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Author(s): Mark E. Hay, Joseph R. Pawlik, J. Emmett Duffy, William Fenical
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Seaweed-herbivore-predator interactions: host-plant specialization reduces predation on small herbivores

Mark E. Hay[1], **Joseph R. Pawlik**[2, *], **J. Emmett Duffy**[1], **and William Fenical**[2]

[1] University of North Carolina at Chapel Hill, Institute of Marine Sciences, Morehead City, NC 28557, USA
[2] University of California at San Diego, Scripps Institution of Oceanography, La Jolla, CA 92093-0228, USA

Summary. Because feeding specialization among marine herbivores is rare, marine communities provide a simplified system for identifying factors selecting for specialization. On Australia's Great Barrier Reef, we investigated interactions among the chemically-defended seaweed *Chlorodesmis fastigiata*, herbivores specialized on this alga, and potential predators of these herbivores. *Chlorodesmis* is a low preference food for reef fishes but appears to be the only food of the crab *Caphyra rotundifrons* and the ascoglossan gastropods *Elysia* sp. and *Cyerce nigricans*. The crab is found only in patches of *Chlorodesmis*, feeds solely on the alga, and selectively shelters in it in laboratory choice experiments. Crab grazing on the red seaweed *Acanthophora spicifera* was stimulated when this alga was coated with increasing concentrations of the cytotoxic diterpenoid chlorodesmin, the major secondary metabolite of *Chlorodesmis*. Crabs did not sequester *Chlorodesmis* metabolites but avoided predators by sheltering in the unpalatable alga. All crabs tethered on the reef without access to *Chlorodesmis* patches were rapidly eaten; those with access to *Chlorodesmis* patches were much less susceptible to predation. The cryptic ascoglossan *Elysia* sp. was found exclusively in patches of *Chlorodesmis* and sequestered metabolites from the alga. Living *Elysia* were unpalatable to the common wrasse *Thalassoma lunare* in laboratory assays, but the crude organic extract of *Elysia* did not significantly deter feeding by *Thalassoma*. *Elysia* sequestered chlorodesmin, which deterred feeding by reef fishes in field assays but was ineffective against *Thalassoma* in laboratory assays at 5% food dry mass. Unlike *Elysia*, the aposematically colored ascoglossan *Cyerce nigricans* sequestered *Chlorodesmis* metabolites in relatively small amounts, but produced larger amounts of unrelated polypropionate compounds. *Cyerce* were never attacked by fishes and the crude organic extract of this slug strongly deterred feeding by wrasses in laboratory assays. The dorid nudibranch *Gymnodoris* sp. was found only in *Chlorodesmis* patches and appeared to be a specialized predator on *Elysia;* it would not prey on *Cyerce*. Data from this and other recent investigations demonstrate that some small marine herbivores feed selectively or exclusively on seaweeds that are chemically defended from fishes. This association reduces predation on the herbivores and suggests that escape from and deterrence of predation may be a dominant factor selecting for specialization among these herbivores.

Key words: Ascoglossans – *Caphyra rotundifrons* – Chemical defense – *Chlorodesmis* – Plant-herbivore-predator interactions

In terrestrial communities, insects comprise the large majority of all herbivores and specialization on a few closely related host plants is the rule rather than the exception (Strong et al. 1984; Bernays and Graham 1988 and references therein). There is disagreement about what factors select for host-plant specialization but attempts to explain the high proportion of specialized insects have focused on resource partitioning, coevolution between insects and chemically defended plants, the limited ability of invertebrates to recognize more than a few potential host plants, and more recently the possibility that specialization may allow insects to escape their natural enemies (see Price et al. 1980, 1986; Strong et al. 1984; Bernays and Graham 1988 and other references in the same volume).

Determining important factors selecting for host-plant specialization may be easier in marine than in terrestrial communities because there are many fewer specialists in marine communities (Lubchenco and Gaines 1981; Hay and Fenical 1988). Additionally, the rarity of specialist herbivores may mean that specialization occurs only under a limited number of intense selective regimes. If this is the case, then common factors selecting for specialization may be more obvious in marine than in terrestrial communities.

In this investigation we studied herbivores that specialize on the filamentous green alga *Chlorodesmis fastigiata*, which is one of the only abundant and obvious macrophytes on coral reefs along the northern portion of Australia's Great Barrier Reef. At our study sites near the Australian Museum's Lizard Island Research Station, *Chlorodesmis* was the only abundant macrophyte on shallow reefs where grazing fishes were common. The alga produces several diterpenoid metabolites (see Paul and Fenical 1987 for structures) that are unique among all diterpenoids in that they possess ketone functionalities and enol acetate groups on both ends of the linear skeleton. Both the crude organic extract from *Chlorodesmis fastigiata* and the pure compound chlorodesmin significantly deter feeding by herbivorous reef fishes (Paul 1987; Wylie and Paul 1988). Although the diterpenes contained in *Chlorodesmis* are cytotoxic, ich-

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Offprint requests to: M.E. Hay

[*] *Present address:* Friday Harbor Marine Laboratories, University of Washington, Friday Harbor, WA 98250, USA

thyotoxic, antifungal, and antibacterial (Paul and Fenical 1987), the plant and its compounds are not avoided by all herbivores. We found one herbivorous crab (*Caphyra rotundifrons*), two ascoglossan gastropods (*Cyerce nigricans* and *Elysia* sp.), and one dorid nudibranch (*Gymnodoris* sp.) that appeared to occur only in patches of *Chlorodesmis*.

Because other studies on small marine herbivores hypothesized that specialization and strong feeding preferences for chemically noxious plants could function as adaptations to avoid or deter predation (Hay et al. 1987, 1988a, b, 1989b; Hay and Fenical 1988), we focused our attention on the role of algal chemical defenses in mediating plant-herbivore-predator interactions.

This study addressed the following questions: (1) How susceptible is *Chlorodesmis fastigiata* to grazing by reef fishes versus the invertebrates mentioned above? (2) How specialized are the invertebrates? (3) Are feeding and microhabitat selection by the crab cued by the alga's chemical defenses? (4) Do the specialist herbivores sequester algal defenses for use against their own predators? (5) For herbivores that do not sequester algal compounds, does physical association with *Chlorodesmis* confer protection from predators?

