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RESEARCH ARTICLE

Anti-grazing activity and seasonal variation of dimethylsulfoniopropionate-associated compounds in the invasive alga *Codium fragile* ssp. *tomentosoides*

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Abstract In this study, we present evidence that the invasive alga *Codium fragile* ssp. *tomentosoides* is chemically defended against grazing by a wound-activated chemical defense involving dimethylsulfoniopropionate (DMSP) and the products of its cleavage, dimethylsulfide (DMS), and acrylic acid (AA). DMSP in *C. fragile* ssp. *tomentosoides* was present throughout the year, but concentrations varied seasonally and were highest in the winter. Intra-thallus variation was neither large, nor consistent over time. High DMSP concentrations were uncommon among northwestern Atlantic macrophytes. Of 26 other species tested, only two, *Ulva lactuca* and *Polysiphonia harveyi* contained concentrations comparable to, or higher than, those of *C. fragile* ssp. *tomentosoides*. DMS and AA, both individually and together, deterred grazing by the green sea urchin *Strongylocentrotus droebachiensis* at "natural" concentrations. These results suggest that DMS and AA contribute to the avoidance of *C. fragile* ssp. *tomentosoides* by *S. droebachiensis*. As a result, the production of DMSP and its subsequent cleavage, upon injury, may reduce herbivory on *C. fragile* ssp. *tomentosoides* and contribute to its success.

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Introduction

Codium fragile ssp. *tomentosoides* is one of the world's most widespread invasive algal species, having spread from Japan to the shores of Europe, Australia, New Zealand, and North and South America over the past century (Silva 1955; Bouck and Morgan 1957; Dromgoole 1975; Trowbridge 1999; Castilla et al. 2005). The dramatic success of *C. fragile* ssp. *tomentosoides* has been attributed to aspects of the alga's ecophysiology and life history, its association with human activities such as shipping and aquaculture, and characteristics of invaded communities (reviewed by Trowbridge 1998). In the Northwest Atlantic, where *C. fragile* ssp. *tomentosoides* has been particularly successful, a combination of biological disturbance and facilitation is believed to have played an important role. Urchin mass mortality combined with defoliation of kelp by the invasive bryozoan *Membranipora membranacea* have provided *C. fragile* ssp. *tomentosoides* with opportunities to colonize, expand, and competitively exclude other macrophytes in former urchin barrens and kelp beds in the rocky subtidal zone (Harris and Tyrell 2001; Chapman et al. 2002; Levin et al. 2002; Scheibling and Gagnon 2006).

Many exotic plants are suppressed by native herbivores, particularly generalists (Parker et al. 2006). Although *C. fragile* is susceptible to some saccoglossan sea slugs that are specialized to feed upon it (Trowbridge 1995, 2004), the alga is generally a low preference food among generalist herbivores (Trowbridge 1998). In the northwest Atlantic, the green sea urchin *Strongylocentrotus droebachiensis* and the gastropod *Lacuna vincta* prefer *Laminaria* spp. and turf algae over *C. fragile* ssp. *tomentosoides.* This is likely to reinforce the shift from kelp beds to meadows of *C. fragile* ssp. *tomentosoides* (Scheibling and Anthony 2001; Levin et al. 2002; Sumi and Scheibling 2005). While the specific

reasons why herbivores avoid the alga have not been identified, it has been suggested that odourous volatile compounds may be involved (Trowbridge 1998). One such chemical is dimethylsulfoniopropionate (DMSP), a methionine-derived natural product found in many macroalgae (Van Alstyne and Puglisi 2007), including *C. fragile* and other *Codium* spp. (Van Alstyne et al. 2001; K. L. Van Alstyne, unpublished data). DMSP can serve as the precursor of an activated anti-grazing defense in some macroalgae (Van Alstyne et al. 2001; Van Alstyne and Houser 2003). Both acrylic acid (AA) and dimethylsulfide (DMS), the products of DMSP-cleavage, deter grazing by *S. droebachiensis* (Van Alstyne et al. 2001; Van Alstyne and Houser 2003).

In this study, we examine the potential role of DMSP and associated compounds in protecting *C. fragile* ssp. *tomentosoides* against grazing. We show that DMSP is present in *C. fragile* ssp. *tomentosoides* in Nova Scotia, but is undetectable or in very low concentrations in most co-occurring macrophytes. We examine both seasonal and intra-thallus variation in DMSP concentration in *C. fragile* ssp. *tomentosoides*. Finally, we demonstrate that AA and DMS deter grazing by *S. droebachiensis* at concentrations that are ecologically relevant for *C. fragile* ssp. *tomentosoides* in Nova Scotia.

