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# BioScience.

Organisms from Molecules to the Environment

November 2011

American Institute of Biological Sciences

Vol. 61 No. 11

\$9.99 • \$9.99 Canada



www.biosciencemag.org

Cover: Sponges dominating a reef off Little Inagua, Bahamas, at a depth of 15 meters. The large, dead coral head is now home for at least seven species of sponges, including one that is burrowing into the limestone skeleton. Sponges are now the dominant habitat-forming animals on Caribbean reefs. The production of secondary metabolites (chemical compounds) by sponges plays an important role in the ecology of such ecosystems, a role discussed in the article by Joseph R. Pawlik that starts on p. 891. Photograph: Joseph R. Pawlik

Sponges' Chemical Defenses

## The Chemical Ecology of Sponges on Caribbean Reefs: Natural Products Shape Natural Systems

#### JOSEPH R. PAWLIK

Sponges are now the dominant habitat-forming animals on Caribbean reefs, where the combined effects of climate change, pollution, and disease have decimated reef-building corals. Natural products chemists have been isolating novel secondary metabolites from Caribbean sponges for many decades, but relevant studies of the ecological functions of these compounds have been more recent. Bioassay-guided surveys have revealed sponge chemical defenses against predators, competitors, and pathogens, but many common sponge species lack chemical defenses and appear to have followed a different evolutionary trajectory, investing instead in greater reproduction or growth. The emerging conceptual model predicts that changes in the abundances of fish- and sponge-eating fishes on Caribbean reefs will have a cascading impact on the sponge community, with indirect effects on the broader community of corals and seaweeds. Caribbean sponges provide an important alternative to terrestrial plant and insect communities for testing basic ecological theories about chemical defenses and resource allocation.

Keywords: marine ecology, chemical defense, trophic cascades, resource trade-offs, indirect effects

Natural products are chemical compounds found in the cells and tissues of plants, animals, and microorganisms that are not involved in primary metabolic pathways; they are therefore also referred to as secondary metabolites. The discovery of natural products by humans predates recorded history, with the medicinal use of plants containing compounds such as morphine, quinine, and digitalis. Organic chemists subsequently determined the structures of these compounds, and more recently, ecologists have considered the selective processes that led to their evolution, founding the discipline of chemical ecology (Eisner and Meinwald 1995). Natural product defenses of terrestrial plants rely on a combination of bad taste and toxicity to deter insect and vertebrate herbivores. For example, the plant alkaloid caffeine, which is such an important ingredient in your morning coffee, is also a potent inhibitor of insect feeding, and the coffee plant invests higher quantities of the metabolite in more valuable shoots and fruits (Nathanson 1984). Some insects have evolved the ability to thwart plant chemical defenses-in some cases, storing and using the metabolites to protect themselves from their own predators. One well-known example is the monarch butterfly, which retains the alkaloid defenses of the milkweed plant that it eats as a caterpillar. After metamorphosis, the bright color and distinctive pattern on its wings warn birds and other predators of the acquired toxicity (van Zandt Brower 1958).

This warning coloration is the basis for the evolution of mimicry by other butterflies, whether they are chemically defended or not. The involvement of natural products in plant defenses and predator–prey relationships, as well as inter- and intraspecific chemical communication by plants, insects, and vertebrates, is all the domain of chemical ecology (Eisner and Meinwald 1995).

The advent of scuba diving in the mid-twentieth century opened a new realm to chemists and ecologists alike. For the first time, researchers could gain access to the marine benthos in much the same way that they had studied terrestrial habitats. The result was a "golden age" for marine natural products chemists as they discovered a wealth of new secondary metabolites from previously unexamined benthic marine invertebrates and seaweeds (Faulkner and Fenical 1977). One of the richest sources of new chemical compounds was sponges, and although the chemists speculated on the ecological functions of the unusual metabolites that they were discovering, it would be over a decade before ecologically relevant bioassays would be used to test these functions.

#### **Sponges on Caribbean reefs**

Sponges have always been an important component of Caribbean coral reef communities, but they have become increasingly dominant as reef-building corals have declined

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because of stressors associated with climate change, disease, and pollution (Maliao et al. 2008). Unlike corals and some seaweeds that secrete calcium carbonate skeletons, sponges have skeletons of glass or protein (if they have them at all), and they are less likely to be affected by ocean acidification, which may tip the competitive balance in their favor. Populations of the giant barrel sponge (*Xestospongia muta*) increased by 46% over six years, beginning in 2000, on reefs off the Florida Keys (McMurray et al. 2010), where this sponge is now the most important habitat-forming animal on the reefs (figure 1). Moreover, sponges are long-lived organisms, with age estimates in the hundreds to thousands of years for *X. muta* (McMurray et al. 2008).

