



# Nutritional preferences override chemical defenses in determining food choice by a generalist herbivore, *Littorina sitkana*

Kathryn L. Van Alstyne<sup>\*</sup>, Karen N. Pelletreau<sup>1</sup>, Anna Kirby<sup>2</sup>

Shannon Point Marine Center, Western Washington University, 1900 Shannon Point Road, Anacortes, Washington 98221, USA

## ARTICLE INFO

### Article history:

Received 6 March 2009

Accepted 3 August 2009

### Keywords:

Activated defenses

Chemical defenses

Food choice

*Littorina sitkana*

Nutritional quality

## ABSTRACT

In this study, we investigated why a littorinid snail, *Littorina sitkana*, preferentially consumes the ulvoid green algae *Ulva lactuca* and *Ulva linza*, even though these algae contain high concentrations of dimethylsulfoniopropionate (DMSP), which has been shown to be the precursor to an activated defense that deters feeding by sea urchins (*Strongylocentrotus droebachiensis* and *S. purpuratus*). When urchins consume algae containing DMSP, the DMSP is rapidly cleaved into DMS and acrylic acid, which are feeding deterrents to the urchins. In multiple-choice feeding-preference assays, *L. sitkana* consumed *U. lactuca* and *U. linza* at a greater rate than six other algal species. In experiments to confirm that DMSP was being cleaved by the snails, DMS was generated when snails were fed either *U. lactuca* or *U. linza*, but was not present in containers with snails alone or algae alone. In laboratory bioassays, acrylic acid reduced feeding by snails when it was incorporated into agar-based diets at concentrations of 0.25% and 0.50% of the food's fresh mass, demonstrating that the animals are deterred by one of the products of DMSP cleavage. When feeding rates from multiple-choice feeding-preference assay were compared to the carbon to nitrogen ratio (C:N) of the macroalgae, there was a strong negative linear relationship between grazing rates and C:N until a C:N of ~8 was reached. Algae with a C:N higher than 8 were avoided by snails. When the snails were offered agar-based diets containing a range of nitrogen concentrations (1.96–6.85% of the dry mass), feeding rates were positively correlated with the diets' nitrogen concentrations and negatively correlated with their C:N. Because *L. sitkana* forages only during low tides, it may have a limited window of time to obtain sufficient nitrogen. Therefore, it may preferentially select foods with high nitrogen concentrations, even though these foods contain chemical defenses.

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## 1. Introduction

Herbivore food preferences can be important in controlling the structural properties and dynamics of algal communities (Lubchenco, 1978). By preferentially consuming some algae or avoiding others, herbivores can lower the density of preferred algae and give competitively inferior, but less palatable algal species the opportunity to persist. Food preferences of herbivores can be affected by several factors that are extrinsic to the alga, such as the feeding history of the herbivore (Cronin and Hay, 1996) and the availability of other food types (Cubit, 1984; Kennish et al., 1996). However, there are also many intrinsic properties that can affect preferences, including the alga's morphology (Steneck, 1983; Padilla, 1985; Watson and Norton,

1985; Steneck, 1986), cell wall structure (Zemke-White and Clements, 1999a,b; Zemke-White et al., 2000), chemical defenses (Paul, 1992; McClintock and Baker, 2001; Amsler, 2008), and nutritional value, especially its nitrogen content (Nicotri, 1980; Duffy and Hay, 1991; Wakefield and Murray, 1998).

Chemical defenses are one of the best-studied intrinsic properties that affect food choice and they are produced by numerous species of marine macroalgae (Paul, 1992; McClintock and Baker, 2001; Amsler, 2008). Yet, producing large quantities of them does not guarantee that an alga will remain ungrazed. Many chemical defenses are effective towards some herbivores, but not others, suggesting some herbivores may have physiological mechanisms that allow them to tolerate or detoxify the compounds. For example, it has been hypothesized that small “mesograzers”, which use macroalgae as habitat and food, are more tolerant of their hosts' defenses than larger, more mobile grazers (Hay et al., 1989; Hay et al., 1990a; Hay et al., 1990b; Duffy and Hay, 1994). Although the physiological mechanisms by which marine organisms tolerate or detoxify algal chemical defenses are largely unknown, there is evidence that predatory fishes can detoxify some secondary metabolites using cytochrome P450 enzymes (Vrolijk et al., 1994; 1995).

<sup>\*</sup> Corresponding author. Tel.: +1 360 293 2188; fax: +1 360 293 1083.

E-mail address: [kathy.vanalstyne@wwu.edu](mailto:kathy.vanalstyne@wwu.edu) (K.L. Van Alstyne).

<sup>1</sup> Present address: Department of Biochemistry, Microbiology, and Molecular Biology, University of Maine, Orono, Maine, 04469, USA.

<sup>2</sup> Present address: Department of Reproductive Medicine, University of California, San Diego, La Jolla, California, 92093, USA.