Materials and methods

DMSP concentrations in *C. fragile* ssp *tomentosoides* and other northwest Atlantic species

Codium fragile ssp. *tomentosoides* was collected from the shallow subtidal zone at Birchy Head (44°35′N, 64°03′W; September 2004, March 2005, May 2005, February 2006), Cranberry Cove (44°28′N, 63°56′W; November 2004), Sandy Cove (44°27′N, 63°42′W; July 2005), and The Lodge (44°33′N, 64°01′W; October 2005), Nova Scotia, Canada. To monitor seasonal variation in DMSP concentrations, tip samples from the distal end of the thallus were taken from at least 20 individuals from each collection. To compare DMSP concentrations in different parts of the frond, branch samples from the area between the second and third bifurcation and stipe samples, which included the holdfast and the section of stipe directly above it, were sampled in November 2004 ($n = 23$) and March 2005 ($n = 19$). In July 2005, only tips and branches were sampled. Samples of 25 other macroalgal species ($n = 1$–3) and one marine vascular plant ($n = 3$) were collected from the subtidal zone in February 2006 at Birchy Head, Cranberry Cove, The Lodge, and Paddy's Head (44°31′N, 63°57′W), Nova Scotia, Canada.

Algal and plant samples were gently shaken to remove excess water, weighed, dried at 60°C for 24 h, and reweighed to calculate a dry-to-wet mass ratio. The dried samples were then sent by overnight courier to the Shannon Point Marine Center (SPMC) in Anacortes, WA, USA for DMSP analysis. DMSP was measured as DMS following alkaline cleavage in 4 N NaOH 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 with 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. The dry-to-wet mass ratio was then used to determine the concentration of DMSP in terms of the % fresh mass (FM). Previous studies with other species of green macroalgae have shown that oven-drying the samples prior to extracting them in NaOH increases the amount of DMSP extracted and that dried samples can be stored for at least a month without loss of the compound (K. L. Van Alstyne, unpublished data; Karsten et al. 1994).

Feeding assays

Because of regulatory restrictions on importing exotic invertebrates to Washington and the lack of facilities for measuring DMS at Dalhousie University, we tested the effects of AA and DMS on *S. droebachiensis* from Washington, rather than Nova Scotia. Small adult (35 ± 2 mm SD test diameter) urchins were collected by hand from Forbes Point, Whidbey Island, WA, USA (48°16′N, 122°38′W) at about 0.3 m depth during a low tide in August 2005. Urchins were kept in flow-through sea water tables at the SPMC and were not fed macroalgae for 1 month prior to beginning feeding assays. Urchins used in multiple experiments were maintained without food for a minimum of 1 day between assays.

Seaweed-based artificial foods were prepared to test the effects of AA and DMS on urchin feeding behavior. The concentrations tested were based on those observed in the Nova Scotia *C. fragile* ssp. *tomentosoides* population (see Results), assuming complete conversion of DMSP into AA and DMS. In experiments with foods that contained both AA and DMS, we attempted to use ratios of DMS to AA that assumed that DMSP cleavage results in a ~1:1 ratio of DMS (MW 72) to AA (MW 62), although the volatility of DMS made this difficult (see below). To create artificial diets, 46.5 ml of de-ionized water was mixed with 1.5 g of high gel strength agar and microwaved for 45 s. After the mixture cooled to 40°C, 2.0 g of freeze-dried, ground *Saccharina latissima* (=*Laminaria saccharina*) and AA (Aldrich, anhydrous, 99%) and/or DMS (Aldrich, anhydrous, 99%) were added. This amount of *S. latissima* was chosen

in order to create artificial foods that roughly mimicked (in terms of energy) the nutritional value of *C. fragile* ssp. *tomentosoides*. The use of *S. latissima*, which is attractive to sea urchins (Van Alstyne and Houser 2003; Van Alstyne et al. 2006), in place of *C. fragile* ssp. *tomentosoides* increased the likelihood that urchins would feed on the artificial foods. This may make our tests of the effect of the defensive compounds slightly more conservative than if *C. fragile* ssp. *tomentosoides* had been used. Control diets were prepared using the same method, except they lacked AA and DMS.

The agar-based food was then poured onto a glass plate evenly sprinkled with 3 g of clean beach sand, which made the food negatively buoyant. The mixture was then pressed flat with a second glass plate, separated from the first by 3 mm spacers. Once the agar had set (~1 min), the food was cut into pieces using "punches" made from short lengths of circular or square pipe, ~1.5 cm across. To distinguish between treatment and control foods in the choice experiments, the food shape was assigned systematically, such that the treatment food would be circular in one experiment, and square the next. In the no-choice experiment, all foods were circular. Preliminary experiments indicated that urchins exhibited no preference for circular or square foods and that food pieces exhibited very little autogenic mass change (average weight change: $+0.01 \pm 0.01$ g SD).

Due to the volatility of DMS, it was difficult to predict the concentration that would be present in food at the start of the experiment. In order to determine the concentration of DMS at the start of an experiment, three pieces of treatment food were placed into 4 N NaOH in gas-tight vials at the same time that the food was first being offered to the urchins. DMS content was analyzed as described above.

