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ALLELOPATHIC INTERACTIONS BETWEEN SPONGES ON A TROPICAL REEF

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Abstract. Competition for space among organisms on tropical reefs has often been hypothesized to be mediated by allelopathic interactions, but the secondary metabolites involved in these interactions have rarely been identified. On Guam, the sponge *Dysidea* sp. overgrows the sponge *Cacospongia* sp. and causes necrosis. Using field assays, we tested the effects of crude organic extracts and a major sesquiterpene isolated from *Dysidea,* 7-deacetoxyolepupuane, on *Cacospongia* and on the production of the major terpenoid metabolites scalaradial and desacetylscalaradial by *Cacospongia.* We also tested whether the amounts of organic compounds produced by *Dysidea* differed in the presence or absence of *Cacospongia.* To determine whether 7-deacetoxyolepupuane had another ecological function, we tested the compound as a predator deterrent. In field experiments, crude extracts of *Dysidea* and pure 7-deacetoxyolepupuane both caused necrosis in *Cacospongia* when they were incorporated into agar strips and placed in contact with *Cacospongia* for 7 d. Organic extract, scalaradial, and desacetylscalaradial concentrations in *Cacospongia* were not affected by the overgrowth of *Dysidea.* However, a greater quantity of organic extract (but not scalaradial or desacetylscalaradial) was found in portions of *Cacospongia* covered by agar strips containing *Dysidea* organic extracts than in portions of *Cacospongia* covered by control agar strips. The production of 7-deacetoxyolepupuane by *Dysidea* occurring on rock substrates and on *Cacospongia* did not differ; thus, the production of this compound is not induced by the presence of competitors. In addition to its role in competition, 7-deacetoxyolepupuane deterred predation by a spongivorous fish, illustrating the multiple ecological roles that a single secondary metabolite may play.

Key words: allelopathy; Cacospongia; *chemical defense;* Dysidea; *interspecific competition;* Pomacanthus imperator; *predation; reef; sponge.*

INTRODUCTION

Predation and competition for space are among the most important processes that influence the structure of marine benthic communities (Menge and Farrell 1989, Paine 1994). Predation is a major source of mortality for benthic organisms and, in the absence of an escape response, it must either be tolerated or reduced (Lubchenco and Gaines 1981). Competition between neighboring organisms for the acquisition and maintenance of space can be especially intense when substrate availability is limited (Jackson and Buss 1975, Branch 1984). Opportunistic life cycles, habitat selection, differential growth rates, and physical or chemical defenses may assist in the fight against both predators and competitors (Connell 1975, Brosnan 1992). Recent studies of the chemical ecology of benthic organisms have provided new insights on the roles that secondary metabolites may play in these processes (Bakus et al. 1986, Paul 1992, Hay 1996).

While predator–prey interactions have been studied extensively from a chemical ecology perspective (Paul 1992, Pawlik 1993, Hay 1996), the roles of secondary metabolites in competition for space have received much less attention (Jackson 1977, Russ 1982, Sammarco et al. 1983, De Nys et al. 1991, Hay 1996). Secondary metabolites from a wide variety of benthic organisms have been reported to deter feeding by natural predators (Paul 1992, Pawlik 1993, Hay 1996). Although competitive interactions among sessile invertebrates have long been hypothesized to be mediated by the production of allelopathic secondary metabolites (Goodbody 1961, Bakus 1971, Jackson and Buss 1975, Porter and Targett 1988), the compounds responsible for these competitive effects have rarely been isolated and identified (Sullivan et al. 1983, De Nys et al. 1991). The activities of allelochemicals have been implied by several studies of spatial competition, including the prevention of settlement by competitors (Bak and Borsboom 1984, Thompson et al. 1985, Davis et al. 1989, Woodin et al. 1993, Maida et al. 1995, Schmitt et al. 1995, Slattery et al. 1995) and the exertion of toxic effects on nearby organisms (Sullivan et al. 1983, Leone et al. 1995). Compounds that influence spatial competition need not be limited to this single ecological function. In soft corals, terpenoid compounds may play roles in spatial competition, predator deterrence, and

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antifouling (Sammarco and Coll 1990, Sammarco 1996), while diterpene alcohols produced by the brown seaweed *Dictyota mentrualis* deter predators and inhibit fouling (Schmitt et al. 1995).

Sponges have yielded a large diversity of biologically active compounds (Thompson et al. 1985, Faulkner 1996), which may function as deterrents to predation (Bakus and Green 1974, Schulte and Bakus 1992, McClintock et al. 1994, Pennings et al. 1994, Pawlik et al. 1995, Wulff 1995), as antifoulants (Davis et al. 1989, 1991, Sears et al. 1990), or in competition for space (Ayling 1983, Sullivan et al. 1983, Porter and Targett 1988, Turon et al. 1996*a*, *b*). Sponges containing substances that are biologically active in laboratory toxicity assays are rarely overgrown (Thompson 1985). Sullivan et al. (1983) identified a secondary metabolite, siphonodictidine, which produced allelopathic effects in laboratory assays but performed no field manipulations. Walker et al. (1985) measured the rates of exudation of two potential allelochemicals in the field, but did not determine the effects of these compounds in the field. Other studies of sponge competition have made field manipulations (Porter and Targett 1988, Bingham and Young 1991, Turon et al. 1996*b*), but have not characterized the compounds involved in these interactions.

Since both competition for space and predation strongly affect the structure of tropical reef communities, these communities present numerous opportunities to examine multiple ecological roles of secondary metabolites. We focused our study on the compounds involved in spatial competition and predator deterrence in the sponge *Dysidea* sp., which causes necrosis in sponges that it overgrows, and the sponge *Cacospongia* sp., the sponge most often found overgrown by *Dysidea* sp. on Guam. We used field-based assays to ask the following questions: (1) Do crude extracts and a pure compound isolated from *Dysidea* sp. cause necrosis in *Cacospongia* sp. in the field? (2) Do secondary metabolites produced by *Cacospongia* sp. change in concentration in the presence of *Dysidea* sp.? (3) Do compounds produced by *Dysidea* sp. change in concentration in the presence of *Cacospongia* sp.? (4) Since compounds produced by *Cacospongia* are known to deter predation, do compounds produced by *Dysidea* sp. also affect predators?

Methods

Study organisms

Dysidea sp. (Order Dictyoceratida, Family Dysideidae; hereafter referred to as *Dysidea*) is an undescribed species of encrusting sponge with a reticulate skeleton supporting delicate soft tissues (P. Bergquist and M. Kelly-Borges, *personal communication*; voucher deposited at the British Museum of Natural History, London, BMNH 1997.5.13.1). On Guam, *Dysidea* is found on Sponge Mound, a pinnacle in Apra Harbor, at depths of 20–30 m. At Sponge Mound, *Dysidea* occurs primarily in the presence of *Cacospongia* sp. (hereafter referred to as *Cacospongia*). This *Cacospongia* (Order Dictyoceratida, Family Thorectidae; identified as *Hyrtios erecta* in Rogers and Paul 1991) is an undescribed species that forms thick, erect lobes and is extremely compressible with a highly conulose surface (M. Kelly-Borges, *personal communication*; voucher: BMNH 1995.6.22.23). *Cacospongia* is found in two large areas on the eastern and western sides of Sponge Mound. The secondary metabolites produced by *Cacospongia* on Sponge Mound have been described previously (Rogers and Paul 1991). Two of these metabolites, scalaradial and scalarin, deter feeding by predators at relatively high concentrations (Rogers and Paul 1991, Avila and Paul 1997).