Methods

Study site and organisms. Our work was conducted from the Australian Museum's Lizard Island Research Station located toward the northern end of the Great Barrier Reef (14°38′S latitude). During November and December of 1986 and February and March of 1988, we estimate that we closely investigated more than one thousand *Chlorodesmis* patches for crabs, ascoglossans, and nudibranchs. Because many of these invertebrates are extremely cryptic in *Chlorodesmis*, we used our bare hands to explore each patch and pull out the invertebrates we could find by feel. The cryptic green crab *Caphyra rotundifrons* (see Mead and Beckett [1984] page 209 for a photograph) was very common and we had little difficulty collecting several hundred individuals for our experiments. The black and orange, aposematically colored ascoglossan *Cyerce nigricans* (see Mead and Beckett [1984] page 196 for a photograph) was much less common but we found enough individuals (~50) to perform numerous laboratory and chemical assays.

Individuals of *Elysia* sp. were very cryptic, found only among the basal portions of *Chlorodesmis*, and easy to miss in our search of the plant. We found only about 20 *Elysia* and several of them were subsequently eaten by the dorid nudibranch, *Gymnodoris* sp., that was also associated with *Chlorodesmis*. We found about 15 *Gymnodoris* sp.

Although we extensively searched many other types of microhabitats on the reefs around Lizard Island, we found the invertebrates discussed above only in, or within 1–2 cm of (for *Cyerce*), patches of *Chlorodesmis*. Publications on the natural history of Great Barrier Reef organisms also suggest that these invertebrates are found only in close association with *Chlorodesmis* (Mead and Beckett 1984).

Fish feeding experiments. Susceptibility of 8–9 different seaweeds to herbivorous fishes was assessed on two separate days by weaving one 5 cm long thallus of each species into a 3-strand rope that was fastened to the reef slope at a depth of 3 m ($N=28$–29 ropes during each assay). Seaweed thalli within a rope were separated from one another by about 5 cm and ropes were separated from one another by 3–4 m. At the end of each 19–22 h assay, each species in each rope was recorded as absent or still present. See Hay (1984) for details on this method.

The effects of chlorodesmin on feeding by reef fishes was evaluated following procedures described by Harvell et al. (1988). Carrageenin (2.5 g), freeze-dried brine shrimp (0.6 g), and chlorodesmin (0.163 g or 0.080 g for the 5% and 2.5% treatments, respectively) were mixed and poured into molds to produce 5 × 1 × 0.5 cm carageenin strips with a string embedded in the center of each. One treatment strip and one control strip (i.e. without chlorodesmin) were tied onto opposite ends of a 50 cm length of 3-strand rope and 18 of these ropes were placed at 2–4 m depth along the reef slope. Ropes were placed at intervals of 3–4 m and monitored every 5–10 min during the 1.5–2 h assay period. Fishes seen feeding on the carrageenin strips were noted and ropes were removed whenever more than 50% of either strip had been eaten. Amount eaten was measured by the change in area of the broad side of the strip.

Crab feeding experiments. To determine the feeding preference of the crab *Caphyra rotundifrons*, 15 crabs were placed in separate 1 L containers along with 5 common seaweeds (*Chlorodesmin fastigiata, Chondrococcus hornemannii, Halymenia durvillaei, Acanthophora spicifera,* and *Halophila ovalis*). Each container held one crab and an average of 26–28 mg (blotted wet mass) of each plant species. Five containers with seaweeds but without crabs were used as controls to estimate the change in algal mass that was unrelated to herbivory. Containers were held in the lab and water was changed daily. After 57 h, algae were blotted dry, weighed, and their final mass compared to their initial mass. Significant effects of crab grazing were assessed using a t-test or Mann-Whitney U-test to make within-species comparisons between plants exposed to or protected from crabs.

An assessment of field diets was made by collecting 20 *Caphyra* from patches of *Chlorodesmis* on the reef flat at North Point, Lizard Island and examining their gut contents microscopically. Because we found only two categories of food in the gut, we simply estimated visually the volume of the total gut content that each comprised.

The effect of chlorodesmin, the major metabolite of *Chlorodesmis*, on feeding by the crab *Caphyra* was tested by confining individual crabs with 100 mg (±10 mg) of the red alga *Acanthophora spicifera* that had been treated with chlorodesmin so that it comprised 0%, 0.25%, 0.5%, or 1.0% of *Acanthophora's* dry mass ($N=16$ for each concentration). Major metabolites in green algae like *Chlorodesmis* may vary in concentration but often occur as 0.5% to 1.5% of algal dry mass (Paul and Fenical 1987). Chlorodesmin was applied by dissolving it in diethyl ether at known concentrations and applying 60 µl of the appropriate mixture to an *Acanthophora* branch that had been blotted dry. After the ether evaporates, hydrophobic compounds like chlorodesmin adhere to the alga and the plant can be placed back into seawater without appreciable loss of compounds (for methods, see Hay and Fenical 1988 and references therein). For each of our 4 concentrations of chlorodesmin, we also set up 5 control containers holding chlorodesmin-treated *Acanthophora* but no crabs. These control containers were used to estimate changes in algal mass that were unrelated to crab grazing (e.g. respiration, cell disruption caused by blotting or by coating the alga with ether, etc.). After 50 h, crabs were removed from all containers and each alga was blotted and reweighed. If a crab died during the experiment, the alga in that container was excluded from the analyses.

Crab habitat preference. We performed 3 separate experiments to determine *Caphyra's* preference for various algal habitats. In the first, approximately equal volumes of 5 seaweeds found on the reef flat at North Point (*Chlorodesmin fastigiata, Chondrococcus hornemannii, Turbinaria* sp., *Halymenia durvillaei*, and *Acanthophora spicifera*) were randomly positioned around the periphery of each of ten 14 cm diameter containers and one *Caphyra* was dropped into the center of each container (the crab was thus less than 7 cm from each of the 5 seaweeds). We recorded the seaweed to which each crab immediately moved. The second experiment was similar but used 27 × 50 cm rectangular containers (the crab was now approximately 25 cm from each seaweed when released) and 4 seaweeds from the reef flat at North Point (*Chlorodesmis fastigiata, Halimeda* sp., *Amphiroa* sp., and *Gracilaria* sp.). One seaweed species was placed in each corner (position was randomized for each container), a crab was released in the center of each container ($N = 20$), and each crab's position was monitored 15 min, 2 h, 4 h, and 20 h later.