Feeding bioassays were conducted in circular plastic containers (20 cm diameter) with flow-through seawater (10°C). Individual urchins were placed in each container and provided with a food or choice of foods. The amount of food consumed was determined as the difference between the estimated initial mass of the food (circles: 1.01 ± 0.02 g SD; squares: 0.96 ± 0.02 g SD) and the actual mass of food remaining at the end of the experiment. Estimates of the initial masses were used to reduce the time between preparing the foods and introducing them to the urchins so that DMS evaporation from the foods would be kept to a minimum.

Choice experiments

The effect of three concentrations of AA, four concentrations of DMS, and nine combinations of AA and DMS were tested in choice experiments. In each experiment, a single urchin in each container was placed in contact with two artificial foods that were placed next to each other in the center of the container: a food containing AA, DMS, or both AA and DMS, and a control food. Urchins were allowed to feed for 4 h.

Because DMS is very volatile and can evaporate or diffuse from foods during the course of an experiment (Van Alstyne and Houser 2003), we assessed its loss during two experiments (0.02% AA and 0.007% DMS versus Control, 0.04% AA and 0.028% DMS versus Control). Extra pieces of treatment food were placed into containers without urchins. Two to three pieces were removed from the containers and placed into gas-tight vials after 0.5, 1, 2, and 4 h. These samples were analyzed for DMS, as described above. During these two experiments we also monitored the position of each urchin relative to the two food types. The number of urchins contacting each food after 0.5, 1, 2, and 4 h was recorded.

No-choice experiment

Nine urchins were randomly assigned to each of seven diet groups containing different concentrations of AA and DMS (ranging from 0% AA and 0% DMS to 0.10% AA and 0.11% DMS). Each of the 63 urchins was placed in a container and provided with a single piece of artificial food corresponding to its diet group and allowed to feed for 3 h. One replicate food from the treatment containing 0.02% AA and 0.035% DMS was mislaid upon collection, and thus excluded from analysis.

Statistical analyses

The concentration of DMSP in tips of *C. fragile* ssp. *tomentosoides* in the different months was compared by one-way ANOVA. The concentration of DMSP in different parts of the thallus in November 2004 and March 2005 was compared by two-way ANOVA with individual as a random (blocking) factor. Multiple comparisons were then conducted with a Tukey HSD test. The DMSP concentrations in paired tip and branch samples collected in July 2005 were compared with a paired *t*-test. According to statistical theory percentages, such as our DMSP concentrations, form a binomial, rather than a normal distribution. As a result, the arcsine transformation (Zar 1999) was applied to all DMSP concentration data prior to analyses so that they would approximate a normal distribution. For the no-choice experiment, the mass of food consumed by the urchins in each group was compared by one-way ANOVA. Levene's test was used to confirm that the data used in ANOVA conformed to the assumption of homogeneity of variance ($P > 0.39$). The masses of treatment and control foods consumed in the choice experiments were compared with two-tailed, paired *t*-tests.

Results

DMSP concentrations in *C. fragile* ssp. *tomentosoides* and other macrophyte species

Dimethylsulfoniopropionate concentrations in individual thalli of *C. fragile* ssp. *tomentosoides* ranged from 0.03 to 0.18% FM. Tips exhibited distinct variation in DMSP content (Fig. 1; $F_{6,}145 = 87.7$, $P < 0.001$). Average DMSP concentrations were lowest in boreal fall (September–November; 0.04-0.05%) but underwent a marked increase in winter, reaching a peak of 0.13% in March. Concentrations then declined gradually through the spring and summer.

The concentration of DMSP in different parts of the thallus (Fig. 2) differed significantly in both November 2004 ($F_{2,44} = 7.96$, $P = 0.001$) and March 2005 ($F_{2,36} = 15.7$, $P < 0.001$). However, the pattern of differences was not the same in both months. In November 2004, branches contained less DMSP than stipes and tips, which were not significantly different (Tukey HSD, $P < 0.05$). In contrast, stipes contained significantly less DMSP than branches and tips in March 2005. In July 2005 there was no significant difference between the tips and the branches ($t_{19} = 1.68$, $P = 0.11$).

Substantial concentrations of DMSP were detected in *Ulva lactuca* and *Polysiphonia harveyi*, with both species containing more DMSP (0.78 and 0.19%, respectively; Table 1) than *C. fragile* collected at the same time (0.10%; Fig. 1). Low concentrations (≤0.01%) of DMSP were detected in seven other species and no DMSP was detected in the remaining 17 species (Table 1).

