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**Iris Schnitzler · Georg Pohnert · Mark Hay
Wilhelm Boland**

Chemical defense of brown algae (*Dictyopteris* spp.) against the herbivorous amphipod *Ampithoe longimana*

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Abstract Terpenoids, polyphenols, and C_{11} metabolites are broadly distributed among brown seaweeds. Terpenoids and polyphenols have often been investigated as chemical defenses against herbivores, while there are only few investigations of the fatty-acid-derived C_{11} hydrocarbons and C_{11} sulfur compounds as potential defenses. We investigated effects of C_{11} sulfur metabolites from the cosmopolitan brown alga *Dictyopteris membranacea* on feeding and fitness of the herbivorous amphipod *Ampithoe longimana*. In choice tests between freshly collected thalli of *D. hoytii* (which lacks C_{11} sulfur compounds) and *D. membranacea* (which contains C_{11} sulfur compounds) amphipods consumed about 4 times more of the species lacking the C_{11} sulfur compounds. The same feeding preference was observed when these plants were finely ground and embedded in an agar matrix to destroy morphological differences. When a diet made from field-collected thalli of *D. membranacea* containing C_{11} sulfur compounds was tested against a diet made from a laboratory culture of *D. membranacea* that had lost the ability to produce C_{11} sulfur compounds, the same magnitude of preference was observed for the population lacking the sulfur compounds. In addition to the C_{11} sulfur compounds, a water-soluble C9-oxo acid that appears to be a by-product in the biosynthesis of the C_{11} metabolites also suppressed amphipod feeding to a comparable extent. Both classes of compound may contribute to the effective chemical protection of *D. membranacea*. When juvenile amphipods were reared for 28 days on artificial diets containing the above compounds, their survivorship (≤10%) closely resembled that of a starved treatment, but differed dramatically from a control treatment (60%) consisting of the same food, but without the metabolites. Most other classes of brown algal secondary metabolites are defensive against a broad spectrum of larger herbivores, but relatively ineffective against the amphipod studied here. In contrast, the fatty-acid-derived sulfur compounds and the C9-oxo acid strongly deter *Ampithoe*-like mesograzers but appear less effective against other herbivores, suggesting that these metabolites could be ecologically important in defending zygotes and germlings against these small consumers.

Keywords Lipoxins · Sulfur metabolites · Marine chemical ecology · Plant-herbivore interactions · (5*Z*,*E*)-9-oxonona-5,7-dienoic acid

I. Schnitzler · G. Pohnert · W. Boland (✉)
Max-Planck-Institut für Chemische Ökologie,
Carl-Zeiss-Promenade 10, 07745 Jena, Germany
e-mail: boland@ice.mpg.de
Fax: +49-3641-643670

M. Hay
School of Biology, Georgia Institute of Technology, Atlanta,
GA 30332–0230, USA

Introduction

Seaweeds can be exposed to extremely high rates of grazing (Carpenter 1986; Hay 1991; John et al. 1992), and many species have developed structural, morphological, or chemical defenses that significantly lessen their susceptibility to herbivores (Duffy and Hay 1990; Hay 1996). Chemical defenses have been particularly well studied, especially functionalized terpenoids, acetogenins (Hay and Fenical 1988, 1996; Paul 1992; Hay 1996), cyclic peptides containing uncommon amino acid residues (Faulkner 1994), and phlorotannins (Ragan and Glombitza 1986; Steinberg 1992; Steinberg and van Altena 1992). The ecological roles of structurally simple C_{11} hydrocarbons as defensive metabolites, e.g. **1** (Fig. 1), are less well known although **1** and a number of isomeric C_{11} hydrocarbons are known to attract male to female gametes for several brown seaweeds (Boland 1995; Maier 1995; Pohnert and Boland 1996). The genus *Dictyopteris* seems unusual in that C_{11} hydrocarbons occur not only in the gametes, but are also produced in rather large concentrations in the thalli. The few ecological studies of this class of compounds show that some can deter grazing by tropical fishes (Hay et al. 1988b), but that several other simple C_{11} hydrocarbons and C_{11} sulfur compounds derived from *Dictyopteris* spp. (Moore 1976, 1977) strongly deter feed-

