we examined spatial variation in DMSP in *U. lactuca* at two scales, among sites and within individuals. To examine among site differences, we collected *U. lactuca* from six sites in the Puget Sound/Northwest Straits region of Washington, USA (Fig. 1). Although concentrations of DMSP in *U. lactuca* and other northeastern Pacific algae are known to differ among sites and over time (Van Alstyne et al. 2001), this variation has not been systematically evaluated. DMSP was also measured in three areas of each blade: at the base of the thallus near the holdfast, midway up the blade, and at the distal edges. Growth in *Ulva* spp. is diffuse (Bold and Wynne 1985), making it difficult to assign a relative age or growth status to a particular tissue. However, these tissues can experience different microenvironments in terms of water

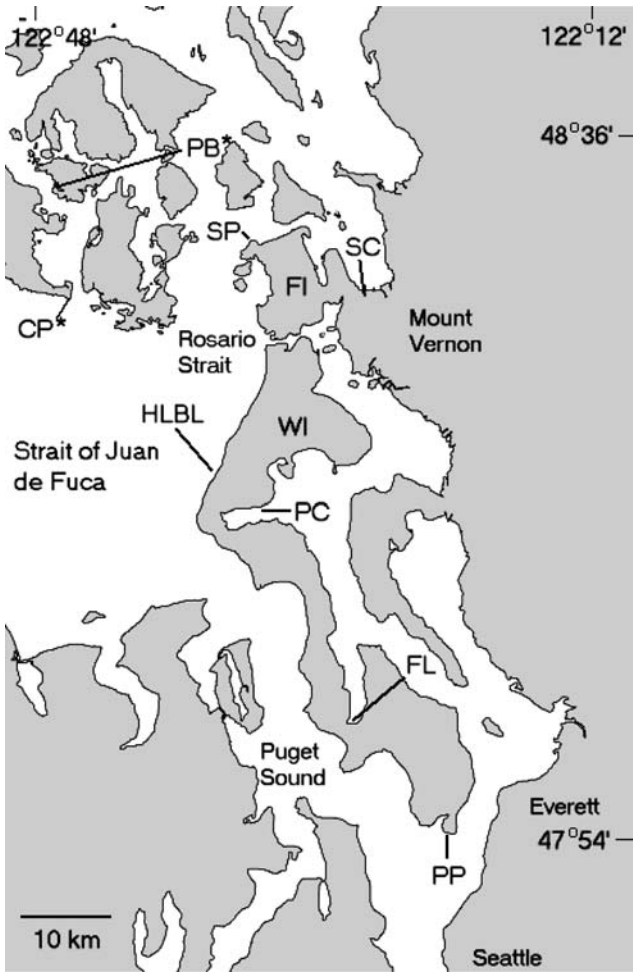

Fig. 1 Sites on Fidalgo Island (FI) and Whidbey Island (WI), Washington, USA where *Ulva lactuca* was collected for spatial analyses of variation in DMSP concentrations. *SP* Shannon Point, *SC* Swinomish Channel, *PC* Penn Cove, *HLBL* Hastie Lake Road boat launch, *FL* Freeland Park, and *PP* Possession Point. Sites designated with asterisks are where algae were collected for laboratory experiments. *PB* Parks Bay, *CP* Cattle Point

flow, light, and possibly nutrient availability and herbivory, which might influence DMSP production.

Because our collection sites had noticeably different levels of water flow, turbidity, and herbivore abundances, we hypothesized that among-site differences in DMSP concentrations could be affected by differences in seawater nutrient concentrations, levels of UV and visible light, and grazing pressures from the gastropod *Lacuna vincta*. These hypotheses were tested by conducting laboratory manipulations of nutrients, light quality and quantity, and *L. vincta* densities.