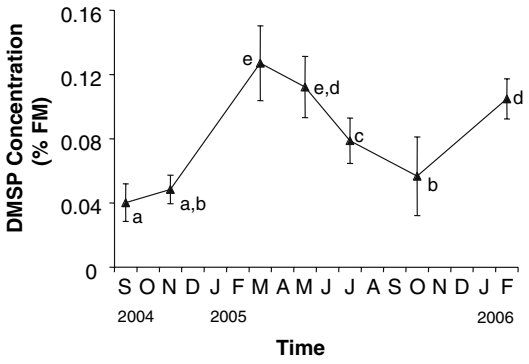

Fig. 1 Seasonal variation of DMSP concentration (% fresh mass ±1 SD) in the branch tips of *Codium fragile* ssp. *tomentosoides* collected between September 2004 and February 2006. *Points* with *different letters* are significantly different from one another (Tukey's HSD, $P < 0.05$)

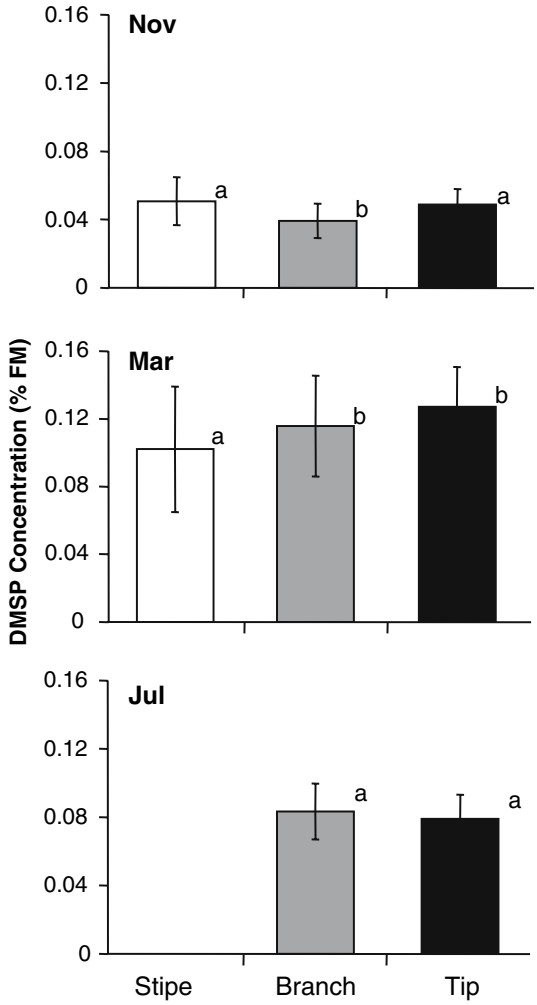

Fig. 2 DMSP concentrations (% fresh mass ±1 SD) in different parts of the *Codium fragile* ssp. *tomentosoides* frond in **a** November 2004, **b** March 2005, **c** July 2005. *Bars* labeled with *different letters* within each *plot* are significantly different from one another (ANOVA; paired *t*-test, $P < 0.05$). Concentrations in the stipes were not measured in July

Choice experiments: individual chemicals versus control

Urchins provided with a choice between a food containing 0.08% AA and a control consumed significantly less of the treated foods (Fig. 3a), while there was not a significant difference between consumption on food containing 0.02 or 0.04% AA and control foods. Urchins provided with a choice between a food containing 0.009, 0.020, or 0.034% DMS and a control consumed significantly less of the treated food (Fig. 3b). There was no significant deterrent effect of DMS at the lowest concentration tested (0.006%).

Choice experiments: AA and DMS versus control

The presence of both AA and DMS in treatment foods tended to discourage urchin feeding. Urchins consumed

Table 1 Mean DMSP (±1 SD) concentrations in marine macroalgae and one marine plant from Nova Scotia in February 2006

Species	*n*	% DMSP (FM)
Chlorophyta		
Chaetomorpha linum	3	nd
Chaetomorpha melagonium	3	nd
Codium fragile	20	0.10 ± 0.01
Spongomorpha aeruginosa	3	nd
Ulva lactuca	3	0.78 ± 0.07
Rhodophyta		
Ahnfeltia plicata	3	nd
Antihamnionella floccosa	3	0.002 ± 0.004
Ceramium rubum	3	nd
Chondrus crispus	3	0.001 ± 0.002
Corallina officinalis	3	nd
Palmaria palmata	2	nd
Phycodrys rubens	2	nd
Polyides rotundus	3	nd
Polysiphonia harveyi	3	0.19 ± 0.14
Ptilota serrata	3	nd
Rhodomela confervoides	2	0.011 ± 0.0003
Phaeophyta		
Agarum clathratum	3	0.01 ± 0.01
Alaria esculenta	3	nd
Ascophyllum nodosum	3	nd
Desmarestia aculeata	3	nd
Desmarestia viridis	2	nd
Fucus distichus	3	0.002 ± 0.003
Fucus vesiculosus	3	0.010 ± 0.004
Halosiphon tomentosus	1	nd
Laminaria digitata	3	nd
Saccharina longicrurus (=*Laminaria longicruris*)	3	nd
Magnoliophyta		
Zostera marina	3	0.01 ± 0.01

