Herbivore Resistance to Seaweed Chemical Defense: The Roles of Mobility and Predation Risk

J. Emmett Duffy, Mark E. Hay

HERBIVORE RESISTANCE TO SEAWEED CHEMICAL DEFENSE: THE ROLES OF MOBILITY AND PREDATION RISK[1]

J. Emmett Duffy[2] and Mark E. Hay
University of North Carolina at Chapel Hill, Institute of Marine Sciences,
Morehead City, North Carolina 28557 USA

Abstract. Numerous small sedentary herbivores (mesograzers such as amphipods, small crabs, and gastropods) are resistant to seaweed secondary metabolites that deter larger, more mobile herbivorous fishes. In addition, specialist mesograzers experience reduced predation from fishes when living on seaweeds that produce these compounds. In this study we tested the hypothesis that generalist, as opposed to specialist, mesograzers can also benefit from reduced predation when they occupy chemically defended plants. Secondly, we assessed the hypothesis that low herbivore mobility, unconfounded by herbivore size or specialized feeding, selects for tolerance of seaweed chemical defenses, by comparing responses to the chemically defended brown seaweed *Dictyota menstrualis* of three sympatric, generalist amphipods that differ in mobility (*Ampithoe longimana, Ampithoe valida,* and *Gammarus mucronatus*).

Responses to *Dictyota*'s chemical defenses varied as much among these three amphipods as among the phylogenetically distant fishes and mesograzers studied previously and supported the hypothesis that less mobile herbivores should be most tolerant of plant chemical defenses. In laboratory experiments, *A. longimana* moved little, preferentially consumed *Dictyota* over other seaweeds, and was unaffected by all *Dictyota* secondary metabolites tested. In contrast, *G. mucronatus* was active, it did not feed on *Dictyota,* and two of three *Dictyota* secondary metabolites deterred its grazing. Distribution of amphipods in the field suggested that these feeding patterns affected amphipod risk of predation. *A. longimana* reached its highest abundance on *Dictyota,* which is unpalatable to omnivorous fish predators, during the season when fish are most abundant. At the same time, the highly active *G. mucronatus* decreased to near extinction. Like *G. mucronatus, A. valida* was deterred by two *Dictyota* secondary metabolites, did not eat *Dictyota,* and disappeared when fishes were abundant.

Experiments confirmed that *A. longimana* was less vulnerable to fish predation when occupying a chemically defended seaweed than when occupying a palatable seaweed. This decreased predation resulted primarily from a decreased frequency of encounter with predators when amphipods were on chemically defended plants. When we experimentally equalized encounter rates between omnivorous pinfish (*Lagodon rhomboides*) and the seaweeds *Dictyota menstrualis* and *Ulva curvata* (unpalatable and palatable, respectively, to pinfish) in the laboratory, amphipods occupying these two plants were eaten at similar rates. In contrast, when live amphipods were affixed to *Ulva* and *Dictyota* and deployed in the field, amphipods survived only on *Dictyota.* Heavy fish grazing on *Ulva* in the latter experiment suggests that poor survival of amphipods on *Ulva* may have resulted from greater detection and/or incidental ingestion of amphipods on this plant, due to frequent visitation by fishes. Infrequent visitation of *Dictyota* by foraging fish also may explain *A. longimana*'s persistence through the summer on this chemically defended seaweed while the two *Ulva*-associated amphipods declined precipitously. These results (1) confirm that association with chemically defended plants can reduce predation on generalist, as well as specialist, herbivores and (2) suggest that preferential feeding on chemically defended plants is most likely for sedentary mesograzers because low mobility enhances the ability to exploit chemically defended seaweeds as refuges from fish predation.

Key words: amphipods; Ampithoe longimana; Ampithoe valida; *chemical defenses;* Dictyota; Gammarus mucronatus; *herbivory; mobility; predation; seaweeds; secondary metabolites; southeastern U.S.; terpenes.*

INTRODUCTION

Secondary metabolites produced by marine algae are important in reducing the impact of grazers on many populations of marine plants (Hay 1991a, Hay and Steinberg 1992). Nevertheless, recent studies have repeatedly documented strong variance in the effectiveness of seaweed chemical defenses against different sympatric herbivores (reviewed by Hay 1992). Specifically, many small herbivorous invertebrates like amphipods, polychaetes, and ascoglossan gastropods (mesograzers) that live on the surfaces of seaweeds are indifferent to secondary metabolites that deter grazing by fishes, which are much larger and more mobile. These patterns have been explained as follows (Hay et al. 1987a, Hay 1991b). Small, relatively sedentary animals that eat macroalgae also live on these plants. Thus, food and habitat are closely linked, particularly for less mobile herbivores, which have longer residence times on a given plant and are more vulnerable to predation if they leave it to forage. Because predation on mesograzers is often intense (Vince et al. 1976, Van Dolah 1978, Young and Young 1978, Nelson 1979a, b, Stoner 1979, 1980a), predation should select for association with substrata that provide both food and shelter from predators. Plants that are chemically defended from fishes could provide such a substratum.

Mesograzers that feed and live on defended seaweeds might experience reduced predation, relative to those on undefended plants, via (1) distastefulness resulting from sequestration of host metabolites, (2) cryptic appearance on the plant (more likely for specialists adapted to a particular plant), and/or (3) infrequent visitation by foraging fish, resulting in lower probability of being detected and/or indirectly consumed by fishes. Some of these situations appear more common than others among marine mesograzers. Unlike sponge-feeding nudibranchs (Faulkner and Ghiselin 1983, Karuso 1987, Pawlik et al. 1988) and herbivorous opisthobranch gastropods (Paul and Van Alstyne 1988, Hay et al. 1989, 1990b, Paul and Pennings 1991) that sequester chemical defenses from their diets, crustacean mesograzers that feed on chemically defended algae are not known to sequester defensive metabolites and are readily eaten by predators when removed from their host plants (Hay et al. 1987a, 1989, 1990a, b). On the other hand, the second and third mechanisms above are well supported for specialist marine herbivores. Experiments demonstrated that palatable specialist mesograzers gained protection from fish predators by living on chemically defended plants because the mesograzers were cryptic (Hay et al. 1990b) or because these plants were rarely visited or consumed by the predators (Hay et al. 1989). An unusual example that combines aspects of all three mechanisms is provided by a specialist amphipod that achieves immunity from fish predation by surrounding itself with a shell made from a chemically defended Dictyota species, which fish will not eat (Hay et al. 1990a).

Clearly then, chemically defended plants can provide safe sites for mesograzers. Moreover, the benefit of such associations should be greatest for mesograzers that can eat the plant, and thus need not leave it to forage. This hypothesis, that predation selects for tolerance of chemical defenses by mesograzers, was based in part on the fact that the generalist mesograzers studied initially were much less mobile than co-occurring fishes; the amphipods and the polychaete worm on which the hypothesis was originally based both spend most of their time in tubes that they build on their host plants (Hay et al. 1987a, 1988c). Although specialist mesograzers have since been shown to escape fish predation by association with chemically defended algae, and a wealth of circumstantial evidence (Hay et al. 1987a, 1988c, Holmlund et al. 1990, Duffy and Hay 1991b) suggests that small, relatively sedentary generalists can gain similar advantages from such associations, a direct demonstration that noxious seaweeds provide a refuge from predation has not been experimentally demonstrated for these generalist mesograzers. Moreover, although several studies have investigated differences between fishes and mesograzers in responses to seaweed secondary metabolites, none have compared taxonomically and ecologically more similar mesograzers that differ in mobility but not size. Consequently it is unclear whether tolerance of seaweed metabolites by mesograzers is really related to low mobility and susceptibility to predation, as suggested above, or is instead some common feature of large vs. small herbivores, or of fishes vs. certain invertebrate taxa. If tolerance of seaweed chemical defenses is indeed related to low mobility and consequent close association with host plants, then this should be as evident in comparisons among related mesograzers that differ in mobility as it is among the unrelated and ecologically different groups (wide-ranging fishes vs. smaller, more sedentary invertebrates) that have been studied previously.

Here we address this hypothesis by comparing the responses of three sympatric amphipod species, which differ in mobility, to the brown seaweed Dictyota menstrualis (formerly D. dichotoma, see Schneider and Searles 1991) and its secondary chemistry. The association between the tube-dwelling amphipod Ampithoe longimana and Dictyota menstrualis provided some of the initial evidence for arguments that predation influences tolerance of seaweed metabolites by mesograzers (Hay et al. 1987a, Duffy and Hay 1991b). This amphipod appears to be resistant to Dictyota secondary metabolites that deter feeding by sympatric fishes (Hay et al. 1987a), it preferentially feeds on Dictyota, survives better on a diet of this plant than on any of four other macroalgae tested, and reaches higher abundances on Dictyota than on other macroalgae in the field when predatory fishes are most active (Duffy and Hay 1991b). In this study we compare distribution, feeding, mobility, and plant-specific predation risk of

A. longimana, the sympatric amphipod *Gammarus mucronatus,* which is not a tube-dweller, and, for some assays, *Ampithoe valida,* which is a tube-dweller. We ask the following questions. (1) Do these herbivorous amphipods differ in their feeding preferences and responses to *Dictyota* metabolites? (2) If so, are the less mobile herbivores more resistant to seaweed chemical defenses? And (3) do amphipods associated with *Dictyota* experience less predation than amphipods associated with an alga that is palatable to fish?

METHODS

The plants and their chemistry

Brown algae identified as *Dictyota dichotoma* have been reported from warm waters throughout the world and, in the western Atlantic, from Brazil to North Carolina and Bermuda (Taylor 1960). Plants in the southeastern USA that were previously known as *D. dichotoma* are now recognized as *D. menstrualis* (Schneider and Searles 1991). Both *D. menstrualis* and *D. ciliolata* occur in the summer in North Carolina, and *D. menstrualis* in particular is abundant on hard substrates, in seagrass beds, and on sand plains and mudflats where it attaches to shell fragments or polychaete tubes.

