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DEFENSE OF ASCIDIANS AND THEIR CONSPICUOUS LARVAE: ADULT VS. LARVAL CHEMICAL DEFENSES[1]

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Abstract. Previous investigations, focused primarily on vertebrates, have noted substantial losses of eggs and embryos to predators and questioned why selection has not more commonly resulted in the evolution of chemically defended eggs or embryos. Hypotheses regarding the apparent rarity of such defenses have emphasized the potential incompatibility of actively developing tissues and toxic metabolites. Alternatively, this apparent pattern could be an artifact of our greater knowledge of vertebrates, which in general show few tendencies for synthesizing defensive metabolites in either juvenile or adult stages. In this study, we investigated adult and larval chemical defenses of a group of benthic marine invertebrates, the ascidians, in which the adults are often chemically rich, and we contrast our findings with what is known about chemical defenses of eggs and embryos from terrestrial and aquatic organisms. Our findings suggest that there is no fundamental incompatibility of rapidly developing juvenile tissues and bioactive metabolites, and that chemically defended eggs and larval stages may be common among some taxonomic groups.

Ascidians are benthic invertebrates that often lack apparent physical defenses against predation, yet are common on coral reefs where predation by fishes is intense. In contrast to most co-occurring invertebrates, many ascidians also release large, conspicuous larvae during daylight hours when exposure to fish predation would be highest. Thus selection by predators might favor the evolution of distasteful larvae. In situ observations indicate that many conspicuous ascidian larvae are distasteful to potential consumers. We investigated the ability of secondary metabolites produced by taxonomically diverse ascidians from geographically distant locales to deter predation on both adults and larvae. Larvae from the Caribbean ascidian *Trididemnum solidum* were distasteful to reef fishes, and when organic extracts of individual larvae were transferred onto eyes of freeze-dried krill (a good larval mimic in terms of size and color), these eyes were rejected by fishes while control eyes (solvent only) were readily eaten. Larvae of the Indo-Pacific ascidian *Sigillina* cf. *signifera* were also distasteful to coral-reef fishes and contained the unpalatable bipyrrole alkaloid tambjamine C. When added to artificial foods at or below their natural mean concentrations and offered to consumers in field and laboratory feeding assays, the secondary metabolites produced by *Trididemnum solidum* (Caribbean Sea), *Sigillina* cf. *signifera* (Indo-Pacific), and *Polyandrocarpa* sp. (Gulf of California) significantly deterred feeding by co-occurring fishes and invertebrates. Secondary metabolites produced by *Trididemnum* cf. *cyanophorum* from the Caribbean Sea, *Lissoclinum patella* from the Indo-Pacific, and *Aplidium californicum* from the temperate Pacific, and the small stellate spicules common to many tropical didemnid ascidians did not significantly affect fish feeding.

High-pressure liquid chromatography (HPLC) analyses of six didemnin cyclic peptides in individual colonies of *Trididemnum solidum* from one patch reef at Little San Salvador, Bahamas found large inter-colony differences in their concentrations. The mean concentration of didemnin B was more than double the concentration needed to significantly deter fish feeding in our field assays, and feeding tests with nordidemnin B showed that it deterred fish feeding across the entire range of natural concentrations. HPLC analysis of the extract from a combined collection of *T. solidum* larvae found adequate concentrations of didemnin B and nordidemnin B to account for their rejection by foraging fishes.

We demonstrate that taxonomically diverse ascidians from habitats characterized by intense predation pressure produce secondary metabolites that significantly reduce predation on both adults and larvae, and suggest that this defensive chemistry may be crucial in allowing the release of large, well-provisioned larvae during daylight periods when larvae have the greatest probability of using photic cues to select physically appropriate settlement sites. Production of defensive secondary metabolites appears widespread among certain groups of ascidians, some of which are also known to concentrate acid and heavy metals as additional defensive strategies.

Key words: *ascidians; chemical defenses; larvae;* Polyandrocarpa *sp.; predation; secondary metabolites;* Sigillina signifera; Trididemnum solidum.

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Introduction

Orians and Janzen (1974) asked "Why are embryos so tasty?" after pointing out that birds, reptiles, amphibians, fish, and insects all lose large proportions of their eggs or larval stages to predators and that the evolution of distasteful eggs and larvae should be favored. They hypothesized that eggs and larvae generally lack chemical defenses due to (1) autotoxicity resulting from a fundamental incompatibility of actively growing tissues and toxic metabolites, (2) energetic constraints that limit investment in toxins, and (3) tradeoffs between deterrence and potential development rates of the young. However, they also noted that much of their focus was on vertebrate eggs and that there was a general lack of chemical defenses among vertebrates. It is thus entirely possible that the "general" tendency for eggs and larvae to be palatable is not general at all but seems to occur due to the inability of most vertebrates to synthesize deterrent compounds in either the adult, juvenile, or embryonic stages. If any of the three hypotheses advanced by Orians and Janzen (1974) are generally applicable to eggs and larvae, then the general palatability of eggs and larvae should be as apparent for marine invertebrates as it was for the terrestrial and aquatic vertebrates that formed most of the focus of the study by Orians and Janzen. In contrast to vertebrates, many marine benthic invertebrates produce a wide variety of secondary metabolites that serve to deter predators (Paul 1992). If these organisms chemically defend eggs and larvae, then patterns discussed by Orians and Janzen (1974) are more likely to result from a focus on taxonomic groups that lack the ability to synthesize unusual secondary metabolites than from any fundamental incompatibility of actively growing tissues and toxic metabolites.

We hope to extend the initial observations of Orians and Janzen (1974) by considering adult vs. larval chemical defenses of marine ascidians, a group of common benthic invertebrates that are known to be chemically rich and that are often abundant on tropical reefs where predation is especially intense.

In tropical reef communities, feeding by fishes strongly affects the distribution and abundance of both seaweeds and benthic invertebrates (Wellington 1982, Lewis 1986, Horn 1989, Littler et al. 1989, Hay 1991). Although most investigations have focused on consumption of adults or developing juveniles, it is becoming clear that fish predation on settling larvae can also play a large part in determining adult distributions (Gaines and Roughgarden 1987, Olson and McPherson 1987). Despite the demonstrated impact of fish predation on soft-bodied reef organisms, invertebrates such as ascidians, octocorals, and sponges are often common in reef habitats characterized by intense levels of fish predation. Both chemical and physical defenses have been hypothesized to contribute to the low susceptibility to predation of some benthic invertebrates. In this study, we focus on potential chemical and structural defenses of ascidians with a special emphasis on contrasting larval vs. adult defenses. We chose ascidians because their large larvae (2–7 mm in length) are amenable to chemical investigation, are likely to have evolved chemical defenses against predators because they are generally conspicuous, and because the larval ecology of these organisms is relatively well known (Davis and Butler 1989, Svane and Young 1989, Bingham and Young 1991) when compared to other benthic invertebrates. In addition, because ascidians and other benthic invertebrates, fishes, frogs, salamanders, and higher plants all have larval or seed stages that differ significantly from the adults, we hoped that this study might provide an initial contrast between ascidians and these other organisms as well as point out potential general patterns in adult vs. larval defenses.

Initial investigations of chemical defenses in ascidians (Stoecker 1980*a*, *b*) focused on the ability of sulfuric acid and the heavy metal vanadium to deter predators. Some ascidians, especially of the order Phlebobranchia, are known to contain extremely high concentrations of vanadium. Other ascidians, including some with high levels of vanadium, possess bladder cells that release highly acidic fluids when the tunic is damaged, and high concentrations of either vanadium or sulfuric acid have been experimentally shown to reduce food consumption by fishes and crustaceans significantly (Stoecker 1980*a*). Stoecker (1980*a*) also identified ascidians with low susceptibilities to predation even though they did not concentrate vanadium or possess bladder cells. These ascidians were hypothesized to be defended by secondary metabolites, but this was not tested. The trophic association between ascidians and various soft-bodied opisthobranch and prosobranch molluscs (Lambert 1980, Morris et al. 1980, Paul et al. 1990) that are believed to use dietary-derived compounds as a chemical defense against potential predators (reviewed by Karuso 1987) also suggests that ascidians may be chemically defended against generalized consumers. The evolutionary trend in numerous opisthobranch molluscs towards a reduction in shell size, or complete loss of the shell, has been correlated with the presence of bioactive secondary metabolites derived from chemically rich prey (Faulkner and Ghiselin 1983).

During the past decade, ascidians have gained recognition among chemists and pharmacologists as a rich source of novel bioactive secondary metabolites (reviewed by Faulkner 1984, 1986, 1987, 1988, 1990, Fenical 1986, and Ireland et al. 1988); however, except for the recent demonstration that several tambjamine-class alkaloids produced by the Indo-Pacific ascidian *Sigillina* cf. *signifera* (=*Atapozoa* sp.) deter feeding by reef fishes (Paul et al. 1990), the ecological role of ascidian secondary metabolites remains untested.

Most ascidians lack physical protection from predation although spicules of various types are found in

the tunic of some species (Monniot 1970, Kott 1980, Lambert and Lambert 1987), and these spicules have been hypothesized to deter potential predators (Olson 1986, Lambert and Lambert 1987). In situ feeding experiments have demonstrated a defensive role for the large sclerites of some gorgonian corals (Gerhart et al. 1988, Harvell et al. 1988, Harvell and Fenical 1989), but the ability of the generally much smaller ascidian spicules to deter predators has not been demonstrated.

The tough tunic produced by most solitary ascidians and some colonial ascidians also could make consumption difficult for certain groups of predators. However, during juvenile stages, reinforcement of the tunic by spicules and structural fibers may be inadequate to deter predation by even small fishes and invertebrates, and even adults with tough tunics may be consumed by larger fishes and crustaceans, or by gastropods that bore through the tunic (Millar 1971 and references therein, Russ 1980, Elner and Campbell 1987). It is conceivable that low nutritive value could minimize predation on ascidians; however, data complied by Conover (1978) indicated that ascidians have intermediate to high levels of protein, carbohydrate, and lipids when compared to other sessile benthic invertebrates. In addition, ascidian larvae are likely to be especially nutritious because they lack much of the physical reinforcement of the adults and because they are provisioned with stores of energy and nutrients that aid their transformation to a feeding juvenile.

Fish predation on larvae has been recognized as a major bottleneck in the recruitment of some marine invertebrates (Gaines and Roughgarden 1987, Olson and McPherson 1987, reviewed by Young and Chia 1987). The larvae of brooding (ovoviviparous) ascidians might be predicted to be especially vulnerable to fish predation because they are large (commonly 2–7 mm in length), lack structural defenses like the spines produced by crab zoeae (Morgan 1989), and are released during daylight hours when visually foraging fishes are most active. Larvae of didemnid ascidians that harbor symbiotic microalgae are released at midday (reviewed by Svane and Young 1989) and would thus seem particularly vulnerable to predation by fishes. Releasing larvae at midday when light is most intense has been hypothesized to facilitate settlement in microhabitats where light is adequate for photosynthesis but ultraviolet intensities are not lethal to the young colonies (Olson 1983, 1986).

Although the large size and daytime release of ascidian larvae has been exploited to make detailed observations of their swimming behavior, effective dispersal distance, and, in some cases, susceptibility to reef predators (van Duyl et al. 1981, Olson 1983, 1985, Young 1986, Olson and McPherson 1987, Davis and Butler 1989), rigorous studies have rarely been conducted to assess mechanisms or effectiveness of larval defense in ascidians. Although Orians and Janzen (1974) suggested that physiological constraints could preclude the chemical defense of animal eggs and larvae, Lindquist and Fenical (1991*a*) found sufficient quantities of tambjamine C in the larvae of *Sigillina* cf. *signifera* to deter feeding by coral-reef fishes (Paul et al. 1990), and Young and Bingham (1987) demonstrated a chemical basis for the unpalatability of larvae from the Caribbean ascidian *Ecteinascidia turbinata,* although no specific chemical deterrent was identified.

In this investigation, we examined: (1) the effects of secondary metabolites produced by several colonial ascidians from geographically distant locales on feeding by reef fishes or invertebrates, (2) the effects of spicules from didemnid ascidians on feeding by reef fishes, (3) the variability of didemnin cyclic peptides among separate colonies of the Caribbean ascidian *Tridemnum solidum,* (4) the susceptibility of larvae from *T. solidum* and the Indo-Pacific ascidian *Sigillina* cf. *signifera* to predation by reef fishes, (5) the palatability of crude lipophilic extracts of individual *T. solidum* larvae to the common predatory wrasse *Thalassoma bifasciatum,* and (6) the concentration of didemnin cyclic peptides in *T. solidum* larvae.

Methods

Organisms and study sites

Caribbean studies.—Tridemnum solidum (Aplousobranchia; Didemnidae) is an abundant encrusting ascidian in many reef habitats throughout the Caribbean Sea. It releases large tadpole larvae (2–3 mm in length) year round that generally swim <15 min before settling (van Duyl et al. 1981). Release occurs at midday when visually oriented predators are active, and the larvae are reported to be unpalatable to reef fishes (van Duyl et al. 1981).

Adult colonies of *Tridemnum solidum,* like its larvae, also appear to experience little predation (Bak et al. 1981, Olson 1986). Low susceptibility to predation could result from the bioactive cyclic peptides the colonies produce (Rinehart et al. 1981), the large quantities of small (20–50 μm in diameter) stellate spicules contained in their tissues (Olson 1986), poor nutritive value, or a tough tunic. These potential defenses against predators are not mutually exclusive and none has been experimentally investigated.

We collected *Tridemnum solidum* from shallow-water habitats at Guadeloupe Island, Eastern Caribbean Sea, in July 1985 and at Little San Salvador, Bahamas in August 1990. At each site, collected colonies were combined and immediately frozen. In addition to the mass collection at Little San Salvador, 12 separate colonies were collected from 1–3 m depth on a single patch reef and frozen in individual plastic bags for later quantification of their secondary metabolites by analytical HPLC methods. From this same patch reef, we also collected various *T. solidum* colonies from which fully developed larvae, appearing as dark spots under the tunic, were obtained by piercing the tunic

over a larva and removing the larva with a glass pipet. Using this procedure, we collected 1101 larvae for chemical analysis in addition to sufficient numbers for use in the feeding studies described below. Prior to lyophilization and extraction, colonies of *T. solidum* collected at Little San Salvador were thawed and their volume measured by displacement of seawater in a graduated cylinder. This allowed us to determine concentrations of secondary metabolites as a function of ascidian volume. Although most solitary ascidians, and some colonial ascidians, are highly contractile and thus likely to change their size after collection and preservation, colonies of *T. solidum* and the other ascidians utilized in our investigations maintained their macroscopic morphology after collection and preservation by freezing or storage in alcohol so that the measured volumes of our preserved collections provided a good estimate of their sizes. The total volume of the 1101 larvae was measured by their displacement of seawater in a 1000-μL syringe.