nd DMSP was not detectable, *FM* fresh mass

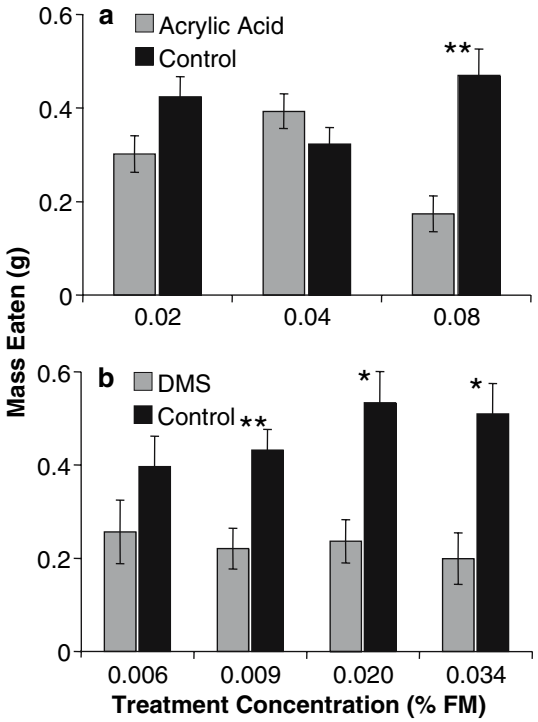

Fig. 3 Mean mass (±SE) consumed by *Strongylocentrotus droebachiensis* given a choice between agar-based foods containing **a** acrylic acid or **b** dimethylsulfide, and a control. *Asterisks* indicate experiments in which consumption differed significantly between treatments (paired *t*-test, *0.01 < $P \leq 0.05$, **0.001 < $P \leq 0.01$)

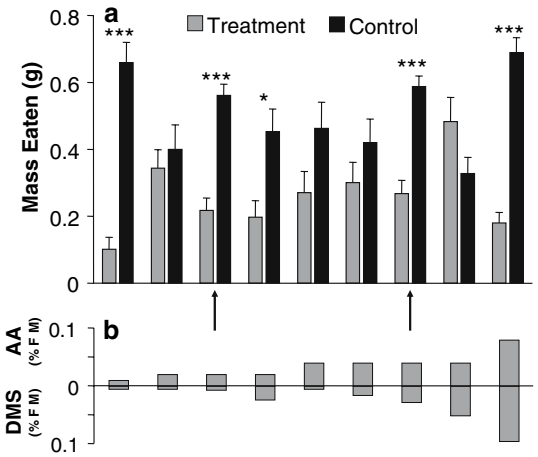

Fig. 4 **a** Mean mass (±SE) consumed by *Strongylocentrotus droebachiensis* given a choice between treatment foods, containing both acrylic acids (*AA*) and dimethylsulfide (*DMS*), and a control. *Asterisks* indicate experiments in which consumption differed significantly between treatments (paired *t*-tests, *0.01 < $P \leq 0.05$, ***$P \leq 0.001$). **b** Concentrations of AA and DMS treatments used in each experiment. *Bars* marked with an *arrow* indicate experiments where loss of DMS and urchin behavior was monitored

significantly less treatment food than control in five of nine experiments (Fig. 4). In three other experiments, urchins consumed less treatment than control food though these differences were not statistically significant. In the one experiment where more of the treatment food was eaten (0.04% AA and 0.051% DMS versus Control), this difference was not statistically significant.

We noticed a tendency for urchins to avoid the treated foods at the beginning of an experiment, often crawling away from them, or pushing them away with their tube feet. However, at the end of the experiment, the same urchins were often on top of and consuming the treatment foods. The number of urchins in contact with treatment and control foods was recorded during two of these experiments (0.02% AA and 0.007% DMS versus Control and 0.04% AA and 0.028% DMS versus Control; Fig. 5a). At the beginning of the experiments, 100% of urchins were placed

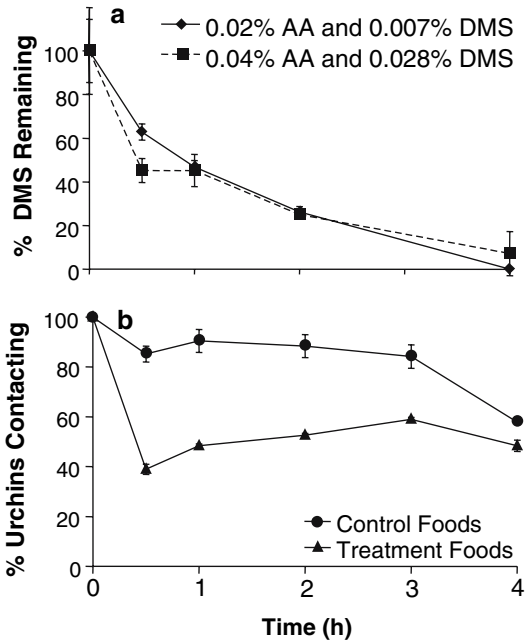

Fig. 5 **a** Percentage (±SE) of dimethylsulfide (*DMS*) remaining in treatment foods in 0.02% acrylic acid/0.007% DMS and 0.04% acrylic acid/0.028% DMS experiments. **b** percentage (±SE) of *Strongylocentrotus droebachiensis* contacting control and treatment foods during the course of the same experiments