When algae produce activated chemical defenses, herbivores have an additional means to avoid chemical feeding deterrents. Activated defenses involve the enzymatic conversion of stored precursor compounds with no or limited biological activity into predator-deterrent defensive compounds (Paul and Van Alstyne, 1992). They are known to be produced by several species of tropical and temperate green seaweeds (Paul and Van Alstyne, 1992; Cetrulo and Hay, 2000; Van Alstyne et al., 2001; Van Alstyne and Houser, 2003). Herbivores may be able to avoid consuming the products of an activated defense if they are able to prevent the defense from being activated and the deterrent products from being produced, although, prior to this study, this possibility has never been tested with marine herbivores.

In this study, we tested several hypotheses to address why littorinid snails (*Littorina sitkana* Philippi) preferentially consume algae (*Ulva lactuca* L. and *Ulva linza* L.) that produce dimethylsulfoniopropionate (DMSP), a multifunctional compound that is a precursor to an activated chemical defense. DMSP and its breakdown products dimethyl sulfide (DMS) and acrylic acid can be involved in osmotic acclimation, cryoprotection, methyl donation, the elimination of excess sulfur or energy, and acting as an antibiotic, antioxidant, or anti-predator defense (Kiene et al., 1996; Steinke et al., 2002; Yoch, 2002; Van Alstyne and Puglisi, 2007; Van Alstyne, 2008). DMSP can be cleaved into DMS and acrylic acid by the enzyme DMSP lyase when algal tissues are physically damaged (Cantoni and Anderson, 1956). Although DMSP is a feeding attractant towards the green sea urchin *Strongylocentrotus droebachiensis*, the products of DMSP cleavage, acrylic acid and DMS, are feeding deterrents (Van Alstyne et al., 2001; Van Alstyne and Houser, 2003; Lyons et al., 2007); however, DMS and acrylic acid do not deter feeding by the tropical urchin *Echinometra lucunter* (Erickson et al., 2006). The effects of DMS and acrylic acid on feeding by littorinid snails are not known.

Our experiments addressed the possibilities that: 1) *L. sitkana* do not activate the breakdown of DMSP into DMS and acrylic acid, 2) *L. sitkana* activate the breakdown of DMSP but are tolerant of the breakdown products, or 3) *L. sitkana* consume *U. lactuca* and *U. linza* despite being deterred by the products of DMSP breakdown because the algae are nitrogen-rich. Our results demonstrate that *L. sitkana* activate the breakdown of DMSP into acrylic acid and DMS and are deterred by at least one of the products of activation; however, they appear to preferentially consume the algae because they contain high concentrations of nitrogen.

## 2. Materials and methods

### 2.1. Study organisms

*L. sitkana* is found in the mid to low intertidal zone of moderately wave-exposed shores from Alaska to Oregon (Behrens, 1972; Behrens Yamada, 1977; McCormack, 1982; Boulding et al., 1993). It consumes both microalgae (Voltolina and Sacci, 2004) and macroalgae (Van Alstyne, 1988; 1990; Edwards et al., 2006) and frequently occurs in macroalgal beds, crevices, and among barnacles (Boulding and Harper, 1998). The *L. sitkana* used in this study were about ~1 cm diameter and were collected from the beach in front of the Shannon Point Marine Center in Anacortes, Washington (48° 31'N, 122°41'W), hereafter referred to as the Shannon Point Beach. After collection, snails were not fed macroalgae for a month; however, they did have access to biofilms on the containers in which they were maintained. From preliminary experiments, we have determined that starved snails tend to be slightly more selective than freshly collected snails (Van Alstyne and Ogden, unpublished data).

*U. lactuca* and *U. linza* are morphologically similar green algae (Phylum Chlorophyta, Order Ulvales) that grow abundantly in mid to low intertidal zones (O'Clair and Lindstrom, 2000; Nelson et al., 2003; Nelson et al., 2008). Like many green algae, they produce high

concentrations of DMSP (Van Alstyne et al., 2007; Van Alstyne et al., 2001). In western Washington's inland marine waters, both species are abundant from late spring through mid fall; during the winter they are uncommon. All of the algae used for this study were collected from the Shannon Point Beach by hand during low tides in July 2001.

### 2.2. Multiple-choice feeding-preference assays

To ensure that food preferences of *L. sitkana* from the Shannon Point Beach are similar to preferences seen in littorinid snails elsewhere, we conducted a multiple-choice feeding-preference assay in which snails were offered a choice of eight species of macroalgae. The methods used were similar to those described in Van Alstyne et al. (1999). Snails were simultaneously offered a choice of similarly sized (~0.1 g) pieces of three green algae: *Acrosiphonia saxatilis* (Ruprecht) K.L. Vinogradova, *U. linza*, and *U. lactuca*, three red algae: *Mastocarpus papillatus* (C. Agardh) Kützinger, *Mazzaella splendens* (Setchell et N.L. Gardner) Fredericq, and *Porphyra* sp., and two brown algae: *Fucus distichus* subsp. *evanescens* (C. Agardh) Powell and *Fucus spiralis* L. The multiple-choice preference experiments were conducted in 9 cm × 9 cm × 9 cm plastic containers from which 5 cm × 7 cm pieces were removed from two of the sides and replaced with 1 mm × 1 mm fiberglass screening. The containers were placed in indoor flow-through seawater tables and each container (N = 8) held one piece of each of the eight algal species and ten *L. sitkana*. An additional set of eight control containers held only pieces of algae that were used to estimate autogenic losses or gains in algal masses. Each of the control containers without herbivores was arbitrarily paired with an experimental container with herbivores.