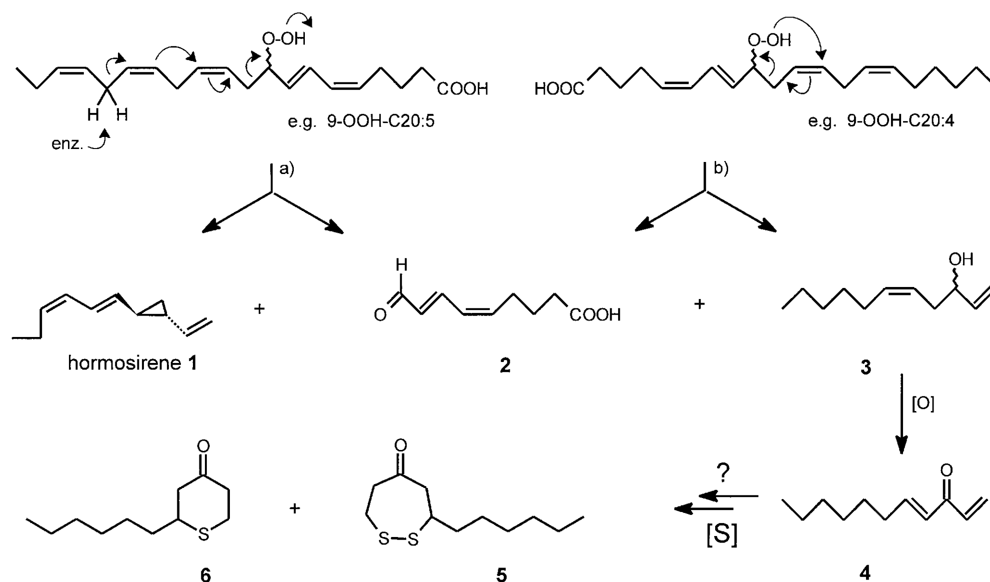

Fig. 1 Proposed pathways for the biosynthesis of C_{11} hydrocarbons and sulfur compounds. The degradative pathway is well studied for the C_{11} hydrocarbons of type **1** By analogy to the formation of the fungal metabolite oct-1-en-3-ol from 13-hydroperoxylinolenic acid (Wurzenberger and Grosch 1985), C_{11} sulfur compounds like **5** and **6** may result from an alternative oxidative cleavage of 9-hydroperoxy-arachidonic acid to yield undeca-1,5-dien-3-ol **3** and 9-oxo acid **2** as second fragment of the fatty acid. Further oxidation and isomerisation of **3** to the unsaturated ketone **4** and introduction of sulfur via as yet unknown mechanisms could account for the simultaneous occurrence of C_{11} hydrocarbons and C_{11} sulfur compounds in certain *Dictyopteris* species. Its water solubility and its highly reactive unsaturated aldehyde function (Michael acceptor) could play an important role in making the 9-oxo acid **2** a potent feeding deterrent

ing by an herbivorous amphipod, but rarely affect feeding by an herbivorous sea urchin (Hay et al. 1998; Schnitzler et al. 1998). The C_{11} hydrocarbons and the C_{11} sulfur compounds might be biosynthetically related and both may result from oxidative degradation of highly unsaturated eicosanoids via oxygenated intermediates as outlined in Fig. 1. The degradative pathway is well studied for the C_{11} hydrocarbons of type **1** and is believed to proceed with concomitant production of (5*Z*,7*E*)-9-oxo-nona-5,7-dienoic acid (Pohnert and Boland 1996; Hombeck and Boland 1998; Hombeck et al. 1999).

To evaluate the potential of C_{11} sulfur compounds and the 9-oxo acid **2** to function as defenses against herbivores, we tested their effects on feeding by the macrophyte-eating amphipod *Ampithoe longimana* (Cruz-Rivera and Hay 2000). *A. longimana* is common in marine and estuarine habitats along the east coast of North America (Bousfield 1973) and selectively consumes dictyotalean brown algae (Nelson 1979; Hay et al. 1987; Duffy and Hay 1991a, 1991b, 1994). Like several marine mesograzers that live on the seaweeds they consume, *A. longimana* selectively feeds and lives on chemically defended brown algae that are avoided by generalist consumers such as fishes and sea urchins (see reviews by Hay 1992; Hay and Fenical 1996). By physically associating with noxious seaweeds that are avoided by fishes, amphipods can decrease their own susceptibility to fish predation; these associational escapes appear to have selected for tolerance of seaweed chemical defenses among less mobile species of amphipods (Hay et al. 1987, 1990; Duffy and Hay 1991a, 1991b, 1994; Cronin and Hay 1996a, 1996b; Sotka et al. 1999). In contrast to this apparently general pattern, our previous investigations of some C_{11} sulfur compounds from *Dictyopteris* spp. suggested that this class of compounds deterred amphipod feeding more strongly but had a minimal effect on sea urchin feeding (Hay et al. 1998; Schnitzler et al. 1998).