The third experiment compared the crab's attraction to *Chlorodesmis* with its attraction to the filamentous green alga *Chaetomorpha* sp., which is visually similar to *Chlorodesmis* but much more susceptible to fish grazing. In this assay, individual crabs were dropped into the center of 27 × 50 cm tanks ($N = 20$) that had similar volumes of the two seaweeds at opposite ends of the tank. We recorded the species to which the crab immediately moved. To assess the importance of visual versus chemical or tactile cues in attracting *Caphyra* to *Chlorodesmis*, this test was repeated with the following modifications. Each crab was dropped into a large clear glass beaker of seawater that was immersed in the tank but that extended above the water's surface and thus had no exchange of water with the aquarium holding the algae (i.e. no chemical or tactile cues). The algae in the tank were moved closer to the beaker so that they could be clearly seen through the sides. We recorded the alga toward which the crab immediately moved.

Crab susceptibility to predation. Two separate experiments were conducted to determine if *Caphyra* experienced reduced predation because it lived in the unpalatable alga *Chlorodesmis fastigiata*. In the first test, 24 *Caphyra* were tethered to small nails using 6–9 cm lengths of 0.18 mm diameter monofilament fishing line. The line was glued to the nail and to the back of the crab using Duro Quick Gel No-Run Super Glue (Locktite). Preliminary lab tests indicated that the tether would hold crabs for more than 4 days. Twelve pairs of tethered crabs were placed 2–3 m deep on the reef in front of Coconut Beach, Lizard Island by driving the nails into the reef structure. One crab in each pair was tethered so that it had access to a small patch of *Chlorodesmis;* the other crab was tethered within 18 cm of the first, but without access to the *Chlorodesmis* patch. Both crabs were monitored continuously for the first few minutes after tethering and all pairs were checked 15 min, 1 h, and 24 h after tethering. Fishes consuming crabs were noted.

A second tethering assay tested whether predation differed between crabs hidden in the palatable (to fishes) green alga *Chaetomorpha* versus the unpalatable green alga *Chlorodesmis*. Patches of each alga were made by affixing 7–8 g (blotted wet mass) of the seaweed to a 10 × 10 cm square of plastic mesh using small plastic cable ties, tethering one crab onto each mesh square, and nailing the squares onto patch reefs (1–2 m deep) in front of the research station. *Chlorodesmis* and *Chaetomorpha* patches were paired within 0.5 m of each other and replicate pairs ($N = 10$) were separated by 4–5 m. Patches were examined after 19 h to determine the number of remaining crabs in each treatment and the status of each alga.

Ascoglossan and nudibranch diets. Since ascoglossans feed by sucking the cytoplasm from algae rather than consuming the thallus itself, their grazing is difficult to measure. We therefore used the frequency with which *Cyerce* was attracted to different plants and chemical analyses of algal metabolites extracted from the ascoglossan to infer its diet. Ascoglossans as a group are known to consume primarily green siphonous seaweeds and seagrasses (Jensen 1980); we therefore placed 20 *Cycerce* in separate 38 L containers, each of which contained approximately equal volumes of *Chlorodesmis fastigiata, Halimeda* sp. no 1, *Halimeda* sp. no 2, and *Halophila ovalis*. At 0.5, 1, 2, 4, and 18 h after initiation of the experiment, we recorded which seaweed *Cyerce* occupied in each tank. All tanks were supplied with flowing seawater.

Thin layer chromatography (TLC) was used to compare the secondary metabolites in the crab *Caphyra rotundifrons*, the ascoglossans *Cyerce nigricans* and *Elysia* sp., and the dorid nudibranch *Gymnodoris* sp. with the secondary metabolites produced by the green alga *Chlorodesmis fastigiata*. These TLC analyses also included pure chlorodesmin that had been isolated by HPLC and structurally confirmed by chemical and spectroscopic methods.

Gastropod chemical defenses. To evaluate the susceptibility of the different gastropods to fish predation, we placed 2–3 individuals of the common wrasse *Thalassoma lunare* in each of 6 separate aquaria and offered each group of fish one *Cyerce*, one *Elysia*, and one *Gymnodoris*. To ensure that the fish were feeding normally, we also offered each group of fish palatable gastropods in the genus *Nerita* from which the shell had been removed.

As an initial assessment of the importance of chemical defenses in protecting *Cyerce* and *Elysia* from fish predation, we extracted each ascoglossan species in acetone, removed the solvent under vacuum, weighed the extract and the remaining dry animal material, dissolved the extract in diethyl ether, and injected the extract into freeze-dried krill that were presented to wrasses held in aquaria. Concentration of the extract per dry mass of the krill was 10%; this is equal to, or below, natural concentrations found in the ascoglossans we studied. Feeding on the treatment pellets was compared to feeding on control pellets that had been injected only with the solvent. Five krill of each type were offered to each of five tanks of *Thalassoma lunare* and 3 krill of each type were offered to mixed species groups of small wrasses that were held in 5 separate tanks. These wrasses included: *Thalassoma jansenii, T. lunare, T. ambycephalus, Halichoeres trimaculatus, H. hoeveni*, and *Coris variegata*. Tanks with fish that refused to feed on both the treatment and control krill were excluded from the analyses. This lowered sample sizes to between 3 and 5 for the different assays.

To determine if sequestered compounds could protect ascoglossans from wrasses, we repeated the above experiment using chlorodesmin at a concentration of 5% of krill dry mass. This approximates concentrations of algal metabolites known to occur in other *Elysia* (Paul and Van Alstyne 1988).

Results

Fish and crab feeding. Chlorodesmis fastigiata was consistently resistant to fish grazing (Fig. 1) but the crab *Caphyra rotundifrons* grazed almost exclusively on *Chlorodesmis* (Fig. 2). Of the 5 seaweeds assayed, only *Chlorodesmis* and *Chondrococcus hornemannii* differed significantly ($P = 0.006$, $p = 0.041$ respectively, Mann-Whitney U-test) in mass between containers with and without crabs. Additionally, the gut contents of 20 crabs taken directly from the field consisted entirely of uniform-length portions of *Chlorodesmis* filaments ($x \pm 1\,\mathrm{SE} = 69 \pm 5\%$) mixed with amorphous green material ($31 \pm 5\%$) that appeared to be the cytoplasm from these filament portions. Thin layer chromatography (TLC) of the lipophilic crude extract from *Caphyra* indicated that crabs were not sequestering algal metabolites.