Ulva lactuca grows as a distromatic flat sheet in the mid intertidal to subtidal zones. It has an isomorphic alternation of generations (O'Clair and Lindstrom 2000) and the two stages can only be distinguished visually by examining spores and/or gametes from reproductive thalli. Concentrations of DMSP in *U. lactuca* are approximately 75 μmol g^{-1} fresh mass (FM) (Van Alstyne et al. 2001). Because *U. lactuca* is structurally simple and DMSP is enzymatically cleaved into DMS and acrylic acid before being used for its two most likely functions in this species, deterring herbivores (Van Alstyne et al. 2001; Van Alstyne and Houser 2003) and absorbing reactive oxygen species (ROS) (Ross and Van Alstyne, submitted), this system is notably different from that in many algae and compounds used in past studies of phenotypic variation in bioactive algal natural products.

Until recently, *U. lactuca* was known as *U. fenestrata* in the northeastern Pacific (Hayden and Waaland 2004). This was changed as a result of molecular studies, which also prompted changes to the classification of many other species and genera in the Ulvaceae (Hayden and Waaland 2002, 2004 Hayden et al. 2003). The authors also recognized that there may be other cryptic species in this region with morphologies that are similar to that of *U. lactuca*; however, it is not currently known how common these cryptic species are.

Materials and methods

Field collections

Ulva lactuca L. ($N = 10$ individuals) were collected from each of six sites (Fig. 1) located on Whidbey Island or Fidalgo Island, Washington. All collections were made at low tide on 23rd–24th May 2005. The sites included (from North to South): the beach in front of the Shannon Point Marine Center (hereafter referred to as the Shannon Point beach; 48°31′N, 122°41°W), the Swinomish Channel boat launch (48°27′N, 122°31°W), the Hastie Lake Road boat launch (48°16′N, 122°45°W), the Penn Cove Park boat launch (48°14′N, 122°41°W), the Freeland County Park (48°02′N, 122°32°W) at the south end of Holmes Harbor, and Possession Point (47°55′N, 122°23°W) at the southern end of Whidbey Island. The Shannon Point, Hastie Lake Boat Launch, and Possession Point sites are in locations that are strongly influenced by the water flow through Rosario Strait, the Strait of Juan de Fuca, and Admiralty Inlet, respectively. These sites are characterized by having beaches that are generally composed of cobbles. The other three sites are in more sheltered areas where the substratum generally consists of small pebbles to mud.

The algae were transported on ice to Shannon Point Marine Center where analyses of DMSP concentrations were conducted. Six samples of ~0.01–0.02 g FM were taken from each individual: two at the base near the holdfast, two from the mid-region of the blade, and two from the distal tips. The samples were weighed, dried at 60°C in a drying oven overnight, reweighed, then extracted in 4 ml of 4 N NaOH in gas-tight vials at 4°C in darkness overnight. DMSP was measured as DMS following alkaline cleavage by injecting 10-μl headspace samples onto a Chromosil 330 column (oven temperature 90°C) in an SRI gas chromatograph equipped with a flame photometric detector (detector temperature 125°C). DMSP standard additions using commercially obtained DMSP (Center for Analysis, Spectroscopy and Synthesis, University of Groningen; purity >98%) added to equal volumes of NaOH were used to generate standard curves. The detection limit of the analysis was 12.5 μg DMSP.

A repeated measures analysis of variance (SPSS 12.0) was used to assess differences in DMSP concentrations among sites and among tissues with tissue type being the repeated factor. The sites were coded by latitude (north-Shannon Point and Swinomish Channel, mid-Hastie Lake and Penn Cove, and south-Freeland and Possession Point) and by exposure (high-Shannon Point, Hastie Lake, and Possession Point, and low-Swinomish Channel, Penn Cove, and Freeland). Data were log transformed to ensure normality. Tukey's post hoc tests were used to compare means among sites by latitude.

