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Palatability of Macroalgae that Use Different Types of Chemical Defenses

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Abstract This study compared algal palatability and chemical defenses from subtropical green algae that may use different types of defense systems that deter feeding by the rock-boring sea urchin *Echinometra lucunter*. The potential defense systems present include (1) the terpenoid caulerpenyne and its activated products from *Caulerpa* spp., and (2) dimethylsulfoniopropionate (DMSP)-related defenses in *Ulva* spp. Secondary metabolites from these chemical groups have been shown to deter feeding by various marine herbivores, including tropical and temperate sea urchins. Live algal multiple-choice feeding assays and assays incorporating algal extracts or isolated metabolites into an artificial diet were conducted. Several green algae, including *Ulva lactuca*, *Caulerpa prolifera*, and *Cladophora* sp., were unpalatable. Nonpolar extracts from *U. lactuca* deterred feeding, whereas nonpolar extracts from *C. prolifera* had no effect on feeding. Polar extracts from both species stimulated feeding. Caulerpenyne deterred feeding at approximately 4% dry mass; however, dimethyl sulfide and acrylic acid had no effect at natural and elevated concentrations. *E. lucunter* is more tolerant than other sea urchins to DMSP-related defenses and less tolerant to caulerpenyne than many reef fish. Understanding the chemical defenses of the algae tested in this study is important because they, and related species, frequently are invasive or form blooms, and can significantly modify marine ecosystems.

Keywords Chemical defense · Herbivory · *Caulerpa* · Caulerpenyne · *Cladophora* · *Ulva* · DMS · DMSP · Acrylic acid · *Echinometra lucunter* · Algal blooms

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Introduction

Marine algae are defended from herbivory by a variety of secondary metabolites (Paul et al., 2001), including polyphenolics, acetogenins, terpenes, amino-acid-based and halogenated compounds, which can influence palatability. These metabolites deter feeding by marine herbivores in the field and in laboratory assays (Hay et al., 1987a, b; Van Alstyne et al., 2001; Van Alstyne and Houser, 2003). Because algal chemical defenses influence herbivore preference, and herbivores, such as sea urchins, can structure marine algal communities (Carpenter, 1986; Wright et al., 2005), chemical defenses indirectly structure algal populations and communities in coral reefs and other nearshore habitats.

Numerous green algae (Chlorophyta) are especially deterrent to coral reef herbivores. Rhizophytic algae in the order Bryopsidales, including *Caulerpa*, *Halimeda*, *Udotea*, *Penicillus*, and *Rhipocephalus* spp., are less palatable than other species commonly found in reef habitats (Paul and Hay, 1986; Wylie and Paul, 1988; Meyer et al., 1994). Their extracts deter feeding in artificial assays (Paul and Van Alstyne, 1992). Algae also produce higher concentrations or additional defensive compounds in areas subject to high herbivore pressure (Paul and Fenical, 1986). In addition, algae in the order Ulvales and Cladophorales, such as *Ulva*, *Enteromorpha*, and *Cladophora* spp., are less palatable than other algae in the field and in live algal and artificial feeding trials toward some temperate herbivores (Paul and Hay, 1986; Van Alstyne et al., 2001; Van Alstyne and Houser, 2003), although less is known about their secondary chemistry.

Some green algae use an activated defense system whereby damage, which could result from feeding, results in the conversion of a stored secondary metabolite, which may have biological activity, into a product with greater bioactivity (Paul and Van Alstyne, 1992). Two notable examples of activated defenses occur in the Bryopsidales, which commonly produce bioactive terpenoids (mainly sesquiterpenes and diterpenes). These are caulerpenyne from *Caulerpa* spp. and halimedatetraacetate from *Halimeda* spp., which, upon wounding, are transformed into the more toxic and deterrent oxytoxins and halimedatrial, respectively (Paul and Van Alstyne, 1992; Cimino et al., 1990; Gavagnin et al., 1994; Jung and Pohnert, 2001). Another activated system used by some macroalgae involves the conversion of dimethylsulfoniopropionate (DMSP) into acrylic acid and dimethyl sulfide (DMS). Many genera of green macroalgae have high DMSP concentrations that deter feeding by marine herbivores (Van Alstyne et al., 2001; Van Alstyne and Houser, 2003). For instance, both conversion products deter feeding by temperate sea urchins at relatively low concentrations (Van Alstyne et al., 2001; Van Alstyne and Houser, 2003; Lyons et al., unpublished data).