The secondary metabolites of a related Caribbean ascidian, *Trididemnum* cf. *cyanophorum* (Aplousobranchia; Didemnidae) were also investigated for their potential defensive properties. *T.* cf. *cyanophorum* possesses an algal symbiont and likely releases its larvae at midday. The susceptibility of this ascidian or its larvae to fish predators is unknown. *T.* cf. *cyanophorum* grew as small encrusting colonies on blades of the seagrass *Thalassia testudinum* and on various green algae, primarily species of *Halimeda*. This ascidian was collected in August 1984 in a seagrass bed on the northwest corner of Shroud Cay in the Bahamas and in August 1987 in the mangrove channels of Sawyer Key located north of Big Pine Key, Florida in the Gulf of Mexico. Collections were rapidly frozen to preserve their chemical composition. Both collections yielded didemnenones A and B as the major secondary metabolites of this ascidian (Lindquist et al. 1988*a*). These epimeric compounds exist as an $\approx$1:1 mixture and effectively inhibited the growth of two marine bacteria and the pathogenic marine fungus *Lagenidium callinectes* (Lindquist et al. 1988*a*).

Indo-Pacific studies.—From the Indo-Pacific, we studied two common colonial ascidians, *Lissoclinum patella* (Aplousobranchia; Didemnidae) and *Sigillina* cf. *signifera* (Aplousobranchia; Polycitoridae). *L. patella* is abundant throughout vast regions of the tropical Indo-Pacific growing primarily on exposed substrate in shallow reef habitats. This didemnid ascidian, like those we investigated from the Caribbean Sea, possesses symbiotic microalgae. Although the relatively large tadpole larvae of this ascidian are often consumed by small reef fishes, predation on the adult colonies has not been observed (Olson and McPherson 1987). Previous chemical investigations of geographically diverse collections of *L. patella* have yielded four distinct classes of secondary metabolites with each collection possessing compounds from only one or two of the structural types (Faulkner 1984, 1986, 1987, 198 1990, Davidson and Ireland 1990). Although sever of these compounds are cytotoxins, their ecologic significance is uninvestigated. We collected colonies *L. patella* in May 1987 from a depth of 3 m at Sabtar Island, Batanes, the northernmost group of Philippir Islands. This collection was preserved in methanol fc later extraction of its secondary chemistry. The volum of this collection was determined by its displacemer of methanol in a graduated cylinder.

Sigillina cf. *signifera* (=*Atapozoa* sp. in Paul et a 1990) is a soft fleshy ascidian with dark green colo ation that is not due to the presence of an algal sym biont, as it is in some didemnid ascidians, but rathe to pigments and the tambjamine-class alkaloids se questered within its granular amebocyte blood cell (Lindquist and Fenical 1991*a*). This ascidian is com mon on shallow exposed substrates throughout th western tropical Pacific and produces tambjamine-clas alkaloids, some of which deter feeding by coral-ree fishes on Guam (Paul et al. 1990). Specifically, tamb jamines C and F significantly reduced feeding by ree fishes, but tambjamine E, the major compound in th adult colonies at several study sites in the central Phil ippines, did not significantly affect food consumption Because *S.* cf. *signifera* is not known to occur on Guam we decided to test the feeding effects of tambjamine E on fishes inhabiting patch reefs where *S.* cf. *signifera* was common, near the Silliman University Marine Laboratory, Dumaguete City, Philippines.

The large (5–7 mm in length), blue larvae of *Sigillina* cf. *signifera* are released from the adults beginning $\approx$2 h after sunrise (N. Lindquist, *personal observation*). Their bright color and daytime release should make them conspicuous to foraging reef fishes, but in situ observations indicate that the larvae are avoided by reef fishes (N. Lindquist, *personal observation*). That this avoidance might be chemically mediated is suggested by the fact that the larvae contain high concentrations of tambjamine C (Lindquist and Fenical 1991*a*). The combined volume of 183 fresh *S. signifera* larvae was measured by their displacement of seawater in a 1000-μL syringe.

Collections of *S.* cf. *signifera* for our study were obtained from a small region within the central Philippines including Siquijor, Apo, and Sumilon Islands and patch reefs offshore from the Silliman University Marine Laboratory. The volumes of several collections of this ascidian were measured by their displacement of seawater in a graduated cylinder before the animals were frozen or preserved in methanol.

Gulf of California studies.—Near Guaymas, Mexico in January 1986, we commonly found the encrusting colonial ascidian *Polyandrocarpa* sp. (Stolidobranchia; Styelidae) from the low intertidal zone to a depth of 10 m. The conspicuous red ascidian was peeled from the substrate and preserved in isopropyl alcohol. Initial chemical investigations of this ascidian yielded large

quantities of the novel bioactive alkaloids polyandrocarpidines A–D (Cheng and Rinehart 1978, Carté and Faulkner 1982). Because separation of the individual compounds is exceedingly difficult, we worked with a naturally occurring mixture of polyandrocarpidines A–D. These compounds exhibit potent antimicrobial activity and cytotoxicity against several cancer cell lines (Cheng and Rinehart 1978); their ecological function has not been investigated.

Temperate Pacific studies.—The only ascidian from a temperate habitat examined in this study was *Aplidium californicum* (Aplousobranchia; Polyclinidae). This ascidian is a common member of many shallow benthic marine communities along the west coast of California and Baja California, Mexico. A chemical study of *A. californicum* collected near San Francisco yielded several prenylated hydroquinone derivatives reported to have antimutagenic activity and cancer-protective properties (Howard et al. 1979). We collected *A. californicum* near Punta Baja, Mexico in July 1985 and isolated prenyl hydroquinone as its major secondary metabolite. The effect of these prenylated hydroquinones on feeding by consumers has not been investigated, but structurally more complex hydroquinone derivatives from algae have shown variable effects on feeding by herbivorous fishes (Hay et al. 1987, 1988).

Feeding assays

In August 1987, at a 5 m deep reef ≈1 km east of Looe Key, Florida, we performed feeding assays to investigate the effect of various ascidian secondary metabolites on feeding by coral-reef fishes. The assays were performed using agar-carrageenan pellets incorporating freeze-dried brine shrimp as the feeding attractant. These pellets were made by stirring 0.5 g of agar, 0.6 g of carrageenan, and 0.4 g of freeze-dried brine shrimp into 40 mL of distilled water. This mixture was heated in a boiling water bath until all the agar and carrageenan had dissolved. It was then transferred to a 35°C water bath to prevent premature gelling while the test compound, dissolved in 100 μL of absolute ethanol (treatment pellets), was stirred into the mixture. Control pellets were made with an equal amount of ethanol but no compound. The mixture was transferred using a pasteur pipet to brass rings (0.73 mL volume) lying on a flat plastic tray. Each ring was filled to half its volume with either the test or control mixture and allowed 15 s to begin solidifying. The remainder of the ring was then filled with an excess volume of the food preparation, which solidifed as a dome on top of the ring. After cooling for 20 min, the excess food above the edge of the ring was cut off with a razor blade and the pellets were slid out of the rings. Treatment and control pellets were placed in separate containers and stored on ice until starting an assay. Typically, 20 treatment and 20 control pellets were taken to the test site.

The test site was a flat rock shelf dominated by various gorgonian corals. Neither *Tridemnum solidum* nor *Tridemnum* cf. *cyanophorum* was present at this reef site. The bluehead wrasse *Thalassoma bifasciatum* and the bicolor damselfish *Eupomacentrus partitus*, both broadly distributed throughout the Caribbean Sea and reported to feed on a great variety of benthic invertebrates and zooplankton (Randall 1968), were abundant at this site and were responsible for virtually all consumption that occurred during these assays. The feeding assays were performed by two divers using SCUBA. A control pellet and a treatment pellet were released one pair at a time in a haphazard order, and each pair of pellets was released at a new location several metres away from the previous one. New release locations were chosen in a haphazard manner, attempting to avoid previously used areas. As pellets were released ≈1 m above the substrate, *Thalassoma* would quickly swim in to investigate. After the first bite was taken from a pellet, we allowed 60 s for feeding to proceed and then recovered any uneaten portion of the pellet. Because preliminary trials testing the feasibility of this assay procedure at this site showed that a palatable pellet would be completely consumed within 60 s after feeding commenced, we felt confident that differences in the consumption of treatment and control pellets within 60 s produced a reasonable comparison of their palatabilities. For assays in which both treatment and control pellets were completely consumed in <60 s, we also recorded the time fish took to consume each pellet or the number of bites taken from each pellet and whether or not the bites were swallowed or rejected. These procedures allowed us (using a *t* test) to determine if control pellets were treated differently than treatment pellets even though both were eaten rapidly.

At the end of an assay, treatment and control pellets exposed to fish grazing and only partially consumed and those not exposed (i.e., kept in the same containers but not offered to the fish on the reef) were returned to the laboratory. The pellets were weighed after blotting away excess water with a paper towel. Pellet mass data in the assay with two treatments and a control were analyzed for a significant difference in pellet consumption using the Kruskal-Wallis test followed by the nonparametric equivalent of the Tukey test (Zar 1984: 199–201). For assays with one treatment and a control, significant differences in pellet consumption were determined by the Mann-Whitney test. Mass changes in the treatment and control pellets that were not exposed to fish feeding differed by no more than 5% and were not factored into the statistical analyses because this change was trivial compared to our among-treatment differences.

Potential defensive substances tested by this procedure at Looe Key, Florida and their concentrations in these assays were didemnin B (0.41 mg/mL), nordidemnin B (0.20 mg/mL and 0.020 mg/mL), and the stellate spicules (300 mg/mL) from the Caribbean as-

cidian *Tridemnum solidum*; didemnenones A and B (2.2 mg/mL) from the Caribbean ascidian *Tridemnum* cf. *cyanophorum*; and prenyl hydroquinone (0.27 mg/mL) from the temperate Pacific ascidian *Aplidium californicum*. These concentrations ranged from 6 to 100% of natural concentrations.

A similar field feeding assay was conducted in May 1988 at a patch reef adjacent to the Silliman University Marine Laboratory near Dumaguete City, Philippines using natural concentrations of tambjamine E (0.22 mg/mL) and patellamide C (0.10 mg/mL) from the Indo-Pacific ascidians *Sigillina* cf. *signifera* and *Lissoclinum patella*, respectively. This assay differed from the one previously described by employing a different pellet formulation. Agar was the only gelling agent used and the feeding attractant was squid mantle flesh that had been liquified in a blender and strained. The pellet formula consisted of 16 mL of distilled water, 4 mL of squid, 0.4 g agar, and 200 μL of ethanol (control) or test compound dissolved in 200 μL of ethanol. The "squid paste" was mixed with 6 mL of distilled water and the test compound in ethanol (or ethanol alone). This mixture was warmed to 35°C, and then combined with 0.4 g agar dissolved in the remaining 10 mL of boiling water. The combined mixtures were thoroughly stirred at 35°C and the pellets formed as previously described.

The assay site offshore from the Silliman University Marine Laboratory was a shallow patch reef, 0.5–2.5 m in depth, with numerous species of small carnivorous and omnivorous fishes. *Sigillina* cf. *signifera* was common in this community while *Lissoclinum patella* was not encountered there in 1986 or 1987, but several small colonies were found in 1988. The major consumers of the pellets released at this site included the wrasses *Cheilinus trilobatus* and *Thlassoma lunare*, and the pomacentrids *Abudefduf saxatilis*, *Pomacentrus popei*, *Pomacentrus flavicauda*, and *Amphiprion melanopus*. These fishes are reported to feed on a great variety of benthic invertebrates, zooplankton, and algae (Myers 1989) and are distributed throughout the known range of *S. signifera*. The uneaten portions of the treatment and control pellets were frozen, returned to Scripps Institution of Oceanography, and weighed. Mass changes in treatment and control pellets not exposed to fish were not determined but were likely unimportant relative to the amount of food consumed (65–100%, see *Results: Indo-Pacific ascidians*) because experiments with the polyandrocarpidines using the same squid-based food showed no detectable changes in mass of either treatment ($P > .5$, $N = 30$, t test) or control ($.5 > P > .2$, $N = 30$, t test) pellets after 5.5 h in seawater. The pellet mass data were analyzed for significant differences in consumption using the Kruskal-Wallis test followed by the nonparametric equivalent of the Tukey test.

To examine the palatability of the polyandrocarpidine A–D mixture, isolated from the ascidian *Polyandrocarpa* sp. collected in the Gulf of California, laboratory feeding assays were conducted with three omnivorous invertebrates that co-occur with *Polyandrocarpa* sp.: the snails *Tegula rugosa* and *Crassipira pluto*, and the hermit crab *Clibanarius digueti*. These animals were collected from the lower intertidal zone at Bahia de Los Angeles where *Polyandrocarpa* sp. was also abundant, transported to Scripps Institution of Oceanography, and maintained in flowing seawater aquaria. The hermit crab *Pagurus granosimanus* and the rock shrimp *Lysmata californica*, collected intertidally at La Jolla, California, do not co-occur with *Polyandcrocarpa* sp. but were also tested for their avoidance of food containing these compounds. The squid-based food used in these feeding assays was prepared in the same manner as the food used in the feeding assay of tambjamine E and patellamide C and fed daily to the test organisms. Treatment pellets contained the natural concentration of 2.0 mg/mL of the polyandrocarpidine A–D mixture. On days when a feeding experiment was performed, test and control pellets were substituted for that day's feeding. The pellets were formed in brass or plastic rings placed over a circular piece of velcro glued to a polyvinyl chloride (PVC) disk. Pellets formed over velcro in this fashion were securely fastened to the PVC disk. Brass rings (0.73 mL) were used to make pellets on PVC disks 7.8 cm in diameter and offered to *Pagurus granisomanus* and *Tegula rugosa*, while plastic rings (0.20 mL) were used to make pellets on PVC disks 5.2 cm in diameter and offered to *Crassipira pluto*, *Clibanarius digueti*, and *Lysmata californica*. A treatment and control pellet were formed on opposite edges on the upper side of each disk and a waterproof mark on the edge of the disk identified each pellet. One disk and one test animal were placed in each of 16 600-mL glass beakers filled with seawater. Each beaker was covered with fiberglass screen to prevent the animal from escaping and placed back into flowing seawater. Individual animals were allowed to feed until $\approx$50% of either pellet on a disk was consumed. Remaining portions of treatment and control pellets were cut away from the disk at the velcro interface and weighed. The paired pellet mass data were analyzed for significant differences in consumption using the paired-sample t test. The mass of treatment and control pellets not exposed to consumers did not differ.

