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Chemical warfare on coral reefs: Sponge metabolites differentially affect coral symbiosis in situ

Abstract—Coral reef ecosystems are characterized by high species diversity and intense levels of biotic interaction, particularly competition for space among sessile benthic invertebrates. Using in situ pulse amplitude modulated fluorometry, we demonstrate that secondary metabolites present in the tissues of some Caribbean sponge species have rapid allelopathic effects on the symbiotic algae (zooxanthellae) that live in coral tissues and provide the energy for coral growth and reef formation. When incorporated into stable gels at natural concentrations and placed in contact with brain coral heads on shallow reefs for ~18 h, secondary metabolites of some sponge species caused a decrease in the photosynthetic potential of the symbiotic algae and bleaching of the coral tissue, whereas those of others interfered with algal photosynthesis without causing bleaching. Some sponges produce metabolites with different modes of action for competing with reef corals.

The global decline in the health of coral reefs has been ascribed to a variety of possible reasons (Hughes 1994; Pandolfi et al. 2005). Among the hypotheses include those of ''indirect effects'' due to the loss of coral reef predators or herbivores. For example, the mass mortality of the urchin *Diadema antillarum* on Caribbean reefs has been blamed for greater growth of macroalgae, which in turn compete for space with reef-building coral species (Hughes 1994). More recently, it has been demonstrated that sponge-eating fishes control the abundance of some sponge species on reefs (Pawlik 1998), so that decreases in fish abundance may permit the growth of otherwise cryptic sponge species, some of which may more effectively compete for space with corals (Plucer-Rosario 1987; Hill 1998). One sponge species, *Chondrilla nucula,* was recently observed to overtake reefs in Belize (Aronson et al. 2002). Similar trophic cascades have been implicated in the restructuring of other marine ecosystems (Franks et al. 2005).

Mechanisms for competition between coral species have been well described (shading, sweeper tentacles, gastric filaments; Lang 1973; Richardson et al. 1979), but those that permit the overgrowth of corals by sponges have not. Although it has long been hypothesized that chemical warfare is important in competitive interactions among benthic invertebrates on coral reefs (Bakus 1981), ecologically relevant tests of allelopathy are scarce. Past laboratory-based respirometry measurements of the effects of sponge metabolites on corals were neither properly controlled nor ecologically realistic (Sullivan et al. 1983). Because of the diluting effects of turbulent flow, allelopathic interactions on coral reefs are most likely to occur on contact or at very short distances (Kubanek et al. 2002). Although the negative effects of sponges in close proximity to corals have been clear (e.g., Plucer-Rosario 1987; Hill 1998), experimental demonstration of allelopathy, as opposed to a physical effect of smothering, has been lacking.

We began developing a field assay for assessing sponge allelopathy against corals over a decade ago, adapting techniques developed for performing field antifouling assays (Henrikson and Pawlik 1995) in which crude organic extracts and purified secondary metabolites were incorporated into stable gels that slowly released metabolites (1–2% per day; Engel and Pawlik 2000) and could be deployed in the field. Although our early attempts showed promise in that we could see obvious effects on corals that had been exposed to gels treated with sponge extracts in the field, there was no simple or nondestructive technique available to quantify these effects until the advent of the diving pulse amplitude modulated (PAM) fluorometer.

PAM fluorometry is a noninvasive technique that has been used to assess the photosynthetic properties of the tissues of photosymbiont-containing invertebrates (Beer et al. 1998; Jones et al. 1999). The diving-PAM fluorometer (Heinz Walz) can be used to measure the maximal quantum yield of photosystem II (Y) of zooxanthellae present in dark-adapted coral tissue in the field. After application of a saturating light flash, fluorescence rises from the ground-state value (F_o) to its maximum value, F_m. The potential quantum yield (Y) is then calculated as $Y = (F_m - F_o) F_m^{-1}$ (also termed $F_v F_m^{-1}$). For our purposes, PAM fluorometry of adjacent, dark-adapted coral tissue that has been exposed to control gels versus metabolite-treated gels yields two useful parameters: F_o, the ground-state fluorescence, a proxy for the number of zooxanthellae in the coral tissue, or relative bleaching as zooxanthellae are lost (*see below*), and Y, a proxy for the photosynthetic efficiency, or

health, of zooxanthellae. Although there are ongoing controversies about the limitations of PAM fluorometry for intra- and interspecific comparisons of coral health (Beer et al. 1998; Ralph et al. 2002), comparisons of adjacent coral tissues exposed to metabolite-treated and control gels on the same coral head are not subject to these concerns. F_o was determined to be directly proportional to chlorophyll *a* (Chl *a*) content for brain coral heads from the same patch reef (*see Methods*). Chl *a* content on a per area basis can change both as a consequence of the amount of zooxanthellae present inside the coral tissue and the Chl *a* content of each zooxanthella. In this case, the gel treatments caused a reduction in irradiance (which, if it had any photoadaptive effect, would cause an increase in Chl *a* content per zooxanthella), and this reduction was the same in all cases, including the control gels. Therefore, any observed decrease in F_o was an indication of a reduction in the number of zooxanthellae in the coral tissue due to experimental treatments.