Sponges are important to the overall ecology of coral reefs for many reasons: They are very efficient filter feeders, providing an important link in benthic–pelagic coupling (Southwell et al. 2008), and their bodies provide shelter for large numbers of invertebrates and fishes (Westinga and Hoetjes 1981). Sponges harbor microbial symbionts, which may be important carbon and nitrogen fixers in oligotrophic tropical waters (Diaz and Ward 1997). Sponges are aggressive competitors for space (Aerts 1998) and are primary agents of carbonate bioerosion on coral reefs (Zundelevich



Figure 1. Sponges are now the dominant habitat-forming animals on Caribbean reefs. The giant barrel sponge, Xestospongia muta, is prominent in many locations; on reefs off Key Largo, Florida, populations increased 46% over a six-year period beginning 2000. Photograph: Joseph R. Pawlik.

et al. 2007) but may also be important in holding the reef framework together (Wulff 1984).

The ecology of Caribbean coral reef sponges is comparatively simple and may represent a better system for testing ecological theory than studies of terrestrial plant and insect communities that make up the principal source of theoretical validation for chemical ecologists (Núñez-Farfán et al. 2007). First, sponge community composition on Caribbean reefs is remarkably consistent across a large biogeographic region, which extends from the Florida Keys to the north coast of South America and from the Gulf of Mexico to the Lesser Antilles. This homogeneity results from the clockwise ocean currents that maintain the connectivity of populations across the broad Caribbean area through the dispersal of planktonic eggs, spores, and larvae (López-Legentil and Pawlik 2009). Second, space is the primary limiting resource for filter-feeding sponges, which have few of the complicating interactions common to plants, such as effects due to variations in light, rainfall, humidity, nutrients, soil chemicals, and so on. Third, sponge communities are controlled largely by predation and competition. Only a few readily identifiable fish species eat sponges, and there is little or no effect from insect-equivalent mesograzers that are major consumers of many terrestrial plants. Finally, all of the component competitors, predators, and prey are still extant on Caribbean reefs, with no missing megaconsumers, as is the case for many terrestrial communities that now lack mastodons, sloths, bison, and other species formerly important to these ecosystems (Gill et al. 2009); even sponge-eating hawksbill turtles are still abundant at some sites in the Caribbean (Diez and van Dam 2002).

## Overturning conventional ideas about sponge ecology

Until the mid-1990s, the conventional view of sponge ecology on Caribbean reefs was that predation had little impact on sponge populations, because only a few fish species, primarily angelfishes, spread their predatory activities over a wide variety of sponge species (Randall and Hartman 1968, Wulff 1994). It was presumed that some combination of chemical defenses, structural defenses, or poor food quality among sponge species resulted in predators' adopting a "smorgasbord" feeding strategy, in which they consumed small amounts of many different sponge species, with little resulting impact on any of them. Furthermore, it was suggested that sponge-eating fishes choose their prey on the basis of color and switch their preferences frequently in order to avoid excessive exposure to the defenses of any one species, as has been described for some fruit-eating primates (Wulff 1994).

Feeding experiments designed to decouple chemical defenses from other factors that may affect the palatability of sponge tissue to potential consumers did much to alter our understanding of fish predation on Caribbean sponges. With the common predatory bluehead wrasse (*Thalassoma bifasciatum*) as an experimental subject, a laboratory

feeding assay protocol was developed in which the secondary metabolites from sponge tissue were extracted using organic solvents, reconstituted in artificial foods at the same volumetric concentration, and then offered to replicate sets of blueheads to gauge palatability (Pawlik et al. 1995). A survey of over 70 Caribbean sponge species revealed that the majority of the species were chemically defended (69%), but many common reef species were not. Interestingly, these common, chemically undefended species had earlier been identified from fish gut-content analyses as constituting a majority of the diet of sponge-eating fishes (Pawlik 1997). Therefore, feeding experiments with generalist predatory fishes revealed the preferences of the specialized sponge-eating fishes, and the specialists were eating chemically undefended sponge species.