At the beginning of the experiment, each algal piece was briefly blotted dry with a paper towel and weighed. After the snails had consumed approximately half of the algae in an experimental container (or after one week if half the algae was not eaten), the snails were removed from the container and all the algae in that container and in its paired control container were blotted dry and reweighed. Consumption was calculated as the difference of the initial minus the final masses for the experimental and control algae. Differences among consumption rates were compared with a Yao's *R* test, a multivariate analysis that is appropriate for analyzing feeding experiment data in which the consumption rates are not independent within arenas (Manly, 1993).

### 2.3. DMS release during grazing

To determine whether the defenses were activated when *L. sitkana* grazed upon green macroalgae, we fed *U. linza* and *U. lactuca* to snails and measured the amount of DMS generated while the snails fed. The methods used were similar to those described by Van Alstyne and Houser (2003). Three snails were placed in each of 10 gas-tight 25 ml flasks with approximately 0.05 g of algae. Additional sets of flasks contained either algae alone (N = 10) or snails alone (N = 10). The flasks were placed in an indoor flow-through seawater table to keep the containers cool. After 2 h, DMS concentrations were measured by withdrawing a 100 µl gas sample from the headspace of the container and injecting it into an SRI gas chromatograph (Chromasil 330 column, flame photometric detector; oven temperature 90 °C). Standards consisted of flasks that contained known amounts of DMS without snails or algae, which received the same treatment as the flasks with snails and/or algae. Statistical analyses could not be conducted on the data generated by this experiment because of a lack of variation in the *L. sitkana* only and alga only controls.

### 2.4. Bioassays with acrylic acid

We used laboratory bioassays to assess the effectiveness of one of the products of the activated defense, acrylic acid, as a feeding

deterrent. Diets composed of lyophilized, ground *Saccharina latissima* (L.) C.E. Lane, C. Mayes, Druehl, et G.W. Saunders and agar were made in a manner similar to that described in Van Alstyne et al. (2001). *S. latissima* was chosen as the base food for all the diets used in this study because it is readily consumed by *L. sitkana*, has very low concentrations of known chemical defenses (phlorotannins), and has a relatively low tissue nitrogen concentration making it an ideal base food for examining the effects of nitrogen on feeding. Although *L. sitkana* occurs too high in the intertidal zone to co-occur with *S. latissima*, it is frequently found feeding on *S. latissima* blades that wash in during storms.

Diets were made by heating 1.5 g of agar and 43.75 ml of deionized water in a microwave oven until boiling and then placed in a 40 °C water bath to cool. When the mixture had cooled but before it could solidify, 5 g of *S. latissima* and acrylic acid were added. Diets without acrylic acid served as controls. The diet was then evenly distributed across a piece of fiberglass screening (1 mm mesh) and allowed to cool. Pieces (1 cm<sup>2</sup> comprised of 10 × 10 mm squares) of the mesh were cut and offered individually to 3 snails in 10 cm diameter glass dishes that were kept cool by being submersed in a flow-through seawater table to a depth of approximately one cm. Snails were given a choice of diet with acrylic acid and control diet lacking acrylic acid. The snails were allowed to feed for 2 h; then, the pieces of mesh with the remaining diets were removed, and the number of squares of diet that had been consumed was counted. Feeding rates on diets with acrylic acid and control diets were compared with a paired *t*-test ( $\alpha = 0.05$ ).

### 2.5. Chemical measurements

To examine chemical characteristics of the foods used in the multiple-choice feeding-preference assay, we measured concentrations of DMSP, Folin-reactive compounds (phlorotannins), carbon, and nitrogen. All algae used in these analyses were collected from the Shannon Point Beach.

DMSP was measured as DMS following alkaline cleavage by drying approximately 100 mg pieces of algae at 60 °C overnight. The dried algae were then weighed and placed into 4 N sodium hydroxide in gas-tight vials. After 24 h, DMS concentrations in the headspaces were measured by direct injection with a SRI gas chromatograph as described in Van Alstyne et al. (2007). Commercially-obtained DMSP (Center for Analysis Spectroscopy and Synthesis, University of Groningen; purity > 98%) was used as a standard.

To measure carbon and nitrogen concentrations, pieces of tissues were excised from the centers of the blades and dried in an oven for 48 h at 60 °C. The dried tissues were ground with a CPEX mixer/mill to a fine powder. Carbon and nitrogen measurements were made with a CE Elantech 1112 Elemental Analyzer using atropine as a standard. Similar methods were used to measure the concentration of carbon and nitrogen in artificial diets.