More than 20 terpenoid secondary metabolites have been reported from *D. dichotoma* (Faulkner 1987). Quantitative analysis, via high-pressure liquid chromatography (HPLC), of the terpenoid metabolites in *Dictyota* species from North Carolina (G. Cronin, *personal communication*) reveals that the major metabolite in *D. menstrualis* is the diterpene alcohol dictyol E (see Fig. 1 for structures), which averages ≈0.2% of plant dry mass, and that the major metabolite in *D. ciliolata* is the structurally related dictyol B acetate, averaging ≈0.5% of dry mass. A third compound, pachydictyol A, occurs at ≈0.06% of dry mass in both species. Each of these compounds deters feeding by some tropical and temperate fishes and sea urchins; however, neither dictyol E nor pachydictyol A deterred feeding by the tube-building amphipod and polychaete studied previously (Hay et al. 1987*a, b,* 1988*a, c*). We tested all three compounds at natural concentrations against three sympatric amphipods.

The animals

The herbivorous amphipods, *Ampithoe longimana, Ampithoe valida,* and *Gammarus mucronatus* co-occur on a sheltered sandflat at Lennoxville Point, near Beaufort, North Carolina, USA (34°42′ N, 76°41′ W). Like other members of the family Ampithoidae, *A. longimana* and *A. valida* live in tubes that they construct from detritus, plant material, and glandular secretions; both species appear to be rather sedentary, spending much of their time within their tubes. *A. longimana* is a common species in both exposed and estuarine situations, occurring from the lower intertidal to a depth of ≈10 m, and ranges from Florida and Bermuda to southern Maine (Bousfield 1973). *A. longimana* occurs
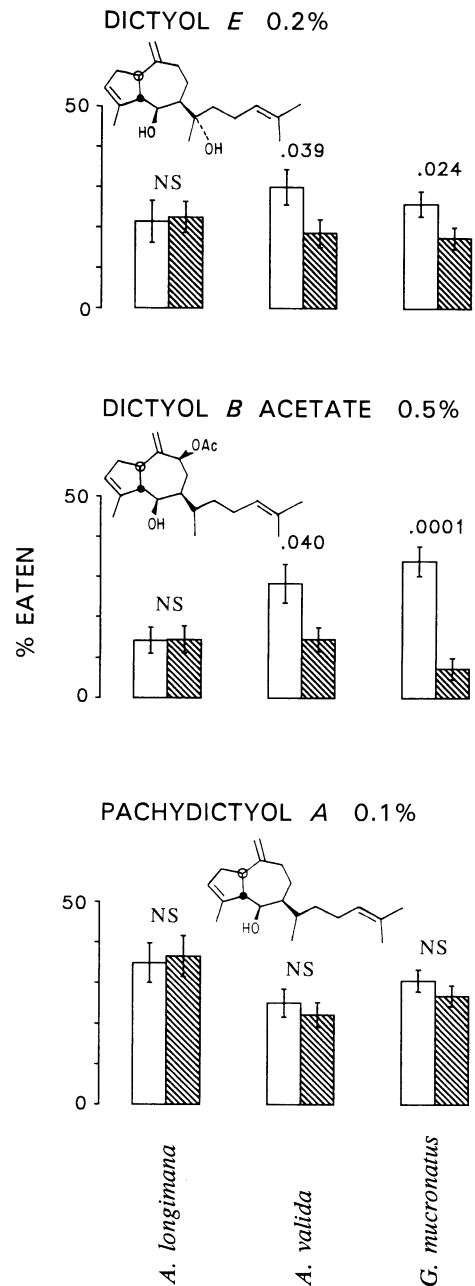
FIG. 1. Effects of three terpenoid secondary metabolites from brown seaweeds, *Dictyota* spp., on feeding by three amphipod species. Hatched bars represent algal discs coated with natural concentrations (as indicated) of a metabolite; open bars are solvent-coated control discs. Treated and control discs were offered together in choice assays (*n* = 27–30 replicates for each assay). Two-tailed *P* values from paired-sample *t* tests appear above each pair of bars. NS (nonsignificant) denotes *P* > 0.100.

on a variety of algae and seagrasses (Nelson 1979*a*, Lewis 1987, Duffy 1990, Duffy and Hay 1991*b*) and on fouled substrates.

Ampithoe valida occupies sheltered, often brackish, waters (Conlan and Bousfield 1982). In the western

Atlantic it occurs between New Hampshire and Cape Canaveral. It is also found on the west coast of North America and Japan. *A. valida* inhabits algae from "low water level to depths of a few meters" (Bousfield 1973). This species was uncommon during our study, and it was impossible to collect enough animals for some experiments.

Gammarus mucronatus is common in estuarine and marine habitats, at salinities from 4 g/kg to full seawater ($\approx$36 g/kg). It ranges from the Gulf of St. Lawrence to the Gulf of Mexico, occurring both intertidally and subtidally (Bousfield 1973) in seagrass beds, macroalgal fouling communities (Fredette and Diaz 1986), and on sandy bottoms. In our area, *G. mucronatus* is abundant on algae in shallow protected water. It does not build tubes and is frequently collected in the water column (Williams and Bynum 1972), suggesting that it may be much more mobile than the tube-building species.

As is true for most amphipods, the field diets of these three species are poorly known. Most ampithoids, including the two *Ampithoe* species studied here, feed primarily on macro- and/or microalgae (Duffy 1990, Brawley 1992), but detritus can also form a substantial component of the diet (Nelson 1979*a*, Zimmerman et al. 1979). *G. mucronatus* feeds on algae, seagrass debris, and detritus (Zimmerman et al. 1979, Smith et al. 1982).

Field sampling of phytal amphipods

To assess plant use patterns and seasonal changes in abundance of the three amphipods, we sampled seaweeds and their associated faunas at Lennoxville Point from May through September 1988 (*n* = 5–7 plants of each species each month), a period that bracketed the sharp summertime increase in biomass and feeding activity of omnivorous fishes in North Carolina estuaries (Adams 1976*a, b*). We focussed our sampling on green algae in the family Ulvaceae (mostly *Ulva curvata* and *Enteromorpha linza*), and on the brown seaweed *Dictyota menstrualis* for two reasons: (1) these plants dominated the flora at our site during spring and summer, respectively (visually estimated to comprise 60–90% of seaweed cover at this site), and therefore are likely to support much of the phytal amphipod community there, and (2) *Dictyota* and *Ulva* are low- and high-preference foods, respectively, for pinfish (Hay et al. 1988*c*), the predator we hypothesized was most likely to mediate amphipod distribution patterns because it is the most abundant fish in local inshore waters during warmer months of the year (Adams 1976*a*, Darcy 1985).

We sampled both intertidal and subtidal ulvaceans (hereafter termed "greens") during each month they were present. *Dictyota menstrualis*, which was exclusively subtidal at this site, was sampled from July, when it first appeared, through September when it comprised almost all of the algal cover. Samples were collected once a month, on a low tide within 2 d of the full moon. Plants were plucked from the substrate (submerged subtidally and exposed intertidally), sealed in plastic bags, and fixed in formalin within 2 h.

Amphipods were removed from seaweed samples by washing the plant repeatedly in tap water, pouring the water through a 163-μm mesh sieve, and sorting the sieve contents. Amphipods comprised 93% of macroscopic animals, and were identified to species. Each plant sample was blotted dry and weighed, and amphipod abundances were standardized to plant wet mass. Prior to statistical analysis, Cochran's test was employed to assess homogeneity of variances among treatments (i.e., plant species). Data that failed this test were transformed by $\log(1000X + 1)$ because of the high frequency of zero and near-zero values (see Sokal and Rohlf 1981), and differences among treatments were assessed with t tests or ANOVA. When the F test from an ANOVA was significant, specific treatment means were compared using Ryan's Q test, or Games-Howell tests when treatment variances remained unequal after transformation, following recommendations of Day and Quinn (1989). For all statistical analyses reported in this paper, we present P values when the null hypothesis was rejected ($P < 0.05$); in all cases where the null hypothesis was not rejected we report the results as nonsignificant (NS) or as $P > 0.10$ (none of the tests we performed resulted in P values between 0.05 and 0.10).

Amphipod feeding preferences

To determine the amphipods' feeding preference for *Dictyota* relative to other common macroalgae, we simultaneously offered amphipods approximately equal masses (except for *Codium*) of the brown algae *Dictyota menstrualis* and *Sargassum filipendula*, the red algae *Hypnea musciformis*, *Agardhiella subulata*, *Rhodymenia pseudopalmata*, *Gracilaria tikvahiae*, *Calonitophyllum medium*, and *Chondria dasyphyllum*, and the green algae *Ulva* sp. and *Codium fragile*.

One preweighed piece (90–110 mg) of each algal species (except *Codium*, for which we used 180–220 mg because of its high mass to volume ratio) was placed in a 500-mL bowl with amphipods of a single species (either 20 *A. longimana*, 5 *A. valida*, or 12 *Gammarus mucronatus*). Different numbers of the three amphipods were used because we could not collect enough *A. valida* and *G. mucronatus* to include 20 amphipods per replicate. Sizes of the amphipods used in this and the other experiments were not measured but were not noticeably different. For each amphipod species, 18 replicate bowls received amphipods and 7 bowls containing algae but no amphipods were used to estimate mass changes unrelated to herbivory. The amphipods were allowed to feed for 39–63 h, depending on how rapidly they fed, after which the remaining algae were blotted dry and weighed.

Tests of chemical feeding deterrence

We tested the major terpenoid compounds produced by *Dictyota* spp. in North Carolina for feeding deterrence against the three species of amphipods. Dictyol E was tested at 0.2%, dictyol B acetate at 0.5%, and pachydictyol A at 0.1% of plant dry mass. These concentrations approximate natural concentrations found in North Carolina specimens of *Dictyota* as determined by analytical HPLC (G. Cronin, *personal communication*). The lipophilic compounds were dissolved in diethyl ether and applied to 12-mm diameter discs of the green alga *Ulva fasciata* with a microlitre pipette. Each compound-coated disc was paired with a second disc coated only with ether, which served as the control.