Additional feeding assays were performed to investigate the role of the sponge skeleton, both mineral (glass) and organic, on feeding preferences. For terrestrial plants, physical or structural defenses, such as thorns on stems, shells on seeds, and mineralized inclusions, represent important mechanisms for deterring herbivores (Reynolds et al. 2009). On coral reefs, however, most fishes bear strong jaws and pharyngeal teeth that can grind the limestone skeletons of corals, molluscs, and worms to dust. Even when feeding assays were performed with blueheads, which lack the strong jaws and teeth of angel- and parrotfishes, the sponges were found to lack the physical defenses associated with their mineral and organic skeletons (Chanas and Pawlik 1995, 1996), although the glass spicules did provide a synergistic effect in combination with chemical defenses for some sponge species (Jones et al. 2005). Analyses of the nutritional quality of sponge tissue revealed that all of the species surveyed were rich in protein and caloric value, which makes sponges a potentially valuable source of food (Chanas and Pawlik 1995). Unlike terrestrial plants, then, Caribbean reef sponges rely primarily on chemical defenses (when they have them at all) and not on defenses associated with their skeletons or on low nutritional quality.

But what about color as the basis for fish selecting sponge prey? Using the feeding assay survey results (Pawlik et al. 1995), chemically defended and undefended sponge species with similar colors and shapes were collected from diverse habitats (reefs, mangroves, seagrass beds) and offered together, attached to weighted panels, to the natural population of fishes on Conch Reef near the Aquarius habitat, an undersea research laboratory off Key Largo, Florida. Surprisingly, fishes and hawksbill turtles unfailingly chose to eat the chemically undefended sponge species, regardless of color, and quickly consumed them (Dunlap and Pawlik 1996, Pawlik 1998). These sponge species had been collected from habitats in which sponge-eating fishes are seldom found (mangroves, seagrass beds), but these sponges could also be found under rubble and in cracks and crevices on the reef itself. Moreover, in addition to angelfishes, it was discovered that parrotfishes-among the most common grazers of corals and algae on coral reefs—were also major sponge predators (Dunlap and Pawlik 1996, 1998).

A new understanding of Caribbean sponge ecology had emerged that is based on sponge chemical defenses. Rather than a minimal effect, predation by sponge-eating fishes (including parrotfishes) and turtles has a profound influence on sponge distributions and abundances, which relegates *preferred* prey species to refuge habitats in reef interstices or in mangrove or seagrass habitats in which these predators are seldom found (Pawlik 1997, 1998). This leaves two categories of sponges on the reef: chemically *defended* species that are avoided by fish predators and *palatable* species that bear the brunt of fish grazing, yet persist.

## Structure–activity relationships of sponge chemical defenses

Identification of the secondary metabolites responsible for defending the sponge species in the chemically defended category has been an ongoing task for over a decade. For any given species, the crude organic extract of the sponge tissue is subjected to solvent partitioning and chromatographic fractionation combined with laboratory feeding assays to separate the deterrent metabolites from those that are not. These investigations often include field experiments in which artificial foods are infused with crude extracts, partitions, or fractions at volumetrically natural concentrations and placed side by side with control foods so that differential feeding by a natural population of Caribbean reef fishes can be observed. Defensive metabolites represent a wide range of structural classes and polarities, including the pyridinium salt amphitoxin from Amphimedon spp. (Albrizio et al. 1995), the brominated alkaloids oroidin and stevensine from Agelas and Axinella spp. (Wilson et al. 1999, Assmann et al. 2000, Lindel et al. 2000), the terpenoid glycosides formoside, ectyoplaside and feroxoside from Erylus and Ectyoplasia spp. (Kubanek et al. 2000, 2002), and the brominated-tyrosine derivative fistularin from Aplysina spp. (figure 2; Puyana et al. 2003). A long-standing assumption was laid to rest when it was discovered that the "putrid stench" volatile metabolites from sponges of the genus Ircinia were not the basis for their potent chemical defense; rather, it is a group of furanosesterterpene tetronic acids (Pawlik et al. 2002). Also noteworthy are the novel secondary metabolites discovered during the bioassay-guided fractionation process that were not deterrent in feeding assays, such as the amaroxocanes and amaranzoles from Phorbas amaranthus (Morinaka et al. 2009, 2010).