To measure phlorotannin concentrations, pieces of tissues were excised from the centers of the blades, lyophilized, and stored at –70 °C. The dried tissues were ground to a fine powder with a CPEX mixer/mill, then extracted in 80% aqueous methanol. Phlorotannin concentrations were measured with a Folin–Ciocalteu assay as described in Van Alstyne (1995). Phloroglucinol-dihydrate was used as a standard.

### 2.6. Protein addition experiments

To determine whether the protein concentration of foods affected food choice by *L. sitkana*, we conducted a series of feeding experiments in which a single protein source, casein, was added to artificial diets. Diets were prepared as described above except that casein was added to three of four diets to increase the protein concentration from 1.96% (control diet) to 3.11% (low-nitrogen

addition diet), 4.90% (medium nitrogen addition diet), and 6.69% nitrogen by dry mass (high nitrogen addition diet), giving the diets carbon to nitrogen ratios (C:N) of 17.60, 10.49, 7.42, and 5.99, respectively (Table 1). The amounts of agar and water added to the diets were adjusted so that the agar concentrations and dry to wet weight ratios (D:W) of all four diets were 3% agar and 0.13 D:W.

The prepared diets were embedded into fiberglass mesh and cut into 1 cm<sup>2</sup> pieces (10 × 10 mm squares). Three snails were added to 10 cm diameter glass Petri dishes that each contained a piece of the mesh/diet (*N* = 10 for each diet type). After 2 h, the snails were removed and the mesh squares were photographed with a digital camera. The number of squares lacking diets in each piece of mesh was later counted and the differences were compared with analysis of variance (ANOVA).

## 3. Results

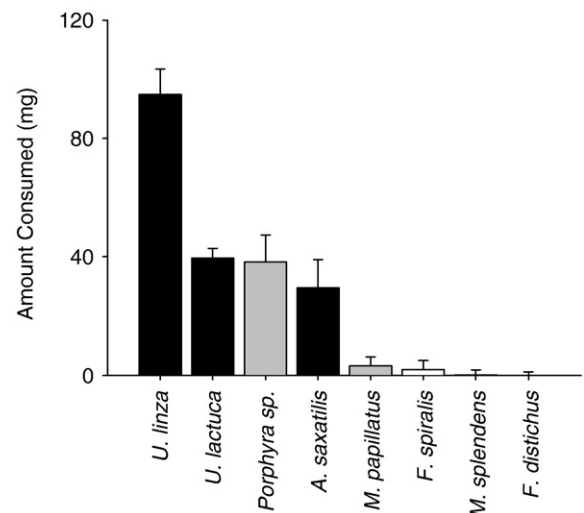
The dietary choices made by *L. sitkana* were similar to those reported previously (Van Alstyne et al. 2006). The snails consumed greater amounts of ulvoid green algae *U. lactuca* and *U. linza* than all other species (Fig. 1). Feeding was high on *A. saxatilis*, the other green alga the snails were offered, as well as on the red alga, *Porphyra* sp., which is morphologically similar to *U. lactuca* and *U. linza*. Feeding rates were much lower on the other red algae, *M. papillatus* and

**Table 1**

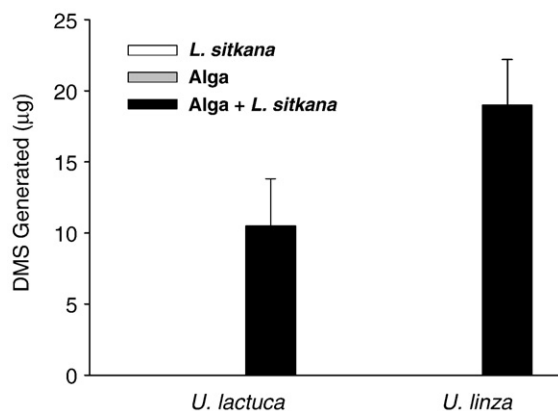
Nitrogen (N) concentrations, carbon (C) concentrations, and C to N ratios (C:N) of diets used in feeding experiments.

	[N] (% DM)	[C] (% DM)	C:N
Control	1.96 ± 0.15 ( <i>N</i> = 3)	30.36 ± 0.37 ( <i>N</i> = 3)	15.55 ± 1.06 ( <i>N</i> = 3)
Low N addition	3.21 ± 0.32 ( <i>N</i> = 3)	32.72 ± 1.01 ( <i>N</i> = 3)	10.24 ± 0.76 ( <i>N</i> = 3)
Medium N addition	4.89 ± 0.04 ( <i>N</i> = 3)	36.46 ± 0.57 ( <i>N</i> = 3)	7.45 ± 0.05 ( <i>N</i> = 3)
High N addition	6.85 ± 0.70 ( <i>N</i> = 3)	41.07 ± 2.88 ( <i>N</i> = 3)	6.01 ± 0.21 ( <i>N</i> = 3)

The control diet consisted of freeze-dried ground *S. latissima* in agar. The remaining diets were supplemented with casein to increase their N concentrations and lower their C:N. DM: dry mass.