We first found *Dysidea* when we observed it overgrowing several colonies of *Cacospongia* on the western side of the mound. Areas of *Cacospongia* underneath *Dysidea* were often necrotic, clearly recognized by a loss of the ectosome, revealing the underlying tan-colored portion of the sponge. These areas lacked the dark pigmentation of surrounding cells and appeared softer than uncovered areas. In a pilot study, we determined that this necrosis was most likely not mediated by a waterborne chemical cue. Three 60-mL plastic syringes were filled with seawater taken from <1 cm above undisturbed *Dysidea* in the field. The seawater-filled syringes were returned to the laboratory where they were extracted with an equal volume of ethyl acetate in a separatory funnel. The ethyl acetate was removed by rotary evaporation and the extract compared to *Dysidea* organic extract using thin-layer chromatography (TLC). No *Dysidea* secondary metabolites were detected in the seawater extracts. However, we noticed that upon contact or handling, *Dysidea* rapidly exuded darkly colored compounds into seawater. Therefore, we hypothesized that any allelopathic interactions would be mediated by contact between the two sponges and not by waterborne allelochemicals.

Distribution of Dysidea

To determine if *Dysidea* was more frequently found on *Cacospongia* than other potential substrates on Sponge Mound, we determined what substrates were available on the mound. We sampled 20 points spaced 0.5 m apart along a depth contour at depths of 21, 23, 25, 27, and 29 m, for a total of 100 points on the western side of the mound. At each point we noted the substrate available. We also looked for *Dysidea* in this same area and noted the substrate on which each colony occurred. The distributions of the available substrates and the substrates on which *Dysidea* occurred were compared with a G test for goodness-of-fit, using William's correction (Sokal and Rohlf 1995).

To determine what proportion of *Cacospongia* on Sponge Mound was attacked by *Dysidea,* we counted the number of *Cacospongia* colonies on the eastern and

western sides of the mound that were covered by *Dysidea* or not. Two divers swam over each area until each diver had encountered ~25 *Cacospongia* colonies.

Response of Cacospongia *to overgrowth by* Dysidea

Field observations.—To determine if contact with *Dysidea* caused variation in the production of *Cacospongia* metabolites, we collected five colonies of *Cacospongia* that had some portion covered by *Dysidea* and five that were not covered. Covered or uncovered areas were then dissected from the same basal regions of the *Cacospongia* colonies.

Cacospongia fragments were exhaustively extracted in a 1:1 mixture of dichloromethane : methanol. These crude extracts were dried in a rotary evaporator and weighed. Extracted *Cacospongia* fragments were dried at 50°C for 48 h and weighed. Percentage yield of crude extracts was calculated as the mass of extract divided by the total mass of crude extract and dried fragments, multiplied by 100. The crude extracts were dissolved in 25% ethyl acetate : hexanes, filtered through short silica gel columns to remove salts and polar materials, dried, and weighed again to determine filtered masses. The percentage yield of filtered extracts was calculated as the mass of filtered extract divided by the total mass of crude extract and dried fragments. Twenty-five percent ethyl acetate : hexanes was added to each filtered extract to reach equal concentrations in each sample (1 mg/20 μL). Each sample was then injected into an analytical high performance liquid chromatograph (HPLC, Econosphere silica 5 μ [Alltech Associates Incorporated, Deerfield, Illinois], 250 × 4.6 mm column, 25% ethyl acetate : hexanes, 10 μL injections, refractive index detection). Amounts of the two major metabolites, scalaradial and desacetylscalaradial, were quantified using standard curves of known quantities of these compounds. The percentage dry masses of scalaradial and desacetylscalaradial were calculated for each sample as the amount of compound divided by the total mass of crude extract and dried fragments. The percentage yield of crude and filtered extracts and the percentage dry mass of scalaradial and desacetylscalaradial were compared between covered and uncovered areas using paired *t* tests.

Field manipulation with crude extract.—To determine if *Dysidea* metabolites play a role in the development of necrosis in *Cacospongia,* we incorporated crude extracts of *Dysidea* into agar strips and placed them in contact with *Cacospongia* colonies in the field. The wet volume of colonies of *Dysidea* collected at Sponge Mound was measured, these colonies were exhaustively extracted, and the percentage dry mass yield of crude extract was calculated as previously described. The percentage wet volume yield of crude extract was calculated as the mass of extract divided by the wet volume of the colonies, multiplied by 100.

We suspended crude extracts from *Dysidea* in an agar strip in the same volumetric proportion as found in the sponge. From the above measurements, we found a crude extract yield of 2.82 g in 110 mL of *Dysidea* (25.6 mg/mL). To make the agar strips, we modified a method we have used to make food strips for feeding assays (described in Hay et al. 1994, Lumbang and Paul 1996) that is similar to the method described by Henrickson and Pawlik (1995) for conducting antifouling assays. To obtain 100 mL of agar strips we mixed 4 g of agar with 93 mL of water, boiled the mixture, then added 2.56 g of *Dysidea* crude extract dissolved in dichloromethane after the mixture began to cool. We added an equal volume of dichloromethane to control strips. The mixture was then spread into Formica molds backed with fiberglass window screening to form four treatment and four control strips of agar measuring 2.6 by 25 cm. The strips were allowed to cool and harden onto the window screening before being removed from the molds and cut in half, for a total of eight treatment and eight control strips.

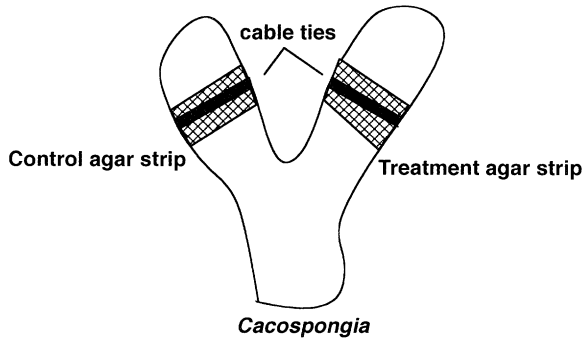

FIG. 1. Agar strips were placed around two branches of a single *Cacospongia* colony. One branch was exposed to a treated strip and the other to a control strip.

We then took the agar strips to Sponge Mound and placed them around erect *Cacospongia* colonies that were branched, so that one branch of the colony was partially covered by a control strip and the other by a treated strip (Fig. 1). The strips were fastened around the branches using plastic cable ties. After 7 d, the *Cacospongia* colonies were retrieved from Sponge Mound. No loss of agar was observed from any of the strips. To determine if the crude extract was still present in the treated strips, the strips were extracted with dichloromethane and analyzed by thin-layer chromatography.

The regions of *Cacospongia* under agar strips were quantified for percentage necrosis by placing window screening around the area covered by the strip. The number of squares of screening covering this area was counted, as was the number of squares covering necrotic areas. Percentage necrosis was then calculated as the number of necrotic squares divided by the total number of squares, multiplied by 100. Sections of *Cacospongia* covered by control and treated strips were then extracted as described above. Paired *t* tests were

used to compare the percentage necrosis, the percentage yield of crude and filtered extracts, and the percentage dry mass of scalaradial and desacetylscalaradial between control and treated areas.