For many chemical cues that influence animal behavior, such as insect sex pheromones, minor changes in the structure of the stimulatory molecules diminishes or eliminates their activity (Plettner 2002). A similar phenomenon was demonstrated for the chemical defenses of sponge species in the genus *Agelas*, which are among the most prominent on Caribbean reefs. These sponges are all defended from fish predation by a group of brominated-pyrrole-containing alkaloids ranging from the most simple 4,5-dibromopyrrole-2-carboxylic acid to the imidizole-containing

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Figure 2. Secondary metabolites responsible for chemical defenses against predators and competitors have been isolated and identified from many common species, including (a) amphitoxin for Amphimedon spp., (b) oroidin and derivatives for Agelas spp., (c) ectyoplaside-A and similar triterpene glycosides for Ectyoplasia ferox, (d) aeroplysinin-1 and related brominated-tyrosine-derived alkaloids for Aplysina spp., and (e) variabilin and related sesterterpenoids for Ircinia spp. Photographs: Joseph R. Pawlik.

compound oroidin (figure 2) or to dimeric metabolites, including sceptrin and ageliferin (Chanas et al. 1997, Assmann et al. 2000, Lindel et al. 2000). The relationship between metabolite structure and feeding-deterrent activity was investigated with a range of naturally occurring compounds, derivatives, and synthetic analogs by using blueheads to conduct laboratory feeding assays (Assmann et al. 2000, Lindel et al. 2000). The pyrrole portion of the molecule was required for activity but was not necessarily sufficient, whereas the imidazole portion was insufficient but enhanced activity (figure 3). Changing the pyrrole heteroatom from nitrogen to oxygen or sulfur had no influence on activity, but greater bromination of the pyrrole enhanced it. Finally, dimerization and modification to increase compound polarity both enhanced activity. It is likely that these metabolites interact with receptors on the gustatory epithelia of fishes and other predators in highly specific ways, initiating signal processing that results in behavioral food rejection. This concept has been better explored for the defensive compound formoside, a triterpene glycoside from the sponge Erylus formosus (Kubanek et al. 2001). A novel coreceptor responsible for signaling in response to formoside was characterized from a zebrafish cDNA library and appears to be structurally and functionally similar to receptoractivity-modifying proteins known from other animals (Cohen et al. 2010).

## Novel bioassays reveal other defensive roles

Secondary metabolites from sponges may have alternative ecological functions, and the same survey approach that was used to assess chemical defenses against fish predation was used to investigate defenses against invertebrate predators, as well as allelopathic (anti-overgrowth), antifouling, and antimicrobial defenses. In each case, the bioassays were designed to test each defensive function in an

ecologically relevant manner and with the metabolites at naturally occurring volumetric concentrations.

Invertebrates—particularly seastars—are major sponge predators in other marine habitats (McClintock et al. 2005). There is little evidence that they have an important predatory effect on Caribbean reef sponges, although invertebrates are often active on the reef at night and hide in crevices in the reef during the day. Assays were designed to test feeding preferences of hermit crabs (*Paguristes puncticeps*) and seastars (*Echinaster* spp.) for artificial foods treated with skeletal components and organic extracts of Caribbean sponges (Waddell and Pawlik 2000a, 2000b). As had been



Figure 3. Relationship between compound palatability in fish-feeding experiments and molecular structure for pyrrole-imidazole alkaloids from sponges of the genus Agelas. The pyrrole portion (left) of the molecule is required to deter fish feeding, whereas the imidizole portion is not required but enhances distastefulness. Changing the nitrogen atom in the pyrrole portion to oxygen or sulfur had no effect, but replacing hydrogen with bromine or increasing compound polarity enhanced the distastefulness of the compound, as did dimerization. A close-up of Agelas clathrodes is shown in the background. Photograph: Joseph R. Pawlik.

observed for fishes, neither hermit crabs nor seastars were deterred from feeding on foods containing sponge skeletal components, but they were both deterred by crude organic extracts of the same species of sponges that had deterred fish predators. Although the specific compounds responsible for chemical defense against hermit crabs and seastars were not determined, it seems likely that sponge chemical defenses may be broadly effective against vertebrate and invertebrate predators.