In this study we extend these investigations by asking (1) how amphipods use different species of *Dictyopteris* and different populations of *D. membranacea* that differ in production of these metabolites, (2) how extracts from these plants affect feeding, (3) how defensive chemistry, as opposed to morphology or nutritional value, affects feeding, (4) how feeding is affected by the specific metabolites 9-oxo acid **2** and the two C_{11} sulfur compounds **5** and **6**, and (5) how compounds **2** and **5** affect fitness of amphipods maintained on diets containing these compounds.

Materials and methods

The organisms

Field collections of seaweeds were made from several reefs (depths of 1–27 m) along the North Carolina coast during August and September 1997. Assays using fresh *Dictyopteris* were run immediately after collection. Plants to be used for artificial foods were frozen immediately after harvesting and stored at –70°C until they were freeze-dried, ground to fine powder, and incorporated into agar-based experimental foods using the methods of Hay et al. (1994, 1998). Individuals of *D. membranacea* that do not produce C_{11} sulfur compounds were obtained from a laboratory culture collected at Villefranche-sur-Mer in 1987 by D.G. Müller. This material is maintained as an unialgal, clonal culture at the University of Konstanz, Germany. This same strain was used for

initial investigations of the C_{11} hydrocarbons in *D. membranacea* (Boland and Müller 1987).

The herbivorous amphipod *A. longimana* was collected from the rock jetty at Radio Island, North Carolina, United States (34°34′N, 76°40′W) where it co-occurred with *D. membranacea* and other dictyotalean seaweeds. Before and between bioassays, amphipods were maintained in 20 l flow-through aquaria at the University of North Carolina's Institute of Marine Sciences and fed a mixed diet of freshly collected seaweeds (*Sargassum filipendula, Padina gymnospora, Dictyota ciliolata, D. menstrualis, Ulva* sp., *Enteromorpha* sp. and *Hypnea musciformis*). Amphipods were not starved prior to being used in feeding assays.

Assays of feeding behavior

To evaluate amphipod feeding preference for fresh plants, amphipods were offered approximately equal masses (50–60 mg, blotted wet weight) of freshly collected *Dictyopteris membranacea* and *D. hoytii*. One preweighed piece of each species was placed in a bowl (10 cm in diameter) with 150 ml of seawater and two amphipods. Equivalent-sized portions of these same individual plants were also placed in paired bowls that did not receive amphipods. These bowls served as controls for changes in algal mass due to factors other than herbivory. Thus, 20 replicate bowls received amphipods and 20 paired bowls did not. Amphipods were allowed to feed for 24–48 h (depending on how rapidly they fed) after which the remaining algae were blotted dry and weighed. The amount of each species consumed was calculated using the equation: consumption$=[(H_0 \times C_f/C_0)-H_f]$, where H_0 and H_f are the masses of algal portions exposed to herbivory before and after the assay, and C_0 and C_f are the masses of the controls for autogenic changes before and after the assays, respectively. Differences in feeding in the above assay could have resulted from differences in algal morphology, nutritional content, or chemical defenses. By freeze-drying each alga, grinding it to a fine powder, and incorporating each powder into an agar matrix, we could test feeding preferences in the absence of structural or morphological differences. To accomplish this, we used methods described in detail by Hay et al. (1994, 1998). In this method, the powdered freeze-dried algae are evenly mixed with heated agar and water, and poured onto strips of plastic screen mesh. The agar-based food adheres to the screen mesh, which is then cut to desired sizes and offered to the experimental animals. Feeding rates are measured by counting the number of mesh squares from which the food is removed. These assays were run in 10 cm diameter bowls holding 150 ml of seawater and three amphipods (n=30). Equal amounts of food made from *D. hoytii* or *D. membranacea* were offered in each bowl. Feeding was monitored periodically, and food was removed from individual replicates when half or more of either food had been consumed, or at the end of the experimental period (48 h). If there was no feeding in a replicate, or if both samples were consumed completely between our monitoring intervals, these replicates were excluded from analyses. This happened rarely. In this and the similar assays described below, final sample sizes ranged from 24 to 30.