Field manipulation with isolated metabolite.—To examine the metabolites produced by *Dysidea,* crude extracts were separated using a Bond Elut silica column with a gradient of 5–30% ethyl acetate : hexanes. The major metabolite was further purified using HPLC (Econosil silica 10 μ [Alltech Associates, Incorporated, Deerfield, Illinois], 250 × 22 mm column, 15% ethyl acetate : hexanes, refractive index detection) and its structure was confirmed by ^{1}H-NMR and ^{13}C-NMR to be 7-deacetoxyolepupuane, a drimane sesquiterpene previously isolated from *Dysidea* sp. (Garson et al. 1992). This compound comprised 12% of the *Dysidea* crude extract. To determine if this compound was involved in the necrotic activity of the crude extract, we repeated our previous field experiment using this compound, at a concentration of 12% of the amount of crude extract used in the previous experiment (0.12 × 2.56 g = 308 mg/100 mL).

At Sponge Mound, eight control and eight treated agar strips were placed in pairs on eight *Cacospongia* colonies as previously described. After 7 d, the *Cacospongia* colonies were retrieved from Sponge Mound. No loss of agar was observed from any of the strips. The strips were extracted with dichloromethane and analyzed by thin-layer chromatography to determine if 7-deacetoxyolepupuane was still present. Regions under the agar strips were quantified for percentage necrosis using the techniques described above. *Cacospongia* were then extracted as described above. Paired *t* tests were used to compare the percentage yield of filtered extracts and the percentage dry mass of scalaradial and desacetylscalaradial. A Wilcoxon signed-ranks test was used to compare the percentage yield of crude extracts, as these data were not normally distributed. The amount of necrosis generated by the crude extract was compared to the amount generated by 7-deacetoxyolepupuane by an independent *t* test on the differences in percentage necrosis between pairs of control and treated strips.

Response of Dysidea *to* Cacospongia

To determine if the amount of organic extract found in *Dysidea* varied when occurring on *Cacospongia* compared to other substrates, we collected four colonies of *Dysidea* from rock substrates and five colonies from *Cacospongia.* These colonies were extracted exhaustively as previously described. The percentage yields of *Dysidea* collected from rock and *Cacospongia* were compared using a *t* test.

To determine if the relative concentrations of secondary metabolites in *Dysidea* varied when occurring on *Cacospongia* compared to other substrates, each extract was examined by 300 MHz proton nuclear magnetic resonance (NMR) spectroscopy. The peak heights of signals corresponding to three major components of the *Dysidea* extracts, triglycerides, 7-deacetoxyolepupuane, and an unidentified dialdehyde, were compared. All extracts were dissolved in deuterated chloroform ($CDCl_3$) at a concentration of 30 mg/mL. The peak heights for methylene signals of triglycerides (1.2 ppm), an olefinic proton of 7-deacetoxyolepupuane (6.0 ppm), and an aldehyde proton (9.85 ppm) of a dialdehyde were measured. The ratios of triglyceride to 7-deacetoxyolepupuane, 7-deacetoxyolepupuane to dialdehyde, and triglyceride to dialdehyde were calculated and compared between *Dysidea* covering *Cacospongia* and *Dysidea* covering rock by *t* tests (if normally distributed) or the Mann-Whitney test (if not normally distributed). While these NMR analyses were not intended to be quantitative, they did provide a good indication of the relative concentrations of different compounds in *Dysidea.* These data were then used to determine if any apparent patterns warranted further quantitative analyses.

Dysidea *metabolites as feeding deterrents*

To determine if *Dysidea* metabolites could also function as feeding deterrents against potential predators, we conducted three feeding assays. We first conducted a field test for feeding deterrence using methods similar to those of Schupp and Paul (1994). *Dysidea* crude extracts were added at a concentration of 6% dry mass (one-half the natural dry mass concentration, one-fourth the natural volumetric concentration) to an artificial diet consisting of 5 g of ground catfish pellets (Kruse's Perfection Brand, El Monte, California) and 2.5 g of carrageenan per 80 mL of water. The crude extracts were used at a concentration lower than their natural concentration because of the limited availability of extract. This diet contained ~22 mg/mL protein, similar to that measured for *Dysidea etheria* (25 mg/mL) and *D. janiae* (14 mg/mL) by Chanas and Pawlik (1995). The carrageenan and water were heated to boiling in a microwave oven, then the ground pellets and crude extract (dissolved in dichloromethane) were added to treated foods, while an equal volume of dichloromethane was added to control foods. The mixture was poured into 1-cm^3 molds to cool. A rubber O-ring was embedded in each cube to attach it to a yellow polypropylene rope with a safety pin. Four control or four treated food cubes were attached to each rope. The ropes were placed on the reef at Western Shoals, Apra Harbor, Guam, in pairs of one control and one treated rope. Twenty replicate pairs were tested. The pairs were removed when approximately half the cubes were eaten, and the number of cubes of control and treated foods eaten was recorded. These data were analyzed with a Wilcoxon signed-rank test for paired comparisons.

Second, we conducted laboratory feeding assays using a common spongivore on Guam, *Pomacanthus imperator,* the emperor angelfish. Fish were collected by net from reef flats around Guam and held individually

in 20-L aquaria, with running seawater and airstones. The fish were fed a base diet consisting of 35 g Prime Reef fish food (Prime Reef, Ocean Nutrition, San Diego, California), 2.5 g carrageenan, and 50 mL water. For preference tests, artificial foods were presented to fish using the methods described in Lumbang and Paul (1996). The foods consisted of 36 mL of water, 0.72 g of agar, and 4 g of ground catfish pellets. This diet contained 36 mg/mL protein, higher than protein concentrations measured in other *Dysidea* (Chanas and Pawlik 1995). A crude extract from *Dysidea* was added to the artificial food at a concentration of 6% dry mass (7 mg/mL volumetric concentration). To incorporate the extract into the artificial food, an appropriate amount of compound was dissolved in dichloromethane and added to the ground pellets. Solvent alone was added to control foods. Solvent was removed from the pellets by rotary evaporation. Foods were then prepared by boiling the agar and water in a microwave, adding the pellets after the agar and water cooled slightly, mixing the diet, then pouring the diet into a mold backed with fiberglass window screening as previously described for making agar strips. After cooling, the molds were removed and the screening was cut so that a length of screening contained both control and treated foods. Food strips were 2 cm wide, with 12 × 15 square openings in the screen for both control and treated foods. Food strips were offered to the angelfish by suspending them from the top of the tank and were monitored periodically and removed when a fish had eaten 50–75% of one of the foods. Consumption was measured by counting the number of squares of screening from which control or treated food was removed. Consumption of control and treated foods was compared using a paired t test.

Finally, we used the laboratory feeding assay described above to test the deterrent activity of pure 7-deacetoxyolepupuane at a natural concentration of 1.5% dry mass. We repeated this test over 2 d to examine any effects of learning by the fish on consumption of the control and treated foods. We analyzed these data using a paired t test to compare the consumption of control and treated foods on each day. We used a Wilcoxon signed-ranks test to compare the difference in consumption of control and treated food between the two days.

Results

Distribution of Dysidea

Dysidea occurs more frequently on *Cacospongia* than expected given the distribution of available substrates on Sponge Mound (Fig. 2, $G = 30.63$, $P < 0.001$). Other sponges on the mound belonged to a variety of families, including Ancorinidae, Ianthellidae, Dysideidae, and Halichondriidae. Three (9.7%) *Dysidea* occurred on the sponge *Melophlus isis* (Family Ancorinidae). Other substrates included rock, coral rubble, live coral, and *Halimeda* spp. On the western side of the mound, 14 of 51 *Cacospongia* colonies (27.5%) were under attack by *Dysidea,* while on the eastern side, none of 29 colonies (0%) were attacked. Effects of *Dysidea* overgrowth on *Cacospongia* were sometimes severe; *Dysidea* could completely cover and destroy the bases of *Cacospongia* and dislodge them from the substrate.