Nitrogen addition experiments

Ulva lactuca was grown at the Friday Harbor Laboratories, Friday Harbor, Washington in two separate experiments in July and August 2001. In Experiment 1, *U. lactuca* was collected from Cattle Point, San Juan Island, Washington (48°27′N, 122°58°W) and disks (~6.5 cm diameter) were cut from the middle of the

thalli ($N = 10$ per treatment). Cutting the algae does not cause changes in DMSP concentrations (Fig. S1). The disks were placed in 200 ml of each of three media: (1) seawater, (2) seawater supplemented with 10 μM NH_4NO_3, and (3) seawater supplemented with 20 μM NH_4NO_3 (Table 1) and incubated in an outdoor sea-table (temperature 14–18°C, noon PAR ~2,200 μmol photons m^{-2} s^{-1}). After 10 days, tissue DMSP, nitrogen, and carbon concentrations were measured. To measure tissue carbon and nitrogen concentrations, pieces (~0.1 g FM) of each alga were dried at 60°C overnight, homogenized on a CPEX mixer/mill for 5 min, and analyzed with a CE Elantech 1112 Elemental Analyzer. Atropine was used as a standard. DMSP was measured as described above.

In Experiment 2, algae were collected from Parks Bay, Shaw Island, Washington (48°34′N, 122°59°W), cut into ~6.5-cm diameter disks ($N = 10$ per treatment), which were placed in each of the following media: (1) Instant Ocean®, (2) seawater, and (3) seawater supplemented with 10 μM NH_4NO_3 (Table 1). The algae were maintained as described above except that the dishes were kept in a lighted incubator (temperature 12°C, PAR: ~80 μmol photons m^{-2} s^{-1}, which is just above the level needed to saturate photosynthesis in *U. lactuca* (Nelson et al., submitted). After 10 days, thallus DMSP, nitrogen, and carbon concentrations were measured as described above.

The effects of nutrient enrichment on tissue nitrogen, carbon, and DMSP concentrations, and carbon to nitrogen (C:N) ratios were examined separately for Experiments 1 and 2 with a factorial multivariate analyses of variance (MANOVA). Tukey's tests were then used to examine differences in the effects of the nutrient treatments. Pearson's correlation coefficients were conducted separately for each experiment to examine correlations between nitrogen, carbon, and DMSP concentrations, and C:N ratios (SPSS, version 10.0).

Light experiments

To examine the effects of ultraviolet radiation (UVR) on DMSP production by *U. lactuca*, 12 individual algae were collected from the low intertidal zone of the Shannon Point beach. Five disks (1.65 cm^2) were punched from the middle of each alga with a cork borer. The disks from each alga were assigned to five treatments: (1) open containers, (2) containers covered by a single sheet of cellulose acetate, which decreased the intensity, but not the quality, of the light, (3) containers covered by a single sheet of mylar, which screened most of the UV-B light, (4) containers covered by Plexiglas, which screened UV-B and 70% of the UV-A light, and (5) containers covered by black fiberglass window screening (1-mm mesh), which reduced all wavelengths of light ($N = 12$ for each treatment). The containers consisted of half a tea infuser with mesh sides that allowed for continuous water flow. Each container was then randomly assigned to one of four cinderblocks that was placed in continuously flowing seawater in an outdoor sea-table. The containers were submerged to a depth of 3 cm and were cleaned regularly to remove diatoms. The algae remained in the sea-table for 10 days (July 8–July 18, 2003). At the end of the experiment, the algae were removed and tissue DMSP concentrations were measured as described above. The data were analyzed with a two-way analysis of variance (ANOVA) with cinder block number and the screen type as factors.

Light penetration under each type of filter was measured with a Li-Cor 1800 spectroradiometer. The intensities of UV-B radiation (300–320 nm), UV-A radiation (320–400 nm), and photosynthetically active radiation (PAR 400–700 nm) were determined for each filter type and compared to unfiltered light. The water temperature in each cage was measured at noon half-way through the experiment to determine if temperature differed among blocks.