To evaluate the role that chemical defenses might play in the feeding patterns documented above, we performed several additional assays. First, we repeated the experiment using powdered algae from field collections of *D. membranacea* (these tissues known to contain C_{11} metabolites) versus *D. membranacea* that had been in culture for many years and was no longer producing C_{11} metabolites. Second, we directly tested the effects of lipid-soluble and water-soluble extracts from *D. membranacea* and *D. hoytii* on amphipod feeding by incorporating these extracts into artificial foods. Third, we tested how individual metabolites from *D. membranacea* affect amphipod feeding by incorporating ecologically realistic concentrations of these metabolites into the artificial foods. After these last assays, uneaten treatment food was extracted and analyzed with thin-layer chromatography to ensure that the test compound was still present in the treated food. We initially tested each metabolite at a concentration of 0.2% of food dry mass (about 2 times the yield found for a population of this alga that we investigated previously: Schnitzler et al. 1998). This doubling of the known yield was to account for both the probable loss of metabolite during the separation and purification process, and variation among individuals or different plant parts. If the metabolite was deterrent at this concentration, we reduced the concentration and retested at concentrations of 0.1 and 0.05% of plant dry mass.

Preparation of algal extracts and artificial diets containing extracts or metabolites

Algal extracts were obtained by grinding and extracting a volume of frozen alga that was equivalent to the volume of artificial food into which the extract was to be incorporated (thus ensuring that extracts were tested at approximately natural concentrations). For lipid extracts, the alga was extracted in 2:1 dichloromethane:methanol. Solvents were eliminated using rotary evaporation, the crude extract was partitioned using 1:1 water:dichloromethane, and the organic solvent was again removed by rotary evaporation. For water-soluble extracts, an appropriate volume of alga was ground and extracted in 7:3 methanol:water. The methanol was removed by rotary evaporation, followed by partitioning between water and dichloromethane. The aqueous extract was frozen at –70°C and concentrated to less than 5 ml in a Savant Speed Vac.

To test the effects of each extract on amphipod feeding, we incorporated the extract, at natural concentrations (by volume), into freeze-dried, finely powdered algae (equal masses of *Enteromorpha linza*, *Ulva fasciata*, and *Hypnea musciformis*) and incorporated this algal powder into an agar matrix. Treatment foods were prepared by dissolving the lipid-soluble extract in 10 ml of diethyl ether, adding it to the food, and eliminating the solvents by rotary evaporation. Control foods were treated similarly, but with diethyl ether alone. For water-soluble extracts, the proper amount of extract was diluted to 5 ml with distilled water and mixed into the powdered algae before this was added to the agar binding agent. Controls were treated with equivalent amounts of water alone. Tests with known metabolites were conducted in a similar manner.

These experimental foods were offered to the amphipods as agar-based food strips, as described by Hay et al. (1994, 1998). This technique has been successfully used to test diverse foods and feeding deterrents in a variety of laboratory assays (reviewed in Hay et al. 1998).

All above assays were evaluated using two-tailed, paired sample *t*-tests, if differences in feeding were normally distributed. If not, we used the Wilcoxon signed-ranks test.

Assays of amphipod fitness

The effects of specific metabolites on amphipod fitness were studied by maintaining juvenile *A. longimana* on artificial diets containing either: (1) 0.2% dry mass of compound **2**, (2) 0.1% of compound **5**, (3) a control diet treated only with the solvent that was used to add the compounds to the other diets, or (4) agar strips alone (i.e., no added algal material). Ovigerous amphipods (n=30) were obtained and held in individual petri dishes until offspring were released. Four young from each female were selected and randomly assigned to one of the four treatment groups. The young were raised in 60×15 mm petri dishes, with food and water changed daily. Food was always given in excess. Survival was monitored for 28 days, and differences were evaluated using the *G*-test.