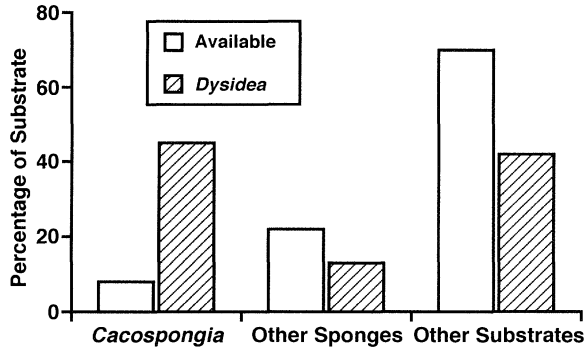

FIG. 2. The distribution of substrates available on Sponge Mound (open bars) and substrates occupied by *Dysidea* (hatched bars), represented as percentage *Cacospongia,* percentage other sponges, and percentage other substrates. A significant difference between these distributions ($G = 30.63$, $P < 0.001$) indicates that *Dysidea* is more frequently found on *Cacospongia* than expected by a random model of substrate selection.

Response of Cacospongia *to overgrowth by* Dysidea

Field observations.—Cacospongia regions, which were covered or not covered by *Dysidea,* did not differ significantly in their percentage yield of crude extract (covered: 13.09 ± 1.79% (mean ± 1 SE), uncovered: 12.14 ± 1.53%, $t = 0.664$, 4 df, $P = 0.543$) or percentage yield of filtered extract (covered, 10.01 ± 2.57%; uncovered, 5.39 ± 2.01%; $t = 1.065$, 4 df, $P = 0.347$). Covered and uncovered regions also did not differ significantly in their percentage of scalaradial (covered, 0.69 ± 0.20%; uncovered, 0.98 ± 0.28%; $t = 1.499$, 3 df, $P = 0.231$) or desacetylscalaradial (covered, 0.55 ± 0.10%; uncovered, 0.57 ± 0.04%; $t = 0.834$, 3 df, $P = 0.465$).

Field manipulation with crude extract.—TLC analyses showed that the agar strips still contained some of the crude extracts after 1 wk and that the secondary metabolites present in the extract were still present. No decomposition products were observed. *Cacospongia* areas underlying agar strips containing crude *Dysidea* extracts developed significantly more necrosis than areas under control strips (Fig. 3, $t = 18.16$, 7 df, $P < 0.001$). Areas exposed to *Dysidea* also had a significantly higher percentage yield of crude organic extract (control, 11.12 ± 1.04%; treated, 19.10 ± 2.36%; $t = 2.74$, 7 df, $P = 0.029$), but filtered extract yields were not significantly different (control, 5.22 ± 0.57%; treated, 6.15 ± 0.60%; $t = 1.015$, 7 df, $P = 0.344$). No

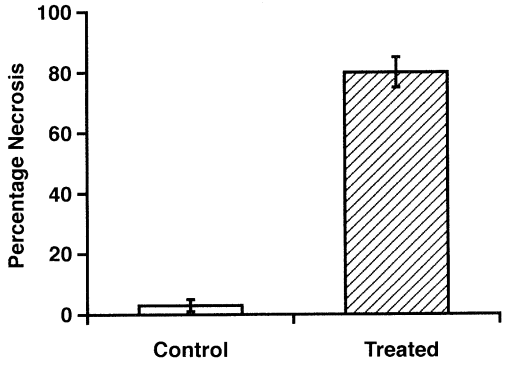

FIG. 3. The percentage of *Cacospongia* areas (mean ± 1 SE) underlying agar strips that developed necrosis after 1 wk of exposure in the field. Strips treated with *Dysidea* crude extracts (hatched bar) caused a much greater amount of necrosis than control strips (open bar, $t = 18.16$, 7 df, $P < 0.001$).

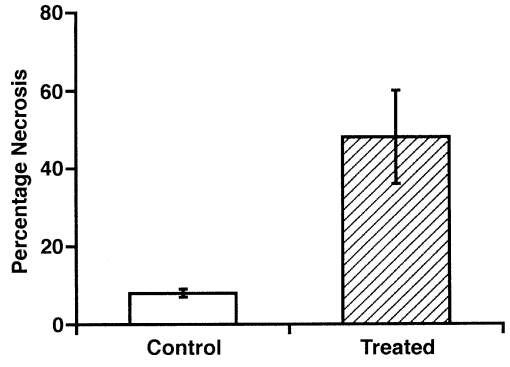

FIG. 4. The percentage of *Cacospongia* areas (mean ± 1 SE) underlying agar strips that developed necrosis after 1 wk of exposure in the field. Strips treated with 7-deacetoxyolepupuane (hatched bar) caused significantly more necrosis than control strips (open bar, $t = 3.52$, 7 df, $P = 0.010$).

significant differences were observed in the percentage yields of scalaradial (control, 0.92 ± 0.18%; treated, 0.69 ± 0.10%; $t = 1.273$,7 df, $P = 0.244$) or desacetylscalaradial (control, 0.49 ± 0.06%; treated, 0.48 ± 0.06%; $t = 0.247$, 7 df, $P = 0.812$).

Field manipulation with isolated metabolite.—TLC analyses showed that the agar strips contained 7-deacetoxyolepupuane after 1 wk. Agar strips containing the compound generated significantly more necrosis in underlying *Cacospongia* areas than control strips (Fig. 4, $t = 3.52$, 7 df, $P = 0.010$). The percentage yield of crude extracts of the underlying *Cacospongia* areas did not differ significantly (control, 40.2 ± 1.7%; treated, 44.4 ± 5.2%; Wilcoxon $T = 0.42$, $n = 8$, $P = 0.674$), nor did the percentage yield of filtered extracts (control, 9.7 ± 3.5%; treated, 6.3 ± 1.7%; $t = 0.73$, 7 df, $P = 0.490$). No significant differences were observed in the percentage yields of scalaradial (control, 0.54 ± 0.15%; treated, 0.35 ± 0.11%; $t = 0.83$, 7 df, $P = 0.436$) or desacetylscalaradial (control, 0.47 ± 0.16%; treated, 0.29 ± 0.09%; $t = 0.89$, 7 df, $P = 0.404$). The crude extract generated more necrosis than pure 7-deacetoxyolepupuane (crude extract difference [treated − control] = 76.54 ± 11.92 [mean ± 1 SE], pure compound difference [treated − control] = 39.67 ± 31.87, $t = 3.07$, df = 14, $P = 0.008$).

Response of Dysidea *to* Cacospongia

The percentage yields of crude extracts from *Dysidea* collected from *Cacospongia* and rock substrates were not significantly different (*Cacospongia,* 26.7 ± 4.0%; Rock, 30.6 ± 6.1%; $t = 0.551$, 7 df, $P = 0.599$). No significant differences in the relative concentrations of major metabolites were found between *Dysidea* covering *Cacospongia* and rock (Table 1), but large amounts of variation were observed among specimens. For most colonies, 7-deacetoxyolepupuane was the major secondary metabolite, while the amount of dialdehyde varied considerably among individuals. One colony covering rock had almost undetectable levels of 7-deacetoxyolepupuane and much higher levels of dialdehyde than the other specimens.