Table 1 Chemical composition of nutrient media used in acclimation experiments

	Phosphate	Silicate	Nitrate	Nitrite	Ammonium	Total DIN
Experiment 1						
Seawater	1.63	44.59	15.80	0.23	1.07	17.10
Seawater + 10 μM NH_4NO_3	1.63	44.59	25.80	0.23	11.07	37.10
Seawater + 20 μM NH_4NO_3	1.63	44.59	35.80	0.23	21.07	57.10
Experiment 2						
Instant ocean®	4.95	12.13	16.18	0.45	21.60	38.23
Seawater	1.84	46.51	15.33	0.27	0.92	16.52
Seawater + 10 μM NH_4NO_3	1.84	46.51	25.33	0.27	10.92	36.52

Values for seawater and Instant Ocean were determined by automated nutrient analyses (described by Whitledge et al. 1981) by the Chemistry Laboratory, School of Oceanography, University of Washington. Values for nutrient addition experiments were estimated from seawater values. All values given in μM DIN (dissolved inorganic nitrogen)

To further examine the effect of shading, another experiment was performed in August 2004. Algae were collected from the Shannon Point beach and cut into disks as described above. The disks were placed in half tea infusers that were uncovered or covered with a single layer of 1-mm fiberglass mesh, two layers of mesh, or four layers of mesh. This shading resulted in reductions of PAR of 34, 58, and 76%, resulting in noon PAR levels of 411, 719, 1,258, and 2,200 μmol photons m^{-2} s^{-1}, respectively. The algae were randomly assigned to cinderblocks in the outdoor sea-table as described above. After 10 days, the algae were removed and tissue DMSP concentrations were measured. The data were analyzed with a two-way ANOVA with block number and number of screen layers as factors.

Herbivory experiments

To determine whether herbivores affect DMSP concentrations, *U. lactuca* that lacked obvious grazer damage and 2–3 mm diameter *L. vincta* snails were collected from the Shannon Point beach in February 2004. Thirty disks (~1.65 cm^2) of *U. lactuca* were placed individually in seawater in 8-cm diameter glass bowls that were set in an outdoor sea-table. The seawater level in the table was high enough to surround, but not cover, the bowls in order to maintain a constant temperature. Five *L. vincta* were placed in each of half the bowls. After 10 days, the algae were removed and tissue DMSP concentrations were measured as described above. DMSP concentrations were compared between grazed and control algae with a Student's *t*-test.

Results

Spatial variation

The mean concentration (±1 SD) of DMSP (as μmol g^{-1} FM) across all six sites for all tissues was 104 ± 37 μmol g^{-1} FM (N = 180). DMSP concentrations differed significantly among sites along a North–South gradient, but were not different at sites with higher levels of exposure (Table 2). Concentrations were higher in the algae from the two northernmost sites (Fig. 2; Tukey's post hoc test $P < 0.05$) than the mid-latitude sites which, in turn, were significantly higher than the southern sites (Fig. 2; Tukey's post hoc test $P < 0.05$). The concentrations were also significantly different among tissues (Pillai's Trace = 0.768, hypothesis $df = 2$, error $df = 113$, $F = 1{,}86.64$, $P < 0.001$)

Table 2 *Ulva lactuca*. Between-subjects effects from a repeated measures analysis of variance table for log-transformed DMSP concentrations (SPSS 12.0)

Source	DF	SS	MS	F	P
Exposure	1	0.009	0.009	0.322	0.572
Latitude	2	2.343	1.171	41.997	<0.001
Exposure × latitude	2	0.395	0.198	7.083	<0.001
Error	114	3.180	0.028		