One ether-coated control disc and one compound-coated treatment disc were offered together in 100-mL plastic cups, filled with seawater, to monospecific groups of each of the three amphipod species. Number of replicate cups varied between 27 and 30 for these assays. To distinguish treatment from control discs, white cotton threads of slightly different lengths were tied through each disc. After 24–48 h, the remaining plant portions were removed and the area eaten from each disc was measured by counting the number of points that fell on the remaining portion when looking through an ocular grid mounted in a stereoscope (intact discs averaged ≈60 points each).

In order to minimize nonexperimental variation, assays of a given compound were conducted for all three amphipod species at the same time, using algal discs from the same individual plants. Amphipods used in the feeding deterrence assays were collected <7 d prior to the assays from mixed algae growing at Lennoxville Point and in outdoor running seawater tanks at the Institute of Marine Sciences in Morehead City, North Carolina, USA.

Mobility experiments

We assessed differences in mobility among the amphipod species by measuring, in the laboratory, movement between two of the most abundant seaweed species at Lennoxville Point, *Ulva curvata* and *Dictyota menstrualis*. We measured (1) the net movement, during an 18-h period in the laboratory, of *A. longimana* and *G. mucronatus* from *Dictyota* to *Ulva* and vice versa (*A. valida* was unavailable for these experiments), and (2) the influence of visual and chemical cues from a predatory pinfish on these movements.

In these experiments, we used approximately equal surface areas of *Dictyota* and *Ulva*. Surface areas for each species were estimated from a surface area/wet mass relation calculated as follows. Five pieces (1.5–2.2 g) each of *Dictyota* and *Ulva* were blotted dry, weighed, and their surface areas measured with a LI-COR 3100 area meter. Mean surface area per unit wet mass (mean ± 1 SE) of *Dictyota* was 121.1 ± 3.3 cm²/g, and of *Ulva* was 272.0 ± 5.5 cm²/g.

Separate experiments were performed for *A. longimana* and *G. mucronatus*. Both experiments consisted of four treatments, each of which was replicated 10 times. The first treatment measured movement from *Dictyota* to *Ulva* in the absence of cues from a fish predator. To accomplish this, 10 amphipods were placed in a 500-mL bowl with a piece of *Dictyota*, ≈200 cm² in total surface area (1.49–1.83 g), and allowed to settle for 27 h. A second bowl received ≈200 cm² (0.67–0.81 g) of *Ulva* but no amphipods. After 27 h both of the plants, including the associated amphipods, were carefully transferred into a container (34 × 29 × 13 cm) with ≈5 L of seawater such that the two plants were ≈20 cm apart. This distance is similar to, or much less than, the average spacing between plants at our study site (J. E. Duffy, *personal observation*) and should conservatively estimate the likelihood of voluntary movement (i.e., excluding dislodgement by turbulence) between plants in the field.

After 18 h, the amphipods on each plant were counted as follows. In the *A. longimana* experiment, each plant was carefully removed from the tray and dipped in fresh water, which caused the amphipods to leave their tubes and fall to the bottom. This procedure was insufficient for *G. mucronatus*, which tended to swim when disturbed. Thus in the *G. mucronatus* experiment, plants were quickly scooped from the container with a dipnet and placed in tap water; the associated amphipods were counted. For both species, amphipods were scored as being associated with *Dictyota*, *Ulva*, or elsewhere (i.e., on the water surface or plastic container).

The other three treatments differed from the first as follows. Treatment 2 measured movement from *Ulva* to *Dictyota* in the absence of fish. Ten amphipods were placed on *Ulva*, no amphipods on *Dictyota*; the procedures described above were repeated. Treatment 3 measured movement from *Dictyota* to *Ulva* in the presence of visual and chemical cues from a pinfish. In this treatment, 10 amphipods were placed on *Dictyota*, and none on *Ulva*. After 27 h, both plants were transferred to a tray filled with 5 L of seawater in which a pinfish had been kept for 2 h and removed prior to amphipod addition. A pinfish in a clear plastic bag with seawater and air was then floated in the container so that amphipods could see, but not be eaten by, the fish. We used a clear plastic bag rather than a mesh bag to avoid the possibility of amphipods passing through the mesh and being eaten. This treatment allowed amphipods potentially to sense the fish both visually and chemically, but prevented the fish from consuming the amphipods. When compared with the treatments lacking fish, this and the following treatment allowed us to identify changes in net movement between the two algae (i.e., final amphipod abundances on each plant) caused by the amphipods sensing their predator. Treatment 4 measured movement from *Ulva* to *Dictyota* in the presence of cues from a pinfish. Ten amphipods

were placed on *Ulva*, none on *Dictyota*; bagged fish and fish water were present. These four treatments allowed us to assess differences in mobility of each amphipod species as a function of plant species initially occupied, presence of visual and chemical cues from a predator, and interactions between these two factors.

Predation experiments: laboratory

Mesograzers living on plants that are chemically defended from fishes might experience reduced predation, relative to mesograzers on palatable plants, because (1) mesograzers are less frequently detected or captured on unpalatable plants, relative to palatable plants, even when both plants are encountered at equal rates by fish, or because (2) fishes visit defended plants less frequently, and thus detect and/or incidentally ingest the mesograzers less frequently.

We tested the first of these hypotheses with laboratory experiments. We compared the relative vulnerability of amphipods on *Dictyota menstrualis* vs. *Ulva curvata* when the seaweeds were simultaneously confined with pinfish in small containers in the laboratory. We assumed that pinfish in these containers would have constant access to both seaweeds due to the small size (34 × 29 × 13 cm) and structural simplicity of the containers, and therefore that seaweed–fish encounter rates would be equal for both algae.

We used the pinfish as a predator in these experiments because it is one of the most common inshore fishes of the southeastern USA (Darcy 1985) and is a major predator on amphipods (Carr and Adams 1973, Nelson 1979*a, b,* Stoner 1980*b*). *Dictyota* is a low-preference food, and *Ulva* a high-preference food, for pinfish (Hay et al. 1988*c*). Thus we expected that amphipods occupying *Dictyota* might be less vulnerable to attack by pinfish than those occupying *Ulva*.

Separate experiments employing the same design were performed for *Ampithoe longimana* and for *Gammarus mucronatus* (*A. valida* was unavailable when these experiments were conducted). One piece of *Dictyota*, ≈200 cm² in total surface area (1.49–1.83 g), was placed in a shallow bowl with 10 amphipods of one species. A similar-sized piece of *Ulva* was placed in a separate bowl with 10 amphipods of the same species. The amphipods were allowed to settle on the plants for 2.5 h, by which time most *A. longimana* had at least begun building tubes and had stopped moving around the bowls. Plants and associated amphipods from both bowls were then carefully transferred to a single plastic container filled with ≈5 L of seawater. Thus all replicates contained one piece of *Dictyota* and one piece of *Ulva* with each alga initially carrying 10 amphipods.

Ten of these containers were then assigned to each of three treatments, which allowed us to distinguish between fish predation and amphipod movement in influencing amphipod distribution on the two experimental plants: (1) "free fish" containers received one pinfish that was allowed to forage on the amphipods,

(2) "bagged fish" containers received a bagged pinfish and water in which a fish had been held for 2 h (as described above for the mobility experiments); this allowed amphipods potentially to sense the fish visually and chemically without being eaten, and (3) control containers received no fish and were filled with seawater in which a pinfish had not previously been held (i.e., no chemical or visual cues). Sizes of pinfish used in these experiments were not individually measured, but we estimated that they ranged between ≈6 and 10 cm standard length. The fish were allowed to feed for 2 h and then removed. The number of remaining amphipods associated with each plant was measured as described above for the mobility experiments.

The *A. longimana* experiment was conducted over the course of 2 d. The 1st d produced seven usable replicates of the free-fish treatment (i.e., in which more than zero but less than all amphipods were eaten), and 10 replicates each of the bagged-fish and control treatments. The experiment was repeated the next day to increase replication. However, because the first day's results showed no tendency toward a difference in percentage of amphipods on *Dictyota* between the two treatments without fish predation (i.e., the control and the bagged fish, $P = 0.267$, $n = 10$, t test), we used only the bagged-fish and the free-fish treatments on the 2nd d. We present only the data from the bagged-fish and free-fish treatments. Data from both days of the experiment were pooled prior to analysis.

The *G. mucronatus* experiment was also conducted on two different days. Since the mobility experiment showed no tendency toward differential movement in the presence vs. absence of cues from pinfish (see *Results: Mobility experiments*), we used only the bagged-fish and free-fish treatments in this experiment, as we had on the 2nd d of the *A. longimana* experiment.

Predation experiments: field

The second hypothesis to explain reduced predation risk of mesograzers on chemically defended plants proposes that omnivorous fishes visit the defended plants less often and are thus less likely to detect, and/or incidentally consume, mesograzers living on them. We tested this by measuring the survival of amphipods attached to *Ulva* and *Dictyota* with cyanoacrylate glue (Super Glue), and deployed in the field. We performed this experiment only for *A. longimana* since we expected the experimental procedure would eliminate differences between the amphipod species in activity and mobility levels.

First, to determine whether amphipods would be differentially dislodged from the two plants in the absence of predation, we conducted a laboratory experiment. We glued four live *Ampithoe longimana* to each of 20 pieces of *Dictyota* and 20 pieces of *Ulva*, and placed these plants in a seawater table in the laboratory for 24 h. The amphipods were attached by their backs via a small droplet of Super Glue, after the alga had

been patted dry with a paper towel. Three "Little Gi- ant" water pumps circulated the water in the table to simulate currents present in the field. To estimate the degree of water movement in this experiment relative to that in the field, we used a technique similar to that of Muus (1968): we measured mass loss, due to dis- solution, of plaster-of-Paris blocks ($n = 5$) enclosed in nylon hose and placed haphazardly in the water table during the experiment. The dry mass of the plaster blocks was measured before and after deployment in the water, and the change in mass was calculated as a rough estimatè of average flow.