Dysidea *metabolites as feeding deterrents*

Crude extracts of *Dysidea* were significant feeding deterrents in the field (Fig. 5, Wilcoxon $T = 3.901$, $n = 20$, $P < 0.001$). Crude extracts also significantly deterred feeding by *P. imperator* in the laboratory (Fig. 6, $t = 6.43$, 7 df, $P < 0.001$). 7-deacetoxyolepupuane significantly deterred feeding by *P. imperator* in the laboratory, but was significantly more deterrent on the second day of testing than on the first (Fig. 7, Day 1, $t = 2.77$, 8 df, $P = 0.02$; Day 2, $t = 3.86$, 8 df, $P = 0.005$; Day 1 vs. Day 2, Wilcoxon $T = 2.31$, $n = 9$, $P = 0.02$).

DISCUSSION

Space on which to live can be an important limiting resource for sessile marine organisms (Jackson and Buss 1975, Jackson 1977, Branch 1984, Buss 1986,

TABLE 1. Relative concentrations of secondary metabolites found in *Dysidea* occurring on *Cacospongia* and on rock. The ratios (mean ± 1 SE) of the NMR peak heights of three major components of *Dysidea* extracts were not significantly different between the two substrates.

Secondary metabolites	On *Cacospongia*	On rock	*P*
Triglycerides: 7-deacetoxyolepupuane	8.02 ± 2.05	23.45 ± 16.65	0.806 (Mann-Whitney)
7-deacetoxyolepupuane: dialdehyde	8.72 ± 2.22	11.11 ± 6.59	0.749 (*t* test)
Triglycerides: dialdehyde	75.75 ± 30.33	47.51 ± 19.26	0.459 (*t* test)

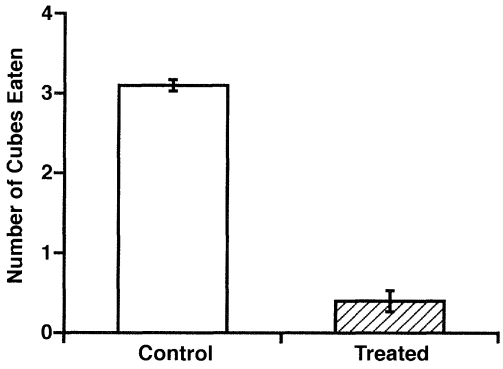

FIG. 5. The number of food cubes (mean ± 1 SE) eaten during the Western Shoals field assay. Significantly more control cubes (open bar) were eaten than cubes containing crude extracts of *Dysidea* (hatched bar, Wilcoxon $T = 3.901$, $P < 0.001$).

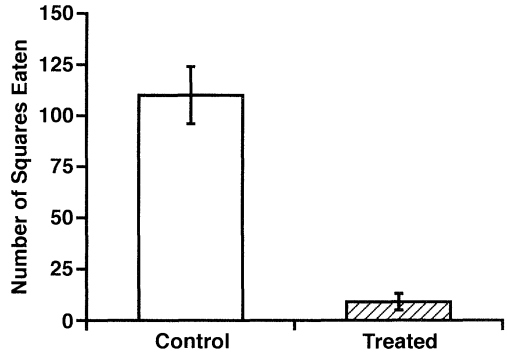

FIG. 6. The number of squares (mean ± 1 SE) consumed by *P. imperator* of either control food (open bar) or food treated with *Dysidea* crude extract (hatched bar). The crude extract significantly deterred feeding ($t = 6.43$, 7 df, $P < 0.001$).

Turon et al. 1996*b*). This may be particularly evident on coral reefs where competition for space is reported to be intense (Jackson 1977, Benayahu and Loya 1981, Branch 1984). Benthic coral reef organisms compete for space through a variety of mechanisms, including overgrowth (Wahle 1980, La Barre and Coll 1982), extracoelenteric digestion of neighbors (Lang 1973), sweeper tentacles (Richardson et al. 1979, Wellington 1980, Sebens and Miles 1988), and chemical interactions (Porter and Targett 1988, De Nys et al. 1991).

The potential importance of allelochemical effects in mediating spatial interactions among benthic marine invertebrates was first demonstrated over twenty years ago (Jackson and Buss 1975). Since that time, other studies have supported these seminal findings, but little progress has been made in understanding both the chemistry and ecology involved in allelopathic interactions. This lack of progress in studies of allelopathy is not unique to the marine environment. Researchers studying allelopathic interactions in terrestrial plant and aquatic communities have also lamented the lack of understanding of the chemical ecology of these interactions and the reliance on laboratory assays for studies of allelopathy (Fischer 1991, Gopal and Goel 1993, Fischer et al. 1994, Inderjit and Dakshini 1994, 1995).

A considerable amount of indirect evidence suggests that allelochemicals mediate competitive interactions among many coral reef organisms. Hard coral species relocated in the vicinity of the soft corals *Sinularia flexibilis* and *Sarcophyton crassocaule* showed growth retardation, necrosis, and mortality (Sammarco et al. 1983, 1985), effects ascribed to the release of secondary metabolites by the soft corals (Coll et al. 1982). The release of allelochemicals by the soft coral *Xenia puertogalerae* influenced patterns of succession and community structure by inhibiting the settlement of some, but not all, scleractinian coral species (Atrigenio and Aliño 1996). Hard corals provoked the secretion of a protective layer in soft corals (Sammarco et al. 1985) and reduced algal growth rates even under noncontact situations (De Ruyter van Stevenick et al. 1988), suggesting the production of allelochemicals by scleractinian corals. A waterborne exudate of the sea anemone *Condylactis gigantea* limited the nearby accumulation of filamentous algal biomass (Bak and Borsboom 1984). Despite this indirect evidence for allelochemical effects, only one prior study characterized a compound involved in spatial competition among reef organisms and demonstrated that the compound had an effect on a neighboring organism. De Nys et al. (1991) used field experiments to demonstrate that the monoterpene chloromertensene produced by the red alga *Plocamium hamatum* mediates its competitive interactions with the octocoral *Sinularia cruciata.*

Although the production of a surrounding zone of inhibition by sponges has been previously attributed to

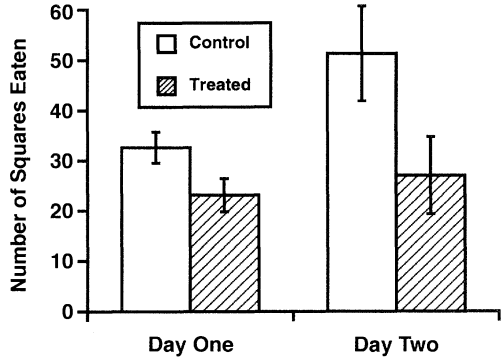

FIG. 7. The number of squares (mean ± 1 SE) consumed by *P. imperator* of either control food (open bars) or food treated with 7-deacetoxyolepupuane (hatched bars) on two consecutive days of testing: 7-deacetoxyolepupuane deterred feeding but was significantly more deterrent on the second day of testing than on the first (Day 1, $t = 2.77$, 8 df, $P = 0.02$; Day 2, $t = 3.86$, 8 df, $P = 0.005$; Day 1 vs. Day 2, Wilcoxon $T = 2.31$, $n = 9$, $P = 0.02$).