Feeding by *Caphyra rotundifrons* was stimulated by increasing concentrations of the cytotoxic compound chlorodesmin (Fig. 3). Figure 3 A shows the change in mass of *Acanthophora* thalli coated with varying concentrations of chlorodesmin and exposed to, or protected from, crab grazing. Because of difficulties in rigorously analyzing these types of data (Peterson and Renaud 1989), the data were analyzed by two different methods. First, changes in the mass of *Acanthophora* thalli exposed to crabs versus those protected from crabs (=controls) were analyzed for each concentration of chlorodesmin. A *t*-test indicated that crabs caused a significant ($p = 0.0245$) loss of *Acanthophora* mass only in the 1.0% chlorodesmin treatment. However, these analyses have limited power because of the low number of replicates without crabs ($N = 5$ at each concentration) and the relatively low rates of feeding by *Caphyra*.

Because the mass in the controls (i.e. without crabs) showed no tendency to change as a function of chlorodesmin concentration ($p = 0.687$, ANOVA) we pooled all of the controls (i.e. $N = 20$) to provide a second, and more powerful, way to analyze the data. Comparisons were made among this control mean and the various treatments exposed to crabs. Results of ANOVA ($P = 0.0043$) and Student-Newman-Keuls tests are shown by the letters below the histograms in Fig. 3A. Mass changes for plants coated with 0% or 0.25% chlorodesmin and exposed to crabs did not differ from ungrazed plants; *Acanthophora* treated with 0.5% or 1.0% chlorodesmin experienced significantly ($P \leq 0.05$) greater losses of mass than ungrazed plants or those treated with 0% chlorodesmin. The algal mass actually eaten in each of the treatments (i.e. after correction for the mass change unrelated to grazing – the far left bar in Fig. 3A) is shown in Fig. 3B. Crab feeding increased with increasing concentration of chlorodesmin.

Crab habitat preference and susceptibility to predation. When 10 crabs were released in small containers (14 cm diameter)

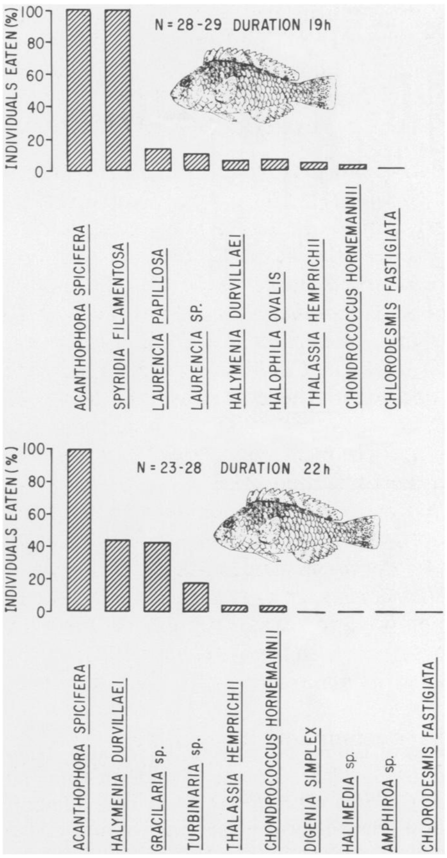

Fig. 1. The susceptibility of various seaweeds to removal by fishes when transplanted into a reef-slope habitat. Susceptibility was measured as the proportion of transplants that were consumed completely. The two graphs represent assays conducted on separate days

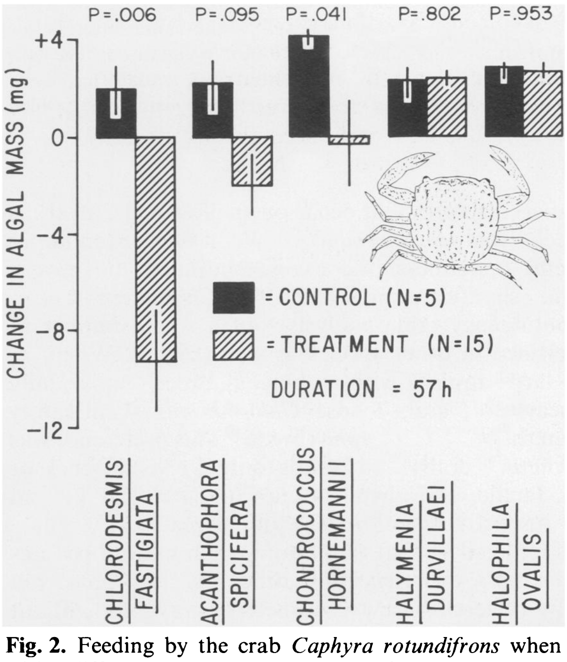

Fig. 2. Feeding by the crab *Caphyra rotundifrons* when confined with 5 different seaweeds. Mean mass of each species was 26–28 mg at the initiation of the experiment. Vertical bars through histograms show ±1 SE. *P*-values are from *t*-tests or Mann-Whitney U-tests in cases of heterogeneous variances

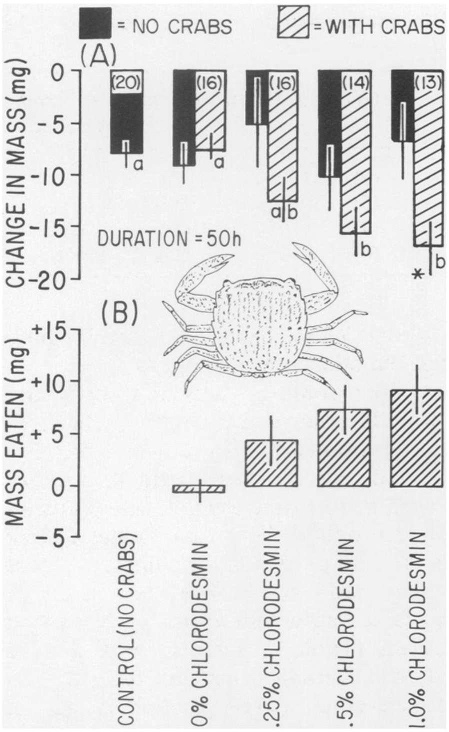

Fig. 3. **A** Changes in mass of 100 mg portions of the red alga *Acanthophora* coated with differing concentrations of chlorodesmin and subjected to grazing by *Caphyra rotundifrons*. Dark bars adjacent to the hatched bars show changes in controls (i.e. without crabs) for each concentration of chlorodesmin ($N=5$). * indicates a treatment in which mass change in the presence of crabs differed significantly ($P \leq 0.05$, *t*-test) from that in controls (without crabs, $N=5$) at that concentration of chlorodesmin. Because mass change of controls at each concentration of chlorodesmin showed no tendency to differ ($P=0.687$, ANOVA), all controls were pooled (far left histogram, $N=20$) to provide a more accurate estimate of change that was unrelated to herbivory. ANOVA and Student Newman-Keuls tests were used to compare mass changes among the treatments and this pooled control; bars with the same letter below them do not differ significantly ($P \leq 0.05$). **B** Illustrates the estimated mean mass of each treatment that was actually eaten by the crabs (i.e. these data have been corrected using the pooled control data)