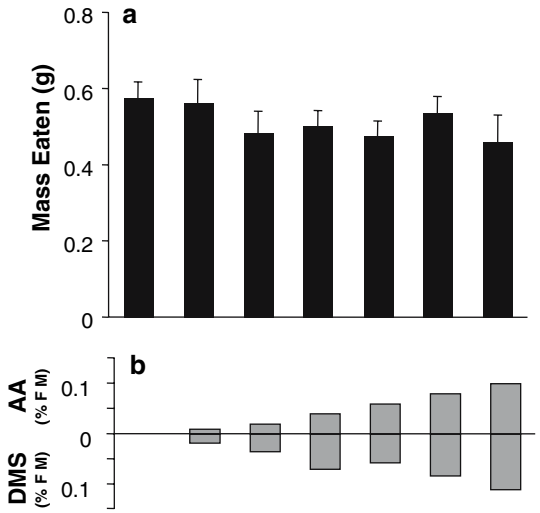

Fig. 6 **a** Mean mass (±SE) of prepared foods containing different concentrations of acrylic acid (*AA*) and dimethylsulfde (*DMS*) eaten by *Strongylocentrotus droebachiensis* in no-choice experiment. **b** Concentrations of AA and DMS in each prepared food

in contact with both foods (Fig. 5b). After 0.5 h, the percentage of urchins contacting the control food had dropped slightly to 85% and then remained relatively stable over the first 3 h before falling to 57% at the end of the 4–h experiment. In contrast, the percentage of urchins contacting the treatment food dropped to 38% after 0.5 h, and then gradually increased to 59% after 3 h, before dropping slightly to 47% at the end of the experiment.

Over the course of each feeding experiment, significant amounts of DMS were lost from treatment foods (Fig. 5a). Treatment foods lost about half of the DMS within 0.5 h, about 75% within 2 h, and more than 90% at the end of the 4-h experiments.

No-choice experiment

There was no significant effect of AA and DMS on the amount of food consumed by the urchins over concentrations ranging from no AA or DMS to 0.10% AA and 0.11% DMS (Fig. 6; $F_{6,}55 = 0.91$, $P = 0.49$).

Discussion

DMSP variation in *Codium fragile* ssp. *tomentosoides*

Our results demonstrate that the population of *C. fragile* ssp. *tomentosoides* in Nova Scotia undergoes substantial temporal variation in DMSP concentration, with concentrations peaking during winter and then decreasing through to the fall. During winter, many large thalli of *C. fragile* ssp. *tomentosoides* die back and undergo marked morphological/physiological changes, including loss of utricle hairs, a darkened thallus (Benson et al. 1983), and increased fragmentation (Fralick and Mathieson 1972). It is also during this period that water temperatures can fall below 0°C.

Similar temporal variation in secondary metabolite concentrations have been found in Australasia, where phenolic levels in brown algae tend to peak in spring (Steinberg and Van Altena 1992), and in the Mediterranean, where caulerpenyne concentrations in *Caulerpa taxifolia* peak in autumn (Dumay et al. 2002). Some natural products and morphological changes in macroalgae are induced in response to herbivory (Van Alstyne 1988; Yates and Peckol 1993; Cronin and Hay 1996a; Pavia and Toth 2000; Taylor et al. 2002; Macaya et al. 2005), or change in response to environmental factors such as temperature (Amade and Lemee 1998; Dethier et al. 2005), nutrients (Van Donk and Hessen 1993; Van Donk et al. 1997), UV radiation (Van Donk and Hessen 1995; Pavia et al. 1997), or desiccation (Renaud et al. 1990; Cronin and Hay 1996b; Ross and Van Alstyne 2007).