The field experiment assessed differential survival of amphipods on *Dictyota* and *Ulva* due to the combined effects of differential detection and/or incidental in- gestion of amphipods on those plants. In preparation for the experiment, we glued four live *A. longimana* to each of 20 preweighed pieces of *Dictyota* and 20 preweighed pieces of *Ulva,* as described above, and then attached each alga to a heavy nail with a cable tie. As in the laboratory experiment, amphipods were attached to the plant by their backs so that the glue would not foul their legs or mouthparts. This close spatial association with the plant mimics that of an amphipod in its tube on the alga's surface; however, the amphipods' exposed and moving head and legs made them much more conspicuous to us (and pre- sumably to fish) than when they were nestled in their tubes. We deployed the algae, with attached amphi- pods, in the field by pushing the nails into the sub- stratum, which was submerged under shallow water. Twenty replicate pairs, consisting of one *Dictyota* and one *Ulva* plant, were deployed in a line parallel to the shore. At the same time, to estimate changes in algal mass unrelated to grazing by fish, we deployed 10 pre- weighed pieces each of *Dictyota* and *Ulva* (with no amphipods attached) inside separate, cylindrical plas- tic mesh cages, 30 cm long × 20 cm in diameter with 1.9-cm mesh. Finally, to estimate the degree of water movement around the plants, we deployed five pre- weighed plaster-of-Paris blocks by placing them in ny- lon hose and nailing these into the substratum at hap- hazardly selected positions along the line of experimen- tal plants. After 24 h we retrieved the plants and the plaster blocks, counted the number of amphipods remaining on each plant, and reweighed the plants and blocks.

RESULTS

Amphipod field abundances

Seasonal and spatial patterns of abundance for *Am- pithoe longimana* differed greatly in comparison with the other two amphipod species (Fig. 2). Initially absent in May, *A. longimana* reached moderate abundance on subtidal greens in June, but remained absent on intertidal greens. In mid-June *Dictyota menstrualis* ap- peared at the site and increased in abundance during the following months, comprising most of the subtidal
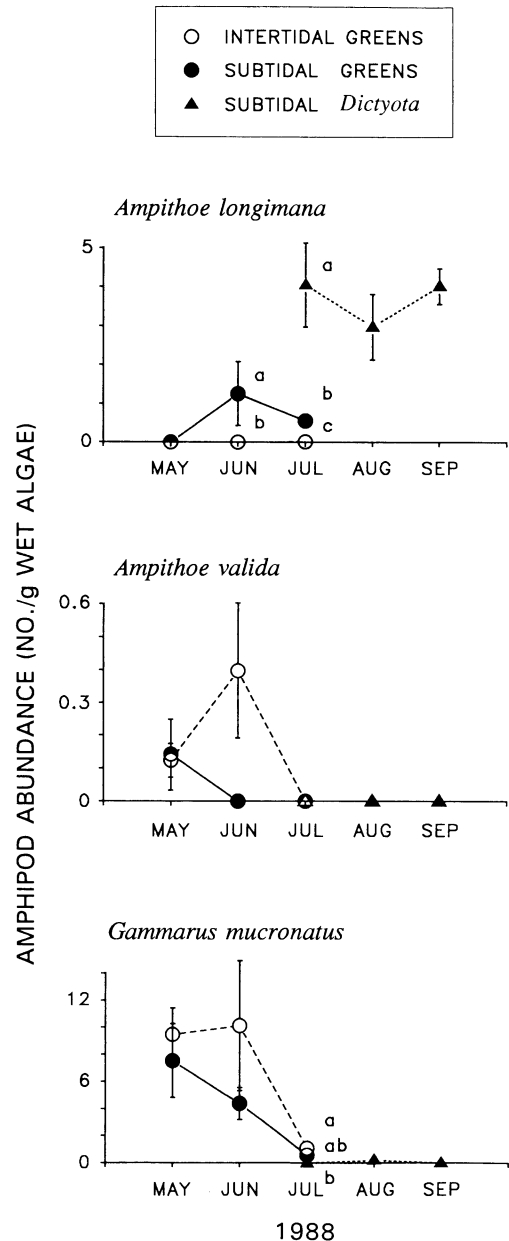

FIG. 2. Abundances of three amphipod species on green seaweeds (mostly *Ulva curvata* and *Enteromorpha linza*) and *Dictyota menstrualis* at Lennoxville Point, North Carolina, USA, during summer 1988. Each symbol represents the mean ± 1 SE of 5–7 samples. Means of zero (i.e., symbols on the x axis) indicate that the plant was present and was sampled but no amphipods of this species were present; absence of a sym- bol in a given month means that the plant was not present, and not sampled (green algae were present only from May to July, and *Dictyota* only from July onward; thus July was the only month in which both algae were present). Means with the same letter in a given month, or no letter, do not differ significantly ($P > 0.05$, *t* test, see *Results: Amphipod field abundances*). Note different scales.
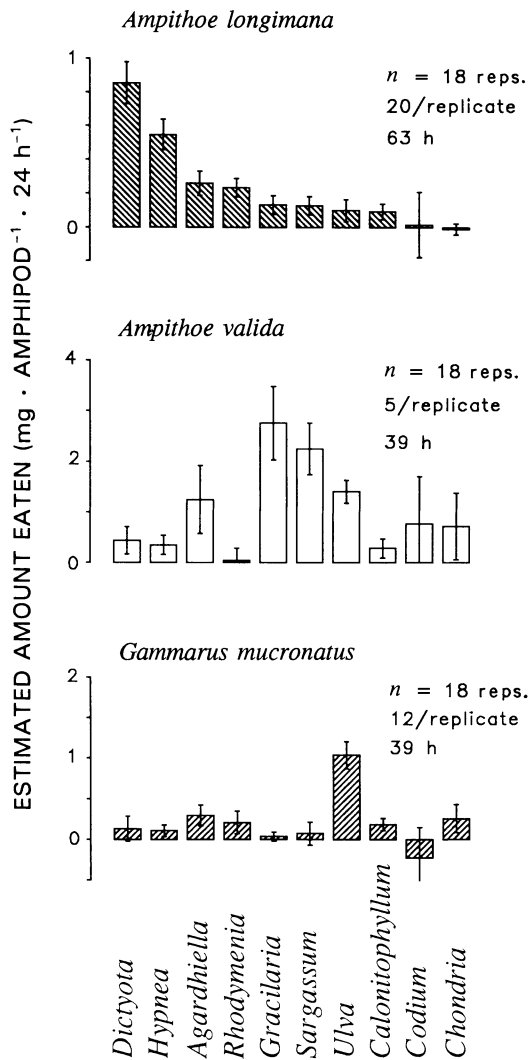
FIG. 3. Estimated algal consumption by three amphipod species when given a choice of 10 seaweeds. All plants were available in excess. Data show means ± 1 SE; estimated amount eaten = mean mass change in n = 18 replicates with herbivores corrected by mean mass change in n = 7 control replicates without herbivores, with SE calculated from the formula for the SE of the difference between two means (Zar 1974). Grazing rates of the three species are not strictly comparable since the duration and number of amphipods per replicate differed among the three assays.

algal cover by August (J. E. Duffy, *personal observation*). *A. longimana* reached its highest abundance on *Dictyota* during this time (Fig. 2). In July, the only month when all three sampled plants were present, *A. longimana* was most abundant on *Dictyota*, significantly less abundant on subtidal greens, and least abundant on intertidal greens ($P < 0.05$, ANOVA and Ryan's Q tests on log-transformed data). After July subtidal greens and all intertidal algae disappeared from the study site. *A. longimana*'s abundance remained high on *Dictyota* through the last sampling period in September.

Ampithoe valida was the least abundant of the three amphipods (Fig. 2). Its abundance was low but similar ($P > 0.100$, t test) on intertidal and subtidal greens in May. In June it remained present on intertidal greens but was absent from subtidal greens (though differences between tidal heights were not significant, $P > 0.100$, t test). *A. valida* was absent from all plants sampled during the following 3 mo (Fig. 2).

Gammarus mucronatus was the most abundant herbivorous amphipod in our samples and showed a distribution similar to that of *A. valida* (Fig. 2). *G. mucronatus* was equally abundant on subtidal and intertidal greens in May ($P > 0.100$, t test) and June ($P > 0.100$, t test on log-transformed data). By the July sampling date, *G. mucronatus* abundance had declined greatly, although it was significantly more abundant on intertidal greens than on *Dictyota* at this time ($P < 0.05$, ANOVA and Games-Howell tests on log-transformed data). *G. mucronatus* remained at very low density in August, when nine individuals were found on *Dictyota*, and was absent from *Dictyota*, the only plant sampled, in September.

Abundances of the three amphipods differed most dramatically on the chemically defended seaweed *Dictyota*. *A. longimana* was by far the most abundant herbivorous amphipod on this plant in all 3 mo (July, August, and September) in which *Dictyota* was sampled ($P < 0.0001$ for each month, ANOVAs on log-transformed data, Fig. 2). Only nine individuals of *G. mucronatus* (out of 1085 total) were found on *Dictyota* during the entire study, whereas 369 out of 410 *A. longimana* were found on *Dictyota*. We found no *A. valida* on *Dictyota* (all 20 *A. valida* in our samples occurred on *Ulva*).

Amphipod feeding preferences

Feeding preferences among macroalgae clearly differed for the three amphipod species (Fig. 3), although we were unable to analyze these preference rankings rigorously because all treatments (i.e., different algal species) were simultaneously available to each replicate group of grazing amphipods and were therefore not independent (Peterson and Renaud 1989). *Ampithoe longimana* consumed primarily *Dictyota menstrualis* and *Hypnea musciformis* (see also Duffy and Hay 1991*b*), which were hardly grazed by the other two amphipods. *A. valida* ate *Gracilaria tikvahiae* and *Sargassum filipendula* most rapidly, and ate several other species in substantial but smaller amounts. *Gammarus mucronatus* ate only *Ulva* sp. in substantial amounts.