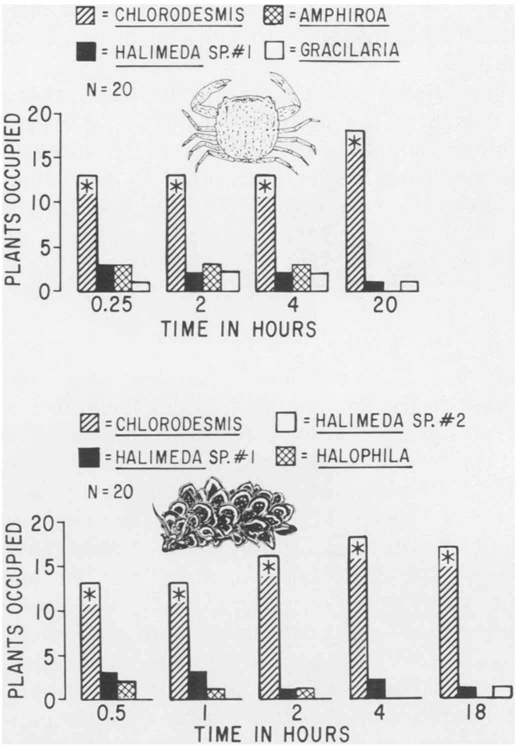

Fig. 4. Plant occupancy patterns of the crab *Caphyra rotundifrons* and the ascoglossan *Cyerce nigricans* when placed in separate aquaria with a choice of 4 seaweeds. * indicates a species that was occupied significantly more often than all other species ($P < 0.05$, contingency table analyses by the simultaneous test procedure)

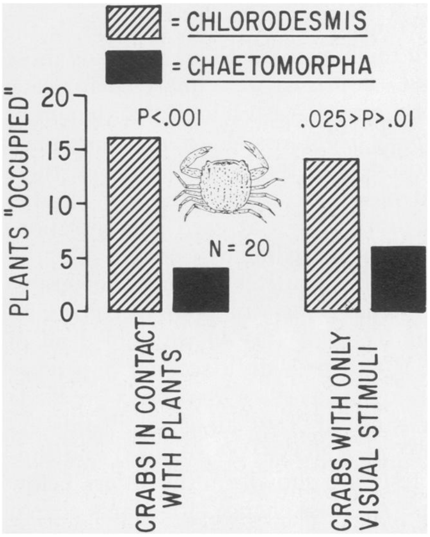

Fig. 5. The number of *Caphyra* immediately moving to *Chlorodesmis* or to the visually similar alga *Chaetomorpha* when the crabs were released in aquaria with the plants (*left*) or in beakers submerged in the aquaria (*right*). In the latter experiment, crabs could see the plants but could not contact them or sense them chemically. *P*-values are from contingency table analyses

with 5 seaweed species that occur on shallow reefs, all crabs immediately went to *Chlorodesmis*. When released in larger (27 × 50 cm) containers, the crabs continued to show a strong and significant preference for *Chlorodesmis* ($P < 0.001$, contingency table analysis) but a small number of crabs sheltered in other species as well (Fig. 4). When released in large aquaria with a choice between two visually similar seaweeds, crabs chose *Chlorodesmis* significantly more often than *Chaetomorpha* (Fig. 5). This preference was similar whether crabs had access only to visual cues or to visual, tactile, and chemical cues ($0.025 > P > 0.01$ and $P < 0.001$ respectively, contingency table analyses).

Crabs tethered so that they could reach natural patches of *Chlorodesmis* experienced significantly less predation than crabs tethered a few centimeters away but without access to *Chlorodesmis* (Fig. 6, $P < 0.001$ for all time periods, Fisher's Exact test). All crabs without access to *Chlorodesmis* were consumed in less than 15 min; 67% of the crabs in *Chlorodesmis* patches were still alive after 24 h. We observed all but one of the predation events on crabs without access to *Chlorodesmis*; these crabs were consumed by groups of wrasses in the genus *Thalassoma* joined by a few pomacentrids.

When we constructed algal patches from *Chlorodesmis* or from the more palatable (to fishes) green alga *Chaeto-*

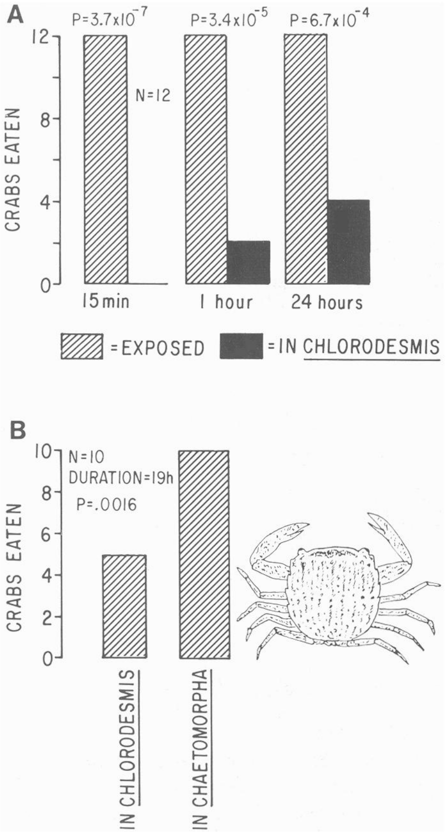

Fig. 6 A, B. The fate of *Caphyra* tethered (**A**) in natural patches of *Chlorodesmis* versus those tethered in the open, or (**B**) tethered in algal patches made from *Chlorodesmis* versus the morphologically similar but palatable (to fishes) alga *Chaetomorpha*. *P*-values are by Fisher's exact tests. By the end of this experiment, 9 of 10 *Chaetomorpha* patches had been completely consumed by fishes