In shipboard feeding assays in the Bahamas, we examined the susceptibility of larvae from *Tridemnum solidum* to predation by the bluhead wrasse *Thalassoma bifasciatum* and how their crude organic extract affected feeding by *Thalassoma*. *Thlassoma bifasciatum* is one of the most abundant small carnivores on Caribbean reefs, and the importance of this wrasse as a potential larval predator was illustrated in a field feeding assay conducted at San Salvador, Bahamas during which 19 of 21 larva-sized pieces of artificial food released between 0.5 and 1 m above the substrate

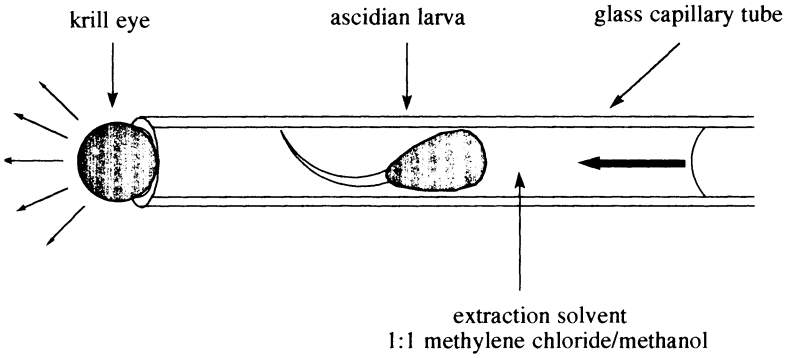

FIG. 1. The "micromethod" employed to extract the organic soluble compounds from an individual ascidian larva and impregnate that extract into a single krill eye. After inserting the larva 0.25 cm into a capillary tube, the left end containing the larva (as shown in the figure) was dipped into the extraction solvent, which was drawn past the larvae ≈2.5 cm into the tube by capillary forces. The right end of the tube was sealed with parafilm and a single krill eye placed on the open end. Evaporation of the solvent from the krill eye drew the solvent past the stationary larva, extracted the organic soluble compounds, and impregnated them into the krill eye. As the last of the solvent evaporated, the krill eye fell from the end of the tube.

were consumed by *Thalassoma* (N. Lindquist, *unpublished data*).

Eight groups of 5–7 wrasses and one larger group of 20–25 fish were kept in separate flow-through seawater aquaria. The fish were fed an ample amount of food 1.5 h prior to each feeding assay so they were not starved. To test the palatability of *T. solidum* larvae, we used the eyes from freeze-dried krill as a palatable larval mimic because these were similar in size and shading to the *T. solidum* larvae and were readily eaten by the fish. Each of the nine groups of wrasses were offered in order: a krill eye, a *T. solidum* larva, and a second krill eye. Susceptibility was scored as: (1) consumed, if the fish ultimately ate the food regardless of the number of rejections or (2) not consumed, if after being rejected it settled to the bottom of the aquarium. The second krill eye offered to the fish was always eaten, indicating the fish were still foraging when the larva was offered. The Fisher exact test was used to determine if consumption of larvae differed significantly from consumption of krill eyes.

The unpalatability of the crude organic extract from individual *Tridemnum solidum* larvae was investigated by impregnating the extract from a single larva into a single freeze-dried krill eye. The extraction of an individual larva was accomplished by inserting it ≈0.5 cm into a capillary tube (see Fig. 1). The tube was dipped into a mixture of 1:1 dichloromethane–methanol and capillary action drew the solvent approximately 2.5 cm into the tube. The opposite end of the tube was sealed with parafilm and a krill eye was placed on the open end of the tube. Evaporation of solvent from the krill eye drew the solvent past the larva, thus extracting its lipophilic organic compounds and impregnating the krill eye with this crude organic extract. After all the solvent evaporated, the krill eye would fall off the end of the capillary tube. Control krill eyes were treated in the same manner but with solvent alone. A treatment and control krill eye were offered to each of the nine groups of *Thalassoma bifasciatum* and susceptibility recorded as previously described. Data were analyzed by the Fisher exact test.

The in situ susceptibility of *Sigillina* cf. *signifera* larvae was experimentally determined by releasing a larva and a similar-sized piece of squid at a site on a patch reef where this ascidian was abundant. Fully developed larvae were dissected from colonies collected shortly after dawn, but by the time the assay was conducted, larvae did not swim when released. Twenty-one larvae and 21 squid pieces were offered to the fish and the order of release was varied haphazardly. The larvae and squid pieces were observed until they traveled ≈3 m from the release site. Two larval data points were not included in the analysis, since the fate of these larvae was not adequately determined. Susceptibility was recorded as described above and the data were analyzed by the Fisher exact test.

Extraction, isolation, and concentrations of assay materials

Our usual procedure for isolating lipophilic secondary metabolites from ascidians began by lyophilizing the frozen animal or decanting off the alcohol from collections preserved in methanol or isopropyl alcohol. Lyophilized and alcohol-preserved ascidians were then ground in a blender with organic solvents, usually a 2:1 mixture of dichloromethane : methanol. The ground animal was soaked in the extraction solvent for 3–6 h. The extraction solvent was then decanted and fresh solvent added to the ground tissues. This procedure was repeated 3–4 times and the solvents were removed from the combined extractions by rotary evaporation under reduced pressure. Residual water was removed from this organic residue by high-vacuum evaporation. Vacuum-flash chromatography over silica gel was generally the first fractionation procedure used, with the solvent elution scheme tailored to the polarity of the compounds being isolated. Next, size exclusion chro-

TABLE 1. Ash content and concentration of didemnin cyclic peptides from 12 separate colonies (A–L) of the ascidian *Tridemnum solidum* and from a single collection of 1101 *T. solidum* larvae obtained from multiple colonies. Concen-

Colony	Ash (% dry mass)	Didemnin B		Nordidemnin B		Didemnin D	
		mg/mL	%	mg/mL	%	mg/mL	%
A	86.9	0.239	0.342	0.037	0.053	0.080	0.115
B	81.8	0.368	0.379	0.044	0.046	0.005	0.005
C	85.5	0.542	0.699	0.080	0.103	0.127	0.163
D	87.4	0.624	0.927	0.056	0.083	0.260	0.386
E	82.4	0.588	0.625	0.074	0.079	0.494	0.526
F	81.6	0.983	1.001	0.097	0.098	0.306	0.311
G	82.4	0.950	1.011	0.138	0.147	0.306	0.326
H	82.9	0.916	1.003	0.130	0.142	0.404	0.443
I	82.4	1.088	1.157	0.121	0.129	0.306	0.326
J	81.1	1.105	1.095	0.162	0.160	0.360	0.357
K	82.4	1.234	1.313	0.124	0.132	0.360	0.384
L	79.6	1.197	1.100	0.173	0.159	0.522	0.479
	Mean ± SE	Mean ± SE		Mean ± SE		Mean ± SE	
Mass/volume		0.820 ± 0.097		0.103 ± 0.013		0.294 ± 0.045	
%	83.0 ± 0.7	0.888 ± 0.089		0.111 ± 0.012		0.318 ± 0.044	
Larvae		0.777	0.638	0.109	0.089	0.181	0.149

matography using Sephadex LH-20 was generally used before the final purification of the secondary metabolites performed by silica or C-18 reversed-phase HPLC. Structures of the purified metabolites used in this study were confirmed by comparing their proton and carbon-13 nuclear magnetic resonance (NMR), ultraviolet, infrared, and mass spectral features, and optical rotations, with values previously reported in the following references: didemnin cyclic peptides from *Trididemnum solidum* (Rinehart et al. 1981, McKee et al. 1989); didemnenones A and B from *Trididemnum* cf. *cyanophorum* (Lindquist et al. 1988*a*); cyclic peptides from *Lissoclinum patella* (Sesin et al. 1986, Zabriske et al. 1988); tambjamine-class alkaloids from *Sigillina* cf. *signifera* (Carté and Faulkner 1983, Lindquist and Fenical 1991*a*); polyandrocarpidines A–D from *Polyandrocarpa* sp. (Carté and Faulkner 1982); prenyl hydroquinone from *Aplidium californicum* (Howard et al. 1979).

The small stellate spicules of *Trididemnum solidum* were obtained by digesting 50.4 g of dried, extracted ascidian tissue with full-strength chlorine bleach for 3 d. The remaining material (47.2 g) was then washed with distilled water, dried, and weighed. Microscopic examination of the residue revealed that only the stellate spicules, common to many didemnid ascidians, were present. From this spicule yield, the total mass of spicules in this collection of *T. solidum* was determined.

Several of the ascidians were collected and extracted before deciding to test compound palatability on a volumetric basis rather than a dry mass basis. For those ascidians, the volume of the collection was estimated by the methods described below. The collection volume of *Trididemnum* cf. *cyanophorum* from the Bahamas was estimated from the volume it occupied in the collection container. The volume of *Trididemnum solidum* collected at Guadeloupe was estimated using the volume-to-dry-mass ratio (1.87 mL/g) measured for *T. solidum* collected at Little San Salvador, Bahamas. The collection volume of *Polyandrocarpa* sp. from the Gulf of California was estimated by multiplying its dry mass by the volume-to-dry-mass ratio (7.63 mL/g) for the morphologically similar ascidian *Metandrocarpa dura* collected at La Jolla, California.

Quantitative HPLC analysis of didemnin cyclic peptides in Tridemnum solidum

Quantification of didemnin cyclic peptides in 12 separate colonies of *Trididemnum solidum* and one pooled group of larvae collected at Little San Salvador, Bahamas was accomplished by the analytical HPLC methods described in the Appendix.

RESULTS

Caribbean ascidians.—Trididemnum solidum collected at Guadeloupe Island yielded 0.13 and 0.06% by dry mass of didemnin B and nordidemnin B, respectively, corresponding to volumetric concentrations of 0.69 and 0.33 mg/mL, respectively. The analyses of 12 separate colonies of *Trididemnum solidum* from Little San Salvador showed didemnin B ($\bar{X} \pm 1$ SE = 0.82 ± 0.10 mg/mL), didemnin E (0.55 ± 0.08 mg/mL), and didemnin D (0.29 ± 0.05 mg/mL) to be the major secondary metabolites. Smaller quantities of nordidemnin B (0.10 mg/mL), didemnin X (0.18 ± 0.02 mg/mL), and didemnin M (0.08 ± 0.01 mg/mL) were also detected (Table 1). The range of concentrations for individual peptides in the separate colonies varied as follows: didemnin B: 0.239 to 1.234 mg/mL, nordidemnin B: 0.037 to 0.173 mg/mL, didemnin D: 0.005 to 0.522 mg/mL, didemnin E: 0.120 to 0.991 mg/mL, didemnin X: 0.051 to 0.294 mg/mL, and didemnin M: 0.028 to 0.155 mg/mL. In general, colonies rich in one

trations are listed both as mass of compound per unit volume of ascidian tissue, and with mass of the compound given as a % of the ash-free dry mass of the colony.

Didemnin E		Didemnin X		Didemnin M	
mg/mL	%	mg/mL	%	mg/mL	%
0.139	0.198	0.051	0.073	0.034	0.049
0.120	0.123	0.242	0.249	0.089	0.092
0.381	0.492	0.078	0.101	0.054	0.070
0.354	0.526	0.088	0.130	0.028	0.040
0.711	0.757	0.197	0.210	0.063	0.068
0.603	0.614	0.190	0.193	0.067	0.068
0.584	0.621	0.191	0.203	0.115	0.123
0.704	0.771	0.143	0.157	0.086	0.095
0.638	0.679	0.216	0.230	0.078	0.084
0.681	0.675	0.250	0.248	0.108	0.107
0.743	0.790	0.203	0.216	0.065	0.069
0.991	0.910	0.294	0.270	0.155	0.143
Mean ± SE		Mean ± SE		Mean ± SE	
0.554 ± 0.075		0.179 ± 0.021		0.079 ± 0.010	
0.596 ± 0.068		0.190 ± 0.018		0.084 ± 0.030	
0.412	0.339	0.0 ± 0		0.0 ± 0	

compound were rich in all compounds (Table 1). As an example, simple rank correlations between the concentration of didemnin B and each of the other compounds were positive and always significant (nordidemnin B, $r_s = 0.82$; didemnin D, $r_s = 0.71$; didemnin E, $r_s = 0.86$; didemnin X, $r_s = 0.62$; didemnin M, $r_s = 0.59$; $P < .05$, two-tailed Spearman rank correlation coefficient).

At 60% of their natural mean concentration in the Guadeloupe collection of *Trididemnum solidum*, didemnin B and nordidemnin B both depressed feeding by reef fishes at Looe Key, Florida by a significant 73% ($P < .001$, Kruskal-Wallis test followed by the nonparametric equivalent of the Tukey test, Fig. 2). Nordidemnin B remained a significant feeding deterrent when tested at 6% of its natural concentration ($.025 > P > .01$, Mann-Whitney test, Fig. 2). Because these compounds also deter feeding by three temperate consumers common to coastal North Carolina, the pinfish *Lagodon rhomboides*, the sea urchin *Arbacia punctulata*, and the sea anemone *Aiptasia pallida*, a potential larval predator (N. Lindquist, *unpublished data*), they may be unpalatable to a wide variety of potential fish and invertebrate predators on Caribbean reefs. The mean concentrations of didemnin B and nordidemnin B in colonies of *T. solidum* from Little San Salvador in the Bahamas were +19 and −68%, respectively, of their concentrations in this ascidian from Guadeloupe. The concentration of didemnin B used in our assay was only 50% of the mean concentration in *T. solidum* colonies from Little San Salvador; the two assay concentrations of nordidemnin B bracketed the entire range of concentrations found for this cyclic peptide in colonies of *T. solidum* from Little San Salvador (Fig. 3). Significant quantities of several other didemnin-class peptides were present in both collections of *T. solidum*, and although their potential as feeding deterrents has not been investigated, didemnin B and nordidemnin B together afford this ascidian significant chemical protection against predation by coral-reef fishes. Didemnin B and related cyclic peptides have also been isolated from *T. solidum* collected at several sites along the western coast of the Caribbean Sea (Rinehart et al. 1981).