This study tested the relative effectiveness of different types of defenses that are commonly found in green algae on feeding by a common tropical and subtropical reef herbivore, the rock-boring sea urchin *Echinometra lucunter*. Live algal feeding assays were conducted to assess palatability of green algae relative to co-occurring red and brown macroalgae, found in the Indian River Lagoon, and at offshore sites near Fort Pierce, Florida. In addition, relative palatability was tested among unpalatable green algae that may use different defense systems (caulerpenyne-related terpenoids vs. products from the activation of DMSP). Finally, artificial feeding assays were conducted, where algal extracts or isolated metabolites were incorporated into agar-based foods, to assess if trends in palatability were related to algal chemistry. This study is the first to examine how DMSP-related defenses influence feeding by a subtidal, subtropical herbivore, and to assess the relative susceptibility of this herbivore to components of these different systems.

Methods and Materials

Collection of Organisms

Algae used in live algal feeding assays were collected from Fort Pierce, Florida, USA, in the Indian River Lagoon (27°27.769′N, 80°19.291′W), offshore at Pepper Park (27°29.566′N, 80°17.796′W), and from the Smithsonian Marine Ecosystems Exhibit from August to October, 2003. Green algae included *Caulerpa prolifera*, *Caulerpa racemosa* var. *laetevirins*, *Caulerpa sertularioides*, *Cladophora* sp., *Codium taylorii*, *Halimeda discoidea*, and *Ulva lactuca*. Red algae included *Amphiroa fragilissima*, *Gracilaria caudata*, *Gracilaria cervicornis*, and *Gracilaria tikvahiae*. Brown algae included *Dictyopteris deliculata* and *Lobophora variegata*. The cyanobacterium *Lyngbya confervoides* was obtained from a bloom off Fort Lauderdale, Florida, in the fall of 2003 (26°16.408′N, 80° 03.833′W). *G. tikvahiae* for artificial feeding assays was obtained from cultures maintained at Harbor Branch Oceanographic Institution by D. Hanisak. Sea urchins (*E. lucunter*) were obtained from a rocky sea wall in the Indian River Lagoon (27°27.769′N, 80°19.291′W). This species is found in reef communities and rocky shores throughout the Caribbean, from North Carolina south to Brazil, and on the Atlantic coast of Africa (Hendler et al., 1995).

Live Algal Feeding Assays

Multiple-choice feeding assays were conducted with live algae commonly found in reef and rocky habitats to gain perspective on how algae rank in palatability. Individual sea urchins ($N = 15$–20) were offered pieces of similar volumes of four to six algal species (see figures for species used in each experiment). *G. tikvahiae*, an abundant red drift alga readily consumed in preliminary live algal feeding assays, served as a positive control. Algae were weighed before and after feeding, and assays were stopped once an alga was completely consumed or after 70 hr. Paired sea urchin exclusion controls were run at the same time in each aquarium to account for changes in algal mass unrelated to feeding (Peterson and Renaud, 1989). Consumption of each species by each sea urchin was determined by using the formula $[T_i \times (C_f / C_i]) - T_f$, where T_i is the initial algal mass, T_f is the final algal mass, C_i is the initial control algal mass, and C_f is the final control algal mass. The amount of each algal species consumed was expressed as the percentage of the total algae consumed by an individual sea urchin (Lockwood, 1998). Sea urchins that consumed <10% or >90% of the total algal mass per aquarium^{-1} were excluded from statistical analysis. Friedman's repeated-measures ANOVA and Student–Newman–Keuls multiple comparison test were used to identify significant differences in the percentage of total consumption among algae (Lockwood, 1998). Based on the results from the live algal feeding assays, additional assays were performed, comparing palatability among the green algae *U. lactuca*, *Cladophora* sp., and *C. prolifera* as described above.

Preparation of Algal Extracts

Freshly collected *U. lactuca* and *C. prolifera* were individually homogenized in solvent and extracted ×3 in 1:1 ethyl acetate/methanol to yield a nonpolar extract and then ×2 in 1:1 ethanol/distilled H_2O to yield a polar extract. It is possible that nonpolar extracts might contain some polar compounds that are partially soluble in methanol, and that some polar compounds were excluded from the polar extract due to the presence of 50% ethanol.

Extracts were filtered, dried by rotary evaporator, and stored at 4°C until used in feeding assays. There was not enough *Cladophora* sp. to perform extractions and use in feeding assays.