The broad spectrum of defenses offered by secondary metabolites in chemically defended sponges extends beyond protection from predation. Standard microbial inhibition assays were performed in which crude organic extracts of sponges were spotted onto paper discs that were placed on agar-filled petri dishes streaked with cultures of marine bacteria that were then allowed to grow. "Halos" of inhibited microbial growth around the discs treated with sponge extracts revealed much the same pattern of activity as in the feeding assays, which again suggests that sponge defenses have multifunctional roles (Newbold et al. 1999). More sophisticated experiments on bacterial attachment and swarming-both important components of the process of marine biofouling-corroborated the finding of multiple defensive functions. The same purified metabolites that defended sponge species in the genera Agelas, Amphimedon, and Aplysina, which constitute the dominant chemically defended sponge species on Caribbean reefs, inhibited

bacterial attachment or swarming (Kelly et al. 2003, 2005). Further work on *E. formosus*, a sponge that is well defended against fish predators by a suite of triterpene glycosides, revealed that these compounds were present at sufficient concentration at the sponge surface to inhibit the settlement and growth of fouling algae and invertebrates. This was determined by performing field experiments (figure 4a) in which crude organic extracts and purified metabolites from *E. formosus* were incorporated into stable gels and monitored for biofouling over a 20-day period (Kubanek et al. 2002).

Besides predation, the dominant biotic factor influencing community structure on Caribbean coral reefs is competition. Of course, this is true for terrestrial communities as well, and the use of secondary metabolites by plants to take and hold real estate—a phenomenon known as allelopathy has been a major focus of chemical ecologists (Field et al. 2006). Sponges face competitive overgrowth by reef-building corals, seaweeds, octocorals (sea whips and fans), ascidians (sea squirts), and other sponges, all of which have the capacity to take space by spreading as they grow. To test for allelopathic activity of sponge secondary metabolites, a novel field assay technique was developed in which a living competitor, whether a sponge, ascidian, or bryozoan, was grown onto a 5-centimeter (cm) square of clear acrylic plastic then fixed to the center square of a "tic-tac-toe" plate with 5-cm square wells on each of its four sides and 5-cm plastic squares in each of its four corners (figure 4b; Engel and Pawlik 2000). Two opposing wells were filled with a stable gel containing a natural concentration of the crude organic extract of the test sponge tissue, and the other two wells contained untreated control gels. The entire tic-tac-toe plate was returned to the field, and the competitor invertebrate was allowed to grow outward on the plate for 21 days. Differential lateral growth on the extract-treated and control gels was measured and revealed whether there was an allelopathic effect of the sponge extract. Of the extracts tested, 30% exhibited an allelopathic effect, including those from sponge species in the genera Amphimedon and Aplysina, and the purified triterpene glycosides were identified as the allelopathic agent for Ectyoplasia ferox (Engel and Pawlik 2000, Kubanek et al. 2002). Surprisingly, the extracts of three sponge species promoted the growth of competitors, and these sponges tend to be overgrown by other sponges in the field. One in particular-Geodia gibberosa-is a preferred food of angelfishes and turtles, yet when it is found in the field, it is almost always wrapped in another, chemically defended sponge species (most often Amphimedon viridis)-an example of a symbiosis in which there is defense by association (Engel and Pawlik 2000, Wilcox et al. 2002).

With the decline of reef-building corals on Caribbean reefs, there is particular interest in competitive interactions that may further affect coral abundance. The tic-tac-toe assay described above was not amenable to testing the effects of sponge metabolites on corals, because corals grow slowly. Instead, a field bioassay system was developed in which gels containing crude organic extracts of sponges at

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Figure 4. Gel-based bioassays for testing the allelopathic and antifouling activity of secondary metabolites in organic extracts of Caribbean sponges. Compounds or extracts were added to gels at the concentration at which they are found in the same volume of sponge tissue and were returned to the field to measure (a) the natural recruitment of fouling organisms to control and extracttreated gel surfaces after two weeks, (b) the comparative overgrowth of live sponge (Tedania ignis, center square) on control gels (right and left) and gels containing extracts of Erylus formosus (top and bottom), and (c) the relative changes in the health of algal symbionts (zooxanthellae) after control and extract-treated gels in petri dishes were overturned onto the coral Diploria labyrinthiformis, which mimics sponge overgrowth. Photographs: Joseph R. Pawlik.