Trididemnum cf. *cyanophorum* collected at Shroud Cay, Bahamas, contained didemnenones A and B at 0.7% of dry mass or 2.2 mg/mL of tissue. These compounds had no effect on feeding by coral-reef fishes; both treatment and control pellets were completely consumed within 1 min (Fig. 2), with consumption times of 19.8 ± 7.3 s and 21.6 ± 22.3 s ($\bar{X}$ ± 1 SD) for treatment and control pellets. Consumption times for treatment and control pellets ranged from 11–26 s and 5–60 s, respectively, and likely reflect the variable number of fishes feeding at the points where individual pellets were released. Based on the smaller molecular size of the didemnenones and their assay concentration of 2.2 mg/mL, this treatment food had ≈500 times more molecules per unit of volume than did the unpalatable treatment food incorporating 0.02 mg/mL of nordidemnin B. The edibility of pellets containing didemnenones A and B demonstrates that the fishes feeding in these field assays were not simply deterred by the presence of unusual organic molecules in the food. Thus, the reduced consumption of foods containing didemnin B and nordidemnin B was not an artifact of the assay method alone.

The concentration of stellate spicules in *Trididemnum solidum* from Guadeloupe Island was 500 mg/mL (determined for the mass collection). Using percent ash as an estimate of spicule mass (Table 1), the concentration from 15 separate colonies from Little San Salvador was, at most, 442 ± 11.5 mg/mL ($\bar{X}$ ± 1 SD) and microscopic examination of the ash found it to be

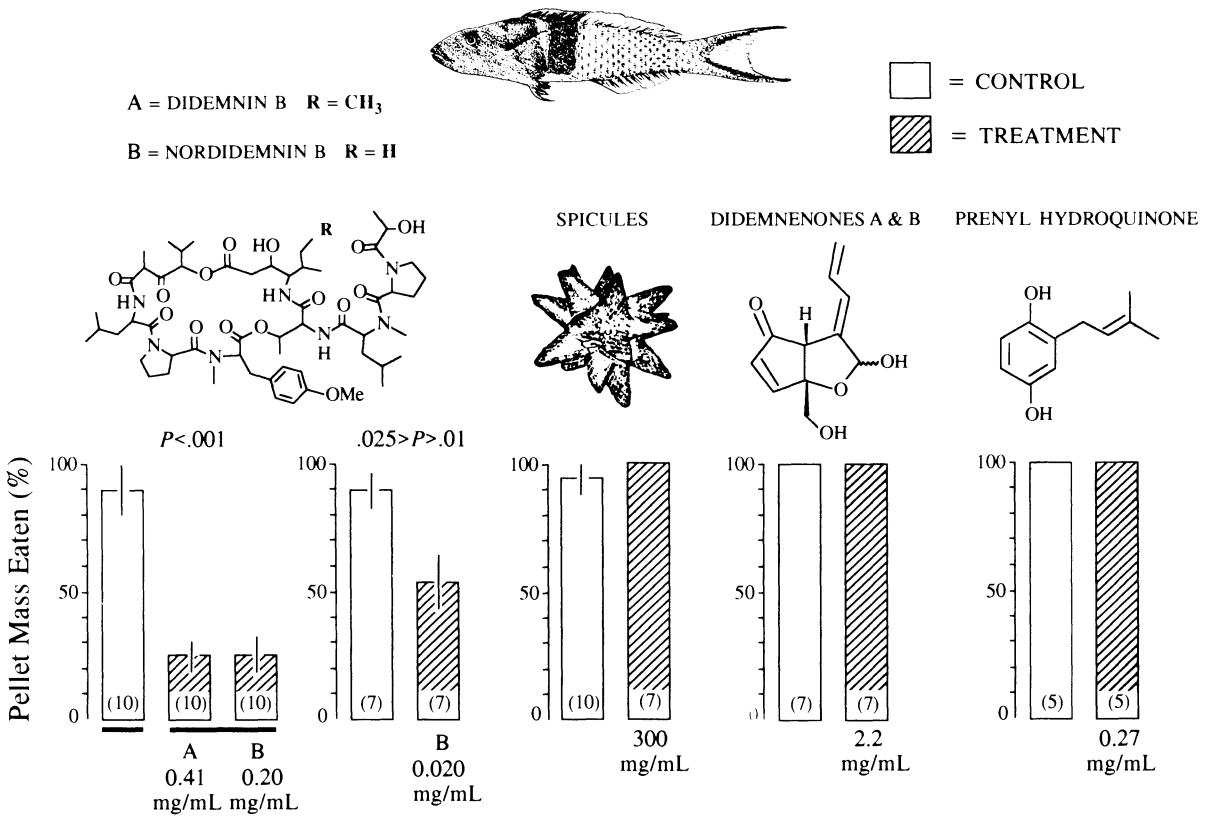

FIG. 2. The effect of four different ascidian metabolites and of stellate ascidian spicules on feeding by coral reef fishes on a reef near Looe Key, Florida. Error bars show ±1 SE. *N* for each assay is in parentheses at the base of each histogram bar. In the assay with three treatments, horizontal bars beneath histogram bars connect treatments that are not significantly different by a Kruskal-Wallis test followed by the nonparametric equivalent of the Tukey test (Zar 1984:199–201). Significance values for assays with two treatments are from the Mann-Whitney test.

almost entirely composed of spicules. These spicules, structurally representative of didemnid spicules in general, had no effect on feeding by coral-reef fishes ($P > .50$, Fig. 2) when tested at 60–68% of their natural volumetric concentration. Treatment pellets were consumed in 15.8 ± 1.8 bites ($\bar{X}$ ± 1 SD, $N = 5$) without a single bite being rejected for the five trials that were counted, while control pellets were completely consumed in 17.4 ± 2.8 bites ($N = 9$) with only one bit being rejected in the nine trials that were counted. Although the volumetric concentration of spicules in the test food was less than the volumetric concentration of spicules in *T. solidum* colonies collected from light-exposed habitats at Guadeloupe and Little San Salvador, Olson (1986) found a significantly lower concentration of spicules in *T. solidum* colonies at Galeta, Panama growing in shaded habitats than in light-exposed habitats. Thus our test concentration likely falls within the range of naturally occurring spicule concentrations for this ascidian.

Indo-Pacific ascidians.—The common didemnid ascidian *Lissoclinum patella* collected at Sabtang Island, Philippines contained 0.10 mg/mL (0.05% dry mass) of patellamide C. The concentration of tambjamine E from the Indo-Pacific ascidian *Sigillina* cf. *signifera* was 0.22 mg/mL.

When tested at a field site where *Sigillina* cf. *signifera* was abundant, the natural concentration of tambjamine E reduced consumption of treatment pellets by coral-reef fishes by a significant 35% ($P < .025$, Kruskal-Wallis test followed by the nonparametric equivalent of the Tukey test, Fig. 4). Patellamide C from *Lissoclinum patella* produced a 14% reduction in feeding by coral reef fishes, but the reduction was not statistically significant ($P > .5$, Kruskal-Wallis test followed by the nonparametric equivalent of the Tukey test, Fig. 4).

Gulf of California ascidians.—*Polyandrocarpa* sp. collected near Guaymas, Mexico yielded 2.03 milligrams of polyandrocarpidines A–D per millilitre of ascidian tissue. In laboratory assays, the mixture of polyandrocarpidines greatly reduced consumption of treatment pellets by the hermit crab *Clibanarius digueti* (consumption reduced by 100%, $P < .0005$) and the

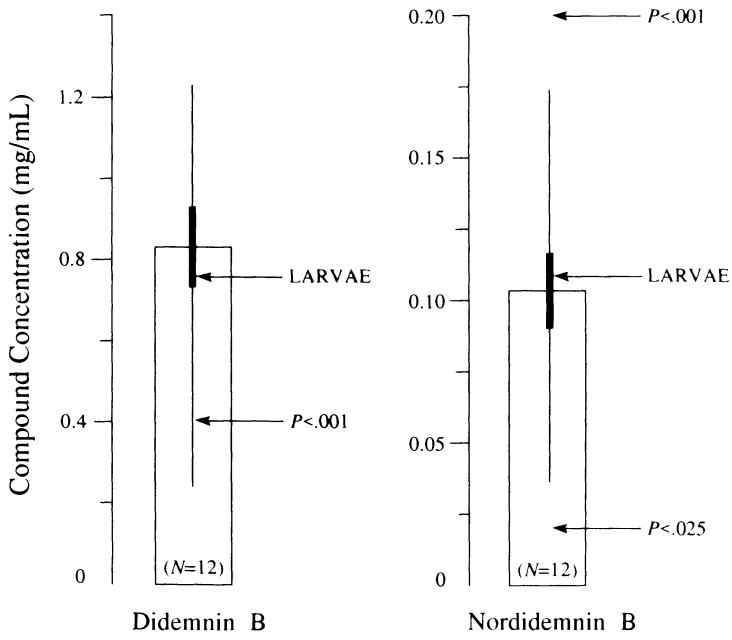

FIG. 3. Concentration mean ± 1 SE (thick vertical line) and range (thin vertical line) of didemnin B and nordidemnin B for 12 separate colonies of the Caribbean ascidian *Tridemnum solidum* collected at Little San Salvador, Bahamas. The concentration of each compound in a pooled collection of 1101 *T. solidum* larvae from multiple colonies collected at Little San Salvador is indicated. *P* values are from field feeding assays (Fig. 2) with the concentration of compound used in the assay indicated by the label's position relative to the vertical axes.

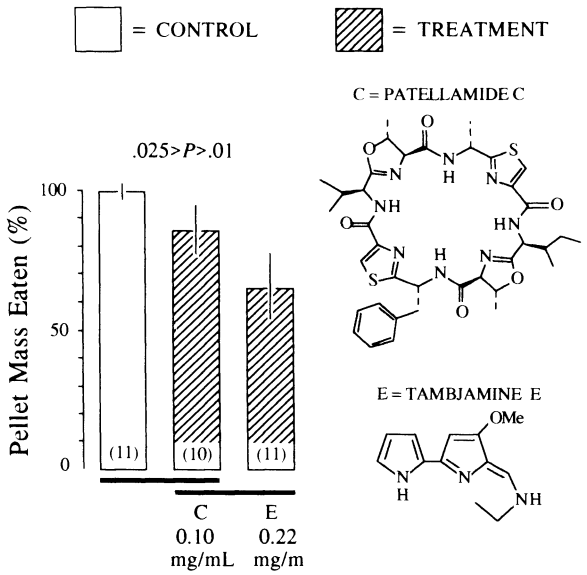

FIG. 4. The effect of patellamide C and tambjamine E from the Indo-Pacific ascidians *Lissoclinum patella* and *Sigillina* cf. *signifera*, respectively, on feeding by coral reef fishes. The assay was conducted on a patch reef near Dumaguete City, Philippines. The *P* value is for the difference between treatments that are not connected by a bar beneath the histograms (nonparametric equivalent of the Tukey test; Zar 1984:199–201). Other symbols are as in Fig. 2.

marine snails *Crassipira pluto* (−79%, $P < .0005$) and *Tegula rugosa* (−82%, $.025 > P > .01$, all data analyzed by the paired-sample *t* test, Fig. 5). These invertebrate consumers co-occur with *Polyandrocarpa*. The ability of these secondary metabolites to deter feeding by two allopatric invertebrate consumers collected at La Jolla, California was more variable. The hermit crab *Pagurus granosimanus* significantly reduced its consumption of pellets containing these compounds (−56%, $.01 > P > .005$, paired-sample *t* test, Fig. 5). Although these compounds produced a 37% reduction in the consumption of treatment food by the shrimp *Lysmata californica*, this reduction was not statistically significant ($.25 > P > .1$, paired-sample *t* test, Fig. 5).

Temperate Pacific studies.—*Aplidium californicum* from the Pacific coast of Baja California contained prenyl hydroquinone at a concentration of 0.27 mg/mL (0.25% dry mass), and at that concentration, had no effect on food consumption by reef fishes at Looe Key, Florida (Fig. 2) with consumption times ($\bar{X}$ ± 1 SD) of 21.0 ± 22.9 s and 23.2 ± 21.6 s for treatment and control pellets, respectively.