Our primary interest was whether the three amphipods ate *Dictyota* when other seaweeds were available. To test this, we compared mass loss of *Dictyota* in control replicates without amphipods to mass loss in replicates with amphipods for each of the three amphipod species. *Dictyota* lost far more mass in the presence of *A. longimana* than in controls, whereas neither

TABLE 1. Feeding by three herbivorous amphipod species on the brown seaweed *Dictyota menstrualis*. Significance of grazing was determined by comparing mass loss of *Dictyota* in the presence vs. absence of amphipods. *P* values are derived from *t* tests (on log-transformed data for *Ampithoe longimana*); *P* values >0.10 are listed as NS (nonsignificant). n = the number of independent, replicate containers in which algal mass change was measured.

| | Mass change of *Dictyota* (mg, mean ± 1 SE) | | |
Amphipod species	Without amphipods ($n = 7$)	With amphipods ($n = 18$)	*P* value
Ampithoe longimana	−0.1 ± 2.2	−42.6 ± 5.8	<0.001
Ampithoe valida	6.6 ± 1.5	3.0 ± 1.7	NS
Gammarus mucronatus	10.3 ± 2.6	7.6 ± 1.7	NS

of the other amphipod species significantly affected mass loss of *Dictyota* (Table 1).

Tests of chemical feeding deterrence

Responses to terpenoid metabolites from *Dictyota* differed substantially among the three amphipod species (Fig. 1) and mirrored their willingness to feed on the plant itself. Dictyol E, the major secondary metabolite in *D. menstrualis* from North Carolina, significantly reduced feeding by both *Ampithoe valida* and *Gammarus mucronatus* at natural concentration, but had no effect on feeding by *A. longimana,* the only one of the three amphipods that feeds on this plant. Similarly, Dictyol B acetate, the major metabolite in *D. ciliolata* from North Carolina, reduced feeding by *A. valida* and was an especially strong deterrent against *G. mucronatus,* but had no effect on *A. longimana* at the same concentration (Fig. 1). Pachydictyol A, which occurs at relatively low concentrations (0.05–0.10% of dry mass) in both species of *Dictyota* in North Carolina, had no significant effect on feeding by any of the three amphipod species at natural concentration (Fig. 1).

Mobility experiments

The tube-building amphipod *Ampithoe longimana* was considerably more sedentary than *Gammarus mucronatus,* which does not build tubes (Fig. 4). Averaging over all treatments, the net movement of amphipods between plants was 5.4 times higher for *G. mucronatus* than for *A. longimana* ($P < 0.0001$, Mann-Whitney U test). On average, 60–69% of *G. mucronatus* individuals moved from the initially occupied plant in all treatments. In fact this estimate is probably conservative since it does not account for amphipods that moved and then returned to the initially occupied plant. Net movement of *G. mucronatus* was unaffected by the presence of visual and chemical cues from predatory pinfish ($P > 0.100$, $F_{1,36} = 0.01$), the plant species initially occupied ($P > 0.100$, $F_{1,36} = 0.98$), or the interaction between these factors ($P > 0.100$, $F_{1,36} = 0.48$, two-way ANOVA on arcsine-transformed data). When the algae and associated *G. mucronatus* were transferred from the settling bowls into the larger plastic containers, *G. mucronatus* immediately began swimming rapidly around the containers, in accord

with our observations of *G. mucronatus* swimming in the field.

Under the same conditions, only 3–18% of *A. longimana* individuals moved to different plants (Fig. 4). These amphipods remained in the tubes they had constructed on the algae and were not observed moving around the containers. Movement by *A. longimana* also was unaffected by fish cues ($P > 0.100$, $F_{1,36} = 1.03$), the plant species initially occupied ($P > 0.100$, $F_{1,36} = 2.40$), or their interaction ($P > 0.100$, $F_{1,36} = 0.33$, two-way ANOVA on arcsine-transformed data).

Predation experiments: laboratory

Our laboratory predation experiments tested the null hypothesis that, when pinfish had equal access to two plants, amphipods occupying those plants experienced equal rates of predation regardless of whether they occupied the plant that was palatable (*Ulva*) or unpalatable (*Dictyota*) to pinfish. We suspected that amphipods might be less susceptible on *Dictyota,* either because they were more cryptic on that plant, or because *Dictyota* interfered with the fish's ability to capture amphipods.

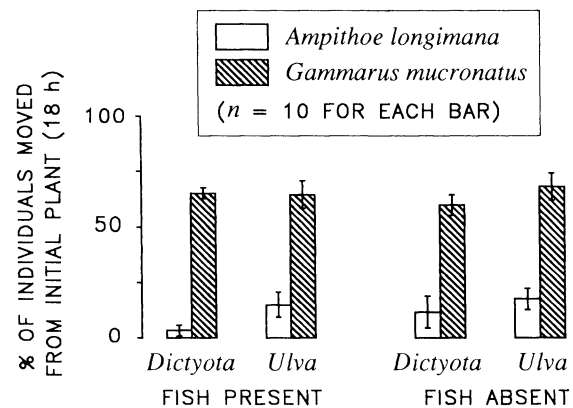We analyzed the results of these experiments as fol-

FIG. 4. Mobilities of two amphipods as a function of the species of alga initially occupied, and presence of visual and chemical cues from a predator, the pinfish *Lagodon rhomboides.* In each replicate container ($n = 10$), 10 amphipods were placed on the initially occupied plant. Histogram bars represent mean ± 1 SE. Statistical analyses are discussed in *Results: Mobility experiments.*

TABLE 2. Results of an experiment examining pinfish (*Lagodon rhomboides*) predation on the amphipod *Ampithoe longimana* occupying two algal substrata. Data are pooled for both days on which the experiment was conducted. After amphipods had been counted at the end of the experiment, the number on *Ulva* was subtracted from the number on *Dictyota* (= D − U) for each replicate (*n* = the number of replicate containers for each treatment). This variable was compared between bagged-fish and free-fish treatments with a *t* test (NS denotes nonsignificance, i.e., *P* > 0.100). All values are expressed as mean ± 1 SE.

	Treatment		
	Bagged fish	Free fish	*P* value
n	24	20	
No. amphipods on *Ulva* (U)	9.0 ± 0.3	2.2 ± 0.8	
No. amphipods on *Dictyota* (D)	10.7 ± 0.2	4.1 ± 0.7	
No amphipods elsewhere	0.4 ± 0.1	0.1 ± 0.1	
% amphipods on *Dictyota*	53 ± 1	71 ± 7	
D − U	1.7 ± 0.5	2.0 ± 0.8	NS

lows. After amphipods had been counted at the end of an experiment, the number of amphipods remaining on *Ulva* in a given replicate was subtracted from the
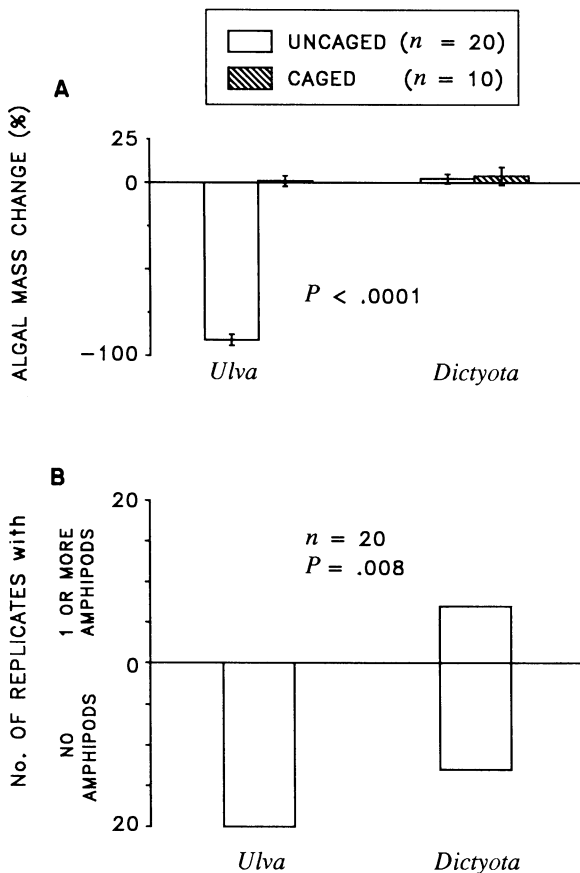

FIG. 5. Results of a field experiment measuring the change in mass of the green seaweed *Ulva* sp. and the brown seaweed *Dictyota menstrualis* (A) and loss of amphipods that had been attached to each alga (B), after 24 h in the field at Lennoxville Point. The *P* value in (A) shows a highly significant effect on algal mass change of the interaction between algal species and caging treatments (two-way ANOVA), confirming that *Ulva* was grazed much more heavily than *Dictyota*. The *P* value in (B) is from Fisher's exact test of the difference between *Ulva* and *Dictyota* in the number of replicate plants retaining at least one attached amphipod at the end of the experiment.

number remaining on *Dictyota* in the same replicate. This variable (*Dictyota* minus *Ulva*, or D − U) was then compared among treatments. If fish ate more amphipods from *Ulva* than from *Dictyota*, then D − U would be significantly greater in the free-fish treatment, where the difference was the combined result of differential predation and amphipod movement, than in the bagged-fish treatment where D − U resulted only from amphipod movement.

When analyzed in this way, there was no significant difference in D − U between the two treatments (*P* > 0.100, *t* test, Table 2). Thus fish ate similar numbers of *A. longimana* from *Dictyota* and *Ulva*. *Gammarus mucronatus* also experienced similar rates of predation on *Dictyota* and *Ulva*; there was no significant difference in D − U between the bagged-fish and free-fish treatments for this amphipod (*P* > 0.100, *t* test, Table 3).

Predation experiments: field

The field predation experiment sought to answer two questions: (1) Do fishes in the field consume the palatable plant *Ulva* more rapidly than the chemically defended *Dictyota*? (2) Are amphipods occupying the palatable *Ulva* more vulnerable to fish predation than those on the unpalatable *Dictyota*? Our experiment provided a positive answer to both questions.