morpha and tethered *Caphyra* within these for 19 h, 100% of the crabs in *Chaetomorpha* patches were consumed while only 50% of those in *Chlorodesmis* patches were consumed (Fig. 6, $P = 0.0016$, Fisher's exact test). At the end of the experiment, all of our *Chlorodesmis* patches were still present and there was no obvious evidence that any had been eaten; all of the *Chaetomorpha* was gone from 9 of our 10 patches. Because all of our algal patches were transplanted onto a protected lagoonal reef and because we directly observed parrotfishes grazing on the *Chaetomorpha* patches but not on the paired patches of *Chlorodesmis*, we assume that the loss of *Chaetomorpha* was due to grazing by fishes. We directly observed only one act of predation; a crab tethered in *Chaetomorpha* was eaten by a parrotfish that started grazing the alga. The quickness and intensity of movement by the fish suggested that the fish preferentially consumed the crab once it became aware of its presence and that the crab was not consumed indirectly while the fish grazed the alga.

Table 1. The fate of different gastropods when dropped into tanks holding the wrasse *Thalassoma lunare*

Species	(*N*)	% Eaten	% spat out	% Never touched
Nerita (shell removed)	(6)	100%	0%	0%
Gymnodoris sp.	(5)	0%	100%	0%
Elysia sp.	(4)	0%	100%	0%
Cyerce nigricans	(6)	0%	0%	100%

Gastropod habitat preference and susceptibility to predation. The black and orange ascoglossan *Cyerce nigricans* occurred on the upper surface of *Chlorodesmis* or on relatively unvegetated substrate immediately adjacent to the alga. *Cyerce* was always obvious and was never hidden within the plant. When placed in aquaria with a choice of 4 types of seaweeds known to be fed upon by ascoglossans, *Cyerce*'s attraction to *Chlorodesmis* was significantly greater than to any of the other species (Fig. 4).

When prodded, *Cyerce* would rock its body back and forth causing the brightly colored cerata on its back to be displayed even more prominently. If prodded forcefully, these structures would detach from the animal, stick to whatever had been used to prod the animal, and convulse while pumping out a milky fluid. When individual *Cyerce* were dropped into tanks with groups of the common wrasse *Thalassoma lunare* that had been trained to eat foods dropped from the surface, fish would approach but never touch these ascoglossans (Table 1). Even when fish were hungry and aroused to a feeding frenzy by the addition of freeze-dried krill, we could never get fish to attack *Cyerce*. TLC analysis of the crude acetone extract from *Cyerce* indicated the presence of chlorodesmin (presumably sequestered from *Chlorodesmis*) and of 4 additional unusual metabolites that were UV-active, more polar than chlorodesmin, and not present in the alga. Our acetone extract of *Cyerce* constituted 12.5% of the total dry mass of the animals; more rigorous extraction methods gave a yield of 23% (Roussis et al. 1989). When injected into freeze-dried krill as 10% of the krill dry mass, our crude acetone extract from *Cyerce* strongly deterred feeding by the wrasse *Thalassoma* and by mixed-species groups of smaller wrasse species (Fig. 7, $P = 0.011$ and $P < 0.001$ respectively, paired t-test).

Elysia sp. were always well hidden in *Chlorodesmis* and could not be seen without disturbing the patch. When *Elysia* were presented to wrasses in aquaria, fish would take the ascoglossans into their mouths but immediately reject them (Table 1); the *Elysia* appeared unharmed by this. TLC analysis of the acetone extract of fresh *Elysia* indicated the presence of two UV-active compounds from *Chlorodesmis* (chlorodesmin and a less polar compound) and one UV-active compound that was more polar than chlorodesmin and not present in *Chlorodesmis*. Our acetone extract of wet animals gave a crude extract yield of 10.5% of whole animal dry mass. When this crude extract was injected into krill at a concentration of 10%, it did not significantly deter feeding by either *Thalassoma lunare* or the smaller wrasses (Fig. 7), although there was a suggestion of mild deterrence ($P = 0.0945$ for both experiments, paired t-test). The pure compound chlorodesmin at 5% of the krill dry mass also did not decrease feeding by these wrasses (Fig. 7). In the field, chlorodesmin at 5% of food dry mass did significantly deter feeding ($P = 0.0273$, paired sample t-test) but it had no deterrent effect at 2.5% (Fig. 8, $P = 0.420$, paired sample *t*-test). The concentration of chlorodesmin in *Cyerce* is less than 1% dry mass (Roussis et al. 1989). We were unable to assess the concentration of chlorodesmin in *Elysia* sp. due to the limited availability of these animals. During our field assays, we observed 58 fish feeding from our carra-