Chemical methods

To isolate the dithiepanone **5**, algal material (500 g fresh weight, *D. membranacea*) was homogenized and stirred for 2 h in methanol:chloroform (2.0 l, 1:1, v:v) at ambient temperature. After filtration, the solvent was removed under reduced pressure (1.2 kPa). The residual brown oil was subjected to repeated column chroma-

tography on silica gel employing a *n*-pentane:diethyl ether gradient (95:5 to 60:40, v:v), followed by rechromatography using *n*-pentane:diethyl ether 90:10 (v:v) for elution. Evaporation of the solvent provided 42.0 mg 3-hexyl-[1,2]dithiepan-5-one **5** as a faint yellow oil. The 9-oxo acid **2** was obtained via synthesis as described by Pohnert and Boland (1996). The 2-hexyl-tetrahydro-thiopyran-4-one **6** was synthesized from the ketone **4** (see Fig. 1) by addition of one equivalent of thioacetic acid followed by hydrolysis of the resulting thioester and ring closure in *c.* 80% overall yield according the protocol of Gockel (1994) [selected spectroscopic data: ^{1}H NMR: δ (ppm) 0.9 (t,3H), 1.6 (m,2H), 1.3 (m,8H), 2.9 (m, 7H); ^{13}C NMR: δ (ppm) 14.01, 22, 50, 26.72, 28.08, 31.55, 35.55, 43.36, 44.73, 50.65, 208.80; MS (70 eV): m/z (%) 201 ($M^{+\cdot}$+1, 26), 183(3), 171(13), 143(10), 129(17), 115(100), 87(62), 81(12), 73(31), 61(24), 55(34), 41(56)].

Results

Effects on herbivore feeding

When simultaneously offered freshly collected *D. membranacea* (which produces C_{11} sulfur compounds) and *D. hoytii* (no C_{11} sulfur compounds), *A. longimana* consumed about 4 times more *D. hoytii* than *D. membranacea* ($P \leq 0.001$, Fig. 2A). When thalli of these species were freeze-dried, ground to fine powder, and incorporated into agar to eliminate morphological and structural differences between the two algal species, *A. longimana* still consumed about 4 times as much *D. hoytii* as *D. membranacea* ($P \leq 0.001$, Fig. 2B).

Lipid-soluble and water-soluble extracts from *D. hoytii* and *D. membranacea* were tested separately after incorporation into artificial foods at natural concentrations. As with the previous test, *A. longimana* consumed foods with extracts from *D. hoytii* much more rapidly than foods with extracts from *D. membranacea*. For both the lipid-soluble and the water-soluble extracts, consumption of foods with *D. hoytii* extracts was more than 4 times greater (Fig. 3) than consumption of foods with *D. membranacea* extracts ($P \leq 0.001$ for both contrasts: for lipid extracts, *D. membranacea* 7.5±2.4, *D. hoytii* 37.5±2.5 squares eaten; for water extracts, *D. membranacea* 10.2±3.0, *D. hoytii* 46.2±1.2 squares eaten). Therefore, *D. membranacea* contained both lipid-soluble and water-soluble metabolites that deterred amphipod feeding.

We also tested individual metabolites to see if these could account for the effects observed above. In particular, the water-soluble 9-oxo acid **2** and the lipophilic dithiepanone **5** strongly deterred feeding by the amphipod. When added to the artificial diet at only 50% of their natural concentrations, both the dithiepanone **5** and the 9-oxo acid **2** reduced feeding by about 60% ($P \leq 0.001$ in both cases, Fig. 4A, B). The potency of the 2-hexyl-tetrahydro-thiopyran-4-one **6** was lower than that of the other metabolites but it significantly deterred feeding by 50% at 0.2% of food dry mass (P=0.011, Fig. 4A).

The observation that *D. membranacea,* grown in laboratory cultures for several years no longer produced C_{11} sulfur metabolites offered the unique chance to assess feeding on a single species of alga when it was not producing the C_{11} sulfur compounds. When *D. membranacea* from the field and *D. membranacea* from laboratory cultures were incorporated into agar-based diets and fed to *A. longimana*, food prepared from cultured *D. membranacea* (lacking the metabolites) was consumed 4 times more than that containing *D. membranacea*