Larval chemical defenses.—Based on an average larval volume of 0.32 μL, the HPLC quantification of didemnin cyclic peptides from 1101 larvae of *Tridemnum solidum* found concentrations of didemnin B, nordidemnin B, didemnin D, and didemnin E com-

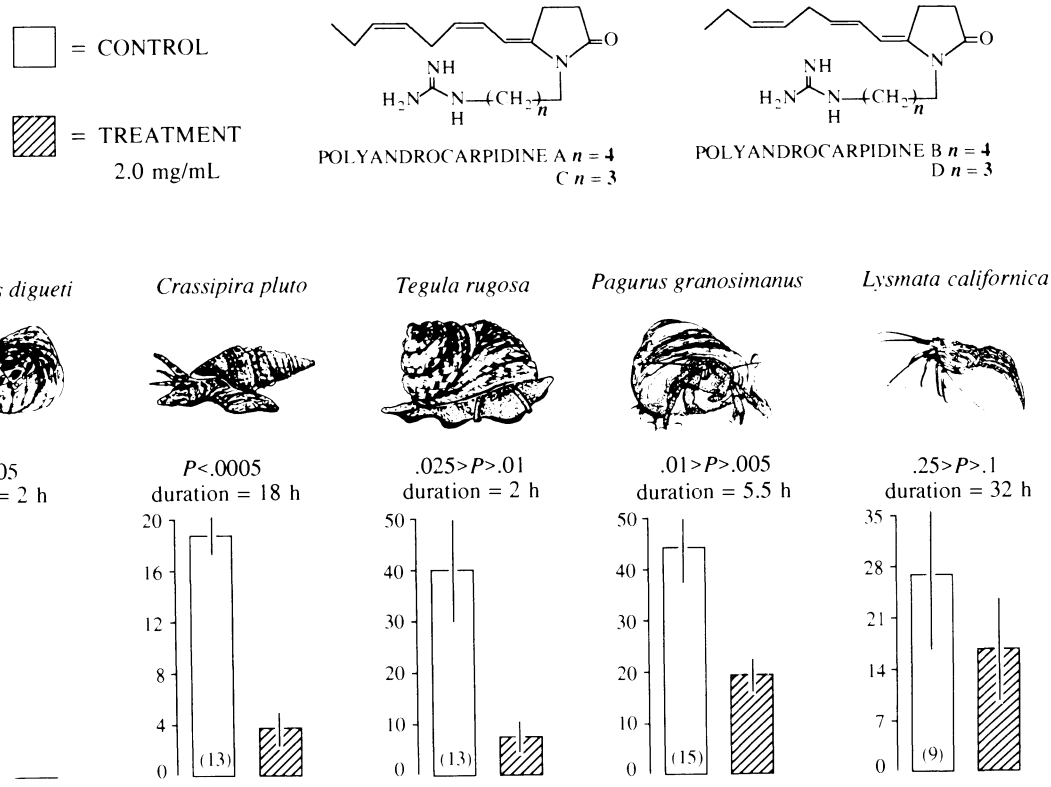

FIG. 5. The effect of a naturally occurring mixture of polyandrocarpidines A–D from the ascidian *Polyandrocarpa* sp., collected in the Gulf of California, on feeding by invertebrate consumers. n = number of -CH_2- groups. Assays were conducted using the natural concentration of the compounds (2.0 mg/mL) in flow-through seawater aquaria at Scripps Institution of Oceanography. Significance values are from the paired-sample t test. The three species on the left side of the figure co-occur with *Polyandrocarpa* sp.; the two species grouped on the right side do not. Symbols are as in Fig. 2.

parable to the mean concentration of these compounds in the adult colonies (Table 1 and Fig. 3); didemnin M and didemnin X were not detected in the larvae.

The tadpole larvae from *Tridemnum solidum* were highly unpalatable to the wrasse *Thalassoma bifasciatum*; eight of nine replicate groups of wrasses rejected an ascidian larva while readily consuming our larval mimic (krill eyes) ($P = 2.1 \times 10^{-4}$, Fisher exact test, Fig. 6). Coating a krill eye with the lipid soluble compounds from a single *T. solidum* larva (Fig. 1) rendered the treated krill eyes as unpalatable as the larvae themselves ($P = 2.1 \times 10^{-5}$, Fisher exact test, Fig. 6). Following a similar pattern, the much larger larvae (5.2 μL) of the Indo-Pacific ascidian *Sigillina* cf. *signifera* were almost always avoided by coral reef fishes that readily consumed similar-sized pieces of squid ($P = 5.7 \times 10^{-10}$, Fisher exact test, Fig. 6).

DISCUSSION

Defenses against predation

Ascidians are common in a variety of marine communities, and in some habitats are dominant members of the benthic invertebrate fauna (Millar 1971, Keough and Butler 1979, Dean 1981, Connell and Keough 1985, Young 1985). Factors affecting their distribution and abundance involve complex interactions of predation (Keough and Butler 1979, Young 1985, Stocker and Berquist 1986), recruitment (Russ 1980, van Duyl et al. 1981, Olson 1983, 1985), competition for substrate (Dayton 1971, Jackson and Buss 1975, van Duyl et al. 1981, Todd and Turner 1988), and disturbance (Dayton 1971). Previous investigators have suggested, but rarely sufficiently demonstrated, that the secondary metabolites of some ascidians may be important defenses against predation (Stoecker 1980*a*, *b*, Young and Bingham 1987, Lindquist et al. 1988*b*, Lindquist and Fenical 1991*b*), fouling organisms (Stoecker 1978, Davis and Wright 1989, 1990), spatial competitors (Jackson and Buss 1975, Bak et al. 1981), and microbial infection (Lindquist et al. 1988*a*, Azumi et al. 1990). These hypotheses remain untested in ecologically relevant assays using known metabolites except for one recent demonstration that several tambjamine-class alkaloids produced by the Indo-Pacific ascidian *Sigillina* cf. *signifera* (=*Atapozoa* sp.) deter feeding by reef fishes (Paul et al. 1990). Our results illustrate that several structurally diverse metabolites from ascidians in the Caribbean Sea, the Indo-Pacific, and the Gulf of Cal-

ifornia deter feeding on both adult and larval ascidians (Figs. 2, 4, 5, and 6). Furthermore, our investigation on the variability of didemnin cyclic peptides in separate colonies of *Tridemnum solidum* from Little San Salvador, Bahamas revealed large differences in the concentration of these chemical defenses among separate colonies. The source and significance of this variance is unknown, although all colonies examined contained sufficient quantities of the unpalatable compounds didemnin B or nordidemnin B to significantly deter fish predators.

Previous studies on defensive adaptations of ascidians examined the palatability of the heavy metal vanadium and the low pH of body fluids exuded from damaged tunic tissue (Stoecker 1980*a*, *b*). In general, vanadium is thought to be concentrated in vacuoles of the vanadocyte blood cells (see Stoecker 1980*a*, *b*), while sulfuric acid is produced and stored in the bladder cells found in the blood and concentrated in the tunics of some ascidians (Stoecker 1978, 1980*a*). High concentrations of vanadium are common among some ascidians of the order Phlebobranchia, but are rare among aplousobranchs, and not reported from stolidobranchs (reviewed by Stoecker 1980*a*, *b*). The presence of acid-filled bladder cells is limited to ascidians of the orders Aplousobranchia and Phlebobranchia (see Stoecker 1980*b*, Parry 1984). Feeding by potential ascidian predators on agar-based food pellets was significantly reduced by incorporating high concentrations of vanadium or acid into the pellets (Stoecker 1980*a*). Several unpalatable ascidians studied by Stoecker (1980*a*) did not exhibit elevated levels of vanadium or possess bladder cells containing acid; these ascidians were hypothesized to be defended by organic secondary metabolites. The chemical literature describing secondary metabolites of ascidians (reviewed by Faulkner 1984, 1986, 1987, 1988, 1990, Fenical 1986, and Ireland et al. 1988) reveals that the phlebobranch ascidians, the order of ascidians most often noted to have elevated levels of vanadium, are the most depauperate in bioactive secondary metabolites. Thus, production of defensive secondary metabolites and concentrating vanadium may be effective, but alternative, strategies of defense.

The defensive roles of vanadium and the acid-filled bladder cells proposed by Stoecker (1980*a*, *b*) were questioned by Parry (1984) on the basis of the pH buffering nature of seawater, the presence of acid-neutralizing spicules of calcium carbonate in some ascidians, and observations of predation on ascidians, primarily by molluscs and sea stars. These arguments against the defensive roles of vanadium and low pH are not convincing for two reasons. First, in questioning low pH as a defensive mechanism, Parry did not consider the instantaneous effects of released acid on a predator that may be sufficient to invoke a taste-induced avoidance response before the acid is neutralized by seawater or carbonate spicules. Second, predation by generalist fishes is tremendously important in structuring tropical benthic communities where ascidians are common (Wellington 1982, Lewis 1986, Horn 1989, Littler et al. 1989, Hay 1991), and the predation by molluscs and sea stars discussed by Parry (1984) may therefore be of less relative importance. The molluscs cited as common ascidian predators included numerous soft-bodied opisthobranchs and lamellarins that specialize on ascidians (Lambert 1980, Morris et al. 1980) and often sequester defensive compounds from their chemically rich prey (Faulkner and Ghiselin 1983, Andersen et al. 1985, Lindquist et al. 1988*b*, Paul et al. 1990). In addition, exudation of acidic fluids has been noted for several of these molluscs that prey on acidic ascidians (reviewed by Karuso 1987). Ecologically similar, but more thoroughly studied, interactions among herbivorous fishes, chemically defended seaweeds, and small herbivores (including metabolite-sequestering gastropods) that specialize on these seaweeds indicate that selective pressures created by the specialists are evolutionarily trivial compared to the selective pressures created by the generalist fishes (Hay et al. 1989, Hay 1991).

The spicules produced by many benthic marine invertebrates are thought to provide physical support

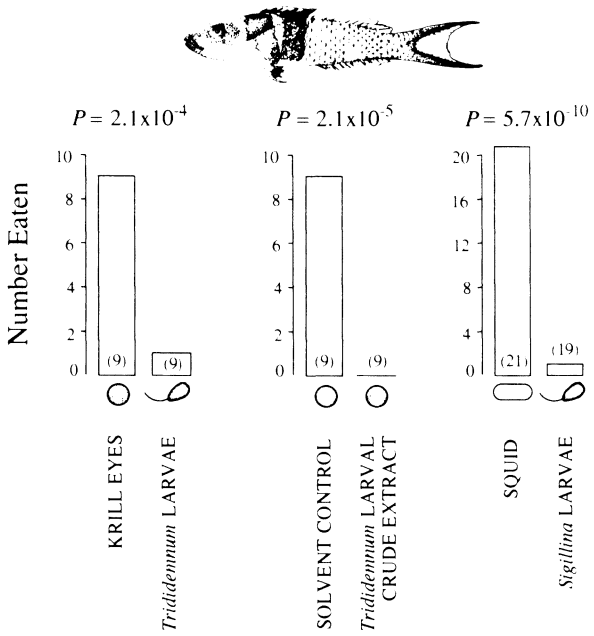

FIG. 6. The susceptibility of *Tridemnum solidum* larvae and larval extract to consumption by the blue-head wrasse *Thalassoma bifasciatum* in shipboard feeding assays conducted at Little San Salvador, Bahamas, and the susceptibility of *Sigillina* cf. *signifera* larvae to coral-reef fishes on a patch reef near Dumaguete City, Philippines. *N* for each treatment is in parentheses at the base of the relevant histogram bar. Significance values are from the Fisher exact test.

TABLE 2. Physical, ecological, and chemical characteristics of ascidians for which data on larval palatability are available. Numbers in superscript refer to papers describing these characteristics; references are listed below by number. ··· = no data are available.

	Ascidian species				
	Trididemnum solidum	*Sigillina signifera*	*Didemnum molle*	*Ecteinascidia turbinata*	*Eudistoma olivaceum*
Adult chemistry	didemnin cyclic peptides[1]	tambjamines C, E, and F[2]	diphenethyl-urea[1]	ecteinascidins[3,4] vanadium[5]	eudistomins[1]
Reported larval chemistry	didemnin cyclic peptides	tambjamines C and E tetrapyrrole[6]	···	vanadium[7]	···
Larval size (mm)	3–4	5–7	2–3[9]	4–5[7]	2–3[10]
Larval color	brown[8]	purple[6]	green[9]	orange[7]	orange[7]
Larval release time	midday[8]	morning	midday[9]	morning[13]	morning[13]
Larval dispersal time (min)	<15[8]	<10	<10[9]	<5[14]	···
Palatability of larvae	**unpalatable**[8]	**unpalatable**	**unpalatable**[9]	**unpalatable**[7]	**unpalatable**[7]
Palatability of compound	didemnin B, nor-didemnin B **unpalatable**	tambjamines C, E, and F, tetrapyrrole **unpalatable**[2]	···	heated, nondialyzed extracts **unpalatable**,[7] vanadium **unpalatable**[5]	···

(1) Faulkner 1984, 1986, 1987, 1988, 1990; (2) Paul et al. 1990; (3) Wright et al. 1990; (4) Rinehart et al. 1990; (5) Stoecker 1980*a*; (6) Lindquist and Fenical 1991*a*; (7) Young and Bingham 1987; (8) van Duyl et al. 1981; (9) Olson 1983; (10) B. Bingham, *personal communication*; (11) Olson and McPherson 1987; (12) Davis 1988; (13) Svane and Young 1989; (14) Bingham and Young 1991; (15) Davis and Butler 1990.

(Koehl 1982) and deter predators; however, only the spicules of the gorgonians *Pseudopterogorgia* spp. (Harvell et al. 1988, Harvell and Fenical 1989) and *Leptogorgia virgulata* (Gerhart et al. 1988) have been experimentally shown to reduce food consumption by potential predators. Although ascidians appear to have few physical defenses against generalist predators, several spicule types are found in some species. Many tropical didemnid ascidians produce small stellate spicules 20–50 μm in diameter (Monniot 1970, Olson 1986). A potential defensive role for these small stellate spicules was hypothesized by Olson (1986), but in our feeding experiments these spicules had no effect on feeding by fishes (Fig. 2). They may, however, substantially lower the food value of the adult ascidian by greatly increasing its ash content (Table 1). Olson (1986) also proposed that spicules in didemnin ascidians harboring symbiotic microalgae could function to shade the algal symbiont from excessive solar radiation (especially ultraviolet), since colonies of these ascidians growing in full sunlight had a greater concentration of spicules than did shaded colonies. Spicule shading may also be important for some didemnid ascidians that do not harbor symbiotic algae, because they also deploy large quantities of spicules in the upper regions of their tunics (Van Name 1945).

A second plate-like spicule form with a diameter up to 500 μm has been described from various species of the genus *Cystodytes* (Monniot 1970). The function of these much larger spicules has not been investigated. Two additional ascidian spicule morphologies have been described from the common tropical ascidian *Herdmania momus* (Lambert and Lambert 1987); large elongated spicules up to 2.5 mm in length are found in its mantle, siphons, and branchial basket while smaller (150–250 μm in length) elongated spicules protrude through the outer surface of its tunic. These spicules, similar in size and morphology to those of gorgonian corals, have been proposed as a physical defense against generalist predators (Lambert and Lambert 1987). In general, however, the limited occurrence of spicules in ascidians and the inability of the small stellate spicules that are common to many didemnid ascidians to directly deter potential fish predators indicate that for the majority of ascidians, spicules do not appear to be an integral aspect of their antipredator defenses.