the production of allelochemicals (Ayling 1983, Sullivan et al. 1983, Porter and Targett 1988, Turon et al. 1996*a*, *b*), our study is the first to explicitly demonstrate the role of a secondary metabolite in spatial competition between reef invertebrates. Other competitive interactions involving sponges are likely to be mediated by secondary metabolites. Turon et al. (1996*b*) examined the small-scale patterns of association among benthic organisms as an indicator of allelochemical interactions. A strong nonrandom pattern was found in the interspecific associations, which was mostly due to negative associations between the sponge *Crambe crambe* and other sponges in its community. The strength of this relationship diminished with distance from the sponge, with no relationship at 5 cm from the sponge border. Field experiments demonstrated that *C. crambe* inhibited the growth of the sponge *Scopalina lophiropoda,* its major competitor (Turon et al. 1996*b*). The formation of a competitor-free zone around *C. crambe* has been attributed to the inhibitory properties of secondary metabolites produced by the sponge. Sullivan et al. (1983) identified a secondary metabolite, siphonodictidine, produced by the burrowing sponge *Siphonodictyon* sp., which was toxic to the coral *Acropora formosa* at 100 mg/kg and inhibited its respiration at concentrations as low as 0.01 mg/kg in laboratory assays. Porter and Targett (1988) observed the liver sponge *Plakortis halichondroides* overgrowing the sheet coral *Agaricia lamarcki* and creating a zone of dead coral. The sponge reduced the number of zooxanthellae, the mass of chlorophyll *a,* and the mass of nitrogen per unit area of the coral whether or not the sponge and coral were in contact. However, only when these species were in contact did coral respiration exceed production and the sponge gain a competitive advantage. Crude organic extracts were isolated from *P. halichondroides,* coated onto synthetic cellulose pads, and tied to living coral in field experiments. The extract-soaked pads caused coral bleaching within 24 h, while control pads had no effect (Porter and Targett 1988).

Our study used methods similar to those of Porter and Targett (1988) and De Nys et al. (1991) to place *Dysidea* extracts and the pure metabolite 7-deacetoxyolepupuane in contact with *Cacospongia.* We incorporated the extract and pure compound into agar strips at natural volumetric concentrations where they remained (based on reextraction of the agar strips and TLC) for the 7 d of our experiments. Both the extract and pure major metabolite caused pronounced necrosis in *Cacospongia,* similar to that observed during overgrowth by *Dysidea.* This necrosis is mediated by contact between *Dysidea* (or *Dysidea* compounds) and *Cacospongia.* Only the portions of *Cacospongia* that were overgrown by *Dysidea* or directly under agar strips containing *Dysidea* compounds were necrotic, supporting our preliminary observations that the allelochemicals were not waterborne.

Our results support the observation that secondary metabolites found in marine sponges can play roles in both competitive interactions and defense against predators (Pawlik 1993, Turon et al. 1996*b*, Becerro et al. 1997). While other sponge compounds are known to deter potential predators (Pennings et al. 1994, Pawlik et al. 1995, Uriz et al. 1996), prior studies have not experimentally demonstrated both an allelopathic and antipredatory role for any sponge natural products. Crude extracts of *Dysidea* and the pure metabolite 7-deacetoxyolepupuane deterred feeding by potential predators in the field and by the spongivore *P. imperator* in the laboratory. *P. imperator* was more deterred by 7-deacetoxyolepupuane on the second day of testing, indicating that predators may initially sample *Dysidea* and learn to avoid it after experiencing some adverse effect. Similar results have been found for herbivorous fishes feeding on compounds from benthic cyanobacteria (Thacker et al. 1997). Our artificial diets contained relatively high levels of protein compared to those measured in other *Dysidea* (Chanas and Pawlik 1995), making our tests of feeding deterrence conservative. Chanas and Pawlik (1995) found few relationships between protein, carbohydrate, and lipid content and the palatability of organic extracts from sponges.

The crude extract of *Dysidea* showed stronger feeding deterrence than pure 7-deacetoxyolepupuane and caused a greater amount of necrosis in *Cacospongia* than the pure compound. At least eight different sesquiterpenoid compounds including sesquiterpene aldehydes (Paul et al. 1997) are also present in *Dysidea.* Although we have not addressed the possible additive or interactive effects of these minor compounds in our experiments, our data strongly suggest that other compounds present in the *Dysidea* extract increase the effectiveness of 7-deacetoxyolepupuane in interactions with predators and competitors.

Given the multiple ecological functions that are played by the *Dysidea* sesquiterpene 7-deacetoxyolepupuane, it is unlikely that this compound evolved in response to either predators or competitors alone. Garson et al. (1992) also reported antibacterial, antifungal, and cytotoxic activities for this compound in laboratory in vitro assays, suggesting that this compound may play a role in defense against microorganisms as well. Multiple functions for secondary metabolites from plants (Bernays et al. 1989, Krischik et al. 1991, Berenbaum 1995), seaweeds (Paul and Fenical 1986, Schmitt et al. 1995), soft corals (Sammarco and Coll 1990), and sponges (Thompson et al. 1985, Becerro et al. 1997) have also been reported.

The multiple functions of 7-deacetoxyolepupuane in competitive interactions and predator defense may yield conflicting evolutionary constraints (Schmitt et al. 1995). Crude extracts of *Dysidea* and pure 7-deacetoxyolepupuane cause necrosis when *Dysidea* overgrows competing sponges. Despite this role in competition, we found similar levels of 7-deacetoxyole-

pupuane in *Dysidea* growing on *Cacospongia* and *Dysidea* growing on rock. Since the production of 7-deacetoxyolepupuane was not induced by the presence of competitors, the constant threat of predation may apply selective pressure to maintain high concentrations of this compound as a chemical defense, regardless of its effects on competitors.

Cacospongia has been previously reported to contain the defensive terpenoid metabolites scalaradial and desacetylscalaradial (Rogers and Paul 1991, Avila and Paul 1997). Despite the presence of these terpenes, *Cacospongia* was the organism most frequently overgrown by *Dysidea* and the concentrations of these compounds did not change in response to overgrowth by *Dysidea.* Thus, our data do not support a role of these compounds in preventing overgrowth by *Dysidea.* In both species, we observed a large amount of individual variation in the production of secondary metabolites. This variation, which could result from differences in food or nutrient availability, current flow, other microhabitat characteristics or genetic differences, may have obscured any differences in production caused by competitive interactions. Yields of crude extracts of *Cacospongia* did increase in the presence of *Dysidea* extracts. Whether this difference reflects an increase in the production of other compounds not investigated in this study that could play a role in competitive interactions or an increase in membrane lipids as a consequence of tissue damage remains unclear.

We have demonstrated that allelochemicals produced by *Dysidea* cause necrosis in *Cacospongia,* which seems to be unable to prevent overgrowth by *Dysidea.* The ability of *Dysidea* to overgrow *Cacospongia* may influence the distribution of *Dysidea.* Our field data indicate that *Dysidea* occurs more frequently on *Cacospongia* than on any other available substrate on Sponge Mound. Whether *Dysidea* preferentially settles on *Cacospongia* or has greater success attacking *Cacospongia* than other species is unknown. *Cacospongia* on the eastern side of Sponge Mound was not attacked and *Dysidea* was not found on this side of the mound. *Dysidea* may not settle or grow in this area because of current flow or other microhabitat characteristics. Cues influencing the settlement of *Dysidea* larvae and the ability of other species or other *Cacospongia* populations to resist overgrowth by *Dysidea* remain to be studied. The effects of antipredatory compounds can be highly species dependent (Schupp and Paul 1994) and such specificity may also be found in the effects of compounds involved in competitive interactions. Further research on the interactions among *Dysidea* and its competitors will provide additional insights into the role of allelochemicals in competition for space on tropical reefs.

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Literature Cited

Atrigenio, M. P., and P. M. Aliño. 1996. Effects of the soft coral *Xenia puertogalerae* on the recruitment of scleractinian corals. Journal of Experimental Marine Biology and Ecology **203**:179–189.

Avila, C., and V. J. Paul. 1997. Chemical ecology of the nudibranch *Glossodoris pallida*: is the location of diet-derived metabolites important for defense? Marine Ecology Progress Series **150**:171–180.