First, in order to verify that our experimental technique was valid, we glued amphipods to algae and measured their loss from the algae after 24 h in a laboratory seawater table. At the end of this period, the number of the original four amphipods remaining on each *Ulva* plant was 2.75 ± 0.20 (mean ± 1 SE), and on *Dictyota* was 2.50 ± 0.22; this difference is not significant (*P* > 0.100, *n* = 20, paired *t* test). The mean mass loss from the plaster blocks in the seawater table was 34.9 ± 8.2%, compared with 22.4 ± 0.9% in the field (*P* > 0.100, *n* = 5, *t* test for unequal variances), indicating that water flow (and the consequent potential for dislodgement of amphipods) was no stronger in the field than in our laboratory seawater table.

When deployed in the field, uncaged pieces of *Ulva* lost nearly all of their mass (mean ± 1 SE = 91.2 ±

3.2%) over 24 h, while pieces of *Dictyota* gained an average of 2.2 ± 2.8%; both species gained mass slightly inside cages (Fig. 5). A two-way ANOVA revealed highly significant effects on algal mass change of caging ($P < 0.0001$, $F_{1,56} = 161$), algal species ($P < 0.0001$, $F_{1,56} = 328$), and the interaction between them ($P < 0.0001$, $F_{1,56} = 148$), confirming that *Ulva* was grazed much more heavily than *Dictyota*.

Most importantly, at the end of the experiment, 7 of 20 replicate *Dictyota* plants still retained at least one amphipod, while no amphipods remained on any of the 20 *Ulva* plants (Fig. 5); thus *A. longimana* had a significantly higher probability of survival on *Dictyota* than on *Ulva* when exposed to the natural assemblage of predators in the field ($P = 0.008$, Fisher's exact test). Given the conspicuousness and leg motion exhibited by the amphipods that were glued to seaweeds, we suspect that the predation on amphipods attached to *Dictyota* in this experiment may have been artificially high relative to predation rates on unfettered amphipods hidden in their tubes.

DISCUSSION

The three sympatric amphipod species we studied showed markedly different responses to terpenoid metabolites from the brown seaweed *Dictyota*. Feeding by *Ampithoe longimana* was unaffected by any of the three compounds tested, whereas both its congener *A. valida* and the more distantly related *Gammarus mucronatus* significantly reduced their feeding in response to two of the same compounds (Fig. 1). The different tolerances of these three amphipods for the pure metabolites mirror their willingness, or lack thereof, to feed on *Dictyota* (Fig. 3, Table 1), and are of similar magnitude to those documented previously among very different types of herbivores, i.e., fishes, sea urchins, amphipods, and polychaetes (Hay et al. 1987a, b, 1988b, c, Paul et al. 1987). These results confirm that secondary chemistry significantly affects herbivore preferences among seaweed species at finer taxonomic scales (among congeneric species of amphipods) as well as at the coarser levels previously described (fish vs. mesograzers). More interestingly, our results suggest that differences among the three amphipod species in food preference and tolerance of secondary metabolites mediate their susceptibility to predators, and consequently their population dynamics in the field.

Functional groups of herbivores?

It is customary, in attempts to make the diversity of nature tractable to ecological analysis, to lump related or ecologically similar species into functional groups that are believed to share important ecological characteristics. This approach has yielded many insights (Littler and Littler 1980, Steneck and Watling 1982, Carpenter 1986); however, it can also obscure important ecological differences among superficially similar species (Paine 1988, Duffy 1990, Duffy and Hay 1991a,

Polis 1991). For example, investigators erecting functional groups of marine herbivores on the basis of feeding apparatus have considered small crustaceans such as amphipods incapable of appreciably damaging fleshy algae (Steneck 1983). While it is undoubtedly true that, as a group, amphipods tend to graze softer algae than fishes or sea urchins, several amphipod species feed on large, rather tough seaweeds, sometimes in preference to structurally simpler forms (Duffy 1990, Duffy and Hay 1991a), and occasionally with dramatic consequences for plant communities (Tegner and Dayton 1987).

The results presented here corroborate previous findings of diverse feeding habits among herbivorous amphipods from three families (Duffy 1990). In contrast to several studies demonstrating similar diets in confamilial (Horn et al. 1982) or congeneric (Coen 1988, Steinberg 1988) herbivores, we found that two amphipods in the genus *Ampithoe* have markedly different feeding preferences (Fig. 2). Since the mandibles of these two species are very similar (see Bousfield 1973: Plates 54 and 55), morphology of feeding structures appears unlikely to explain their divergent food preferences, as it does for some herbivorous mollusks (Steneck and Watling 1982). Instead, the dissimilar responses of the two amphipods to *Dictyota* are apparently mediated by their different tolerances for its secondary metabolites (Fig. 1). These results reemphasize the conclusion (Hay and Steinberg 1992) that ecological effects of seaweed secondary metabolites are highly compound and species specific.

Predation risk, mobility, and tolerance of chemical defenses

The specificity of herbivore responses to seaweed chemical defenses raises questions about what factors are ultimately responsible for these patterns. One of the most pervasive ecological influences on where, when, and what animals eat in the field is risk of predation (Lima and Dill 1990). The frequent compromise of food intake to avoid predation was a major premise for the argument (reviewed by Hay 1992) that fish predation has selected for the ability of many small, sedentary marine herbivores (i.e., mesograzers) to feed on seaweeds that are chemically defended from fish. There is now considerable support for this hypothesis in a variety of taxonomically and geographically diverse systems (Hay et al. 1987a, 1988b, c, 1989, 1990a, b, Duffy and Hay 1991b). In this study we examined two components of this argument: the mechanisms by which association with chemically defended plants reduce predation risk and the suggestion that such associations should be better developed in less mobile herbivores. We consider each of these issues in turn.

First, association with chemically defended plants might reduce a grazer's risk of predation either by (1) providing noxious metabolites that make the grazer distasteful to its own predators, (2) providing cam-

TABLE 3. Results of an experiment examining pinfish (*Lagodon rhomboides*) predation on the amphipod *Gammarus mucronatus* on two algal substrata. Data are pooled for both days on which the experiment was conducted. Analysis and labels as in Table 2.

	Treatment		
	Bagged fish	Free fish	P value
n	36	19	
No. amphipods on *Ulva* (U)	8.9 ± 0.5	4.3 ± 0.7	
No. amphipods on *Dictyota* (D)	9.1 ± 0.5	5.6 ± 0.7	
No. amphipods elsewhere	1.6 ± 0.2	0.4 ± 0.1	
% amphipods on *Dictyota*	46 ± 2	56 ± 5	
D − U	0.2 ± 0.9	1.4 ± 0.7	NS

ouflage, especially for specialist herbivores whose color or morphology renders them cryptic on the plant, or (3) reducing the frequency of visitation by foraging fish and thus the grazer's probability of being detected or indirectly consumed by them. *A. longimana* does not sequester distasteful metabolites from *Dictyota* (Hay et al. 1987*a*), falsifying the first of these hypotheses. As for the second, our laboratory experiments (Tables 2 and 3) showed that amphipods on *Dictyota* were equally susceptible to predation as those on the palatable alga *Ulva* when fish encountered both plants with equal frequency. Our results for these generalist mesograzers contrast with the situation for several specialist mesograzers (Paul and Van Alstyne 1988, Hay et al. 1989, 1990*a, b,* Paul and Pennings 1991) and insects (Bernays 1988, 1989, Bernays and Cornelius 1989) that escape predation either by sequestering noxious metabolites from host plants, or by avoiding detection as a consequence of adaptations in color, morphology, or behavior that interfere with the ability of predators to recognize them when associated with their host plants. Unlike many such specialist herbivores, generalist grazers like *A. longimana* rarely show obvious adaptations that might camouflage them on a specific host plant. For these generalists, therefore, the most likely of the three hypotheses to explain reduced predation in association with defended plants is the third one above, i.e., that fish visit defended plants less often than palatable ones and that as a consequence mesograzers on the former are less frequently detected or consumed incidentally.

Our field experiment (Fig. 5) confirmed that *Ampithoe longimana* was in fact less vulnerable to predation when occupying the chemically defended seaweed *Dictyota* than when on *Ulva,* which is palatable to local omnivorous fishes. To our knowledge, this is the first experimental demonstration that a generalist grazer experiences reduced predation by associating with an unpalatable plant. This is an important result because arguments that predators drive the evolution of association with chemically defended plants (Bernays and Graham 1988, Bernays 1989, Hay 1991*b,* 1992) generally assume, explicitly or implicitly, that generalized feeding habits are ancestral to specialization, and that plant-mediated differences in vulnerability to preda-

tion thus impose selection on the grazers before specific adaptations to a given host plant (such as are common in specialists) evolve. For *A. longimana,* and possibly for other generalist grazers (e.g., Hay et al. 1988*b, c*), association with unpalatable plants appears to provide refuge from predators not as a result of crypticity but via less frequent visitation and/or incidental ingestion by fishes when on unpalatable plants.

Given the severe loss of *Ulva* to fish grazing in our field experiment, the greater vulnerability of amphipods on this plant could have resulted either from incidental ingestion or from enhanced detection, by fish grazing on the plants. We tend to favor the latter explanation because adult pinfish, despite their primarily herbivorous diet (Stoner 1980*b*), prefer amphipods over algae when both are available (Holmlund et al. 1990). In either case, the patterns of amphipod abundance in the field (Fig. 2) support the conclusion from our experiment that mesograzers occupying palatable vs. chemically defended seaweeds are differentially susceptible to predation, as well as its corollary that plant-mediated differences in predation risk contribute to the different seasonal population dynamics of the three amphipods. In coastal North Carolina, inshore fish communities are dominated by omnivorous sparids that eat both seaweeds and small invertebrates (Stoner 1980*b,* Darcy 1985). Particularly abundant are pinfish, which can comprise nearly 100% of total fishes during spring and summer (Adams 1976*a,* Nelson 1979*a*) and feed heavily on amphipods (Adams 1976*b,* Stoner 1980*b*). Thus fish predation on amphipods increases from near zero in winter, when these fish are offshore, to high in late summer.