Little is known about the spatial allocation of secondary metabolites within ascidian tissues. However, ascidians are unique among the major taxa of sessile invertebrates in possessing a relatively well-developed circulatory system that moves various types of blood cells throughout the animal (Goodbody 1974). This unique feature of ascidians may allow the encapsulation of bioactive compounds within blood cells and their circulation throughout the animal, allowing compounds to fulfill various ecological roles while avoiding autotoxicity. Investigations on the response of ascidian tissues to foreign material and tissues (Anderson 1971) and interspecific contact (Koyama and Watanabe 1986) have revealed that the contact zones are heavily infused with various types of blood cells. Three types of blood

TABLE 2. Continued.

Ascidian species		
Lissoclinum patella	*Podoclavella moluccensis*	*Clavelina oblonga*
various cyclic peptides[1]	...	...
...	...	...
5–6[11]	4[12]	4–5[7]
green[11]	...	transparent[7]
midday[11]	midday[13]	all day[13]
<10[11]	<2[15]	...
palatable[11]	**unpalatable**[15]	**palatable**[7]
patellamide C **palatable**	...	...

cells from the ascidian *Phallusia nigra* (=*Ascidia nigra*) have been found to contain virtually all the ascidian's vanadium (Oltz et al. 1988), while its bladder cells (another blood constituent) contain sulfuric acid (Stoecker 1978). More recently, the ascidian *Halocynthia roretzi* was discovered to produce a previously undescribed group of antimicrobial secondary metabolites, the halocyamines, that were detected only in its morula cells (Azumi et al. 1990). It also appears that the Indo-Pacific ascidian *Sigillina* cf. *signifera* concentrates its large quantity (0.5–1.7% dry mass) of defensive tambjamine alkaloids in granular amebocyte blood cells (Lindquist and Fenical 1991*a*).

Larval chemical defenses

Sexual reproduction in brooding ascidians appears to be inexorably tied to a daytime release and settlement of their larvae (reviewed by Svane and Young 1989). The apparent necessity for daytime settlement exposes these larvae to potentially intense levels of predation from visually oriented predators (primarily fishes), and may have had profound effects on selection for chemically based defenses in ascidian larvae. Chemical defenses may be especially important for larvae of didemnid ascidians that harbor symbiotic microalgae because death of juveniles exposed to intense sunlight is thought to select for midday larval release when they would be most apparent to fishes. The high light of midday is hypothesized to allow larvae to seek out appropriate (e.g., shaded) photic environments, thus minimizing their settlement in unsuitable habitats (Olson 1983). Olson (1983) found that larvae and juvenile colonies of the didemnid ascidian *Didemnum molle* lack potential shading adaptations (i.e., spicules and dark pigmentation) that allow the adults to colonize sun-exposed substrates. Newly settled larvae of *D. molle* survived well in shaded microhabitats but died within 4 d if transplanted into an intense photic environment that is readily tolerated by the adults. Olson (1983) hypothesized that after the juvenile colonies develop the appropriate shading adaptations, they migrate into high light environments where adults are most common. *Trididemnum solidum* (van Duyl et al. 1981) and *Lissoclinum patella* (Olson and McPherson 1987) harbor symbiotic microalgae and exhibit a similar pattern of larval release and settlement.

Colonial marine invertebrates, like many ascidians, can lose tissue to predators and survive the attack. However, predators may kill larvae or eggs of marine invertebrates in a single encounter (Olson and McPherson 1987, Westneat and Resing 1988). Thus, one would expect extremely high mortality for large conspicuous larvae or eggs in the absence of defensive mechanisms. In situ observations of invertebrate larval and egg palatabilities are rare; however, the large size of many ascidian larvae and their daytime release has recently been exploited to obtain detailed in situ observations of their swimming behavior, effective dispersal distance, and palatability to potential predators (reviewed by Svane and Young 1989; Table 2). Larvae of several tropical and temperate ascidians were observed to be rejected by fishes and corals (Table 2). These larvae often survived the attack (Olson 1983, Davis and Butler 1989) and in the only study where survivorship was rigorously assessed (Young and Bingham 1987), attacked larvae settled and metamorphosed at a rate equal to unattacked controls. Thus, the common assumption that attacks on small soft-bodied organisms usually kill the prey, even if it is ultimately rejected, needs to be reassessed (see also Wiklund and Jarvi 1982).

Our shipboard feeding assays in the Bahamas with the common predatory wrasse *Thalassoma bifasciatum* and larvae of the Caribbean ascidian *Trididemnum solidum* confirmed (Fig. 6) earlier in situ observations of the larvae's unpalatability to coral-reef fishes (Bak et al. 1981). Larvae of *T. solidum*, unlike the adult colonies, are devoid of stellate spicules; thus, the larvae's unpalatability appears due to chemical rather than structural defenses (Fig. 6) and is well explained by the presence of didemnin cyclic peptides (Table 1, Figs. 2 and 3). Of those ascidians for which data on larval palatability are available, adults of all species except *Podoclavella molluccensis* and *Clavelina oblonga* have been chemically investigated and are known to produce bioactive secondary metabolites. In addition to secondary metabolites, adult colonies of *Ecteinascidia turbinata* contain high concentrations of vanadium

(Stoecker 1980*b*). With the exception of *Lissoclinum patella* and *Clavelina oblonga,* larvae of the ascidians investigated to date are generally unpalatable to fishes (Table 2). The presence of vanadium was noted in larvae of *E. turbinata* but its concentration was not measured (Young and Bingham 1987). The presence of deterrent secondary metabolites in the unpalatable larvae of the tropical ascidians *Tridemnum solidum* (Tables 1 and 2) and *Sigillina* cf. *signifera* (Lindquist and Fenical 1991*a*), coupled with observations of unpalatability among the larvae of other taxonomically diverse brooding ascidians, indicate that large, conspicuous ascidian larvae may, in general, be well defended from predators. Previous investigations of larval and egg chemical defenses against co-occurring consumers have: (1) identified unpalatable saponins in the larvae of the sea star *Acanthaster planci* (Lucas et al. 1979), (2) demonstrated a chemical basis for rejection of larvae from the ascidian *Ecteinascidia turbinata* by the pinfish *Lagodon rhomboides* (Young and Bingham 1987), and (3) identified diet-derived defensive macrolides in the egg ribbons of the nudibranch *Hexabranchus sanguineus* (Pawlik et al. 1988). In addition, McClintock and Vernon (1990) found the eggs of several antarctic sea stars to be distasteful to the temperate marine killifish *Fundulus grandis.*

As an alternative to the production of defensive secondary metabolites or concentrating vanadium to deter predators, larvae of some ascidians, even though they are large, may minimize detection by visually foraging predators by reducing their pigmentation until they are nearly transparent. Reduced apparency to predatory fishes has been shown for various species of transparent zooplankton (O'Brien 1979, Kerfoot 1982). The transparency of the palatable larvae of the ascidian *Clavelina oblonga* (Table 2) may similarly reduce their rate of detection by foraging fishes.

The short swimming phase of brooded ascidian larvae in natural habitats (Table 2) may reduce their exposure to predators and effectively limit their dispersal, often to <10 m from the parent colony (Bak et al. 1981, Olson and McPherson 1987, Davis and Butler 1989, Bingham and Young 1991, reviewed by Svane and Young 1989). This may produce dense aggregations of genetically similar colonies (kin groups) (Grosberg and Quinn 1986). Given that many predatory reef fishes have limited home ranges (reviewed by Sale 1980), clumping of unpalatable prey may increase the rate at which fishes learn to avoid ingesting chemically defended larvae and eggs. If the occasional loss of larvae and of adult tissue to naive predators promotes learned aversion, then consumption of unpalatable larvae from the same colony or nearby related colonies might decline as experienced predators visually recognize the larvae and adults. However, the large size of brooded ascidian larvae will increase their probability of being detected, and possibly attacked, by naive fishes. This may, in part, explain why these well-provisioned larvae settle rapidly after release even though they may be capable of a longer pelagic existence than the smaller, less apparent, and longer lived larvae released by oviparous ascidians. In addition, if producing larger, better provisioned larvae increases the survivorship of newly metamorphosed juveniles, then the evolution of defensive chemistry may have allowed a shift towards producing fewer but much larger and more conspicuous larvae. Thus, the evolution of chemical defenses and the reproductive strategies of some ascidians may be linked to, and influenced by, learned aversion in localized populations of predators.

That fish can learn to associate chemical deterrents with physical characteristics (e.g., color) of larvae and thus avoid chemically defended larvae without tasting them has been demonstrated by Young and Bingham (1987). Their work showed that the common omnivorous fish *Lagodon rhomboides* avoided the orange-colored larvae of the ascidian *Ecteinascidia turbinata* and learned to associate the orange aposematic coloration with unpalatability, probably in association with larval size and form. They argue that the evolution of aposematic coloration in *E. turbinata* likely resulted from selection on individuals, since the majority (89%) of larvae attacked by *Lagodon* were rejected and most (81%) survived to successfully settle and metamorphose with a mortality rate similar to unattacked control larvae. Presently, the relative importance of kin vs. individual selection in the evolution of the orange coloration of *E. turbinata* larvae may be difficult to determine because little is known about their susceptibility to other potential larval predators and the number of attacks a larva might incur under natural conditions. Furthermore, the orange coloration may have evolved in response to other factors, such as the need for photoprotection (Kerfoot 1982 and references therein) prior to the larvae becoming unpalatable (see also Guilford 1988). Young and Bingham (1987) suggest that the bright orange and red coloration of unpalatable larvae and eggs of many marine invertebrates, including other ascidians, might also function as aposematic coloration, but among unpalatable ascidian larvae bright coloration is not universal (Table 2).

An important aspect of investigating larval chemical characteristics is the comparison of the chemistries of the larvae and the colonies that produced them. Such comparisons will allow examination of ontogenetic shifts in secondary metabolite composition in marine invertebrates, and help identify biotic and abiotic factors affecting secondary metabolite composition at different life stages. Chemical differences between colonies of various soft corals and their brooded eggs have been extensively documented for Australian soft corals (Bowden et al. 1985, Coll et al. 1989). These corals synchronously release their yearly production of eggs during only a few nights each year (Harrison et al. 1984, Coll et al. 1989). This characteristic results in eggs

avoiding many daytime predators and swamping predators that are active during the spawning. Because reef fishes readily consumed these eggs (Bowden et al. 1985, Coll et al. 1989), the terpenoid metabolites of the eggs, which are absent in the adult colonies, clearly are not effective feeding deterrents. The effects of the different terpenoid secondary metabolites from the adult colonies on feeding by co-occurring reef fishes are not known.

Ascidians can also exhibit chemical differences between larvae and adults. When compared to adult tissues, competent larvae dissected from colonies of *Sigillina* cf. *signifera* contained more tambjamine C, less tambjamine E, and no tambjamine F (Lindquist and Fenical 1991*a*). Similarly, the larvae of *Trididemnum solidum* contained only four of the six didemnins found in the adult colonies (Table 1). The larvae of these ascidians are not simply endowed with adult chemistries; they either receive a selected transfusion of adult chemistry or synthesize a suite of secondary metabolites that may be better adapted to enhancing larval survival and perhaps the survival of recently metamorphosed juveniles. After settlement and metamorphosis, secondary metabolite compositions may shift to suit the requirements of a benthic rather than pelagic environment. The chemical differences between the larvae and adult colonies of these two ascidians are in contrast to results from a study on the chemical defenses of larvae of the sea star *Acanthaster planci* where the composition of defensive saponins were identical in larval and adult sea stars (Lucas et al. 1979).

Predation can be a major source of mortality for larvae or pelagic juveniles of marine invertebrates (Gaines and Roughgarden 1987, Olson and McPherson 1987, reviewed by Young and Chia 1987); thus it is not unexpected that both benthic marine invertebrates and reef fishes exhibit characteristics that appear to minimize predation on eggs and larvae. Survival of externally fertilized eggs can be enhanced by one or more of the following strategies: (1) Releasing gametes when visually oriented predators are less active, generally at dusk or at night. Evening release is common among various scleractinian corals and octocorals (Harrison et al. 1984, Shlesinger and Loya 1985, Benayahu and Loya 1986), sponges (Hoppe and Reichert 1987), and reef fishes (Sale 1980); (2) Synchronously releasing large numbers of gametes. This strategy, most notably exhibited by some scleractinian corals and octocorals (Harrison et al. 1984, Bowden et al. 1985, Shlesinger and Loya 1985, Benayahu and Loya 1986), is hypothesized to satiate egg predators much as the mass synchronous production of seeds does for some plants (Janzen 1976, Silvertown 1980); (3) Spatial escape from egg predators. This strategy is applicable to fishes that either move to offshore or down-current sections of the reef to spawn, or remain in a home range but release gametes high in the water column (reviewed by Johannes 1978 and Sale 1980). Survival of internally fertilized eggs is enhanced by brooding, which protects the larvae until their release. Once released, these larvae generally have a reduced pelagic stage but are often more conspicuous and apparently more likely to be chemically defended, especially larvae of brooding ascidians. Larval or egg characteristics, other than unpalatability, that may reduce losses to predators include physical defenses (Morgan 1989) and characteristics such as reduced size or increased transparency that have been shown to reduce encounters with predators (O'Brien 1979, Kerfoot 1982).

Brooded larvae of ascidians exhibit both physical and behavioral characteristics that set them apart from brooded larvae and eggs of most other sessile invertebrates. First, brooded ascidian larvae are in general much larger, commonly reaching 4–5 mm in length vs. <2 mm for eggs and larvae of most other benthic invertebrates [e.g., solitary ascidians <1 mm (Svane and Young 1989), hard corals <1.5 mm (Goreau et al. 1981, Szmant 1986), soft corals <1 mm (Alino and Coll 1989), gorgonians <1.5 mm (Grigg 1977, Brazeau and Lasker 1989), sponges <2 mm (Reiswig 1973, Hoppe and Reichert 1987, Ilan and Loya 1990, N. Lindquist, *personal observation*), hydroids <1 mm (N. Lindquist, *personal observation*), and bryozoans <0.5 mm (N. Lindquist, *personal observation*)]. These conspicuous larvae are also released during daylight hours when the majority of reef fishes are most active. The only behavioral characteristic of these ascidians that is likely to reduce losses to fish predators is the short duration of their swimming phase.