Artificial Feeding Assays with Algal Extracts

Methods were similar to those used by Hay et al. (1998). To make the artificial diet, 1 g agar was dissolved in 30 ml distilled H_2O and heated in a microwave. Two grams of dried, ground *G. tikvahiae* were added. Algal extracts were dissolved in 2 ml ethanol and incorporated into the food at natural concentrations on a dry weight basis. Ethanol (2 ml) also was added to the control foods. Artificial food with and without extracts was spread into a mold with parallel, rectangular wells over window screen, cooled, and cut into replicate strips containing one piece of each food type. Sea urchins were fed extract-free artificial food before feeding trials. For artificial assays, sea urchins (N = 15–20) were offered a strip of screen containing three pieces of food of equal size, one with nonpolar extract, one with polar extract, and a control piece without extract (placement of extract-containing and control food was randomized on strips among assays). Sea urchins were allowed to feed until half of the artificial food of one food type was consumed or until 48 hr passed. Preference was quantified as the number of window screen squares revealed after food was consumed. Sea urchins that did not eat or consumed all food were excluded from statistical analysis. Friedman's repeated-measures ANOVA and Student–Newman–Keuls multiple comparison test were used to identify significant differences in the number of squares consumed.

Measurement of DMSP in Green Algae

Tissue concentrations of DMSP in *U. lactuca*, *C. prolifera*, and *Cladophora* sp. were measured with methods similar to those described in Van Alstyne et al. (2001). Dried algae (N = 10) were weighed and placed in 4 N NAOH in 30 ml gas-tight vials that were stored at 4°C in darkness overnight. The next day, DMSP was measured as DMS from the headspaces of the vials by direct injection into an SR1 gas chromatograph (Chromosil 330 column, flame photometric detector; detection limit: 5 μg DMS). Known concentrations of commercially obtained DMSP were used as standards. A Kruskal–Wallis ANOVA and Tukey's multiple comparison test were used to compare concentrations among species.

Artificial Feeding Assays with Algal Compounds

Dimethyl sulfide (Acros Organics) and acrylic acid (Sigma-Aldrich) were incorporated at natural concentrations, and multiples thereof, into artificial foods composed of 2:1 dried *G. tikvahiae*/agar. Because DMS is volatile, it was mixed into artificial food after food cooled to a temperature below 40°C. Equal amounts of distilled H_2O were added to control foods. Evaporative losses of DMS during food preparation were determined through gas chromatography. These numbers were used to adjust the concentration of DMS added to the foods to achieve the desired concentration for the start of the assay. The diet was presented on strips of window screen, as above, for acrylic acid assays. For DMS assays, artificial food was created by spreading food evenly into a thin layer over clean sand (to weight food down), allowing it to cool, and cutting replicate pieces by using different shapes of known size for DMS and controls. This was performed to reduce the time from

incorporation of volatile DMS into food and the start of the assay. Sea urchins were not starved before feeding assays, because starvation reduced feeding rates (A. Erickson, personal observations). Feeding assays (N = 15–20) consisted of pairs of food, with and without a compound, that were offered simultaneously. Sea urchins were allowed to feed until half of one food type was consumed or until 2 hr passed. This time period was based on established diffusion rates of DMS, where 50% was lost from experimental diets after 1 hr and 75% after 4 hr (Van Alstyne and Houser, 2003). Consumption was quantified for acrylic acid as the number of window screen squares revealed when food was consumed and for DMS assays as the number of squares eaten when food pieces were held against window screen. Sea urchins that did not eat or consumed all food were excluded from statistical analysis. Paired t-tests were used to identify significant differences in the number of squares consumed.

Caulerpenyne was semipurified from *C. sertularioides* crude extract with flash column chromatography (Paul and Fenical, 1986). It was found in the 95:5 hexane/ethyl acetate fraction of a florisil column, and nuclear magnetic resonance indicated ~90% purity. The caulerpenyne fraction was dissolved in ethanol and incorporated into artificial food (2:1 dried *G. tikhaviae*/agar) at concentrations that approximate the natural concentration in *Caulerpa* spp. Foods were presented on strips of window screen, as above, along with a control containing ethanol without caulerpenyne. Sea urchins were allowed to feed until half of one food type was consumed or until 48 hr passed. Consumption was quantified as