We conducted our study at Lennoxville Point in part because of its simplicity: *Ulva* and *Dictyota* were the most abundant algae (estimated at 60–90% of algal cover) there during the spring and summer, respectively, and these algae clearly differed in their susceptibility to fish grazing (Fig. 5). Thus, if plant-mediated differences in vulnerability are important in the field, *A. longimana,* which preferentially ate *Dictyota* (Fig. 3, Table 1) and was indifferent to its secondary metabolites (Fig. 1), should be less strongly affected by predation than the other two amphipods. The patterns of amphipod abundance support this hypothesis. Den-

sity of *A. longimana* increased through the summer and peaked on *Dictyota* (Fig. 2) at a time when omnivorous fishes are large and abundant (Adams 1976*a*, Darcy 1985). In contrast, over the same summer period of high fish abundance, *A. valida* and *G. mucronatus,* both of which occupied and fed on plants that were palatable to pinfish, declined to local extinction (Fig. 2).

We believe that plant-mediated differences in susceptibility to predation among the three amphipods provide a strong explanation for these distribution patterns. We can envision two other possibilities, however. First, *A. valida* and *G. mucronatus* may decline in late summer as a result of intrinsic life history characteristics that are unrelated to predation. This explanation is potentially an important part of the story for *A. valida,* which we have found only during a brief period in the spring over several years of collecting in a variety of habitats. It is less likely to explain the summer disappearance of *G. mucronatus,* which thrives and reproduces in our laboratory seawater system during late summer. Second, the disappearance of *A. valida* and *G. mucronatus* in summer may result from emigration to other plants or habitats not sampled here. We consider this improbable for several reasons. As mentioned above, we have not found *A. valida* anywhere during the summer. More importantly, we chose the Lennoxville Point study site, among other reasons, because *Ulva* and *Dictyota* were the dominant plants there during the period of interest. Thus there were few other plants available to amphipods at this site. It is also conceivable that the *G. mucronatus* population migrated en masse to another habitat during the summer, but we know of no evidence for such behavior in benthic amphipods.

Finally, the hypothesis that *A. longimana*'s persistence results in part from refuge on an unpalatable seaweed is also supported by its abundance patterns at a nearby rock jetty supporting a more diverse flora. At that site, density of *A. longimana* declined throughout the summer on a seaweed that is palatable to fishes, while remaining unchanged or increasing on two seaweeds that are unpalatable to fishes (Holmlund et al. 1990). At the same site in a different year, *A. longimana* achieved higher densities on two seaweeds that are unpalatable to fishes than on three palatable species (Duffy and Hay 1991*b*). Taken together, we believe these data are most consistent with the hypothesis that fish predation is the primary cause of differences in persistence among the three amphipods we studied.

The second hypothesis we addressed in this study was that association with chemically defended plants should be better developed in less mobile herbivores. We assessed this idea by comparing three herbivorous amphipod species, with the aim of minimizing the large differences in size, ecology, and phylogenetic relatedness inherent in our earlier comparisons of fishes vs. small invertebrates. These problems have not been

completely eliminated from the present study, however. Specifically, *Ampithoe longimana* and *Gammarus mucronatus,* the two better studied species, probably are not very closely related within the Amphipoda. Although relationships among the families of amphipods are poorly understood, differences in mobility between *Ampithoe* and *Gammarus* are probably fairly ancient, as the tube-dwelling habit (and presumably low mobility) characterizes not only the genus *Ampithoe* but the entire superfamily Corophioidea that includes it (Barnard and Karaman 1991). Indeed, it is precisely because of this conservatism of mobility that we were forced to focus our comparison on members of different families, rather than on sister species. This example illustrates the difficulty, common to comparative biology in general, of determining whether extant character states, such as the differences in food choice among the amphipods studied here, have been molded by current selection pressures or are phylogenetic relics of ancient splitting events.

Despite these caveats, the phylogenetic relation between *Ampithoe* and *Gammarus* is clearly far closer than that between fishes and any of the mesograzers compared in previous studies. Thus, while we could not compare sister species, our comparison of *A. longimana* and *G. mucronatus* controlled for phylogeny more rigorously than any of the previous comparisons and yielded the same result, i.e., that the less mobile herbivore is most tolerant of seaweed chemical defenses. Moreover, the two amphipods are also much more similar in size and other aspects of ecology than are fishes and grazing invertebrates. We consider the consistency of results from these different studies reasonably robust (albeit still circumstantial) support for an important correlation between mobility and food choice in herbivores.

We have argued that low mobility magnifies the selective advantage to small grazers of association with chemically defended plants and, over evolutionary time, for their ability to eat them. The conservatism of mobility within the Amphipoda, and the contrasting diversity in feeding behavior among congeners (Fig. 3, Duffy 1990), supports the view that mobility constrained the evolution of food choice, rather than vice versa, in these amphipods. The hypothesized importance of mobility is due in part to the tight link between habitat and food for sedentary animals, and specifically the difficulty of safely foraging away from the host plant on which they are sheltering. Low mobility may also provide a mechanism for enhanced survival of sedentary mesograzers like the tube-dwelling *A. longimana* in the face of intense predation. Predatory fishes generally orient visually, and prey movement is an important cue in their feeding (Kislalioglu and Gibson 1976, Main 1985). Whereas the tube-dwelling *A. longimana* was cryptic and moved little in our experiments, *G. mucronatus* was very active regardless of the presence or absence of predatory pinfish (Fig. 4). This

conspicuous activity, together with its association with palatable plants, may help explain the rapid decline of *G. mucronatus* when fishes became abundant. Similarly, in several other benthic amphipods, activity increases losses to predation (Bethel and Holmes 1977, Russo 1987, Sudo and Azeta 1992), whereas sedentary, tube-dwelling species are less vulnerable (Nagle 1968, Nelson 1979*b*, Stoner 1982).

In summary, of the two amphipod species for which most data are available, the less mobile species *Ampithoe longimana* is more tolerant of *Dictyota* chemical defenses than the more mobile *Gammarus mucronatus*, and both experiments and distributional data suggest that association with *Dictyota* reduces *A. longimana*'s risk of predation. This pattern parallels a similar and well-documented trend (Duffy and Hay 1990, Hay and Steinberg 1992) at a coarser taxonomic level, i.e., the frequent tolerance by small invertebrate grazers of seaweed chemical defenses that deter larger, mobile fishes. The concordance of these patterns at both coarse and fine taxonomic scales supports the idea that general features of an animal's ecology, such as mobility and susceptibility to predation, can be useful predictors of food choice and community interactions.

LITERATURE CITED

Adams, S. M. 1976*a*. The ecology of eelgrass (*Zostera marina* L.) fish communities. I. Structural analysis. Journal of Experimental Marine Biology and Ecology 22:269–291.

———. 1976*b*. Feeding ecology of eelgrass fish communities. Transactions of the American Fisheries Society 105:514–519.

Barnard, J. L., and G. S. Karaman. 1991. The families and genera of marine gammaridean Amphipoda (except marine gammaroids). Records of the Australian Museum, Supplement 13 (parts 1 and 2):1–866.

Bernays, E. A. 1988. Host specificity in phytophagous insects: selection pressure from generalist predators. Entomologia Experimentalis et Applicata 49:131–140.

———. 1989. Host range in phytophagous insects: the potential role of generalist predators. Evolutionary Ecology 3:299–311.

Bernays, E. A., and M. L. Cornelius. 1989. Generalist caterpillar prey are more palatable than specialists for the generalist predator *Iridomyrmex humilus*. Oecologia 79:427–430.

Bernays, E. A., and M. Graham. 1988. On the evolution of host specificity in phytophagous arthropods. Ecology 69:886–892.

Bethel, W. M., and J. C. Holmes. 1977. Increased vulnerability to predation owing to altered behavior induced by larval acanthocephalans. Canadian Journal of Zoology 55:110–115.

Bousfield, E. L. 1973. Shallow-water gammaridean Amphipoda of New England. Cornell University Press, Ithaca, New York, USA.

Brawley, S. H. 1992. Mesoherbivores. Pages 235–263 *in* D. M. John, S. J. Hawkins, and J. H. Price, editors. Plant-animal interactions in the marine benthos. Systematics Association Special Volume 46.

Carpenter, R. C. 1986. Partitioning herbivory and its effects on coral reef algal communities. Ecological Monographs 56:345–363.

Carr, W. E. S., and C. A. Adams. 1973. Food habits of juvenile marine fishes occupying seagrass beds in the estuarine zone near Crystal River, Florida, USA. Transactions of the American Fisheries Society 102:511–540.

Coen, L. D. 1988. Herbivory by Caribbean majid crabs: feeding ecology and plant susceptibility. Journal of Experimental Marine Biology and Ecology 122:257–276.

Conlan, K. E., and E. L. Bousfield. 1982. Studies on amphipod crustaceans of the northeastern Pacific region. I. 2. Family Ampithoidae. National Museums of Canada Publications in Biological Oceanography 10:41–75.

Darcy, G. H. 1985. Synopsis of biological data on the pinfish, *Lagodon rhomboides* (Pisces: Sparidae). National Oceanic and Atmospheric Administration Technical Reports, National Marine Fisheries Service Circular 19.

Day, R. W., and G. P. Quinn. 1989. Comparisons of treatments after an analysis of variance in ecology. Ecological Monographs 59:433–463.

Duffy, J. E. 1990. Amphipods on seaweeds: partners or pests? Oecologia 83:267–276.

Duffy, J. E., and M. E. Hay. 1990. Seaweed adaptations to herbivory. BioScience 40:368–376.

Duffy, J. E., and M. E. Hay. 1991*a*. Amphipods are not all created equal: a reply to Bell. Ecology 72:354–358.

Duffy, J. E., and M. E. Hay. 1991*b*. Food and shelter as determinants of food choice by an herbivorous marine amphipod. Ecology 72:1286–1298.

Faulkner, D. J. 1987. Marine natural products. Natural Products Reports 4:539–576.

Faulkner, D. J., and M. T. Ghiselin. 1983. Chemical defense and the evolutionary ecology of dorid nudibranchs and some other opisthobranch gastropods. Marine Ecology Progress Series 13:295–301.