Orians and Janzen (1974) noted that embryo chemical defenses against consumption were less common than might be expected given the intense selective pressures imposed by predators. Although many plant seeds were chemically defended, they were able to provide few examples of unpalatable animal eggs. Their hypotheses that autotoxicity, or energetic and developmental constraints prevented the evolution of toxic eggs and larvae seems inadequate given the recent studies showing that egg and larval chemical defenses occur among numerous amphibians (Brodie and Formanowicz 1987), insects (Brower 1984, T. Eisner, *personal communication*), and marine invertebrates (Lucas et al. 1979, DeVore and Brodie 1982, Young and Bingham 1987, Pawlik et al. 1988, McClintock and Vernon 1990, Lindquist and Fenical 1991*a*). The incidence of unpalatability in amphibian eggs and larvae has been closely correlated to habitat use. Species living in streams or ponds with fish are either unpalatable or behaviorally reduce their exposure to predators; species in ephemeral habitats without fish show no such adpatations (Kruse and Francis 1977, Formanowicz and Brodie 1982, Kats et al. 1988). Similarly, chemical protection appears to be an important mechanism for reducing predation on larvae of some ascidians, and perhaps other marine invertebrates, that are competent to settle soon after release and that remain in predator-

rich habitats until settling. Among those marine invertebrates whose larvae become competent to settle after several weeks of development, chemical defenses against fishes and benthic invertebrates may be less common, because macroscale predator avoidance can occur as pelagic eggs and larvae are transported to offshore pelagic habitats believed to be characterized by lower levels of predation (Johannes 1978, Sale 1980, Strathmann 1986).

In contrast to amphibians where both adults and larvae are generally mobile, adult stages of sessile marine invertebrates, like those of plants, are generally non-mobile. Thus it is not unexpected that some plants and marine invertebrates exhibit similar reproductive strategies and patterns of chemical defense. For example, seeds of Central American Leguminosae with low susceptibilities to attack by Bruchidae or "pea weevils" tend to produce fewer but large and better provisioned seeds than the seeds of species that are readily attacked by Bruchidae (Janzen 1969). Numerous compounds have been isolated from the low-preference seeds that significantly affect bruchid growth and survival. Plants with seeds susceptible to Bruchidae attack produce a larger seed crop, but much of it is destroyed by seed predators. Similarly, chemical defenses against predators appear common among ascidians releasing large, conspicuous larvae during daylight hours when exposure to foraging fishes would be greatest. In contrast, marine invertebrates whose larvae have an extended pelagic stage often produce enormous numbers of offspring of which a large proportion fail to reach appropriate settlement sites due to physical forces and predation (reviewed by Young and Chia 1987).

Chemical defenses have been hypothesized to enhance the survival of soft-bodied ascidians that often lack obvious physical defenses and are common in habitats characterized by intense levels of predation (Stoecker 1980*a, b,* Bak et al. 1981, Young and Bingham 1987, Paul et al. 1990). Our investigations demonstrate that numerous secondary metabolites from taxonomically diverse ascidians, in several geographic locations, significantly reduce predation on both adults and larvae. Defensive chemistry in the larvae of these ascidians may be very important in allowing the release of large, well-provisioned larvae during daylight periods when the larvae have the greatest probability of selecting appropriate photic environments, but also the greatest exposure to visually foraging predators.

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Literature Cited

Alino, P. M., and J. C. Coll. 1989. Observations of the synchronized mass spawning and postsettlement activity of octocorals on the Great Barrier Reef, Australia: biological aspects. Bulletin of Marine Sciences **45**:697–707.

Andersen, R. J., D. J. Faulkner, H. Cun-heng, G. D. Van Duyne, and J. Clardy. 1985. Metabolites of the marine prosobranch mollusc *Lamellaria* sp. Journal of the American Chemical Society **107**:5492–5495.

Anderson, R. S. 1971. Cellular response to foreign bodies in the tunicate *Molgula manhattensis* (DeKay). Biological Bulletin (Woods Hole) **141**:91–98.

Azumi, K., H. Yokosawa, and S. Ishii. 1990. Halocyamines: novel antimicrobial tetrapeptide-like substances isolated from the hemocytes of the solitary ascidian *Halocynthia roretzi*. Biochemistry **29**:159–165.

Bak, R. P. M., J. Sybesma, and F. C. van Duyl. 1981. The ecology of the tropical compound ascidian *Tridemnum solidum*. II. Abundance, growth and survival. Marine Ecology Progress Series **6**:43–52.

Benayahu, Y., and Y. Loya. 1986. Sexual reproduction of a soft coral: synchronous and brief annual spawning of *Sarcophyton glaucum* (Quoy & Gaimard, 1833). Biological Bulletin (Woods Hole) **170**:32–42.

Bingham, B. L., and C. M. Young. 1991. Larval behavior of the ascidian *Ecteinascidia turbinata* Herdman; an in situ experimental study of the effects of swimming on dispersal. Journal of Experimental Marine Biology and Ecology **145**: 189–204.

Bowden, B., J. Coll, D. Tapiolas, and R. Willis. 1985. Some chemical aspects of spawning in alcyonacean corals. Pages 325–329 *in* Volume 4. Proceedings of the Fifth International Coral Reef Congress, Tahiti. Antenne Museum–Ephe, Moorea, French Polynesia.

Brazeau, D. A., and H. R. Lasker. 1989. The reproductive cycle and spawning in a Caribbean gorgonian. Biological Bulletin (Woods Hole) **176**:1–7.

Brodie, E. D., Jr., and D. R. Formanowicz, Jr. 1987. Antipredator mechanisms of larval anurans: protection of palatable individuals. Herpetologica **43**:369–373.

Brower, L. P. 1984. Chemical defenses in butterflies. Symposium of the Royal Entomological Society of London **11**: 109–134.

Carté, B., and D. J. Faulkner. 1982. Revised structures for the polyandrocarpidines. Tetrahedron Letters **23**:3863–3866.

Carté, B., and D. J. Faulkner. 1983. Defensive metabolites from three nembrothid nudibranchs. Journal of Organic Chemistry **48**:2314–2318.

Cheng, M. T., and K. L. Rinehart, Jr. 1978. Polyandrocarpidines: antimicrobial and cytotoxic agents from a marine tunicate (*Polyandrocarpa* sp.) from the Gulf of California. Journal of the American Chemical Society **100**:7409–7411.

Coll, J. C., B. F. Bowden, A. Heaton, P. J. Scheuer, M. K. W. Li, J. Clardy, G. K. Schulte, and J. Finer-Moore. 1989. Structures and possible functions of epoxypukalide and pukalide-diterpenes associated with eggs of sinularian soft corals (Cnidaria, Anthozoa, Octocorallia, Alcynoacea, Alcyoniidae). Journal of Chemical Ecology **15**:1177–1191.

Connell, J. H., and M. J. Keough. 1985. Disturbance and patch dynamics: marine animals on hard substrata. Pages 125–151 *in* T. A. Pickett and P. S. White, editors. The ecology of natural disturbance and patch dynamics. Academic Press, New York, New York, USA.

Conover, R. J. 1978. Transformation of organic matter. Pages 404–406 *in* O. Kinne, editor. Marine ecology. John Wiley & Sons, New York, New York, USA.

Davidson, B. S., and C. M. Ireland. 1990. Lissoclinolide, the first non-nitrogenous metabolite from a *Lissoclinum* tunicate. Journal of Natural Products **53**:1036–1038.

Davis, A. R. 1988. Effects of variation in initial settlement on distribution and abundance of *Podoclavella moluccensis*. Journal of Experimental Marine Biology and Ecology **117**: 157–167.

Davis, A. D., and A. L. Butler. 1989. Direct observations of larval dispersal in the colonial ascidian *Podoclavella moluccensis* Sluiter: evidence for closed populations. Journal of Experimental Marine Biology and Ecology **127**:189–203.

Davis, A. R., and A. E. Wright. 1989. Interspecific differences in fouling of two congeneric ascidians (*Eudistoma olivaceum* and *E. capsulatum*): is surface acidity an effective defense. Marine Biology **102**:491–497.

Davis, A. R., and A. E. Wright. 1990. Inhibition of larval settlement by natural products from the ascidian, *Eudistoma olivaceum* (Van Name). Journal of Chemical Ecology **16**:1349–1357.

Dayton, P. K. 1971. Competition, disturbance, and community organization: the provision and subsequent utilization of space in a rocky intertidal community. Ecological Monographs **41**:351–398.

Dean, T. A. 1981. Structural aspects of sessile invertebrates as organizing forces in an estuarine fouling community. Journal of Experimental Marine Biology and Ecology **53**: 163–180.

DeVore, D. E., and E. D. Brodie, Jr. 1982. Palatability of the tissues of the holothurian *Thyone briareus* (Lesueur) to fish. Journal of Experimental Marine Biology and Ecology **61**:279–285.

Elner, R. W., and A. Campbell. 1987. Natural diets of lobster *Homarus americanus* from barren ground and macroalgal habitats off southwestern Nova Scotia, Canada. Marine Ecology Progress Series **37**:131–140.

Faulkner, D. J. 1984. Marine natural products. Metabolites of marine invertebrates. Natural Products Report **1**:551–598.

———. 1986. Marine natural products. Natural Products Report **3**:1–33.

———. 1987. Marine natural products. Natural Products Report **4**:539–576.

———. 1988. Marine natural products. Natural Products Report **5**:613–663.

———. 1990. Marine natural products. Natural Products Report **7**:269–309.

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

Fenical, W. 1986. Marine alkaloids and related compounds. Pages 275–330 *in* S. W. Pelletier, editor. Alkaloids: chemical and biological perspectives. Volume 4. John Wiley & Sons, New York, New York, USA.

Formanowicz, D. R., Jr., and E. D. Brodie, Jr. 1982. Relative palatabilities of members of a larval amphibian community. Copeia **1**:91–97.

Gaines, S. D., and J. Roughgarden. 1987. Fish in offshore kelp forests affect recruitment to intertidal barnacle populations. Science **235**:479–481.

Gerhart, D. J., D. Rittschof, and S. W. Mayo. 1988. Chemical ecology and the search for marine antifoulants. Journal of Chemical Ecology **14**:1905–1917.

Goodbody, I. 1974. The physiology of ascidians. Advances in Marine Biology **12**:2–232.

Goreau, N. I., T. J. Goreau, and R. L. Hayes. 1981. Settling, survivorship and spatial aggregation in planulae and juveniles of the coral *Porites porites* (Pallas). Bulletin of Marine Sciences **31**:424–435.

Grigg, R. W. 1977. Population dynamics of two gorgonian corals. Ecology **58**:278–290.

Grosberg, R. K., and J. F. Quinn. 1986. The genetic control and consequences of kin recognition by the larvae of a colonial marine invertebrate. Nature **322**:456–459.

Guilford, T. 1988. The evolution of conspicuous coloration. American Naturalist **131** (Supplement):S7–S21.

Harrison, P. L., R. C. Babcock, G. D. Bull, J. K. Oliver, C. C. Wallace, and B. L. Willis. 1984. Mass spawning in tropical reef corals. Science **223**:1186–1188.

Harvell, C. D., and W. Fenical. 1989. Chemical and structural defenses of Caribbean gorgonians (*Pseudopterogorgia* spp.): intracolony localization of defense. Limnology and Oceanography **34**:382–389.

Harvell, C. D., W. Fenical, and C. H. Greene. 1988. Chemical and structural defenses of Caribbean gorgonians (*Pseudopterogorgia* spp.). I. Development of an *in situ* feeding assay. Marine Ecology Progress Series **49**:287–294.

Hay, M. E. 1991. 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, San Diego, California, USA.

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

Hay, M. E., W. Fenical, and K. Gustafson. 1987. 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 (Berlin) **81**:418–427.

Hoppe, W. F., and M. J. M. Reichert. 1987. Predictable annual mass release of gametes by the coral reef sponge *Neofibularia nolitangere* (Porifera: Desmospongiae). Marine Biology **94**:277–285.

Horn, M. H. 1989. Biology of marine herbivorous fishes. Oceanography and Marine Biology, Annual Review **27**:167–272.

Howard, B. M., K. Clarkson, and R. L. Bernstein. 1979. Simple prenylated hydroquinone derivatives from the marine urochordate *Aplidium californicum*: natural anticancer and antimutagenic agents. Tetrahedron Letters **46**:4449–4452.

Ilan, M., and Y. Loya. 1990. Sexual reproduction and settlement of the coral reef sponge *Chalinula* sp. from the Red Sea. Marine Biology **105**:25–31.

Ireland, C. M., D. M. Roll, T. F. Molinski, T. C. McKee, T. M. Zabriskie, and J. C. Swersey. 1988. Uniqueness of the marine chemical environment: categories of marine natural products from invertebrates. Pages 41–57 *in* D. G. Fautin, editor. Biomedical importance of marine organisms. Memoirs of the California Academy of Sciences, Volume **13.**

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.

Janzen, D. H. 1969. Seed-eaters versus seed size, number, toxicity and dispersal. Evolution **23**:1–27.

———. 1976. Why bamboos wait so long to flower. Annual Review of Ecology and Systematics **7**:347–391.

Johannes, R. E. 1978. Reproductive strategies of coastal marine fishes in the tropics. Environmental Biology of Fishes **3**:65–84.

Karuso, P. 1987. Chemical ecology of the nudibranchs. Pages 31–60 *in* P. J. Scheuer, editor. Bio-organic marine chemistry. Springer-Verlag, Berlin, Germany.

Kats, L. B., J. W. Petranka, and A. Sih. 1988. Antipredator defenses and the persistence of amphibian larvae with fishes. Ecology **69**:1865–1870.

Keough, M. J., and A. J. Butler. 1979. The role of asteroid

predators in the organization of a sessile community on pier pilings. Marine Biology **51**:167–177.

Kerfoot, W. C. 1982. A question of taste: crypsis and warning coloration in freshwater zooplankton communities. Ecology **63**:538–554.

Koehl, M. A. R. 1982. Mechanical design of spicule-reinforced connective tissue: stiffness. Journal of Experimental Biology **98**:239–267.

Kott, P. 1980. Algal-bearing didemnid ascidians in the Indo-West-Pacific. Memoranda of the Queensland Museum **20**: 1–47.

Koyama, H., and H. Watanabe. 1986. Studies on the fusion reaction in two species of *Perophora* (Ascidiacea). Marine Biology **92**:267–275.