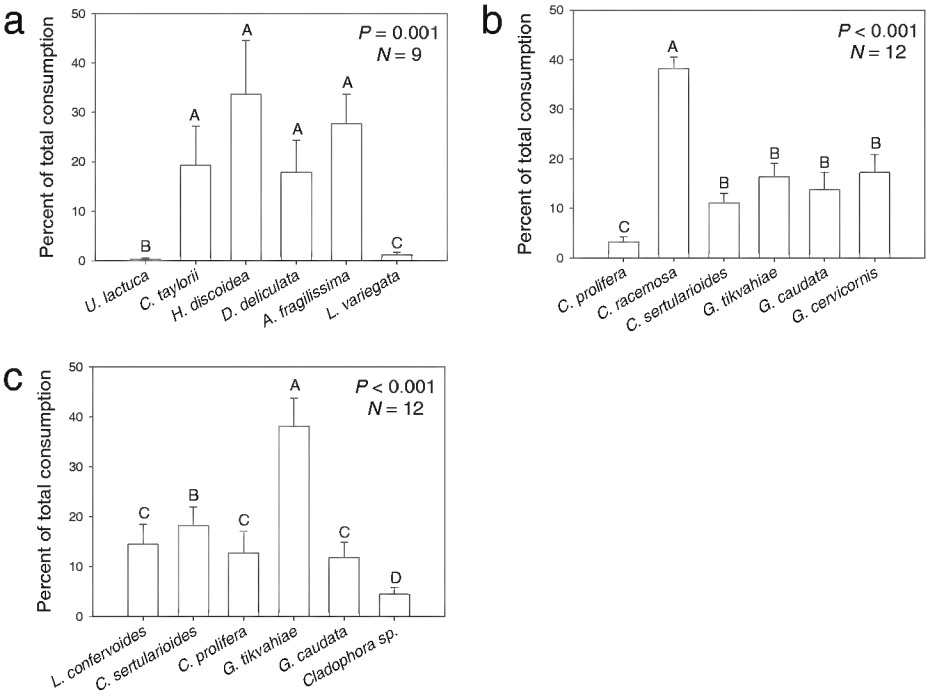

Fig. 1 Percentage of total consumption for a variety of algae offered to *E. lucunter* in live algal multiple-choice feeding assays. Treatments were compared by Friedman's repeated-measures ANOVA followed by the Student–Newman–Keuls *post hoc* test. Error bars represent standard error and letters above bars denote significant differences

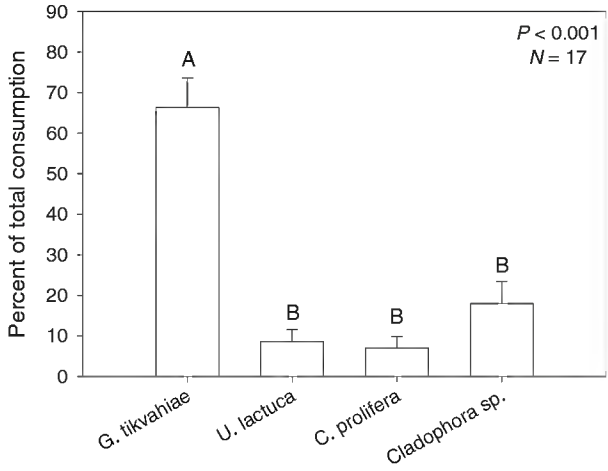

Fig. 2 Percentage of total consumption for select algae offered to *E. lucunter* in a live algal multiple-choice feeding assay. Algae include those deemed highly palatable (*G. tikvahiae*) and unpalatable (*U. lactuca*, *C. prolifera*, and *Cladophora* sp.) from assays in Fig. 1. Treatments were compared by Friedman's repeated-measures ANOVA followed by the Student–Newman–Keuls *post hoc* test. Error bars represent standard error and letters above bars denote significant differences

the number of window screen squares revealed when food was eaten. Sea urchins that did not eat or consumed all food were excluded from statistical analysis. Friedman's repeated-measures ANOVA and Student–Newman–Keuls multiple comparison test were used to identify significant differences in the number of squares consumed.

Results

Live algal multiple-choice feeding assays revealed that certain species of algae were of low preference to *E. lucunter*. *U. lactuca* and *L. variegata* were preferred less compared with other green, brown, and red algae (Friedman's $\chi_r^2 = 19.835$, $P = 0.001$; Fig. 1a). *C. prolifera* was the least preferred species of *Caulerpa* and was eaten less than *Gracilaria* spp. (Friedman's $\chi_r^2 = 32.446$, $P < 0.001$; Fig. 1b). *Cladophora* sp. was fed upon less than *Caulerpa* spp., *Gracilaria* spp., and the cyanobacterium *L. confervoides* (Friedman's $\chi_r^2 = 24.326$, $P < 0.001$; Fig. 1c). Three of the four least preferred algal species were green, suggesting that *E. lucunter* may be more sensitive to secondary metabolites of green algae than those found in other types. Hence, this report concentrates on the influence of

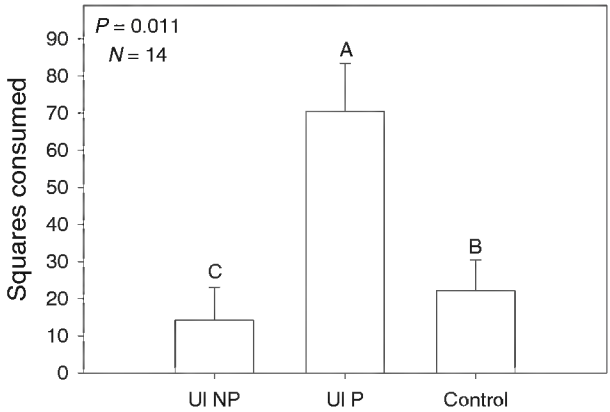

Fig. 3 The number of squares consumed by *E. lucunter* in an artificial feeding assay. Nonpolar (NP) and polar (P) extracts of *U. lactuca* (Ul) were incorporated into artificial food and offered, in conjunction with extract-free controls, simultaneously to sea urchins. Treatments were compared by the Friedman's repeated-measures ANOVA followed by the Student–Newman–Keuls *post hoc* test. Error bars represent standard error and letters above bars denote significant differences