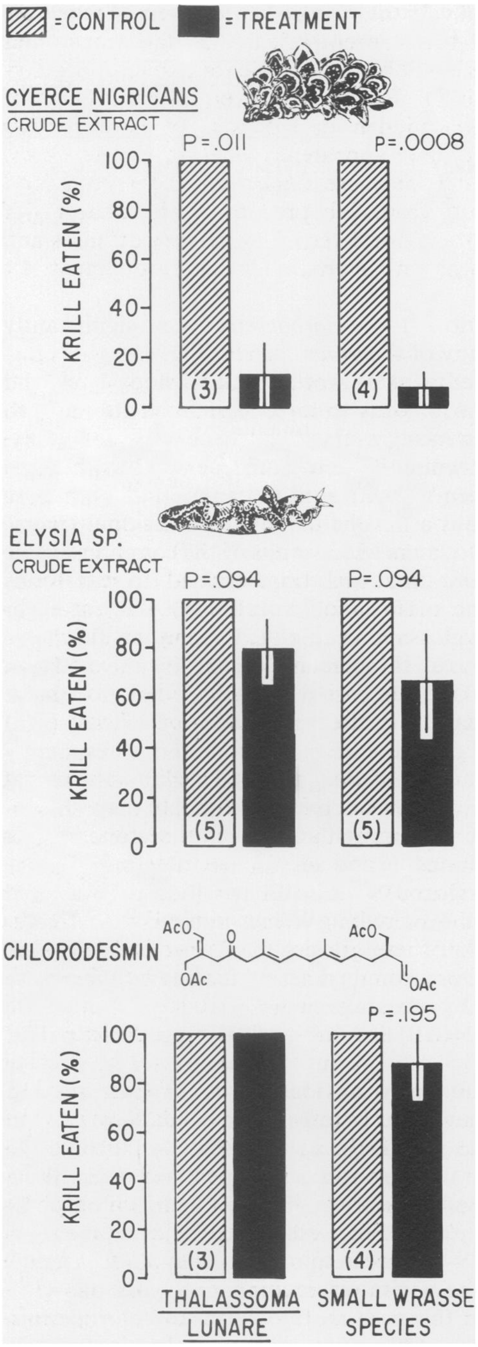

Fig. 7. Effects of the acetone crude extracts from *Cyerce nigricans* and *Elysia* sp. and of the pure compound chlorodesmin on feeding by common wrasses. Five aquaria held *Thalassoma lunare* (2–3/tank) and 5 held mixed species of smaller wrasses (2–3/tank). Each tank of *Thalassoma* was offered 5 freeze-dried krill treated with *Cyerce* extract, *Elysia* extract, or chlorodesmin along with 5 control krill treated only with solvent. Tanks of small wrasses were offered 3 krill of each type. Fish in some tanks occasionally refused to feed; this lowered the sample size below 5 for some tests. Numbers at the base of histogram pairs give the sample size for that test. Crude extracts were assayed at 10% of krill dry mass, which is near or below natural concentrations. Chlorodesmin was assayed at 5% of krill dry mass; this is far above the natural concentration found in *Cyerce* (Roussis et al. 1989), but approximates the concentration of pure metabolites found in some *Elysia* species (Paul and Van Alstyne 1988). Vertical bars through the histograms are ±1 SE. *P*-values (1-tailed) are by the paired sample *t*-test

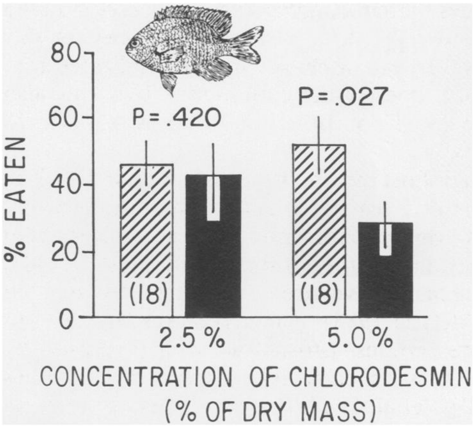

Fig. 8. Effect of adding chlorodesmin to carrageenin strips and exposing these to grazing by reef fishes for 1.5 to 2 h in the field. Analysis and symbols are as in Fig. 7

geenin strips; 91% were pomacentrids, 7% were wrasses, and 2% were butterflyfishes.

Like the *Elysia* species, we found the white dorid nudibranch, *Gymnodoris* sp., hidden only within *Chlorodesmis* patches. When these nudibranchs were dropped into tanks of *Thalassoma lunare*, the fish would take them into their mouths but then spit them out unharmed. No secondary metabolites were apparent when the acetone extract of the nudibranch was evaluated by TLC.

When a *Gymnodoris* and an *Elysia* came into contact in the lab, the *Elysia* would immediately try to move away; the dorid would immediately spread its mouth around the *Elysia* if it was small, or drive its mouth into the body of the *Elysia* if the *Elysia* was larger than the dorid. If a small dorid was placed in a container with an *Elysia* several times its size, it would bite off large chunks of the *Elysia*, "grazing" on it over a period of a few days until it was consumed entirely. In contrast, when *Gymnodoris* contacted *Cyerce*, the dorid immediately withdrew. When placed together in small containers ($N = 4$), *Gymnodoris* never attacked *Cyerce* but always attacked and consumed *Elysia*.

Discussion

Chlorodesmis fastigiata is resistant to grazing by reef fishes on Lizard Island (Fig. 1), and to fishes on Guam where both the crude extract from *Chlorodesmis* and the pure compound chlorodesmin significantly deter herbivory (Paul 1987; Wylie and Paul 1988). Chlorodesmin is not deterrent to all grazers. At natural concentrations of approximately 1% of plant dry mass (V. Paul and W. Fenical personal communication), it does not affect feeding by the rabbitfish *Siganus doliatus* (Hay et al. 1989a), and it significantly stimulates feeding by the crab *Caphyra rotundifrons* (Fig. 3). In fact, in our investigations, *Caphyra* was found only in *Chlorodesmis*, preferentially moved into *Chlorodesmis* when offered several types of algae in different laboratory assays (Figs. 4 and 5), fed almost exclusively on *Chlorodesmis* when offered several seaweeds in the lab (Fig. 2) and nothing but *Chlorodesmis* was found in guts of animals collected from the field. *Caphyra* thus appears to be a highly specialized feeder on this generally toxic alga.

Like many specialist herbivorous insects (see Futuyma

1983 and references therein) *Caphyra*'s feeding is stimulated by a compound that deters generalist herbivores (in this case fishes). However, our chemical analyses indicated that the crabs did not sequester the compound. This was also suggested by the rapid consumption of crabs exposed to reef fishes (Fig. 6).

Sequestration of host metabolites is common in herbivorous ascoglossans and sea hares and in nudibranchs that feed on sponges; this phenomenon is widely interpreted as a defense against predation (Faulkner and Ghiselin 1983) even though few cases have been rigorously investigated (Paul and Van Alstyne 1988). For herbivores that do not sequester host metabolites, protection from predation by physical association with chemically defended plants has been discussed (Hay et al. 1987, 1988a, b; Hay and Fenical 1988) but never rigorously assayed in the field where herbivores are exposed to the natural diversity of potential predators. The data for *Caphyra* clearly show that predation can be greatly reduced by simple physical association with a chemically defended seaweed (Fig. 6). Our data are consistent with recent studies on other marine herbivores suggesting that both feeding preferences and feeding specialization by small herbivores that are common prey for fishes may be driven by the advantages of reducing predation through association with algae that are repellent to, and thus seldom visited by, fishes (Hay et al. 1987, 1988a, b, 1989b; Hay and Fenical 1988).