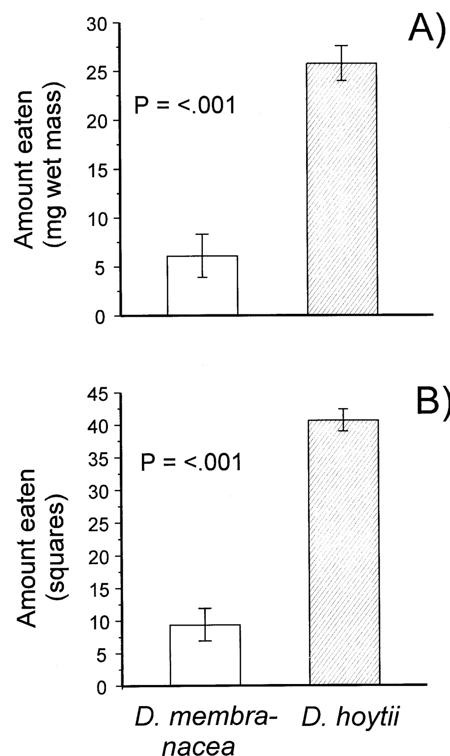

Fig. 2A, B Comparative assay of palatability of thalli of *Dictyopteris membranacea* and *D. hoytii* fed simultaneously to the amphipod *Ampithoe longimana*. **A** freshly harvested thalli, **B** freeze-dried and ground thalli incorporated in an agar based diet (see Materials and methods). *Bars* represent mean (±1 SE) mass of seaweed consumed per replicate during the feeding assay. *P-values* are from paired-sample *t*-tests

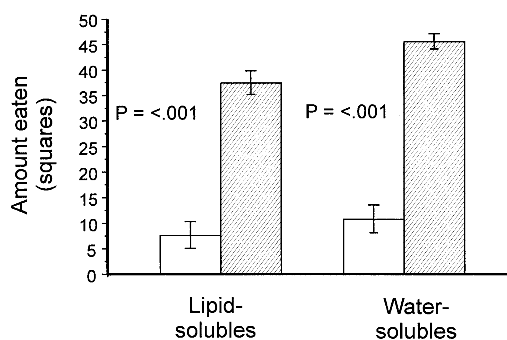

Fig. 3 Comparative assay of palatability of lipid-soluble and water soluble extracts from *D. hoytii* and *D. membranacea* fed simultaneously to the amphipod *A. longimana* (*open bars* extracts from *D. membranacea*, *shaded bars* extracts from *D. hoytii*; for preparation see Materials and methods). *Bars* represent mean (±1 SE) mass of seaweed consumed per replicate during the feeding assay. *P-values* are from paired-sample *t*-tests

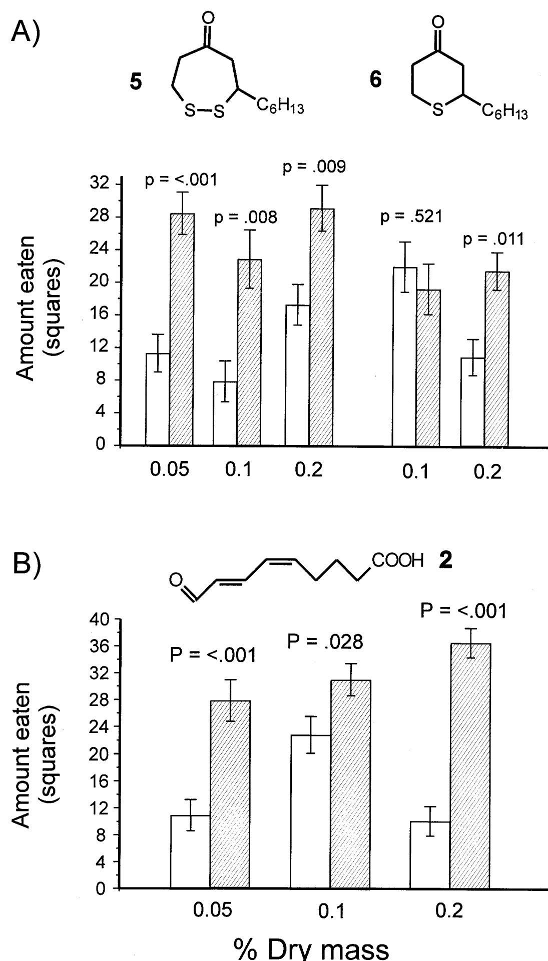

Fig. 4 **A** Effects of different concentrations of the C_{11} sulfur compounds **5** and **6** upon feeding by the amphipod *A. longimana*. *Open bars* represent algal food treated with metabolites, *shaded bars* represent the control (an equivalent food treated with solvent only). The natural concentration of 3-hexyl-[1,2]-dithiepan-5-one **5** in the populations we studied was 0.1% of plant dry mass. **B** Effects of different concentrations of the 9-oxo acid **2** upon feeding of *A. longimana*. *Histograms* show mean percentage of food eaten±1 SE. *P-values* are from paired-sample *t*-tests

from the field (containing the metabolites) ($P \leq 0.001$, Fig. 5).