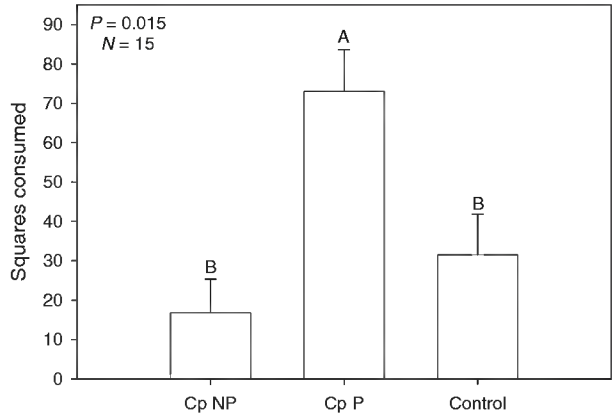

Fig. 4 The number of squares consumed by *E. lucunter* in an artificial feeding assay. Nonpolar (NP) and polar (P) extracts of *C. prolifera* (Cp) were incorporated into artificial food and offered, in conjunction with extract-free controls, simultaneously to sea urchins. Treatments were compared by the Friedman's repeated-measures ANOVA followed by the Student–Newman–Keuls *post hoc* test. Error bars represent standard error and letters above bars denote significant differences

green algal chemistry on feeding by *E. lucunter*. In each case, the low-preference algae composed <5% of the average total consumption. When the three green algae that were the least preferred in previous experiments were offered to sea urchins simultaneously, there was no significant difference in consumption among them. However, they were all consumed less than *G. tikvahiae* (Friedman's $\chi_r^2 = 21.663$, $P < 0.001$; Fig. 2). *U. lactuca* and *C. prolifera* each composed <10% of the average total consumption, whereas *Cladophora* sp. was just below 20% of the average total consumption.

Consumption of artificial food with polar *U. lactuca* extracts was about ×3 greater than for food with nonpolar extract or the control (Friedman's $\chi_r^2 = 9.000$, $P = 0.011$; Fig. 3). Food with nonpolar *U. lactuca* extract also was consumed significantly less than the control. Similarly, artificial food with polar *C. prolifera* extract was consumed two to three times more than food with nonpolar extract or the control (Friedman's $\chi_r^2 = 8.423$, $P = 0.015$; Fig. 4); however, there was no difference in consumption between food with nonpolar extract and the control.

Dimethylsulfoniopropionate concentrations were ~2.76 ± 0.15% of the dry mass (DM) or 0.44 ± 0.01% of the fresh mass (FM) in *U. lactuca*. Trace levels of DMSP (0.14 ± 0.00 DM, 0.02 ± 0.00% FM) were found in *Cladophora* sp., and DMSP was not detected in *C. prolifera*. DMSP concentrations (%DM) were greater in *U. lactuca* than in both *Cladophora* sp. and *C. prolifera* (Kruskal–Wallis ANOVA $H = 26.852$, $P \leq 0.001$).

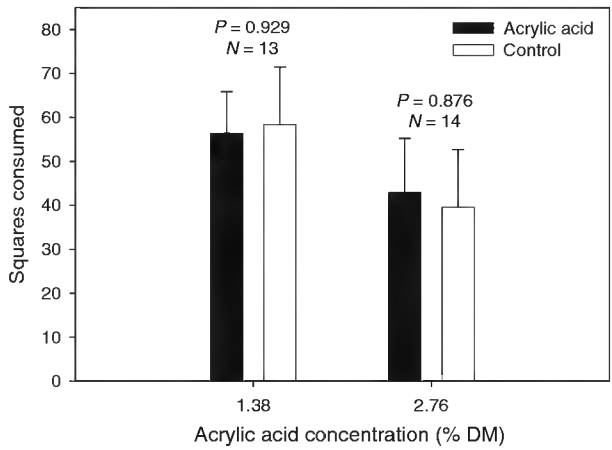

Fig. 5 The number of squares consumed by *E. lucunter* in paired artificial feeding assays. Acrylic acid was incorporated into artificial food at natural concentration (1.38% DM) and twice the natural concentration (2.76% DM). Then, it was offered, in conjunction with acrylic-acid-free controls, to sea urchins. Treatments were compared by paired *t*-tests. Error bars represent standard error and letters above bars denote significant differences