Ayling, A. L. 1983. Factors affecting the spatial distributions of thinly encrusting sponges from temperate waters. Oecologia **60**:412–418.

Bak, R. P. M., and J. L. A. Borsboom. 1984. Allelopathic interaction between a reef coelenterate and benthic algae. Oecologia **63**:194–198.

Bakus, G. J. 1971. An ecological hypothesis for the evolution of toxicity in marine organisms. Pages 57–62 *in* A. de Vries and E. Kochva, editors. Toxins of animal and plant origin. Gordon and Breach, New York, New York, USA.

Bakus, G. J., and G. Green. 1974. Toxicity in sponges and holothurians: a geographic pattern. Science **185**:951–953.

Bakus, G. J., N. M. Targett, and B. Schulte. 1986. Chemical ecology of marine organisms: an overview. Journal of Chemical Ecology **12**:951–987.

Becerro, M. A., X. Turon, and M. J. Uriz. 1997. Multiple functions for secondary metabolites in encrusting marine invertebrates. Journal of Chemical Ecology **23**:1527–1547.

Benayahu, Y., and Y. Loya. 1981. Competition for space among coral reef sessile organisms at Eliat, Red Sea. Bulletin of Marine Science **31**:514–522.

Berenbaum, M. R. 1995. The chemistry of defense: theory and practice. Proceedings of the National Academy of Sciences, (USA) **92**:2–8.

Bernays, E. A., G. C. Driver, and M. Bilgener. 1989. Herbivores and plant tannins. Advances in Ecological Research **19**:263–302.

Bingham, B. L., and C. M. Young. 1991. Influence of sponges on invertebrate recruitment: a field test of allelopathy. Marine Biology **109**:19–26.

Branch, G. M. 1984. Competition between marine organisms: ecological and evolutionary implications. Oceanography and Marine Biology Annual Review **22**:429–593.

Brosnan, D. M. 1992. Ecology of tropical rocky shores: plant–animal interactions in tropical and temperate latitudes. Pages 101–131 *in* D. M. John, S. J. Hawkins, and J. H. Price, editors. Plant–animal interactions in the marine benthos. Clarendon Press, Oxford, UK.

Buss, L. W. 1986. Competition and community organization on hard surfaces in the sea. Pages 517–536 *in* J. M. Diamond and T. J. Case, editors. Community ecology. Harper and Row, New York, New York, USA.

Chanas, B., and J. R. Pawlik. 1995. Defenses of Caribbean sponges against predatory reef fish. II. Spicules, tissue toughness, and nutritional quality. Marine Ecology Progress Series **127**:195–211.

Coll, J. C., B. F. Bowden, D. M. Tapiolas, and W. C. Dunlap. 1982. In situ isolation of allelochemicals released from soft corals (Coelenterata: Octocorallia): a totally submersible sampling apparatus. Journal of Experimental Marine Biology and Ecology **60**:293–299.

Connell, J. H. 1975. Some mechanisms producing structure

in natural communities: a model and evidence from field experiments. Pages 460–490 *in* M. L. Cody and J. M. Diamond, editors. Ecology and evolution of communities. Harvard University Press, Cambridge, Massachusetts, USA.

Davis, A. R., A. J. Butler, and I. van Altena. 1991. Settlement behaviour of ascidian larvae: preliminary evidence for inhibition by sponge allelochemicals. Marine Ecology Progress Series **72**:117–123.

Davis, A. R., N. M. Targett, O. J. McConnell, and C. M. Young. 1989. Epibiosis of marine algae and benthic invertebrates: natural products chemistry and other mechanisms inhibiting settlement and overgrowth. Pages 85–114 *in* P. J. Scheuer, editor. Bioorganic marine chemistry. Volume 3. Springer-Verlag, Berlin, Germany.

De Nys, R., J. C. Coll, and I. R. Price. 1991. Chemically mediated interactions between the red alga *Plocamium hamatum* (Rhodophyta) and the octocoral *Sinularia cruciata* (Alcyonacea). Marine Biology **108**:315–320.

De Ruyter van Stevenick, E. D., L. L. Van Mulekom, and A. M. Breeman. 1988. Growth inhibition of *Lobophora variegata* (Lamouroux) Womersley by scleractinian corals. Journal of Experimental Marine Biology and Ecology **115**: 169–178.

Faulkner, D. J. 1996. Marine natural products. Natural Product Reports **13**:75–125.

Fischer, N. H. 1991. Plant terpenoids as allelopathic agents. Pages 372–413 *in* J. B. Harborne and F. A. Tomas-Barberan, editors. Ecological chemistry and biochemistry of plant terpenoids. Oxford University Press, Oxford, U.K.

Fischer, N. H., G. B. Williamson, J. D. Weidenhamer, and D. R. Richardson. 1994. In search of allelopathy in the Florida scrub; the role of terpenoids. Journal of Chemical Ecology **20**:1355–1380.

Garson, M. J., A. F. Dexter, L. K. Lambert, and V. Liokas. 1992. Isolation of the bioactive terpene 7-deacetoxyolepupuane from the temperate marine sponge *Dysidea* sp. Journal of Natural Products **55**:364–367.

Goodbody, I. 1961. Inhibition of the development of a marine sessile community. Nature **190**:282–283.

Gopal, B., and U. Goel. 1993. Competition and allelopathy in aquatic plant communities. Botanical Review **59**:155–210.

Hay, M. E. 1996. Marine chemical ecology: what's known and what's next? Journal of Experimental Marine Biology and Ecology **200**:103–134.

Hay, M. E., Q. E. Kappel, and W. Fenical. 1994. Synergisms in plant defenses against herbivores: interactions of chemistry, calcification, and plant quality. Ecology **75**:1714–1726.

Henrikson, A. A., and J. R. Pawlik. 1995. A new antifouling assay method: results from field experiments using extracts of four marine organisms. Journal of Experimental Marine Biology and Ecology **194**:157–165.

Inderjit, and K. M. M. Dakshini. 1994. Algal allelopathy. Botanical Review **60**:182–196.

Inderjit, and K. M. M. Dakshini. 1995. On laboratory bioassays in allelopathy. Botanical Review **61**:28–44.

Jackson, J. B. C. 1977. Competition on marine and hard substrata: the adaptive significance of solitary and colonial strategies. American Naturalist **111**:743–767.

Jackson, J. B. C., and L. Buss. 1975. Allelopathy and spatial competition among coral reef invertebrates. Proceedings of the National Academy of Sciences, (USA) **72**:5160–5163.

Krischik, V. A., R. W. Goth, and P. Barbosa. 1991. Generalized plant defense: effects on multiple species. Oecologia **85**:562–571.

La Barre, S. C., and J. C. Coll. 1982. Movement in soft corals: an interaction between *Nepthea brassica* (Coelenterata: Octocorallia) and *Acropora hyacinthus* (Coelenterata: Octocorallia): III. Spacing and aggressive interactions between alyconaceans. Marine Ecology Progress Series **28**: 147–156.

Lang, J. C. 1973. Interspecific aggression by scleractinian corals. 2. Why the race is not only to the swift. Bulletin of Marine Science **23**:260–279.

Leone, P. A., B. F. Bowden, A. R. Carroll, and J. C. Coll. 1995. Chemical consequences of relocation of the soft coral *Lobophytum compactum* and its placement in contact with the red alga *Plocamium hamatum.* Marine Biology **122**:675–679.

Lubchenco, J., and S. D. Gaines. 1981. A unified approach to marine plant–herbivore interactions. I. Populations and communities. Annual Review of Ecology and Systematics **12**:405–437.