Fredette, T. J., and R. J. Diaz. 1986. Life history of *Gammarus mucronatus* Say (Amphipoda: Gammaridea) in warm temperate estuarine habitats, York River, Virginia. Journal of Crustacean Biology 6:57–78.

Hay, M. E. 1991*a*. Fish-seaweed interactions on coral reefs: effects of herbivorous fishes and adaptations of their prey. Pages 96–119 *in* P. F. Sale, editor. The ecology of fishes on coral reefs. Academic Press, New York, New York, USA.

———. 1991*b*. Marine-terrestrial contrasts in the ecology of plant chemical defenses against herbivores. Trends in Ecology and Evolution 6:362–365.

———. 1992. The role of seaweed chemical defenses in the evolution of feeding specialization and in the mediation of complex interactions. Pages 93–118 *in* V. J. Paul, editor. Ecological roles of marine natural products. Comstock, Ithaca, New York, USA.

Hay, M. E., J. E. Duffy, and W. Fenical. 1988*a*. Seaweed chemical defenses: among-compound and among-herbivore variance. Proceedings of the Sixth International Coral Reef Symposium 3:43–48.

Hay, M. E., J. E. Duffy, and W. Fenical. 1990*a*. Host-plant specialization decreases predation on a marine amphipod: a herbivore in plant's clothing. Ecology 71:733–743.

Hay, M. E., J. E. Duffy, W. Fenical, and K. Gustafson. 1988*b*.

Chemical defense in the seaweed *Dictyopteris delicatula*: differential effects against reef fishes and amphipods. Marine Ecology Progress Series **48**:185–192.

Hay, M. E., J. E. Duffy, V. J. Paul, P. E. Renaud, and W. Fenical. 1990b. Specialist herbivores reduce their susceptibility to predation by feeding on the chemically defended seaweed *Avrainvillea longicaulis*. Limnology and Oceanography **35**:1734–1743.

Hay, M. E., J. E. Duffy, C. A. Pfister, and W. Fenical. 1987a. Chemical defense against different marine herbivores: are amphipods insect equivalents? Ecology **68**:1567–1580.

Hay, M. E., W. Fenical, and K. Gustafson. 1987b. Chemical defense against diverse coral-reef herbivores. Ecology **68**: 1581–1591.

Hay, M. E., J. R. Pawlik, J. E. Duffy, and W. Fenical. 1989. Seaweed–herbivore–predator interactions: host-plant specialization reduces predation on small herbivores. Oecologia **81**:418–427.

Hay, M. E., P. E. Renaud, and W. Fenical. 1988c. Large mobile versus small sedentary herbivores and their resistance to seaweed chemical defenses. Oecologia **75**:246–252.

Hay, M. E., and P. D. Steinberg. 1992. The chemical ecology of plant-herbivore interactions in marine versus terrestrial communities. Pages 371–413 *in* G. A. Rosenthal and M. R. Berenbaum, editors. Herbivores: their interactions with secondary plant metabolites. Second edition. Volume II. Evolutionary and ecological processes. Academic Press, New York, New York, USA.

Holmlund, M. B., C. H. Peterson, and M. E. Hay. 1990. Does algal morphology affect amphipod susceptibility to fish predation? Journal of Experimental Marine Biology and Ecology **139**:65–83.

Horn, M. H., S. N. Murray, and T. W. Edwards. 1982. Dietary selectivity in the field and food preferences in the laboratory for two herbivorous fishes (*Cebidichthys violaceus* and *Xiphister mucosus*) from a temperate intertidal zone. Marine Biology **67**:237–246.

Karuso, P. 1987. Chemical ecology of the nudibranchs. Pages 31–60 *in* P. J. Scheuer, editor. Bioorganic marine chemistry. Volume 1. Springer, New York, New York, USA.

Kislalioglu, M., and R. N. Gibson. 1976. Some factors governing prey selection by the 15-spined stickleback, *Spinachia spinachia* (L.). Journal of Experimental Marine Biology and Ecology **25**:159–169.

Lewis, F. G., III. 1987. Crustacean epifauna of seagrass and macroalgae in Apalachee Bay, Florida, USA. Marine Biology **94**:219–229.

Lima, S. L., and L. M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology **68**:619–640.

Littler, M. M., and D. S. Littler. 1980. The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional form model. American Naturalist **116**:25–44.

Main, K. L. 1985. The influence of prey identity and size on selection of prey by two marine fishes. Journal of Experimental Marine Biology and Ecology **88**:145–152.

Muus, B. J. 1968. A field method for measuring "exposure" by means of plaster balls. Sarsia **34**:61–68.

Nagle, J. S. 1968. Distribution of the epibiota of macrobenthic plants. Publications of the Institute of Marine Science, University of Texas **13**:105–144.

Nelson, W. G. 1979a. An analysis of structural pattern in an eelgrass (*Zostera marina* L.) amphipod community. Journal of Experimental Marine Biology and Ecology **39**: 231–264.

———. 1979b. Experimental studies of selective predation on amphipods: consequences for amphipod distribution and abundance. Journal of Experimental Marine Biology and Ecology **38**:225–245.

Paine, R. T. 1988. Food webs: road maps of interactions or grist for theoretical development? Ecology **69**:1648–1654.

Paul, V. J., M. E. Hay, J. E. Duffy, W. Fenical, and K. Gustafson. 1987. Chemical defense in the seaweed *Ochtodes secundiramea* (Montagne) Howe (Rhodophyta): effects of its monoterpenoid components upon diverse coral-reef-herbivores. Journal of Experimental Marine Biology and Ecology **114**:249–260.

Paul, V. J., and S. C. Pennings. 1991. Diet-derived chemical defenses in the sea hare *Stylocheilus longicauda* (Quoy et Gaimard 1824). Journal of Experimental Marine Biology and Ecology **151**:227–243.

Paul, V. J., and K. L. Van Alstyne. 1988. The use of ingested algal diterpenoids by *Elysia halimedae* Macnae (Opisthobranchia: Ascoglossa) as antipredator defenses. Journal of Experimental Marine Biology and Ecology **119**:15–29.

Pawlik, J. R., M. R. Kernan, T. F. Molinski, M. K. Harper, and D. J. Faulkner. 1988. Defensive chemicals of the Spanish dancer nudibranch *Hexabranchus sanguineus* and its egg ribbons: macrolides derived from a sponge diet. Journal of Experimental Marine Biology and Ecology **119**: 99–109.

Peterson, C. H., and P. E. Renaud. 1989. Analysis of feeding preference experiments. Oecologia **80**:82–86.

Polis, G. A. 1991. Complex trophic interactions in deserts: an empirical critique of food web theory. American Naturalist **138**:123–155.

Russo, A. R. 1987. Role of habitat complexity in mediating predation by the gray damselfish *Abudefduf sordidus* on amphipods. Marine Ecology Progress Series **36**:101–105.

Schneider, C. W., and R. B. Searles. 1991. Seaweeds of the southeastern United States. Duke University Press, Durham, North Carolina, USA.

Smith, G. A., J. S. Nickels, W. M. Davis, R. F. Martz, R. H. Findlay, and D. C. White. 1982. Perturbations in the biomass, metabolic activity, and community structure of the estuarine detrital microbiota: resource partitioning in amphipod grazing. Journal of Experimental Marine Biology and Ecology **64**:125–143.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. W. H. Freeman, New York, New York, USA.

Steinberg, P. D. 1988. Effects of quantitative and qualitative variation in phenolic compounds on feeding in three species of marine invertebrate herbivores. Journal of Experimental Marine Biology and Ecology **120**:221–237.

Steneck, R. S., and L. Watling. 1982. Feeding capabilities and limitations of herbivorous molluscs: a functional group approach. Marine Biology **68**:299–319.

Stoner, A. W. 1979. Species-specific predation on amphipod Crustacea by the pinfish *Lagodon rhomboides*: mediation by macrophyte standing crop. Marine Biology **55**:201–207.

———. 1980a. Abundance, reproductive seasonality and habitat preferences of amphipod crustaceans in seagrass meadows of Apalachee Bay, Florida. Contributions in Marine Science **23**:63–77.

———. 1980b. Feeding ecology of *Lagodon rhomboides* (Pisces: Sparidae): variation and functional responses. Fisheries Bulletin **78**:337–352.

———. 1982. The influence of benthic macrophytes on the foraging behavior of pinfish, *Lagodon rhomboides* (Linnaeus). Journal of Experimental Marine Biology and Ecology **58**:271–284.

Sudo, H., and M. Azeta. 1992. Selective predation on mature male *Byblis japonicus* (Amphipoda: Gammaridea) by the barface cardinalfish, *Apogon semilineatus*. Marine Biology **114**:211–217.

Taylor, W. R. 1960. Marine algae of the eastern tropical and subtropical coasts of the Americas. University of Michigan Press, Ann Arbor, Michigan, USA.

Tegner, M. J., and P. K. Dayton. 1987. El Nino effects on

southern California kelp forest communities. Advances in Ecological Research 17:243–279.

Van Dolah, R. F. 1978. Factors regulating the distribution and population dynamics of the amphipod *Gammarus palustris* in an intertidal salt marsh community. Ecological Monographs 48:191–217.

Vince, S., I. Valiela, N. Backus, and J. M. Teal. 1976. Predation by the salt marsh killifish *Fundulus heteroclitus* (L.) in relation to prey size and habitat structure: consequences for prey distribution and abundance. Journal of Experimental Marine Biology and Ecology 23:255–266.

Williams, A. B., and K. H. Bynum. 1972. A ten-year study of meroplankton in North Carolina estuaries: amphipods. Chesapeake Science 13:175–192.

Young, D. K., and M. W. Young. 1978. Regulation of species densities of seagrass-associated macrobenthos: a field experimental study of predation. Journal of Marine Research 36:569–593.

Zar, J. H. 1974. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, New Jersey, USA.

Zimmerman, R., R. Gibson, and J. Harrington. 1979. Herbivory and detritivory among gammaridean amphipods from a Florida seagrass community. Marine Biology 54: 41–47.