Effects on herbivore fitness

After 28 days of culturing juvenile *A. longimana* on food dosed with dithiepanone **5**, with 9-oxo acid **2**, or with only solvents added, only one amphipod (3% survivorship) was still alive in the diet containing dithiepanone **5** and only three (10%) were alive on the diet containing 9-oxo acid **2** (Fig. 6). In contrast, 18 amphipods (60%) were alive on the control diet. The dramatic decrease in survivorship on the compound-containing diets occurred rapidly. By day 7, survivorship of the amphipods exposed to metabolites was less than 20%, while more than 80% of the control animals were alive. All starved amphipods died within 6 days.

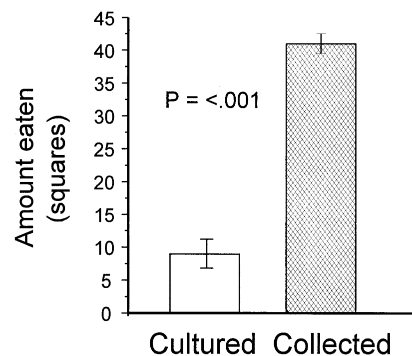

Fig. 5 Palatability of agar-based foods prepared from field-collected *D. membranacea* (*open bar*) and from a laboratory culture of *D. membranacea* (*shaded bar*) that lacked the biosynthesis of C_{11} sulfur compounds. *Bars* represent mean (±1 SE) mass of seaweed consumed per replicate during the feeding assay. *P-values* are from paired-sample *t*-tests

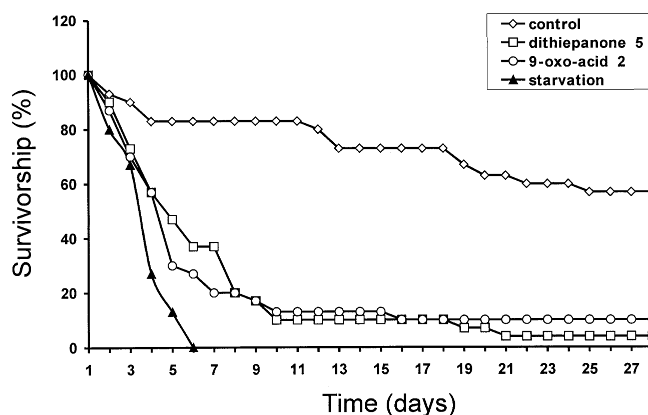

Fig. 6 Survivorship of *A. longimana* on four different diets. Each test series started with 30 newborn amphipods kept individually in separate containers

Discussion

Although feeding by mesograzers is sometimes considered to have little effect on populations of marine macrophytes (Bell 1991), there are several examples of plant-dwelling mesograzers having negative, and sometimes dramatic, effects on populations of marine macroalgae (Tegner and Dayton 1991; Duffy and Hay 1991b, 2000). The strength with which C_{11} sulfur compounds and the 9-oxo acid **2** affected amphipod feeding (Fig. 4A, B) and survival (Fig. 6) suggest that these metabolites could function as very effective defenses against mesograzers. In fact metabolites like **2** and **5** could be specifically targeted against the type of mesograzer studied here. Additional sulfur compounds related to the ones investigated here also deter amphipods, but are relatively ineffective against other invertebrate herbivores such as sea urchins (Hay et al. 1998; Schnitzler et al. 1998). The strong suppression of mesograzer feeding and survival by these metabolites is in contrast to several previous investigations where a variety of seaweed chemical defenses that

deterred feeding by large mobile herbivores like urchins and fishes were relatively ineffective against mesograzers, especially the amphipod *A. longimana* (Hay et al. 1987, 1988a, 1988b; Duffy and Hay 1994; Hay and Fenical 1996). For example, (1) diterpenes from brown algae in the genus *Dictyota* deter a variety of fishes and sea urchins, but have little, if any, effect on feeding by the amphipods *A. longimana*: (Hay et al. 1987, 1988a; Duffy and Hay 1991a, 1991b, 1994; Cronin and Hay 1996a, 1996b), and *Pseudamphithoides incurvaria* (Hay et al. 1990), and (2) a mixture of two C_{11} hydrocarbons of type **1** from the brown alga *Dictyopteris delicatula* deterred feeding by herbivorous reef fishes but failed to deter feeding by plant-dwelling amphipods (Hay et al. 1988a).