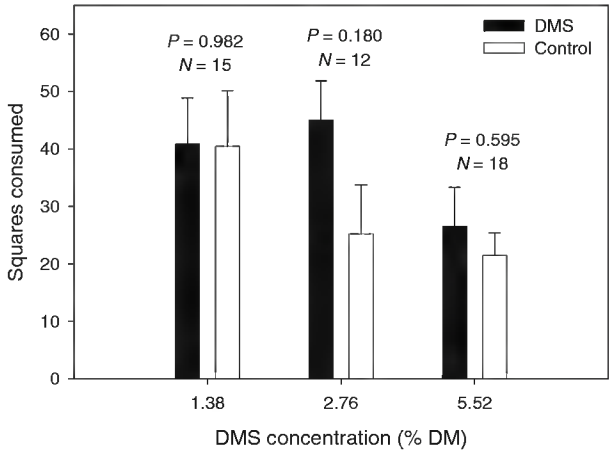

Fig. 6 The number of squares consumed by *E. lucunter* in paired artificial feeding assays. DMS was incorporated into artificial food at natural concentration (1.38% DM), and twice (2.76% DM) and four times (5.52% DM) the natural concentration. Then, it was offered, in conjunction with DMS-free controls, to sea urchins. Treatments were compared by paired *t*-tests. Error bars represent standard error and letters above bars denote significant differences

There was no difference in consumption between controls and artificial food containing either DMS or acrylic acid, the cleavage products of DMSP. Acrylic acid at natural (1.38% DM) and twice the natural concentrations failed to reduce consumption by *E. lucunter* (paired *t*-test $t = 0.092$, $P = 0.929$; paired *t*-test $t = -0.159$, $P = 0.876$; respectively; Fig. 5). DMS at natural (1.38% DM), twice, and four times natural concentrations also failed to reduce consumption (paired *t*-test $t = -0.023$, $P = 0.982$; paired *t*-test $t = -1.432$, $P = 0.180$; paired *t*-test $t = -0.542$, $P = 0.595$; respectively; Fig. 6). There was no significant difference in consumption of artificial food containing caulerpenyne at 2% DM with controls, whereas artificial food containing caulerpenyne at 4% DM was fed upon approximately one third as much as controls (Friedman's $\chi^2_r = 7.115$, $P = 0.029$; Fig. 7).

Discussion

This study examined feeding preferences of the sea urchin *E. lucunter* and whether preferences were chemically mediated. The green algae *U. lactuca*, *C. prolifera*, and

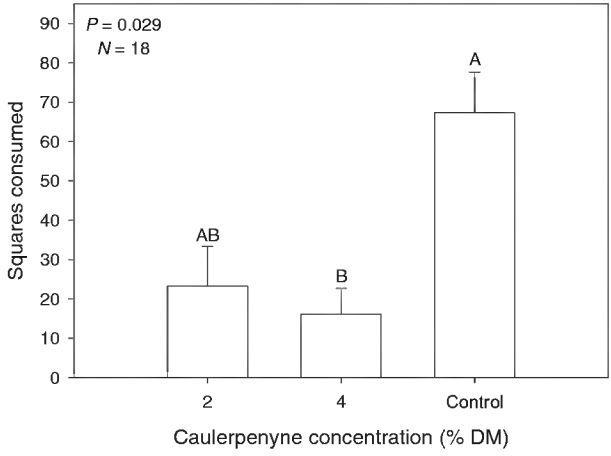

Fig. 7 The number of squares consumed by *E. lucunter* in an artificial feeding assay. Extract from the caulerpenyne-containing fraction was incorporated into artificial food at concentrations of 2% and 4% DM, which approximates what is naturally found in *Caulerpa* spp. Then, it was offered, in conjunction with caulerpenyne-free controls, to sea urchins. Treatments were compared by paired *t*-tests. Error bars represent standard error and letters above bars denote significant differences