**Fig. 1.** Food preferences of *Littorina sitkana* for common intertidal macroalgae in laboratory multiple-choice feeding-preference assays. Data are given as means ± 1 SE. Black bars indicate green algae, grey bars indicate red algae, and white bars indicate brown algae. Feeding rates among algae are significantly different (Yao's *R* = 2932.17, *P* < 0.001).



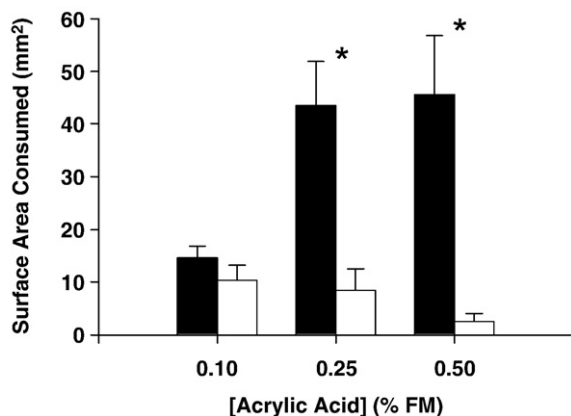
**Fig. 2.** DMS release (mean  $\pm$  1 SE,  $N = 10$ ) during grazing by *Littorina sitkana*. Statistical analyses could not be conducted because of a lack of variation in the *L. sitkana* only and alga only controls. No DMS was detected in containers with only snails or only algae.

*M. splendens*. The snails also avoided feeding on the intertidal brown algae *F. distichus* and *F. spiralis*.

The preference for DMSP-producing algae could not be explained by a lack of defense activation during grazing. Significant concentrations of DMS were produced when snails fed upon *U. lactuca* and *U. linza* (Fig. 2), which indicated that DMSP was being cleaved as a result of the damage. DMS not detected in any of the containers that contained either snails alone or algae alone.

Likewise, the preference for DMSP-producing algae could not be explained by snails not being deterred by acrylic acid, one of the products of the defense activation. When acrylic acid (Fig. 3) was incorporated into diets, snails consumed significantly less of the diet containing the chemical at the two higher concentrations used (0.25% and 0.50% FM acrylic acid) than of the control diets lacking it. Acrylic acid did not significantly deter feeding at 0.10% FM.

The mean feeding rates on whole pieces of algae were positively correlated with the algae's mean nitrogen concentrations (Table 2, Fig. 4; Spearman's  $Rho = 0.786$ ,  $P = 0.021$ ) and mean C:N (Spearman's  $Rho = -0.976$ ,  $P < 0.001$ ). When snails were offered diets with a range of nitrogen concentrations, consumption rates were significantly different among diets (Kruskal–Wallis test:  $H = 8.59$ ,  $P = 0.035$ ). Mean consumption rates were positively correlated with the diets' nitrogen concentrations (Fig. 5; Spearman's  $Rho = 1.000$ ,  $P < 0.001$ ). There was a negative correlation between consumption rates and the diets' C:N (Spearman's  $Rho = 1.000$ ,  $P < 0.001$ ).



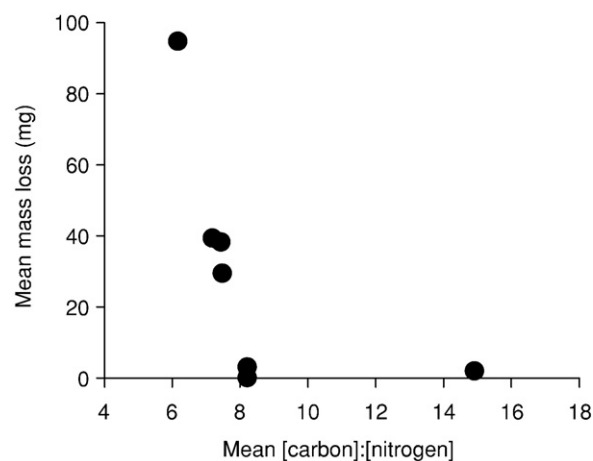
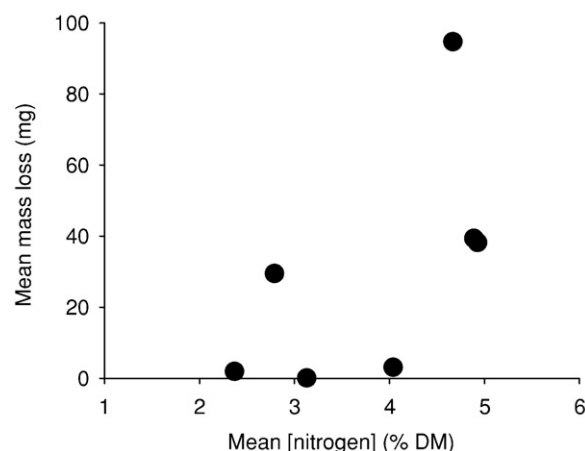
**Fig. 3.** Consumption rates (means  $\pm$  1 SE) of control diets (black bars) and diets containing acrylic acid (white bars). Concentrations of acrylic acid in the diets at the beginning of each experiment are given below each set of bars. Asterisks indicate experiments in which consumption rates differed significantly between control diets and diets with acrylic acid ( $P < 0.05$ , paired  $t$ -test). FM: fresh mass.