Compounds like **2**, **5** and **6** appear to play a central role in defending *D. membranacea* against amphipod feeding. In experiments with cultured versus field populations of *D. membranacea* (the cultured algae produce minor amounts of C_{11} hydrocarbons but no C_{11} sulfur compounds), the cultured material was clearly preferred by the amphipods. In this and all other feeding contrasts, the material lacking the C_{11} sulfur metabolites was consumed at 4–5 times the rate of the material containing these compounds. These differences in preference seem remarkably consistent given that these contrasts varied from fresh plants (Fig. 2A), to powdered plants in agar (Fig. 2B), to chemical extracts and pure compounds coated onto other foods (Fig. 4A, B), to powders of cultured versus field collected plants (Fig. 5). However we cannot exclude the possibility that cultured and field-grown plants may also differ in other aspects that could affect feeding preferences of *Ampithoe*.

Amphipods might differentiate among foods on the basis of negative (e.g., chemical defenses) or positive qualities (e.g., high food value, feeding stimulants). To examine the effect of chemical defense, we tested the effects of both lipophilic and hydrophilic chemical extracts on feeding. While most previous investigations indicate that lipophilic (or nonpolar) secondary metabolites are the major source of tropical and subtropical seaweed chemical defenses (Hay and Fenical 1988; Hay 1991; Paul 1992; Steinberg and van Altena 1992; Bolser and Hay 1996), we found comparable deterrence in both the lipophilic and the aqueous extracts. Whether or not this effect was due solely to the C_{11} sulfur compounds and related metabolites like the unsaturated 9-oxo acid **2** remains to be established.

The natural concentration of the C_{11} sulfur compounds isolated from Mediterranean *D. membranacea* was approximately 0.1% of plant dry mass. However, the few studies addressing intraspecific or intra-plant variation in seaweed secondary metabolites have found considerable variation in compound concentration (reviewed in Hay 1996), suggesting that concentrations in some populations or plant parts could vary considerably from this mean. Concentrations of other C_{11} sulfur compounds in *Dictyopteris* species are not well known. Given this limited information we hoped to bracket the variations in concentration that might occur naturally between different populations or plant parts using concentrations of 0.05–0.2% metabolites per dry mass of algae.

Our investigations showed that C_{11} sulfur compounds from *Dictyopteris*, as well as hypothesized byproducts of their biosynthesis like the 9-oxo acid **2**, may play an important role as chemical defenses against amphipods. Although the 9-oxo acid **2** has not, as yet, been found in extracts of *D. membranacea*, the production of this compound is, according to Fig. 1, linked to the biosynthesis of the C_{11} hydrocarbons. It appears likely that compounds from this pathway represent a class of seaweed secondary metabolites targeting *Ampithoe*-like mesograzers that are often resistant to other defensive metabolites (see also Hay et al. 1998; Schnitzler et al. 1998). Besides the well established terpenoids and polyphenols that are regarded as important classes of chemical defenses against herbivores (Hay and Fenical 1988, 1996; Paul 1992; Steinberg 1992; Hay 1996), the fatty acid derived compounds tested here may be an additional class of potent defensive metabolites.

Secondary metabolites that deter consumption are obviously advantageous for the prey. The reason why consumers recognize and avoid particular compounds are less well understood, and there have been remarkably few investigations on the effects of marine secondary metabolites on consumer physiology or fitness (Boettcher and Targett 1993; Lindquist and Hay 1995). For the majority of deterrent chemicals, nothing is known about their effects on survivorship, growth, or fecundity of consumers. Some studies have focused on the effects of different diets on amphipod fitness (Duffy and Hay 1991a, 1991b; Cruz-Rivera and Hay 2000), but few extended this to determine specific algal traits responsible for affecting fitness. Our study demonstrated a strong effect of *Dictyopteris* secondary metabolites on amphipod fitness. Survival rates on compound-treated diets were similar to the starvation treatment (Fig. 6) during the first few days of feeding. However, the much longer-term survival (28 days) of 6–10% of the individuals exposed to metabolites suggests that either a low frequency of individuals are tolerant of these metabolites, or, if individuals survive past some critical early developmental stage, that they can induce increased tolerance to the effects of the metabolites.

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