then inverted onto the surfaces of large, mound-forming brain corals of the genus Diploria (figure 4c), thereby mimicking the overgrowth of sponge onto coral (Pawlik et al. 2007). Pulse-amplitude modulated fluorometry was used to monitor the health of populations of symbiotic dinoflagellates in the coral tissue (zooxanthellae) under the gels as a proxy for changes in coral health. A reduction in fluorescence of the coral tissue under gels treated with sponge extracts relative to that under control gels was indicative of coral bleaching or loss of symbionts, whereas a reduction in the maximal quantum yield of photosystem II was indicative of reduced photosynthetic efficiency of symbionts. Once again, sponge species that were chemically defended against fish predators also had crude extracts that were the most active in coral bioassays, including species in the genera Agelas, Aplysina, Amphimedon, and Ectyoplasia (Pawlik et al. 2007). Although additional research will be required to isolate and identify the metabolites responsible for these alternative defensive functions, the striking pattern that emerges is one in which sponge species that are chemically defended against fish predators also exhibit defenses against pathogens and competitors, which suggests that either defended sponges produce a suite of chemical defenses that protect them from different predators, competitors, and pathogens, or that the same defensive metabolites play multifunctional roles (Kubanek et al. 2002).

#### Resource allocation and the survival of undefended sponge species

More intriguing than the discovery that one category of sponges on Caribbean reefs, which includes several genera and many species, defends itself from predators, competitors, and pathogens using secondary metabolites was the discovery that another equally diverse category of sponges did not. Among the most common sponges on Caribbean reefs, those in the genera Callyspongia, Niphates, and Iotrochota were undefended by secondary metabolites or physical structures (Pawlik et al. 1995, Pawlik 1997) and were also the largest component of the diet of sponge-eating fishes (Randall and Hartman 1968). This dichotomy was in marked contrast to the pattern observed for gorgonian corals (sea whips and fans) on Caribbean reefs, because all the common species were strongly chemically defended (O'Neal and Pawlik 2002). How were palatable sponge species able to persist and thrive on Caribbean reefs despite grazing from spongeeating fishes and competition from chemically defended sponge species, particularly when the third category of sponges-those preferred by sponge-eating fishes-are removed from the reef and persist only in refuge habitats?

Central to theories of terrestrial plant defenses against consumers is the optimal defense hypothesis, which includes the concept that defenses are costly and come at the expense of other life functions, such as growth and reproduction (Rhoades and Cates 1976). Furthermore, linked to trade-offs in resources is the idea that less-defended plants may adapt

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to tolerate consumer damage (Stowe et al. 2000). Although increasingly complex (Stamp 2003), plant defense theory remains the framework of choice for investigations of defense in other systems and is particularly relevant for understanding the evolution of chemical defenses in Caribbean sponges. Plant defense theory predicts that palatable sponge species should devote greater resources to healing, growth, or reproduction rather than expending them on the production and storage of secondary metabolites for defense.

To test for differences in the rates of healing for chemically defended and palatable sponges, seven species were chosen that have the same tube- or vase-shaped morphology, and 2-square-centimeter holes were cut into the sides of replicate sponges so that two-dimensional healing of the circular wounds could be monitored using digital photography (Walters and Pawlik 2005). The three defended species healed very slowly—0% to 2% of the surface area of the wound per day—whereas the four palatable species healed quickly—6% to 8% per day, and most had completely healed in 10–12 days (figure 5). The results demonstrated that sponge species in the palatable category had evolved mechanisms for more



Figure 5. Examples of different rates of healing among tube- and vase-shaped sponges that are palatable to sponge-eating fishes versus healing rates of those that have chemical defenses. Holes cut in the side of a tube of Callyspongia vaginalis to simulate fish grazing rapidly healed over in less than 12 days (a, before; b, after), whereas similar holes in Ircinia campana were unchanged after the same amount of time (c, before; d, after). Callyspongia vaginalis lacks chemical defenses and is heavily grazed by angelfishes, whereas Ircinia campana is chemically defended by furanosesquiterpene tetronic acids (figure 2). A millimeter scale is shown in each photo. Note the rapid growth of symbiotic polyps, Parazoanthus parasiticus, into the healed tissue in panel (b). Photographs: Joseph R. Pawlik.

rapidly healing wounds than chemically defended species, probably in response to nonfatal grazing by sponge-eating fishes. The rapid rate of healing also explained the field observation that grazing scars on palatable sponge species never persist for very long. The results were also congruent with the hypothesis that palatable sponge species could use their limited resources to rapidly heal wounds, whereas chemically defended species instead expend energy on the production and storage of secondary metabolites (Walters and Pawlik 2005).