**Table 2**

Chemical characteristics of macroalgae used in food preference experiments.

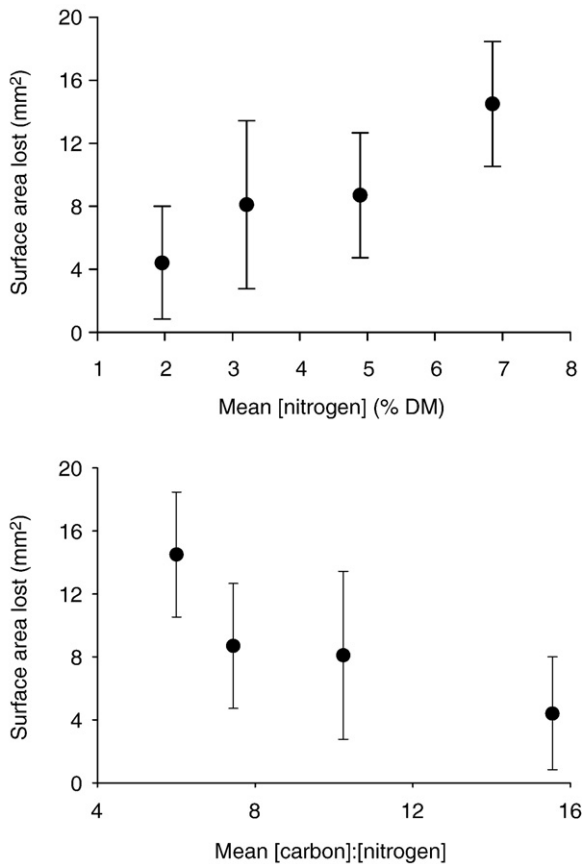
Species	[DMSP] (% FM)	[Folin-reactive compounds] (% DM)	[N] (% DM)	[C] (% DM)	[C]:[N]
<b>Phylum Chlorophyta</b>					
<i>Acrosiphonia saxatilis</i>	0.19 $\pm$ 0.03 ( $n = 5$ )	0.31 $\pm$ 0.05 ( $n = 5$ )	2.79 $\pm$ 0.26 ( $n = 5$ )	20.81 $\pm$ 2.01 ( $n = 5$ )	7.47 $\pm$ 0.31 ( $n = 5$ )
<i>Ulva linza</i>	0.98 $\pm$ 0.13 ( $n = 5$ )	0.41 $\pm$ 0.04 ( $n = 5$ )	4.67 $\pm$ 0.27 ( $n = 5$ )	28.71 $\pm$ 1.25 ( $n = 5$ )	6.16 $\pm$ 0.19 ( $n = 5$ )
<i>Ulva lactuca</i>	1.39 $\pm$ 0.06 ( $n = 5$ )	0.49 $\pm$ 0.06 ( $n = 5$ )	4.89 $\pm$ 0.08 ( $n = 5$ )	35.07 $\pm$ 0.72 ( $n = 5$ )	7.18 $\pm$ 0.18 ( $n = 5$ )
<b>Phylum Phaeophyta</b>					
<i>Fucus distichus</i>	0.01 $\pm$ 0.01 ( $n = 5$ )	8.05 $\pm$ 1.20 ( $n = 5$ )	2.08 $\pm$ 0.07 ( $n = 5$ )	35.66 $\pm$ 1.62 ( $n = 5$ )	17.18 $\pm$ 0.40 ( $n = 5$ )
<i>Fucus spiralis</i>	0.02 $\pm$ 0.02 ( $n = 5$ )	5.62 $\pm$ 1.67 ( $n = 5$ )	2.37 $\pm$ 0.12 ( $n = 5$ )	35.27 $\pm$ 1.42 ( $n = 5$ )	14.91 $\pm$ 1.23 ( $n = 5$ )
<b>Phylum Rhodophyta</b>					
<i>Mastocarpus papillatus</i>	n.d.	0.26 $\pm$ 0.05 ( $n = 5$ )	4.04 $\pm$ 0.13 ( $n = 5$ )	33.16 $\pm$ 0.84 ( $n = 5$ )	8.21 $\pm$ 0.38 ( $n = 5$ )
<i>Mazzaella splendens</i>	n.d.	n.d.	3.13 $\pm$ 0.10 ( $n = 5$ )	27.66 $\pm$ 0.90 ( $n = 5$ )	8.84 $\pm$ 0.34 ( $n = 5$ )
<i>Porphyra</i> sp.	0.01 $\pm$ 0.01 ( $n = 5$ )	1.03 $\pm$ 0.11 ( $n = 4$ )	4.93 $\pm$ 0.41 ( $n = 4$ )	36.51 $\pm$ 1.23 ( $n = 4$ )	7.43 $\pm$ 0.56 ( $n = 4$ )

Data are given as means  $\pm$  1 SD. (n.d.: not detectable: less than 5  $\mu$ g DMS).