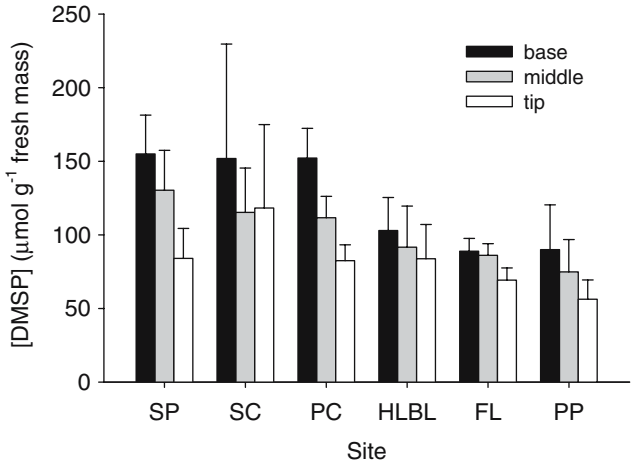

Fig. 2 *Ulva lactuca*. DMSP concentrations (as μmol g^{-1} of fresh mass) from algae collected from six sites in the Puget Sound/Northwest Straits region. Sites are given from north to south. *Bars* are means (±1 SD) from the bases near the holdfast, middle, and distal tips of ten individuals from each site. Two samples were taken from each region of each alga. *SP* Shannon Point, *SC* Swinomish Channel, *PC* Penn Cove, *HLBL* Hastie Lake Road boat launch, *FL* Freeland Park, and *PP* Possession Point

with the bases of the thalli having the highest concentrations and the distal ends of the thalli having the lowest ones.

Effects of nutrients

Nitrogen addition had no significant effect on DMSP concentrations in either Experiment 1 (Fig. 3, MANOVA: nutrients: Wilks $\lambda = 0.201$, $F_{10,90} = 1.455$, $P = 0.229$) or Experiment 2 (MANOVA $F = 1.231$, $P = 0.310$). The nutrient treatments did not have a significant effect (MANOVA $P > 0.05$) on carbon concentrations in either experiment and did not affect nitrogen or C:N in Experiment 1. In Experiment 2, algal tissue nitrogen significantly increased with increasing seawater nitrogen concentrations (MANOVA $F = 12.326$, $P < 0.001$) and C:N decreased (MANOVA $F = 37.730$, $P < 0.001$).

Tissue DMSP concentrations in Experiment 1 were positively correlated with tissue carbon concentrations (Pearson's correlation coefficient = 0.490, $P = 0.007$)

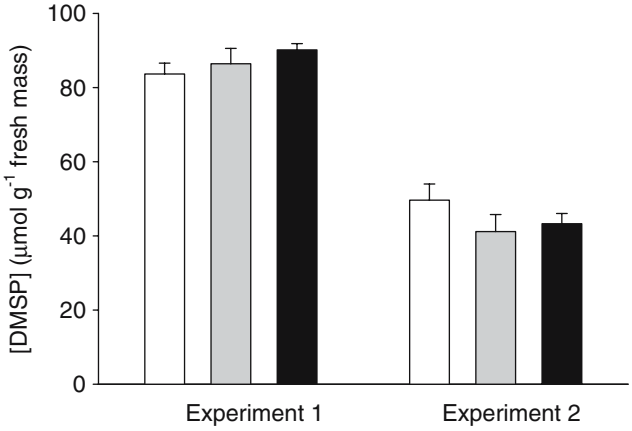

Fig. 3 *Ulva lactuca*. Mean (±1 SE) DMSP concentrations (as µmol g^{-1} of fresh mass) in algae from nutrient addition experiments. Experiment 1 (seawater table experiment): *open bars* seawater, *shaded bars* seawater supplemented with 10 µM l^{-1} NH_4NO_3, *filled bars* seawater supplemented with 20 µM l^{-1} NH_4NO_3. Experiment 2 (incubator experiments): *open bars* seawater, *shaded bars* seawater supplemented with 10 µM l^{-1} NH_4NO_3, *filled bars* artificial seawater. Nutrient addition had no significant effect on DMSP concentrations in either experiment

and negatively correlated with C:N (Pearson's correlation coefficient = −0.917 $P = 0.007$), but were not correlated with nitrogen (Pearson's correlation coefficient = 0.082, $P = 0.685$). In Experiment 2, DMSP was not correlated with tissue carbon or nitrogen concentrations or C:N (Pearson's correlation: $P > 0.05$).