Besides wound healing, could resource trade-offs be demonstrated between chemical defenses and growth or reproduction for Caribbean reef sponges? This would prove to be difficult for multispecies comparisons, because sponges display a range of morphologies and reproductive strategies. Tube- and vase-shaped species generally engage solely in sexual reproduction, but branching species frequently reproduce asexually when their branches are dislodged by currents or storms and reattach to the substratum. Therefore, the growth of branches leads to greater reproduction for branching sponge species, and the two processes are intertwined.

This confounding effect of growth and reproduction was made particularly clear in a study of two equally abundant, closely related palatable sponges of the genus *Callyspongia* on reefs off Florida. The tube-shaped species, *Callyspongia vaginalis*, produced 13 times more larvae over the course of a year than the branching *Callyspongia armigera*, but the latter grew 4 times faster, and had more than twice the number of substratum attachment points, which is indicative of asexual reproduction by fragmentation (Leong and Pawlik 2010a). So, depending on morphology, there are reproductive tradeoffs that may obscure resource trade-offs between chemical defenses and growth or reproduction.

Despite the confounding trade-off between growth and reproduction, a trade-off between chemical defenses and growth was evident when the multispecies comparison was limited to branching sponge species. Thirty growth experiments were performed in the field, each lasting 123-195 days, in which sponge branches of seven species were placed inside and outside of plastic-mesh cages and the sponge mass determined before and after each experiment. In the absence of predation, growth in cages was significantly greater for three palatable than for four chemically defended branching sponge species (Leong and Pawlik 2010b), which supports a resource trade-off between growth and chemical defense despite the fact that these different species may be allocating resources differently to reproduction. The growth of chemically defended sponge species was not different inside and outside of the cages, but the growth of palatable sponge species was significantly greater inside the cages, which confirms that the uncaged palatable sponges were grazed by sponge-eating fishes (Leong and Pawlik 2010b). A study of the same sponge species designed to test for tradeoffs specifically between chemical defenses and reproduction was also undertaken in which replicate sponge samples were collected each month for a year and the surface area of oocytes, embryos, or larvae was determined from histological sections of sponge tissue in a standardized manner (Leong and Pawlik 2011). In this case, however, no significant differences in yearly reproductive output between palatable and chemically defended sponge species were evident, probably because of the confounding trade-off between growth and reproduction (Leong and Pawlik 2011).

#### Sponge recruitment to a shipwreck confirms chemical defense trade-off

An independent and serendipitous test of the resource allocation hypothesis came with the discovery and quantitative survey of a recently formed sponge community on the deck of a 155 meter (m)–long shipwreck four years after it had been intentionally sunk as an artificial reef off Key Largo, Florida. The USS Spiegel Grove was situated in an upright position, with over 1500 square meters of deck area, 19-28 m below the sea surface and 800 m from the nearest reef. The steep vertical sides of the ship, rising 19 m above a sandy bottom, prevented sponge fragments from reaching the horizontal deck, so colonization could only result from larval settlement. Assuming that chemically undefended sponge species invest more in growth or reproduction than do defended species, one would predict that this new habitat would be colonized first by undefended spe-



Figure 6. Sponge community composition off Key Largo, Florida, on (a) Dixie Shoals reef and 800 meters away on (b) the horizontal decks of the four-year-old wreck of the USS Spiegel Grove as a percentage of surface-area coverage. The shades of red in the pie charts indicate chemically defended sponge species; green indicates the undefended species. The red and green categories are further subdivided: The dark tone represents branching species, the middle tone represents vase- and tube-shaped species, and the light tone represents mound-shaped species. The rapid recruitment of chemically undefended sponge species to the wreck is evidence of a resource trade-off between chemical defenses and growth or reproduction among Caribbean sponge species. Photographs: Joseph R. Pawlik.

cies, because they produce more larvae to settle on the deck, because they grow faster once they have colonized the deck, or some combination of these two factors. As was predicted by theory, the chemically undefended sponge species were significantly more abundant (96%) and larger on the shipwreck than were those on the adjacent coral reef (15%) (figure 6; Pawlik et al. 2008). Interestingly, when the wreck was visited 18 months later, the first recruits of several chemically defended sponge species were observed, which suggests that the sponge community on the wreck was in transition to a ratio similar to that observed on adjacent reefs. The pattern of sponge colonization on the shipwreck provided an important confirmation that a resource trade-off exists between chemical defenses and growth or reproduction because colonization combined the effects of both reproduction and growth; this eliminated the confounding effects of the trade-offs between these two factors that made it difficult to test them separately.