The causes of seasonal variation in DMSP concentrations in *C. fragile* ssp. *tomentosoides* in Nova Scotia are not clear. A variety of environmental factors are known affect DMSP concentrations. For example, in several Antarctic and temperate algae, they can change in response to changes in irradiance and salinity (Karsten et al. 1992). However, these effects are not universal. Experimental manipulations of salinity, light quantity and quality, nitrogen

availability, and herbivory failed to influence DMSP production by *U. lactuca* (Van Alstyne et al. 2003, 2007). Our ongoing work suggests that DMSP concentration in *C. fragile* ssp. *tomentosoides* correlates strongly with water temperature (D. A. Lyons, unpublished data). In addition to acting as a feeding deterrent, it has been suggested that DMSP may function as an anti-oxidant (Sunda et al. 2002; Ross and Van Alstyne 2007), a compatible solute (Reed 1983; Edwards et al. 1987, 1988; Kirst 1989; Kirst et al. 1991; Karsten et al. 1992), a cryoprotectant (Karsten et al. 1992, 1996), and a waste molecule used to expel excess sulfur and energy (Stefels 2000). Thus, elevated DMSP concentration in winter may be a physiological response to increased anti-oxidant or cryoprotection requirements during periods of senescence and cold temperature.

We also detected intra-thallus variation, although differences in DMSP concentration between parts of the thallus were small compared to the three-fold seasonal changes and the pattern of the intra-thallus differences was not consistent over time. Relatively small intra-thallus differences in DMSP concentrations have been reported from *U. lactuca* (Van Alstyne et al. 2007). In other algal species, concentrations of both inducible and constitutive natural products within tissues of individual algae can vary greatly (Hay and Fenical 1988; Hay and Steinberg 1992; Hay 1996; Van Alstyne et al. 2003 and references therein). Algae may allocate more compounds toward new, or growing tissues, reproductive structures, or basal structures such as the holdfast or stipe. Growth and reproduction occur throughout the thallus of *C. fragile* ssp. *tomentosoides* (Trowbridge 1998), and its coenocytic structure may allow translocation of secondary metabolites between parts of the alga. This may explain why DMSP is not confined to, or consistently concentrated in, particular parts of the thallus.

DMSP in other species

Dimethylsulfoniopropionate is absent or at very low concentration in the majority of algal species that co-occur with *C. fragile* ssp. *tomentosoides* in the northwest Atlantic. The patterns of DMSP abundance that we measured in these species are similar to those observed in related species from other locations (e.g., Reed 1983; Bischoff et al. 1994; Karsten et al. 1994; Van Alstyne et al. 2001; Van Alstyne and Puglisi 2007; K. L. Van Alstyne, unpublished data). Only *P. harveyi* and *U. lactuca* had DMSP concentrations comparable to those of *C. fragile* ssp. *tomentosoides*. *P. harveyi*, like *C. fragile* ssp. *tomentosoides*, is an invasive species, believed to have originated in Japan (McIvor et al. 2001), and the samples we used in our analysis were found growing epiphytically on *C. fragile* ssp. *tomentosoides*. Although urchin populations in the Northwest Atlantic may have encountered *U. lactuca*, the alga is normally found in the intertidal and upper subtidal zone, above the depth that urchins normally reach (R. E. Scheibling, personal observation). No DMSP was detected in laminarian kelps, which are by far the most abundant macroalgae and potential competitors of *C. fragile* ssp. *tomentosoides* in Nova Scotia and the Gulf of Maine (Levin et al. 2002; Scheibling and Gagnon 2006), as well as the preferred food of urchins (Scheibling and Hatcher 2006). Thus, urchins in this area may be relatively naïve with respect to AA and DMS.

Feeding assays

We found that concentrations of AA as low as 0.08% and concentrations of DMS as low as 0.009% deterred feeding of *S. droebachiensis* in choice experiments with artificial foods that mimicked the nutritional value of *C. fragile* ssp. *tomentosoides*. Urchins also avoided foods containing both chemicals together, although the results were somewhat idiosyncratic. The volatility of DMS and solubility of AA may have contributed to the variability of our results. Although we were able to detect significant effects of AA and DMS in the majority of our choice experiments, the effect was not significant at some intermediate concentrations. The gradual decline in DMS (and presumably AA) during our experiments appeared to cause treated foods to become increasingly attractive as the experiments progressed (Fig. 5b), making our tests for the effects of defensive metabolites conservative. Depriving sea urchins of food for several weeks prior to our feeding assays was done to encourage more rapid feeding, and thus shorten the experimental period (Van Alstyne and Houser 2003), to minimize loss of AA and DMS as the assays progressed. However, increased hunger levels may make herbivores less sensitive to defensive chemicals (Cronin and Hay 1996b), further contributing to the conservative nature of our results. Thus, AA and DMS may be effective deterrents to grazing by *S. droebachiensis* at even lower concentrations than tested in our experiments. AA and DMS have both been shown to deter urchin feeding at concentrations higher than those we tested (Van Alstyne et al. 2001; Van Alstyne and Houser 2003).