**Fig. 4.** Mean mass loss by algae in multiple-choice feeding preference experiments with *Littorina sitkana* versus the algal species' mean nitrogen concentration (Spearman's  $Rho = 0.786$ ,  $P = 0.021$ ) and carbon to nitrogen ratio (Spearman's  $Rho = -0.976$ ,  $P < 0.001$ ). DM: dry mass.





**Fig. 5.** Feeding rates of *Littorina sitkana* (mean  $\pm$  1 SE) on diets in which nitrogen concentration and carbon to nitrogen ratios were manipulated with the addition of casein. Consumption rates are significantly different among the four types of diets (Kruskal–Wallis test:  $H = 8.59$ ,  $P = 0.035$ ) and mean consumption rates are positively correlated with mean nitrogen concentrations (Spearman's Rho = 1.000,  $P < 0.001$ ) and mean carbon to nitrogen ratios (Spearman's Rho = 1.000,  $P < 0.001$ ). DM: dry mass.

#### 4. Discussion

This study provides evidence that food preferences of *L. sitkana* for ulvoid green algae are determined by tissue nitrogen concentration rather than by the presence or absence of an activated chemical defense. In our laboratory assays, *U. linza* and *U. lactuca* were the two most consumed foods when snails were given a choice of eight common intertidal macroalgae (Fig. 1). The presence of other types of chemical or morphological defenses could, in part, account for these preferences. Two of the three least preferred species, *F. distichus* and *F. spiralis*, both contain high concentrations of phlorotannins (Table 2), which are correlated with reductions in grazing by *L. sitkana* in the field (Van Alstyne, 1988). *M. splendens* is covered with a cuticle that has been shown to be a feeding deterrent towards the snail *Lacuna marmorata* and the isopod *Idotea wosnesenskii* (Gaines, 1985).

The preference for ulvoid macroalgae by *L. sitkana* occurred despite the high concentrations of DMSP that were present in the algae. The concentrations that we measured in this study (Table 2) are comparable to concentrations previously reported in *U. lactuca* and *U. linza* in the northeastern Pacific (Van Alstyne et al., 2001; Van Alstyne et al., 2007). Thus, there was a large quantity of DMSP that could be broken down into acrylic acid and DMS. The presence of a lyase enzyme capable of cleaving DMSP into DMS and acrylic acid has also been shown to be present in *U. lactuca* collected from the same location as the *U. lactuca* used in this study (Van Alstyne et al., 2001). Although DMSP lyase activity has not been reported from *U. linza*, it has been shown to be present in several other ulvoid algal species

including *Ulva curvata* (De Souza and Yoch, 1996), *Ulva* (= *Enteromorpha*) *clathrata* (Steinke et al., 1996; Steinke and Kirst, 1996), *Ulva* (= *Enteromorpha*) *intestinalis* (Steinke et al., 1996), *Ulva* (= *Enteromorpha*) *bulbosa* (Steinke et al., 1996), and *Ulva* (= *Enteromorpha*) *compressa* (Steinke et al., 1996), suggesting that it is likely that *U. linza* also contains the enzyme. It should be noted that although the enzymes present in these algae are all reported to be DMSP lyase based on their ability to generate DMS from DMSP, it is not known whether the enzyme is structurally identical in all algal species.

The results of our feeding assays with *L. sitkana* also are consistent with the presence of a lyase enzyme in *U. lactuca* and *U. linza*. The presence of DMS in containers holding both snails and seaweeds and its absence in containers holding snails alone or algae alone demonstrated that DMSP was cleaved as a result of grazing (Fig. 2). Similar results were obtained when sea urchins (*S. droebachiensis*) were fed *U. lactuca* and *U. linza* (Van Alstyne and Houser, 2003). In these experiments, DMS concentrations increased in the headspace of closed containers over a 6 hour period when the urchins were fed the algae. They also continued to increase for an additional 18 h after the algae were removed, suggesting that the breakdown of the algae in the guts of the urchins was one source of the DMS. The breakdown of DMSP into DMS and acrylate or acrylic acid can also occur when coral reef fishes graze upon macrophytes containing DMSP (Dacey et al., 1994) and there are numerous examples of DMS being generated by phytoplankton in response grazing by zooplankton (e.g., Dacey and Wakeham, 1986; Leck et al., 1990; Daly and DiTullio, 1993; Lee et al., 2003). The DMS released by krill grazing can be used as a foraging cue by predatory seabirds (Nevitt et al., 1995; Nevitt, 1999; Nevitt and Haberman, 2003; Nevitt and Bonadonna, 2005). Whether predators of *L. sitkana* can use DMS as a foraging cue is not currently known.