Several lines of evidence suggest that the ascoglossan *Cyerce* is well defended chemically. These include: (1) *Cyerce*'s aposematic coloration, (2) its habit of being clearly exposed (behavior that was never seen in the crab or the *Elysia* sp.), (3) detachment of cerata that convulse, and pump out a milky fluid when the animal is prodded, (4) the strong feeding-deterrent property of *Cyerce*'s lipid soluble extract (Fig. 7), and (5) the complete avoidance of *Cyerce* by predatory fish (Table 1). The deterrence of *Cyerce*'s crude extract did not result from the presence of chlorodesmin. The concentration of chlorodesmin in *Cyerce* is less than 1% of its dry mass (Roussis et al. submitted) and chlorodesmin at a concentration of 5% did not deter common wrasses that were deterred by the crude extract (Fig. 7). In field assays, chlorodesmin did deter feeding by reef fishes at a concentration of 5% but not 2.5% (Fig. 8). At present, the compound or compounds responsible for the deterrent properties of *Cyerce*'s crude extract have not been identified. *Cyerce* contains several polypropionate secondary metabolites that are not chemically related to the terpenoids produced by *Chlorodesmis*; however, the polypropionate compounds do not appear to deter feeding by the species of wrasse used in our experiments (Roussis et al. 1989).

Our assays with *Elysia* were limited. Although *Elysia* were unpalatable to wrasses, they were not as obviously repellent as *Cyerce* since the fish had to taste them before they were rejected (Table 1). Our inability to document a significant deterrent effect of *Elysia* crude extract on fish feeding (Fig. 7) could be due in part to the limited replication of this assay ($N = 5$). However, it is clear that *Cyerce*'s extract was more deterrent than *Elysia*'s because deterrence of the *Cyerce* extract was significant with even less replication ($N = 3$ and 4). The congeneric ascoglossan *Elysia halimedae* is known to sequester green algal metabolites (or slight modifications of the algal metabolites) in concentrations of up to 7% of the ascoglossan's dry mass (Paul and Van Alstyne 1988). If the *Elysia* that we studied sequester chlorodesmin at this concentration, then the sequestered algal metabolites could be responsible for fish rejecting *Elysia* as prey (Table 1, Fig. 8). However, *Elysia*'s habit of always being well hidden in the base of *Chlorodesmis* patches and its cryptic appearence when in *Chlorodesmis*, both suggest that it may use *Chlorodesmis* for both food and spatial escape from fish predation. The nudibranch *Gymnodoris* clearly is not deterred by *Elysia*'s defenses and appears to live only in *Chlorodesmis* and to preferentially consume *Elysia*.

Specialization by marine herbivores. The life history of most marine herbivores includes a pelagic larval stage that drifts in the plankton for hours to months before settling back into a benthic community. The limited ability of this dispersive stage to actively choose the habitat in which it lands (Butman 1987) coupled with a high potential for being preyed upon as it approaches the benthos (Gaines and Roughgarden 1987; Olson and McPherson 1987) may select for generalist herbivores that can utilize a broad range of foods following settlement (Hay and Fenical 1988). Thus, specialization by marine herbivores may occur only under exceptional circumstances. Understanding these circumstances might provide important insights and broaden our understanding of what factors affect the evolution of host-plant specialization in general.

A previous model of host-plant specialization among marine herbivores (Steneck 1982) stressed that specialization should be most common among small herbivores with low energy requirements and small home ranges. Such herbivores were predicted to be most likely to specialize on larger seaweeds that were long-lived and predictable. It was also predicted that these grazers would rarely be fatal to their large algal host. Our data are consistent with Steneck's predictions. Although crabs were relatively common in *Chlorodesmis* patches (at most sites we estimate that 10–30% of the patches we investigated had 1 or 2 crabs in them), we noticed no obvious effects of their grazing. Ascoglossans also appear to inflict minimal damage on their algal hosts (Clark and DeFreese 1987). This probably occurs because they seldom reach high densities, their metabolic rates are low, and they sequester algal chloroplasts from their host plants. Since sequestered chloroplasts can remain functional for weeks to months and can fix enough carbon to meet the respiratory requirements of the ascoglossan (Trench 1975; Clark et al. 1981), chloroplast sequestration could considerably reduce grazing on the plant.

In addition to the herbivore characteristics emphasized by Steneck (1982), a high potential susceptibility to predation also appears to be a characteristic of specialized marine herbivores. Small size and limited mobility should increase susceptibility to predation, and populations of herbivores such as amphipods, crabs, and small gastropods have repeatedly been shown to be controlled or severely affected by predation (Young et al. 1976; Van Dolah 1978; Nelson 1979a, b; Stoner 1980; Edgar 1983; Watanabe 1984). Our findings suggest that specialization by the crab *Caphyra* dramatically decreases its susceptibility to predation. Although less clearly documented, *Elysia* may deter predators with the help of algal-derived compounds, as do other species of *Elysia* that specialize on chemically defended seaweeds (Paul and Van Alstyne 1988). *Cyerce* is the only ascoglossan we know of that is aposematically colored when on its algal host; although its defenses appear to be chemical, it is not clear that they are sequestered from *Chlorodesmis*. *Cyerce* may be unusual among the ascoglossans in that it may no longer need its algal host for defense from predation.

For specialists like *Caphyra* that do not sequester algal defenses, times of migration between host plants could be periods of intense predation (Fig. 6). *Elysia* might face similar problems because its defenses do not appear to be as effective as those of *Cyerce* (Fig. 7 and Table 1) and because it might migrate to a patch containing *Gymnodoris*. Therefore, specialists could be selected to minimize damage to their host plant and thus reduce their need for among-plant migrations. This could be accomplished by lowering their metabolic rates or by developing alternate energy sources such as chloroplast sequestration.

Although small herbivores like those studied here have rarely been rigorously studied under field conditions, a number of correlative and experimental studies suggest that populations of these herbivores are rarely food limited but often limited by predators (Van Dolah 1978; Nelson 1979a, b; Stoner 1980; Edgar 1983; Watanabe 1984). Thus, their feeding preferences are less likely to have evolved due to the need to partition resources than due to the necessity of decreasing losses to natural enemies. Predation may therefore be a major factor selecting for feeding preferences and the evolution of feeding specialization in small marine herbivores. Several terrestrial studies (Price et al. 1980, 1986; Bernays 1988; Bernays and Graham 1988; Bernays and Cornelius 1989) suggest that natural enemies also may play an important role in selecting for feeding specialization among terrestrial insects. Like small marine herbivores, insects are seldom food limited but often subject to intense predation (Strong et al. 1984), and migration among plants that offer adequate food and protection from predators may be a major problem for insects (Kareiva 1982). The apparent parallels between herbivorous insects and some small marine grazers suggest that predation may select for specialization in a variety of systems.

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