### A new ecosystem model of sponge ecology on Caribbean reefs

With a better understanding of the role of secondary metabolites in protecting the chemically defended category

of sponges from predators and competitors and of the alternative strategy of greater growth or reproduction exhibited by sponges in the palatable category, one can diagram a new conceptual model of reef ecology that is likely to become increasingly relevant for contemporary Caribbean coral reefs (figure 7). In this model, fish-eating fishes such as sharks and groupers control populations of sponge-eating fishes (and turtles), which in turn have very different effects on the three categories of sponges: They remove preferred species from the reef, graze on palatable species, and have little impact on defended species. The resulting sponge community on the reef competitively interacts with corals and macroalgae for available space.

The conceptual model is useful because it is predictive. For example, removal of sponge-eating fishes should release preferred and palatable sponges from predation, which would give them a competitive advantage over defended



Figure 7. Conceptual model of ecosystem function on contemporary Caribbean reefs based on sponge chemical defenses. The black arrows indicate predatory control between trophic levels; the white arrows indicate competitive interactions. Ecological concepts discussed in the text are written in italics.



Figure 8. The orange icing sponge, Mycale laevis, growing in association with the hard coral, Montastrea annularis, on reefs off (a) Key Largo, Florida, where sponge-eating fishes are abundant, and (b) Bocas del Toro, Panama, where all fishes are rare because of overfishing. In the absence of fish grazers, this "preferred" sponge species overgrows and kills hard corals. Photographs: Tse-Lynn Loh.

sponges and would allow them to compete with corals and seaweeds. Just this sort of manipulation occurs on many Caribbean reefs today, where overfishing through the use of indiscriminate fish traps greatly reduces the abundance of all fish species. When the sponge community of a heavily fished reef near Bocas del Toro, Panama, was compared with reefs that are better protected from fishing in the Florida Keys, there were clear differences in the relative abundance of defended and undefended sponge species. One species in particular-the orange icing sponge, Mycale laevisgrows only in cryptic locations on Florida reefs, commonly between the branches of living corals. But near Bocas del Toro, this sponge grows into large lobes that smother adjacent coral colonies because of the absence of sponge-eating fishes (figure 8; Loh and Pawlik 2009). When M. laevis was protected from sponge predators on the reefs off Key Largo by placing plastic cages over them, the sponges began growing out from between coral branches. Therefore, it is evident that overfishing results in predator release of a sponge species in the preferred category that has an indirect effect on reefbuilding corals through competitive overgrowth. The Caribbean provides a patchwork of reefs, ranging from heavily overfished to carefully protected from fishing, on which the predictions of the conceptual model can be tested.

#### Conclusions

The field of chemical ecology has been criticized as being overly reductionist, for focusing on the isolation and identification of secondary metabolites with little subsequent interest in the effects of these compounds on individuals, communities, or ecosystems (Brown 1995). For sponge communities on Caribbean reefs, however, chemical ecology provides an important key to understanding the whole ecosystem. The framework provided by the conceptual model of sponge ecology on Caribbean reefs will be particularly useful in the coming decades, as the impacts of global climate change further disrupt marine communities. One forecast might be that sponges will gain greater dominance on Caribbean reefs in the face of ocean acidification, because sponges do not rely on a calcified skeleton as all reef-building corals and some seaweeds do. However, reports of new sponge diseases have been increasing (Cowart et al. 2006, Webster 2007), and the mortality caused by some of them could have a dramatic impact on the populations of susceptible sponge species (McMurray et al. 2010). What remains certain is that Caribbean reefs of the next century will be very different from those of the last.

#### Acknowledgments

Much of the research detailed in this review would not have been possible without the hard work of dedicated undergraduate, graduate, and postdoctoral students. Long-term funding from the National Science Foundation's Biological Oceanography Program (most recently, Grant OCE 1029515) provided support, primarily for student education and training. Invaluable facilities support was provided by the National Oceanic and Atmospheric Administration's Aquarius Reef Base (formerly the National Undersea Research Center). Permission to conduct research on US and Bahamian reefs was provided by the Florida Keys National Marine Sanctuary and the Government of the Bahamas, respectively. Thanks to Melissa Smith for expert help with the illustrations.

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