Lumbang, W. A., and V. J. Paul. 1996. Chemical defenses of the tropical green seaweed *Neomeris annulata* Dickie: effects of multiple compounds on feeding by herbivores. Journal of Experimental Marine Biology and Ecology **201**: 185–195.

Maida, M., P. W. Sammarco, and J. C. Coll. 1995. Effects of soft corals on scleractinian coral recruitment. I: Directional allelopathy and inhibition of settlement. Marine Ecology Progress Series **121**:191–202.

McClintock, J. B., B. J. Baker, M. Slattery, M. Hamann, R. Kopitzke, and J. Heine. 1994. Chemotactic tube-foot responses of a spongivorous sea star *Perknaster fuscus* to organic extracts from antarctic sponges. Journal of Chemical Ecology **20**:859–870.

Menge, B. A., and T. M. Farrell. 1989. Community structure and interaction webs in shallow marine hard-bottom communities: tests of an environmental stress model. Advances in Ecological Research **19**:189–262.

Paine, R. T. 1994. Marine rocky shores and community ecology: an experimentalist's perspective. Ecology Institute, Oldendorf/Luhe, Germany.

Paul, V. J. 1992. Ecological roles of marine natural products. Comstock Publishing Associates, Ithaca, New York, USA.

Paul, V. J., and W. Fenical. 1986. Chemical defense in tropical green algae, order Caulerpales. Marine Ecology Progress Series **34**:255–264.

Paul, V. J., Y. Seo, K. W. Cho, J. Rho, J. Shin, and P. R. Bergquist. 1997. Sesquiterpenoids of the drimane class from a sponge of the genus *Dysidea.* Journal of Natural Products **60**:1115–1120..

Pawlik, J. R. 1993. Marine invertebrate chemical defenses. Chemical Reviews **93**:1911–1922.

Pawlik, J. R., B. Chanas, R. J. Toonen, and W. Fenical. 1995. Defenses of Caribbean sponges against predatory reef fish. I. chemical deterrency. Marine Ecology Progress Series **127**:183–194.

Pennings, S. C., S. R. Pablo, V. J. Paul, and J. E. Duffy. 1994. Effects of sponge secondary metabolites in different diets on feeding by three groups of consumers. Journal of Experimental Marine Biology and Ecology **180**:137–149.

Porter, J. W., and N. M. Targett. 1988. Allelochemical interactions between sponges and corals. Biological Bulletin **175**:230–239.

Richardson, C. A., P. Dustan, and J. C. Lang. 1979. Maintenance of living space by sweeper tentacles of *Montastrea cavernosa,* a Caribbean reef coral. Marine Biology **55**:181–186.

Rogers, S. D., and V. J. Paul. 1991. Chemical defenses of three *Glossodoris* nudibranchs and their dietary *Hyrtios* sponges. Marine Ecology Progress Series **77**:221–232.

Russ, G. R. 1982. Overgrowth in a marine epifaunal community: competitive hierarchies and competitive networks. Oecologia **53**:12–19.

Sammarco, P. W. 1996. Comments on coral reef regeneration, bioerosion, biogeography, and chemical ecology: future di-

rections. Journal of Experimental Marine Biology and Ecology **200**:135–168.

Sammarco, P. W., and J. C. Coll. 1990. Lack of predictability in terpenoid function. Journal of Chemical Ecology **16**: 273–289.

Sammarco, P. W., J. C. Coll, and S. La Barre. 1985. Competitive strategies of soft corals (Coelenterata: Octocorallia). II. Variable defensive response and susceptibility to scleractinian corals. Journal of Experimental Marine Biology and Ecology **91**:199–215.

Sammarco, P. W., J. C. Coll, S. La Barre, and B. Willis. 1983. Competitive strategies of soft corals (Coelenterata: Octocorallia). Allelopathic effects on selected scleractinian corals. Coral Reefs **1**:173–178.

Schmitt, T. M., M. E. Hay, and N. Lindquist. 1995. Constraints on chemically mediated coevolution: multiple functions for seaweed secondary metabolites. Ecology **76**:107–123.

Schulte, B. A., and G. J. Bakus. 1992. Predation deterrence in marine sponges: laboratory versus field studies. Bulletin of Marine Science **50**:205–211.

Schupp, P. J., and V. J. Paul. 1994. Calcium carbonate and secondary metabolites in tropical seaweeds: variable effects on herbivorous fishes. Ecology **75**:1172–1185.

Sears, M. A., D. J. Gerhart, and D. Rittschof. 1990. Antifouling agents from marine sponge *Lissodendoryx isodictyalis* Carter. Journal of Chemical Ecology **16**:791–799.

Sebens, K. P. and J. S. Miles. 1988. Sweeper tentacles in a gorgonian octocoral: morphological modifications for interference competition. Biological Bulletin **175**:378–387.

Slattery, M., J. B. McClintock, and J. N. Heine. 1995. Chemical defenses in Antarctic soft corals: evidence for antifouling compounds. Journal of Experimental Marine Biology and Ecology **190**:61–77.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry. Third edition. W. H. Freeman, New York, New York, USA.

Sullivan, B., D. J. Faulkner, and L. Webb. 1983. Siphonodictidine, a metabolite of the burrowing sponge *Siphonodictyon* sp. that inhibits coral growth. Science **221**:1175–1176.

Thacker, R. W., D. G. Nagle, and V. J. Paul. 1997. Effects of repeated exposures to marine cyanobacterial secondary metabolites on feeding by juvenile rabbitfish and parrotfish. Marine Ecology Progress Series **167**:21–29.

Thompson, J. E. 1985. Exudation of biologically-active metabolites in the sponge *Aplysina fistularis*. I. Biological evidence. Marine Biology **88**:23–26.

Thompson, J. E., R. P. Walker, and D. J. Faulkner. 1985. Screening and bioassays for biologically-active substances from forty marine sponge species from San Diego, California, USA. Marine Biology **88**:11–21.

Turon, X., M. A. Becerro, and M. J. Uriz. 1996*a*. Seasonal patterns of toxicity in benthic invertebrates: the encrusting sponge *Crambe crambe* (Poecilosclerida). Oikos **75**:33–40.

Turon, X., M. A. Becerro, M. J. Uriz, and J. Llopis. 1996*b*. Small-scale association measures in epibenthic communities as a clue for allelochemical interactions. Oecologia **108**:351–360.

Uriz, M. J., X. Turon, M. A. Becerro, and J. Galera. 1996. Feeding deterrence in sponges. The role of toxicity, physical defenses, energetic contents, and life-history stage. Journal of Experimental Marine Biology and Ecology **205**: 187–204.

Wahle, C. M. 1980. Detection, pursuit, and overgrowth of tropical gorgonians by milleporid hydrocorals: Perseus and Medusa revisited. Science **209**:689–691.

Walker, R. P., J. E. Thompson, and D. J. Faulkner. 1985. Exudation of biologically-active metabolites in the sponge *Aplysina fistularis*. II. Chemical evidence. Marine Biology **88**:27–32.

Wellington, G. M. 1980. Reversal of digestive interactions between Pacific reef corals: mediation by sweeper tentacles. Oecologia **47**:340–343.

Woodin, S. A., R. L. Marinelli, and D. E. Lincoln. 1993. Allelochemical inhibition of recruitment in a sedimentary assemblage. Journal of Chemical Ecology **19**:517–530.

Wulff, J. L. 1995. Sponge-feeding by the Caribbean starfish *Oreaster reticulatus*. Marine Biology **123**:313–325.