Cladophora sp., and the brown alga *L. variegata* were unpalatable to *E. lucunter* relative to other algal species. Given that three of four species avoided were green algae, *E. lucunter* may be more sensitive to chlorophyte defenses than those of other types of algae. Feeding responses to these algae, and related species, in past studies have been herbivore specific. For instance, *Caulerpa* spp. were avoided by the surgeonfish *Zebrasoma flavescens* (*C. prolifera*, *C. racemosa*, *Caulerpa serrulata*, *C. sertularioides*; Wylie and Paul, 1988) and the sea urchins *Paracentrotus lividus* (*Caulerpa taxifolia*; Lemee et al., 1996), and *Lytechinus variegatus* (*C. prolifera*, Lowe, 1974; Vadas et al., 1982). In contrast, many reef fish, including parrotfish, surgeonfish, and rabbitfish, consumed *Caulerpa* spp. (*Caulerpa cuppressoides*, *C. prolifera*, *C. racemosa*, and *C. sertularioides*; Paul and Hay, 1986; Paul et al., 1990). The percentage of *C. prolifera* individuals eaten by reef fish has ranged between 8% and 70% in preference experiments (Paul and Hay, 1986). *E. lucunter* also preferred *C. racemosa* var. *laetevirins* (Fig. 1b). Alternatively, *Cladophora* spp. were avoided by parrotfish and some surgeonfish (Paul and Hay, 1986), yet were consumed by *Z. flavescens* (Wylie and Paul, 1988) and the amphipod *Amphithoe longimana* (Hay et al., 1987a). *Ulva* spp. were avoided by the sea urchins *Strongylocentrotus droebachiensis* (Van Alstyne and Houser, 2003), *P. lividus* (Lemee et al., 1996), and *L. variegatus* (Lowe, 1974), the spottail pinfish *Diplodus holbrooki* (Hay et al., 1987a), and *A. longimana* (Duffy and Hay, 1994). However, *Ulva* spp. were fed upon by reef fish (Littler et al., 1983), the sea hare *Aplysia californica* and the shore crab *Pachygrapsus crassipes* (Sousa, 1979), the gastropods *Littorina striata*, *Osilinus atratus* (Granado and Caballero, 2001), *Turbo undulatas* (Davis et al., 2005), and the amphipod *Gammarus mucronatus* (Duffy and Hay, 1994).

It is likely that algal chemistry influenced feeding by *E. lucunter*. In this study, nonpolar *U. lactuca* extract reduced, and polar extract stimulated, feeding by *E. lucunter*. The average DMSP concentration in *U. lactuca* was 0.44 ± 0.01% FM, which was lower than for deterrent *Ulva* spp. from the Pacific northwest (0.49 ± 0.22% FM to 1.58 ± 0.31% FM; Van Alstyne et al., 2001). Previous studies demonstrated that DMS strongly deterred feeding by *S. droebachiensis* at concentrations between 0.04% and 2% FM (Van Alstyne and Houser, 2003), and acrylic acid deterred feeding by *Strongylocentrotus purpuratus* and *S. droebachiensis* at concentrations between 0.1% and 2% FM (Van Alstyne et al., 2001). Although the DMSP concentrations in *U. lactuca* were high enough to generate sufficient DMS and acrylic acid to deter feeding by sea urchins in past studies (Van Alstyne et al., 2001; Van Alstyne and Houser, 2003), no differences in feeding by *E. lucunter* resulted between controls and artificial food containing either acrylic acid or DMS at natural and elevated concentrations. Similarly, no effect of high DMS concentrations was found on feeding by the fish *Thalassoma bifasciatum* (Pawlik et al., 2002), whereas *Ulva* spp. extracts deterred feeding by the amphipod *Gammarus palustris* (Borowsky and Borowsky, 1990), and acrylic acid stimulated feeding by the isopod *Idotea wosnesenskii* at 0.1% to 1% FM (Van Alstyne et al., 2001). DMS may have diffused out of artificial food during this experiment, preventing differences in feeding on experimental food versus controls. However, established DMS diffusion rates (Van Alstyne and Houser, 2003) suggest that DMS should have been present at the end of the assay. *E. lucunter* may be less sensitive to acrylic acid and DMS than temperate sea urchins, as the effects of metabolites can be herbivore specific (Hay et al., 1987b; Paul et al., 2001).

The secondary metabolite caulerpenyne can be converted through activation, degradation, and oxidation to a variety of additional compounds that may have greater toxicity than the precursor, including oxytoxins (1 and 2), epoxycaulerpenynes (6, 7, and 10, 11), 7,7-*C*-

didehydro-6-hydroxy-6,7-dihydrocaulerpenyne, taxifolials (A–D), and taxifolione (Guerriero et al., 1993; Guerriero and D'Ambrosio, 1999; Jung and Pohnert, 2001). During activation, the 1,4-diacetoxybuta-1,3-diene(bis-enol acetate) moiety is hydrolyzed by esterases in seconds and is replaced with 1,4 dialdehydes, which are suggested to be responsible for significant bioactivity. In this study, the chemistry of *C. prolifera* influenced feeding by *E. lucunter*. Artificial food with polar *C. prolifera* extract stimulated feeding by *E. lucunter*; however, there was no effect of food with nonpolar extract on feeding. Despite the inactivity of the nonpolar extract, caulerpenyne deterred feeding by *E. lucunter* at natural concentrations (>2% and 4% DM). Although sea urchins (McConnell et al., 1982) and a few reef fish (Targett et al., 1986; Paul et al., 1987) have been deterred by caulerpenyne, most reef fish were not deterred by *Caulerpa* spp. extracts or caulerpenyne (Paul et al., 1987; Wylie and Paul, 1988; Paul et al., 1990, 1992).