Effects of light

Temperature was not significantly different across blocks or treatments (two-way ANOVA for block: $F = 0.620$, $df = 3$, $P = 0.606$; treatment: $F = 0.706$, $df = 4$, $P = 0.593$) and there was no significant interaction between block and treatment (two-way ANOVA: $F = 1.507$, $df = 12$, $P = 0.162$). While these experiments were being conducted, noon PAR was approximately 2,200 µmol photons m^{-2} s^{-1}. Cellulose acetate decreased intensity of all wavelengths by 80 to 90% (Table 3, Fig. S2). Mylar blocked most of the UV-B, while Plexiglas blocked most of the UV-B and about 70% of the UV-A wavelengths. The fiberglass screen decreased the intensity of all wavelengths by 43%.

Dimethylsulfoniopropionate concentrations did not differ significantly (two-way ANOVA, screen effect: $F = -0.57$, $P = 0.688$) when the algae were uncovered versus covered with cellulose acetate, Mylar, Plexiglas, or a single layer of fiberglass mesh (Fig. 4). Likewise, DMSP concentrations did not differ significantly when the algae were covered with fiberglass mesh that resulted in noon PAR levels of 411, 719, 1,258, or 2,200 µmol photons m^{-2} s^{-1} (Fig. 5; two-way ANOVA, screen effect $F = 0.23$, $P = 0.877$).

Table 3 Relative penetration of light through screens

	UV-B (%)	UV-A (%)	PAR (%)
No filter	100	100	100
Cellulose acetate	81	90	94
Mylar	15	82	90
Plexiglas	0.2	29	94
Fiberglass mesh screening	57	57	57

UV-B 300–320 nm; *UV-A* 320–400 nm; *photosynthetically active radiation (PAR)* 400–700 nm

Effects of herbivores

Lacuna vincta consumed as much as half the algal disks in the bowls. However, the grazer addition had no effect on tissue DMSP concentrations. Mean tissue DMSP concentrations in algae with *L. vincta* (±1 SE) were 46 ± 3 µmol g^{-1} FM and in algae without *L. vincta* were 41 ± 1 µmol g^{-1} FM (Student's *t*-test $t = 1.37$, $P = 0.18$).

Discussion

Dimethylsulfoniopropionate varied in concentration across both the spatial scales examined, among sites

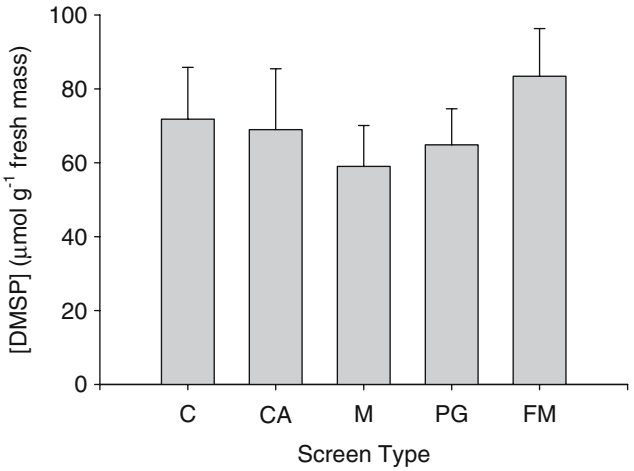

Fig. 4 *Ulva lactuca*. Mean (±1 SE) DMSP concentrations (as µmol g^{-1} fresh mass) from algae under filters that block certain wavelengths or reduce light levels (see Table 3; Fig. S2). *C* unfiltered control, *CA* cellulose acetate (filtered control), *M* mylar (UV-B filtered), *PG* Plexiglas (UV-A and UV-B filtered), and *FM* fiberglass mesh screening. DMSP concentrations did not differ significantly among treatments (two-way ANOVA, screen effect: $F = 0.57$, $P = 0.688$)