We also demonstrated that at least one of the breakdown products of DMSP, acrylic acid, inhibited feeding by the snails at concentrations that could occur naturally (0.50% FM) or at approximately half (0.25% FM) of natural maximal concentrations (Fig. 3). Therefore, snails preferentially consume *U. lactuca* and *U. linza* despite being deterred by at least one compound that is being produced as a result of their cleaving DMSP.

Why then do these snails preferentially consume algal species that contain an activated defense which is both activated by grazing and produces products that are distasteful to the animals? Our data support the hypothesis that the high nitrogen contents of *U. lactuca* and *U. linza* override the deterrent effects of the DMSP breakdown product(s). *U. lactuca* and *U. linza* had the highest nitrogen concentrations and lowest carbon to nitrogen ratios of all the algae offered to the snails in the multiple-choice feeding-preference assays (Table 2). Other marine herbivores are known to tolerate the presence of distasteful metabolites when they occur in nutrient-rich foods. For example, tropical herbivorous fishes have been shown to be less deterred by the presence of defensive compounds (pachydictyol A, manoalide, and a brominated diphenyl ether) when these compounds were offered in high-nitrogen diets than when they were offered in low-nitrogen diets (Duffy and Paul, 1992). Similar effects were seen when several species of amphipods were offered dictyols in higher-quality fish-based food and lower-quality algal-based foods (Cruz Rivera and Hay, 2003).

Nitrogen is frequently considered to be a limiting nutrient to herbivores because the nitrogen concentration within the body of the herbivore is proportionately higher than plant nitrogen concentrations, especially in relative to carbon availability (Mattson, 1980; Sterner and Hessen, 1994). Marine herbivores are known to preferentially select foods with high nitrogen concentrations. *Aplysia californica*, an opisthobranch mollusc, preferentially ate more nitrogen-rich *Gracilaria ferox* than conspecific nitrogen-poor algae and also grew more rapidly on the nitrogen-rich algae (Barile et al., 2004). *Littorina littorea* has also been shown to preferentially feed on *Fucus vesiculosus* from a site with higher seawater nitrogen concentrations than on algae from a site with lower nitrogen concentrations,

although these preferences also could have been due to lower phlorotannin concentrations in the algae from the high-nitrogen site (Yates and Peckol, 1993). Herbivorous snails, particularly species that forage at low tides, such as *L. sitkana* (Van Alstyne, 1988), have a limited window of time to obtain the nitrogen they need, and thus may preferentially select high-nitrogen foods, despite the presence of a chemical defense. This is supported by feeding preferences of other intertidal and subtidal herbivores. *U. lactuca* and *U. linza* were high-preference foods for the intertidal isopod *I. wosnesenskii* (Van Alstyne et al., 2006). In contrast, these algae were lower to mid-preference foods for green sea urchins (*S. droebachiensis*) from subtidal populations (Van Alstyne et al., 2001; Van Alstyne and Houser, 2003; Van Alstyne et al., 2006). Vadas (1977) found that ranked feeding preferences of *S. droebachiensis* for eight algal species were negatively correlated with the caloric value of the algae.

It is not known how *L. sitkana* tolerates the defensive compounds produced by *U. lactuca* and *U. linza*. Sotka and Whalen (2008) suggest that marine herbivores may use detoxification and transport enzymes, such as cytochrome P450s, glutathione S-transferases, and ATP binding cassette transporters, to metabolize ingested secondary metabolites. However, the use of these mechanisms by most marine herbivores, including *L. sitkana*, has not been well documented. The consumption of secondary metabolites can also have positive effects on fitness as some herbivores can take up deterrent secondary metabolites and use them for their own defense (e.g., Paul and Van Alstyne, 1988; Pennings, 1990; Gavagnin et al., 2000; Rogers et al., 2000; Becerro et al., 2001). The effects of DMS and acrylic acid on the growth and reproductive output of *L. sitkana* are not known. *L. sitkana* that consumes *U. lactuca* accumulates measurable amounts of DMSP, whereas DMSP is not detectable in snails fed *M. splendens* and *F. distichus*, which do not contain large amounts of the compound (K. Van Alstyne, unpublished data). Whether the accumulated DMSP serves a defensive function is also not known.

In summary, *L. sitkana* preferentially feeds on the ulvoid green algae *U. lactuca* and *U. linza* despite the high concentrations of DMSP in these algae. Grazing by the snails causes the cleavage of DMSP into acrylic acid and DMS and the snails are deterred by acrylic acid when it is incorporated into an agar-based diet. *U. lactuca* and *U. linza* also have high nitrogen concentrations and low C:N, which appear to be a stronger influence on feeding preferences than the presence of the DMSP-based defense. The preference for high nitrogen foods may have evolved because of the limited amount of time these intertidal snails have for obtaining nitrogen from their diets since their foraging activities to when they are emersed.

## Acknowledgements

We are grateful to A. Nelson for assistance with laboratory assays and chemical measurements. Funding was provided by NSF grants to KLV (IBN-0090825 and IOS-0717631) and NSF Research Experiences for Undergraduates Program grant (OCE-9731144) to the Shannon Point Marine Center of Western Washington University. [SS]

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