Variation in herbivory may relate to caulerpenyne concentration, which varies within and among species (Paul and Hay, 1986; Amade and Lemee, 1998; Dumay et al., 2002). For instance, in *C. prolifera*, it varies from 20% to 40% of the nonpolar extract (Paul and Hay, 1986), and *C. taxifolia* and *C. prolifera* have twice the caulerpenyne concentration of *C. racemosa* (Jung et al., 2002). The discrepancy between the deterrent live algae and nondeterrent, nonpolar *C. prolifera* extract may have resulted from activation of caulerpenyne in the live algae. Conversion to oxytoxins or other metabolites requires active algal esterases no longer present in artificial food. While partial activation may have occurred with extraction (Jung et al., 2002), the effect may not have been as great as that of natural activation through feeding. Finally, the discrepancy between the deterrent caulerpenyne and nondeterrent, nonpolar *C. prolifera* extract may have resulted if the extract contained less caulerpenyne than 4% DM, which was tested. Although feeding assays have not been conducted with isolated algal oxytoxins, due to their unstable and reactive nature, some studies have tested the effect of oxytoxins, mucus, and additional compounds derived from caulerpenyne-sequestering sacoglossans. Compounds and mucus inhibit microbial and protistan growth and feeding by freshwater and carnivorous marine fishes (Cimino et al., 1990; Guerriero et al., 1993; Gavagnin et al., 1994).

Chemical defenses against herbivory have not been explored as well in *Cladophora* spp. Some studies have found numerous sterols, free fatty acids and esters, terpenes, aldehydes, betaines, halogenated, and nitrogen-containing compounds in *Cladophora* spp. (Elenkov et al., 1995; Kamenarska et al., 2004). In addition, some studies have demonstrated antibacterial, anti-inflammatory, and cytotoxic activity of individual compounds or extracts (Kamenarska et al., 2004).

The unpalatable green algae used in this study, and related species, have been implicated in macroalgal blooms and invasions globally (Valiela et al., 1997; Jousson et al., 2000; Meinesz et al., 2001). The presence of such species has led to phase-shifts, where dominant structural species of an ecosystem, such as seagrass and corals, have been replaced by algae (Meinesz et al., 1993; Valiela et al., 1997; Thomas and Bell, unpublished data), leading to concomitant alterations in community and ecosystem parameters. Consequences of phase shifts include habitat loss, reduced biodiversity, and altered environmental parameters, species dominance, and trophic dynamics (Valiela et al., 1997; Thomas and Bell, unpublished data).

Algal chemical defenses have been proposed as a mechanism that promotes phase shifts and invasions due to deterrent, toxic, and allelopathic effects (Guerriero et al., 1993; Paul et al., 2001; Jung and Pohnert, 2001). Toxic effects have influenced a wide range of taxonomic organisms. *Ulva* spp. extracts and caulerpenyne are toxic to larval and adult stages of many marine invertebrates and vertebrates (Paul and Fenical, 1986; Lemee et al.,

1993; Pedrotti et al., 1996; Nelson et al., 2003). Ulvoid extracts have reduced the growth and germination of seagrass epiphytes and macroalgal zygotes (Nelson et al., 2003). Caulerpenyne is also toxic to bacterial and fungal pathogens (Paul and Fenical, 1986). Although studies often test for toxicity by bathing organisms in algal extracts, which may not accurately assess potential for deterring settlement or feeding, these studies demonstrate that bloom-forming and invasive species have the potential to impair or kill organisms if they release metabolites into the water column. Given the toxic and deterrent effects of these algae, it appears that their chemistry has the potential to promote bloom persistence and invasion success.

In summary, algal chemistry is partially responsible for feeding patterns observed in this study. Nonpolar extracts of *U. lactuca* and caulerpenyne reduced feeding by *E. lucunter*, whereas polar extracts stimulated feeding. Further analysis is needed to determine (1) which nonpolar *U. lactuca* compounds deter herbivory; (2) whether nonpolar compounds of *C. prolifera*, in addition to caulerpenyne, deter herbivory; and (3) other chemical defenses in *Cladophora* spp. Finally, it is important to understand how algal chemistry influences herbivory, thus indirectly influencing algal communities, given the current rise in algal blooms and invasions.

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