Although AA and DMS deterred urchin feeding under choice conditions, they did not impair feeding under no-choice conditions. These results are consistent with previous studies where urchins consumed *C. fragile* ssp. *tomentosoides* readily in the absence of other algae, but avoided it when kelps or turf algae were present (Scheibling and Anthony 2001; Sumi and Scheibling 2005). Anti-grazing defenses are unlikely to be equally effective in all situations. Rather, their effects will depend on the nutritional status of the grazer (Cronin and Hay 1996b), the nutritional value of the alga (Duffy and Paul 1992; Hay et al. 1994;

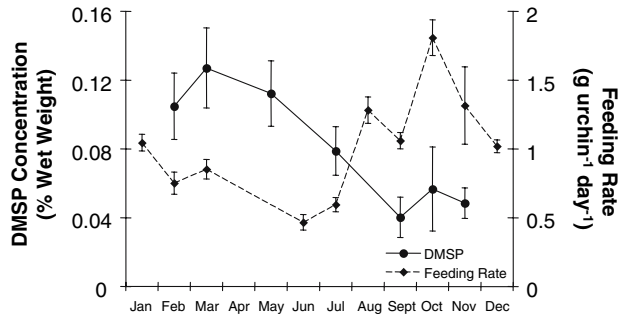

Fig. 7 Seasonal patterns of DMSP concentrations in *Codium fragile ssp. tomentosoides* (this study) and consumption of *Codium fragile ssp. tomentosoides* by *Strongylocentrotus droebachiensis* (Lyons and Scheibling 2007). Note that data are arranged according to the calendar year, not the sequence of sampling

Hemmi and Jormalainen 2002; Cruz-Rivera and Hay 2003), grazer density (Wright et al. 2005), and the availability of more palatable foods (Cruz-Rivera and Hay 2003). Although there was no effect of concentration on feeding rate in the no-choice experiment, patterns of temporal variation in DMSP concentration in *C. fragile* and the feeding rate of urchins on the alga suggest that further research into the influence of DMSP on feeding rate is warranted. Urchins tend to feed on *C. fragile* at a high rate in the late summer and fall when DMSP concentrations are low, and at a low rate during the winter when DMSP concentrations are high (Fig. 7). This pattern of consumption stands in contrast to that of urchins feeding on kelp, which peaks in the winter (Scheibling and Anthony 2001; Lyons and Scheibling 2007).

Due to logistical constraints, we tested the effects of AA and DMS on *S. droebachiensis* from Washington, rather than Nova Scotia, which could affect the applicability of our experimental results to interactions between *S. droebachiensis and C. fragile* ssp. *tomentosoides* in Nova Scotia. Different populations of conspecific herbivores sometimes react differently to algal secondary metabolites (Bricelj et al. 2000; MacQuarrie and Bricelj 2000; Sotka and Hay 2002; Bricelj et al. 2005). However, in these systems, populations with a history of exposure to defended algae tend to be more tolerant of them (Sotka 2003; Sotka et al. 2003; Bricelj et al. 2005). *C. fragile* ssp. *tomentosoides* arrived in Nova Scotia relatively recently, and DMSP is uncommon in the native flora of the rocky subtidal zone. In contrast, *S. droebachiensis* in Washington coexist with an indigenous population of *C. fragile* (subspecies are not recognized within the native range; Trowbridge 1998) and at least seven other macroalgae containing equivalent or greater concentrations of DMSP as *C. fragile* ssp. *tomentosoides* in Nova Scotia (Van Alstyne et al. 2001). Although we cannot be certain that urchins from Nova Scotia respond to AA and DMS in the same way as those from Washington, if urchin populations do differ in their sensitivity to these chemicals, it is more likely that those in Washington have evolved a degree of tolerance for them.

Ecological implications of DMSP production by *Codium fragile* ssp. *tomentosoides*

Previous studies have demonstrated that *C. fragile* is not a preferred food of *S. droebachiensis* (Prince and Leblanc 1992; Scheibling and Anthony 2001; Levin et al. 2002; Sumi and Scheibling 2005) or several other fish (Hay et al. 1987), arthropod (Duffy and Hay 1994; Bruno and O'Connor 2005), polychaete (Hay et al. 1988) and molluscan grazers (Lubchenco 1978; Trowbridge 1995; Chavanich and Harris 2002). Our results suggest that production of relatively high quantities of DMSP by *C. fragile* ssp. *tomentosoides* may be a contributing factor in the avoidance of *C. fragile* ssp. *tomentosoides* by urchins when other macroalgae, such as kelps or turf-forming species, are available.

Codium fragile ssp. *tomentosoides* and another chemically defended alga, *Desmarestia viridis*, were the only erect macrophytes observed in the wake of an urchin grazing front in Nova Scotia (D. A. Lyons, personal observation). Because the effect of AA and DMS is to alter preferences among foods, rather than prevent grazing when only one food source is available, urchins may eventually consume *C. fragile* ssp. *tomentosoides* remaining in barrens. In mixed algal beds with low to moderate densities of urchins, however, preferential grazing of native algae by *S. droebachiensis* may reduce competition with *C. fragile* ssp. *tomentosoides*, allowing populations of the invasive alga to establish and expand.

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