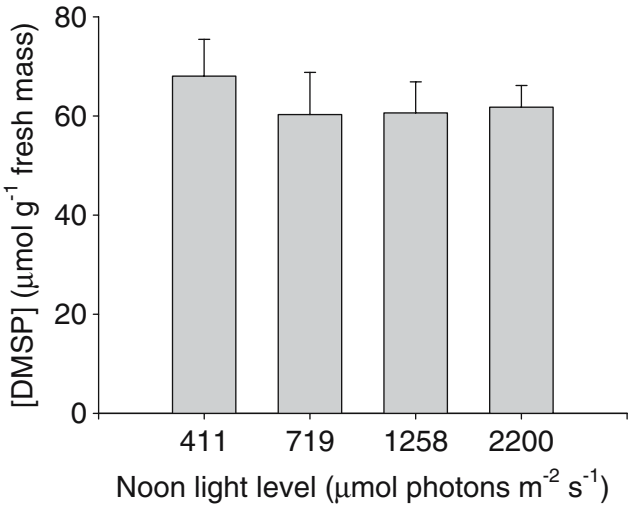

Fig. 5 *Ulva lactuca*. Mean (±1 SE) DMSP concentrations (as µmol g^{-1} fresh mass) from algae under different numbers of layers of fiberglass mesh screening: zero, one, two, and four. DMSP concentrations did not differ significantly among treatments (two-way ANOVA, screen effect: $F = 0.23$, $P = 0.877$)

and within thalli. The presence of a North–South gradient in DMSP concentrations but lack of a difference between more sheltered and exposed sites suggests that factors associated with water flow such as turbidity, which would alter light quantity and quality, are unlikely to be the cause of the differences. Instead, other factors that follow a North–South gradient in the region such as salinity, nutrients, or water temperature may be involved. The Puget Sound basin is an estuary and temperatures and ammonium concentrations tend to increase with decreasing latitude, while salinity tends to decrease from North to South (Newton et al. 2002).

The presence of among-site differences in DMSP concentrations is not surprising, given that concentrations of other bioactive natural products vary over similar spatial scales. Although among-site differences in macroalgal DMSP concentrations have not been well documented, among-site differences occur in phlorotannins produced by brown algae (reviewed by Van Alstyne et al. 2003a) and in furanones produced by red algae (Wright et al. 2000). Some of these differences are reported to be due to herbivore-induced increases (Van Alstyne 1988) or to long-term selection for increased amounts of compounds (Steinberg 1992; Steinberg et al. 1995); however, other studies suggest that unknown environmental differences among sites and unknown genetic differences among populations may be responsible for these patterns (Van Alstyne et al. 1999; Wright et al. 2000). Alternatively, these patterns could be caused by gradients in genetic factors. For example, if our collections from some sites included cryptic species and they have different DMSP concentrations, then site-to-site differences in DMSP concentrations could result.

There is also the possibility that spatial patterns in DMSP concentrations could result from differences in the abundances of *U. lactuca* gametophytes and sporophytes, which are morphologically identical. The distribution and seasonality of different life-history stages for *U. lactuca* in Washington waters is not known and the algae used in this study were not reproductive so we could not distinguish between the two phases. It is also not known whether DMSP concentrations differ between the two stages. Differences in concentrations within a life-history stage (e.g., between juveniles and adults of the same stage) occur in terpenes produced by tropical green algae (Hay et al. 1988; Paul and Van Alstyne 1988) and phlorotannins produced by temperate brown algae (Denton et al. 1990; Van Alstyne et al. 2000), but differences among life-history stages are not well described. In one of the few studies that has examined between-stage differences, there were no significant differences in terpene concentrations in *Dictyota ciliolata* gametophytes and sporophytes (Cronin and Hay 1996a).

This is one of the first studies to document intra-thallus differences in DMSP concentrations; however, intra-thallus differences in concentrations of other bioactive algal metabolites are common. Within a species or higher taxonomic group, the distribution of phlorotannins and terpenes within individuals can be correlated with tissue age, reproductive status, and whether the tissue is actively growing (reviewed by Van Alstyne et al. 2003a). In *U. lactuc*a, growth is diffuse. As a result, actively growing tissues occur throughout the thallus and tissue age is not tightly coupled with location. Nonetheless, basal tissues that were close to the holdfast had significantly higher concentrations of DMSP than more distal tissues. It is possible that the difference could arise from basal tissues experiencing different microenvironmental conditions than distal tissues, rather than from differences in age or growth status. It is also notable that basal tissues also appeared to be tougher and more resistant to tearing than distal tissues. The combination of a higher DMSP concentration and a more robust thallus may make basal tissues less susceptible to damage by grazers than distal tissues, which may ultimately reduce subsequent tissue loss (Padilla 1983) from water motion.