Kruse, K. C., and M. G. Francis. 1977. A predation deterrent in larvae of the bullfrog, *Rana catesbeiana*. Transactions of the American Fisheries Society **106**:248–252.

Lambert, G. 1980. Predation by the prosobranch mollusk *Lamellaria diegoensis* on *Cystodytes lobatus*, a colonial ascidian. Veliger **22**:340–344.

Lambert, G., and C. C. Lambert. 1987. Spicule formation in the solitary ascidian, *Herdmania momus*. Journal of Morphology **192**:145–159.

Lewis, S. M. 1986. The role of herbivorous fishes in the organization of a Caribbean reef community. Ecological Monographs **36**:183–200.

Lindquist, N., and W. Fenical. 1991*a*. New tambjamine class alkaloids from the marine ascidian *Atapozoa* sp. and its nudibranch predators—origin of the tambjamines in *Atapozoa*. Experientia **47**:504–506.

Lindquist, N., and W. Fenical. 1991*b*. Polyclinal, a new polyhydroxy benzaldehyde from the marine ascidian *Polyclinum planum*. Experientia **47**:503–504.

Lindquist, N., W. Fenical, G. D. Van Duyne, and J. Clardy. 1988*b*. New alkaloids of the lamellarin class from the marine ascidian *Didemnum chartaceum* (Sluiter, 1909). Journal of Organic Chemistry **53**:4570–4574.

Lindquist, N., W. Fenical, D. F. Sesin, C. M. Ireland, G. D. Van Duyne, C. J. Forsyth, and J. Clardy. 1988*a*. Isolation and structure determination of the didemnenones, novel cytotoxic metabolites from tunicates. Journal of the American Chemical Society **110**:1308–1309.

Littler, M. M., P. R. Taylor, and D. S. Littler. 1989. Complex interactions in the control of coral zonation on a Caribbean reef flat. Oecologia (Berlin) **80**:331–340.

Lucas, J. S., R. J. Hart, M. E. Howden, and R. Salathe. 1979. Saponins in eggs and larvae of *Acanthaster planci* (L.) (Asteroidea) as chemical defenses against planktivorous fish. Journal of Experimental Marine Biology and Ecology **40**: 155–165.

McClintock, J. B., and J. D. Vernon. 1990. Chemical defense in the eggs and embryos of antarctic sea stars (Echinodermata). Marine Biology **105**:491–495.

McKee, T. C., C. M. Ireland, N. Lindquist, and W. Fenical. 1989. The complete spectral assignment of didemnin B and nordidemnin B. Tetrahedron Letters **21**:2735–2738.

Millar, R. H. 1971. The biology of ascidians. Pages 1–100 *in* Sir F. S. Russel and Sir M. Yonge, editors. Advances in marine biology. Volume 9. Academic Press, London, England.

Monniot, F. 1970. Les spicules chez les Tuniciers Aplousobranches. Archives de Zoologie Expérimentale et Générale **111**:303–311.

Morgan, S. G. 1989. Adaptive significance of spination in estuarine crab zoeae. Ecology **70**:464–482.

Morris, E. H., D. P. Abbott, and E. D. Haderlie, editors. 1980. Intertidal invertebrates of California. Stanford University Press, Stanford, California, USA.

Myers, R. F. 1989. Micronesian reef fishes. Coral Graphics, Territory of Guam, USA.

O'Brien, W. J. 1979. The predator–prey interaction of planktivorous fish and zooplankton. American Scientist **67**: 572–581.

Orians, G. H., and D. H. Janzen. 1974. Why are embryos so tasty? American Naturalist **108**:581–592.

Olson, R. R. 1983. Ascidian–prochloron symbiosis: the role of larval photoadaptations in midday larval release and settlement. Biological Bulletin (Woods Hole) **165**:221–240.

———. 1985. The consequences of short-distance larval dispersal in a sessile marine invertebrate. Ecology **66**:30–39.

———. 1986. Photoadaptation of the Caribbean colonial ascidian–cyanophyte symbiosis *Trididemnum solidum*. Biological Bulletin (Woods Hole) **170**:62–74.

Olson, R. R., and R. McPherson. 1987. Potential vs. realized larval dispersal: fish predation on larvae of the ascidian *Lissoclinum patella* (Gottschaldt). Journal of Experimental Marine Biology and Ecology **110**:245–256.

Oltz, M. E., R. C. Bruening, M. J. Smith, K. Kustin, and K. Nakanishi. 1988. The tunichromes. A class of reducing blood pigments from sea squirts: isolation, structures, and vanadium chemistry. Journal of the American Chemical Society **110**:6162–6172.

Parry, D. L. 1984. Chemical properties of the test of ascidians in relation to predation. Marine Ecology Progress Series **17**:279–282.

Paul, V. J., editor. 1992. Ecological roles of marine secondary metabolites. Cornell University Press, Ithaca, New York, USA.

Paul, V. J., N. Lindquist, and W. Fenical. 1990. Chemical defenses of the tropical ascidian *Atapozoa* sp. and its nudibranch predators *Nembrotha* spp. Marine Ecology Progress Series **59**:109–118.

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.

Randall, J. E. 1968. Caribbean reef fishes. T. F. H., Neptune City, New Jersey, USA.

Reiswig, H. M. 1973. Population dynamics of three Jamaican Demospongia. Bulletin of Marine Sciences **23**:191–226.

Rinehart, K. L., Jr., J. B. Gloer, R. G. Hughes, Jr., H. E. Renis, J. P. McGovern, E. B. Swynenberg, D. A. Stringfellow, S. L. Kuentzel, and L. H. Li. 1981. Didemnins: antiviral and antitumor depsipeptides from a Caribbean tunicate. Science **212**:933–935.

Rinehart, K. L., T. G. Holt, N. L. Fregeau, J. G. Stroh, P. A. Keifer, F. Sun, L. H. Li, and D. G. Martin. 1990. Ecteinascidins 729, 743, 754, 759A, 759B, and 770: potent antitumor agents from the Caribbean tunicate *Ecteinascidia turbinata*. Journal of Organic Chemistry **55**:4512–4515.

Russ, G. R. 1980. Effects of predation by fishes, competition, and structural complexity of the substratum on the establishment of a marine epifaunal community. Journal of Experimental Marine Biology and Ecology **42**:55–69.

Sale, P. F. 1980. The ecology of fishes on coral reefs. Oceanography and Marine Biology: an Annual Review **18**:367–421.

Sesin, D. F., S. J. Gaskell, and C. M. Ireland. 1986. The chemistry of *Lissoclinum patella*. Bulletin des Sociétés Chimiques Belges **95**:853–867.

Shlesinger, Y., and Y. Loya. 1985. Coral community reproductive patterns: Red Sea versus the Great Barrier Reef. Science **228**:1333–1335.

Silvertown, J. W. 1980. The evolutionary ecology of mast seeding in trees. Biological Journal of the Linnean Society **14**:235–250.

Stocker, L. J., and P. R. Berquist. 1986. Seasonal cycles, extrinsic factors, and the variable effects of turfing algae on the abundance of a colonial ascidian. Journal of Experimental Marine Biology and Ecology **102**:1–21.

Stoecker, D. 1978. Resistance of a tunicate to fouling. Biological Bulletin (Woods Hole) **155**:615–626.

———. 1980*a*. Chemical defenses of ascidians against predators. Ecology **61**:1327–1334.

———. 1980*b*. Relationship between chemical defenses and ecology in benthic ascidians. Marine Ecology Progress Series **3**:257–265.

Strathmann, R. R. 1986. What controls the type of larval development? Summary statement for the evolution session. Bulletin of Marine Sciences **39**:616–622.

Svane, I., and C. M. Young. 1989. The ecology and behaviour of ascidian larvae. Pages 45–90 *in* M. Barnes, editor. Volume 27. Oceanography and Marine Biology: an Annual Review. Aberdeen University Press, Aberdeen, Scotland.

Szmant, A. M. 1986. Reproductive ecology of Caribbean reef corals. Coral Reefs **5**:43–54.

Todd, C. D., and S. J. Turner. 1988. Ecology of intertidal and sublittoral cryptic epifaunal assemblages. II. Nonlethal overgrowth of encrusting bryozoans by colonial ascidians. Journal of Experimental Marine Biology and Ecology **115**: 113–126.

van Duyl, F. C., R. P. M. Bak, and J. Sybesma. 1981. The ecology of the tropical compound ascidian *Trididemnum solidum*. I. Reproductive strategy and larval behaviour. Marine Ecology Progress Series **6**:35–42.

Van Name, W. G. 1945. The North and South American ascidians. Bulletin of the American Museum of Natural History **84.**

Wellington, G. M. 1982. Depth zonation of corals in the Gulf of Panama: control and facilitation by resident reef fishes. Ecological Monographs **52**:223–241.

Westneat, M. W., and J. M. Resing. 1988. Predation on coral spawn by planktivorous fish. Coral Reefs **7**:89–92.

Wiklund, C., and T. Jarvi. 1982. Survival of distasteful insects after being attacked by naive birds: a reappraisal of the theory of aposematic coloration evolving through individual selection. Evolution **36**:998–1002.

Wright, A. E., D. A. Forleo, G. P. Gunawardana, S. P. Gunasekera, F. E. Koehn, and O. J. McConnell. 1990. Antitumor tetrahydroisoquiniline alkaloids from the colonial ascidian *Ecteinascidia turbinata*. Journal of Organic Chemistry **55**: 4508–4512.

Young, C. M. 1985. Abundnce pattern of subtidal solitary ascidians in the San Juan Islands, Washington, as influenced by food preferences of the predatory snail *Fusitriton oregonensis*. Marine Biology **84**:309–321.

———. 1986. Direct observations of field swimming behavior in larvae of the colonial ascidian *Ecteinascidia turbinata*. Bulletin of Marine Sciences **39**:279–289.

Young, C. M., and B. L. Bingham. 1987. Chemical defense and aposematic coloration in larvae of the ascidian *Ecteinascidia turbinata*. Marine Biology **96**:539–544.

Young, C. M., and F.-S. Chia. 1987. Abundance and distribution of pelagic larvae as influenced by predation, behavior, and hydrographic factors. Pages 385–463 *in* C. Giese and J. S. Pease, editors. Volume 9. Reproduction of marine invertebrates. Aberdeen University Press, Aberdeen, Scotland.

Zabriskie, T. M., C. L. Mayne, and C. M. Ireland. 1988. Patellazole C: a novel cytotoxic macrolide from *Lissoclinum patella*. Journal of the American Chemical Society **110**:7919–7920.

Zar, J. H. 1984. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, New Jersey, USA.

APPENDIX

The quantification of didemnin cyclic peptides in 12 separate colonies of *Trididemnum solidum* and one pooled group of larvae collected at Little San Salvador, Bahamas was accomplished by the following methods. The frozen colonies were lyophilized to a constant mass and then pulverized to a fine powder with a mortar and pestle. A weighed amount of the pulverized ascidian was placed in a 5-mL filtering syringe and the lipophilic material isolated by sequential extraction with 30 mL of 2:1 dichloromethane : methanol, 5 mL of dichloromethane, and 5 mL of methanol (only HPLC-grade solvents were used in the sample preparation and HPLC analysis). The larvae were not lyophilized but were extracted three times with 10 mL of 2:1 dichloromethane : methanol. The combined extracts for each sample were concentrated by rotary evaporation, redissolved in 5 mL of 2:1 dichloromethane : methanol, and filtered to remove any insoluble material. This filtered solution was reduced by rotary evaporation and the residue dissolved in 2.200 mL of a 10:1 mixture of dichloromethane : methanol. One millilitre of this solution was removed and added to the top of a 1-g silica-gel cartridge column. For larvae, their entire organic extract entered the preparation scheme at this first chromatographic step. Fifteen minutes were allowed for the application solvent to evaporate and any residual solvent was removed by high vacuum applied for 15 min. Two fractions were collected from this column. The first fraction, eluted with 10 mL of diethyl ether, was discarded because it did not contain didemnin cyclic peptides. The second fraction was eluted with 10 mL of methanol to remove the remainder of the organic material including the didemnin cyclic peptides. This second fraction was concentrated by rotary evaporation, dissolved in 0.250 mL of 2:1 dichloromethane : methanol, and applied to the top of a 1-g C18 reversed-phase cartridge column. A second wash of the sample container with 0.150 mL of the same solvent was then applied to the column. The solvent was allowed to evaporate for 10 min and residual solvent was removed under high vacuum applied for 15 min. Three fractions were eluted from this column with the following solvents: 3 mL of distilled water, 6 mL of 85:15 methanol : water, and 5 mL of methanol. The first and third fractions contained no cyclic peptides and were discarded. The second fraction was transferred to a vial, evaporated to dryness, and the residual water removed under high vacuum applied for 2 h. In preparation for injection, 1.000 mL of the HPLC elutant solvent, 8:2 methanol : water, was added to this fraction and the vial sealed with an airtight polyseal cap. This fraction from the extract of the larvae was dissolved in 0.350 mL of the HPLC eluting solvent. The volume of each colony was estimated by multiplying its dry mass by the volume to dry mass ratio determined for the large collection of *T. solidum* from Little San Salvador. This volume was used to determine the volumetric concentration of the cyclic peptides in the separate colonies.

The HPLC system consisted of a solvent delivery system (421A System Controller, 110B solvent pumps, and 210A injector; Beckman Instruments, Incorporated, San Ramon, California, USA) and a detection system (SP8430 RI detector and SP4290 integrator; Spectra-Physics, Incorporated, San Jose, California, USA). For the analytical quantifications, we used an 80-100-C3 silica-gel HPLC column (Rainin Instrument Company, Woburn, Massachusetts, USA). The optimal HPLC conditions for quantifying the didemnin cyclic peptides employed 8:2 methanol : water as the eluting solvent

Stocker, L. J., and P. R. Berquist. 1986. Seasonal cycles, extrinsic factors, and the variable effects of turfing algae on the abundance of a colonial ascidian. Journal of Experimental Marine Biology and Ecology **102**:1–21.

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———. 1980*a*. Chemical defenses of ascidians against predators. Ecology **61**:1327–1334.

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Van Name, W. G. 1945. The North and South American ascidians. Bulletin of the American Museum of Natural History **84**.

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