The absence of treatment effects on DMSP concentrations in our laboratory experiments was surprising given that so many other studies have found that marine bioactive compounds, such as terpenes and phlorotannins, respond to changes in a variety of environmental

factors (Cronin 2003; Van Alstyne et al. 2003a). Because DMSP has to be cleaved into DMS and acrylic acid for some of its functions, it is possible that *U. lactuca* regulates the production of the cleavage products by modifying the activity or amount of DMSP lyase rather than by modulating the amount of DMSP. Environmentally induced changes in DMSP lyase may be more important to the functioning of the DMSP cleavage reaction than changes in DMSP. Protozoan grazing is highest on cells of the marine phytoplankter *Emiliania huxleyi* with low DMSP lyase activity levels (Wolfe et al. 1997), suggesting that regulating enzyme activity may be as, if not more, important than regulating the concentration of DMSP. A similar lack of plasticity in the precursor compound of an activated defense was found in tropical green algae that produce halimedatetraacetate (Paul and Van Alstyne 1992).

The absence of treatment effects on DMSP concentrations in the present study may be species-specific, even within the genus *Ulva*. DMSP concentrations in *U. rigida* (Karsten et al. 1991) and *U. bulbosa* (Karsten et al. 1992) increase in response to increases in light. In *U. rigida* (Karsten et al. 1992), *U. bulbosa* (Karsten et al. 1992), and *U. intestinalis* (Edwards et al. 1987, 1988), DMSP concentrations are affected by changes in salinity (Edwards et al. 1987, 1988; Karsten et al. 1992). Light has been suggested to stimulate the activity of the enzymes involved in DMSP synthesis (Karsten et al. 1992). It also increases oxidative stresses and may induce the cleavage of DMSP into DMS and acrylic acid, which are more effective ROS scavengers than DMSP (Sunda et al. 2002, Ross and Van Alstyne, submitted).

It is possible that decreased light levels in our experiments had no effect because even the lowest levels (noon PAR of ~750 μmol photons m^{-2} s^{-1} under four screens) exceeded the amount of light needed to saturate photosynthesis in *U. lactuca*, which is ~30–50 μmol photons m^{-2} s^{-1} (Nelson et al., submitted). Previous studies conducted with *U. rigida* (Karsten et al. 1991) and *U. bulbosa* (Karsten et al. 1992), which showed an effect of increased radiation on DMSP concentrations, were conducted in the laboratory at light levels of 0–55 μmol photons m^{-2} s^{-1}. It is noteworthy that the DMSP concentrations in Experiment 2 (at ~80 μmol photons m^{-2} s^{-1}) were substantially lower than in Experiment 1 (with noon PAR levels of ~2,200 μmol photons m^{-2} s^{-1}) (Fig. 3); however, the algae used in these two experiments were also collected from different sites, so without further experimentation, we can not determine whether the difference was due to differences in the algae at the two collection sites or to differences in the light levels in the experiments.

In summary, DMSP concentrations in *U. lactuca* varied at spatial scales ranging from cms to ten's of kms. Using laboratory manipulations, we found no evidence that this variation arose from differences in salinity (Van Alstyne et al. 2003b), environmental nitrogen concentrations, light quantity and quality, or herbivory. Although we cannot definitively rule out any effects of environmental variability on DMSP concentrations without further study, the data so far suggest that there is a strong genetic component to the determination of tissue DMSP concentrations in this species. These differences could reside at levels ranging from life history stages to populations to species, since cryptic species of *Ulva* exist within the our study area (Hayden and Waaland 2004) and have not been fully explored.

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