Using in situ PAM fluorometry, we demonstrate herein that secondary metabolites present in the tissues of some sponge species have rapid negative effects on the symbiotic algae (zooxanthellae) that live in coral tissues and provide the energy for coral growth and reef formation. When incorporated into stable gels at natural concentrations and placed in contact with brain coral heads on shallow reefs for ~18 h, secondary metabolites of some sponge species caused a decrease in the photosynthetic potential of the symbiotic algae and bleaching of the coral tissue, whereas those of others interfered with algal photosynthesis without causing bleaching.

Methods—Assay techniques were adapted from past studies of antifouling and overgrowth (Henrikson and Pawlik 1995; Engel and Pawlik 2000). Sponges were collected from reefs in the Bahamas Islands or near Key Largo, Florida. Sponge species were chosen on the basis of past studies that revealed differences in chemical defenses among reef sponges (Pawlik et al. 1995; Engel and Pawlik 2000). Sponge tissue volume was determined by displacement with water, then exhaustively extracted in an equal mixture of dichloromethane and methanol followed by methanol alone. Solvents were removed by rotary evaporation and extracts were combined into a homogeneous mixture. Extracts were incorporated into cooling polysaccharide gels (60°C Phytagel or carrageenan in water) at natural volumetric concentrations then poured into 5-cm plastic petri dishes. Each dish was inverted and fixed in place so that the gel surface gently rested against the top, flat area of a coral head on patch reefs at ~4 m depth at Sweetings Cay, Bahamas. For each experiment, one control and one of each of four treatment gels were placed against one coral head (five gels per coral head, each gel >8 cm from any other gel). This process was replicated on five coral heads on the same patch reef between 09:00 and 11:00 h. By employing a PAM fluorometer specially designed for use while scuba diving (Heinz Walz), readings were performed ~18 h later, before sunrise the next day, in order to take measurements on dark-adapted coral tissue. While red Cylume light sticks were used for minimal working illumination, three to five fluorometer readings were taken in rapid succession on the coral surface, both under each gel and adjacent to each gel at a distance of 3–5 cm with the probe specially designed for coral readings (Heinz Walz). For all readings, the probe was held at a distance of 0.5 cm from the coral surface, and the optimal quantum yield of photosystem II was derived as $Y = (F_m - F_o)/F_m$ (*see* Genty et al. 1989), where F_o is the fluorescence emitted by the dark-exposed zooxanthellae and F_m is the maximal fluorescence emitted by the zooxanthellae as registered during the application of a 0.8-s period of light that is saturating for photosynthesis (>6,000 μmol photons m^{-2} s^{-1}).

To test whether F_o could be used as a proxy for Chl *a* content on the basis of coral area, three F_o readings were taken on each of three surfaces of spherical heads of *D. labyrinthiformis* that were differentially exposed to light: highest on the top, lower on the sides, and lowest on the bottom of the coral. At the location of each reading, replicate 5-cm-diameter circles of tissue were cut out of the coral. Coral tissue was extracted and spectrophotometric measurements and calculations were made by using established protocols (Moran 1982), and a linear relationship was found between Chl *a* content and F_o values ($R^2 = 0.989$).

Results and discussion—We have now performed three sets of field experiments to assess the effects of crude extracts of nine Caribbean sponge species on one coral, *Diploria labyrinthiformis* (Fig. 1). For each experiment, differences in the effects of gels on F_o and Y were analyzed by a type III sum of squares two-factor ANOVA at $\alpha = 0.05$. This analysis accounted for variation between coral heads with a single set of gels per coral head and detected significant differences in F_o and Y between coral heads in the second experiment (Fig. 1b, $p = 0.004$ and 0.012, respectively) and in F_o in the third experiment (Fig. 1c, $p = 0.013$), indicating that different heads of *D. labyrinthiformis* on this patch reef had different densities of zooxanthellae and photosynthetic quantum efficiencies. PAM readings from coral tissue under control gels were also compared with those taken from tissue immediately adjacent to control gels in order to monitor the effect of the physical presence of control gels against coral tissue, and in no case was there a significant effect of control gels on F_o or Y.

Sponge metabolites in gels placed in contact with coral tissues affected the health of zooxanthellae in two different ways relative to control gels: impaired photosynthesis with bleaching, and impaired photosynthesis with little or no effect on bleaching. Metabolites in the extract of *Agelas clathrodes* caused a significant reduction in both F_o and Y (Fig. 1a), and coral tissues exposed to this treatment were also the most visibly bleached. This sponge contains pyrrole–imidazole alkaloids, including the metabolite oroidin, already known as potent antipredatory and antimicrobial defenses (Chanas et al. 1996; Kelly et al. 2005) that may also play a role in sponge–coral allelopathy. Most of the sponge metabolite treatments exhibited impaired photosynthesis with little or no effect on bleaching. Extracts of five species in particular, *Aka coralliphagum, Cliona langae, Xestospongia muta, Ectyoplasia ferox,* and *Aplysina fulva* (Fig. 1b, c), caused

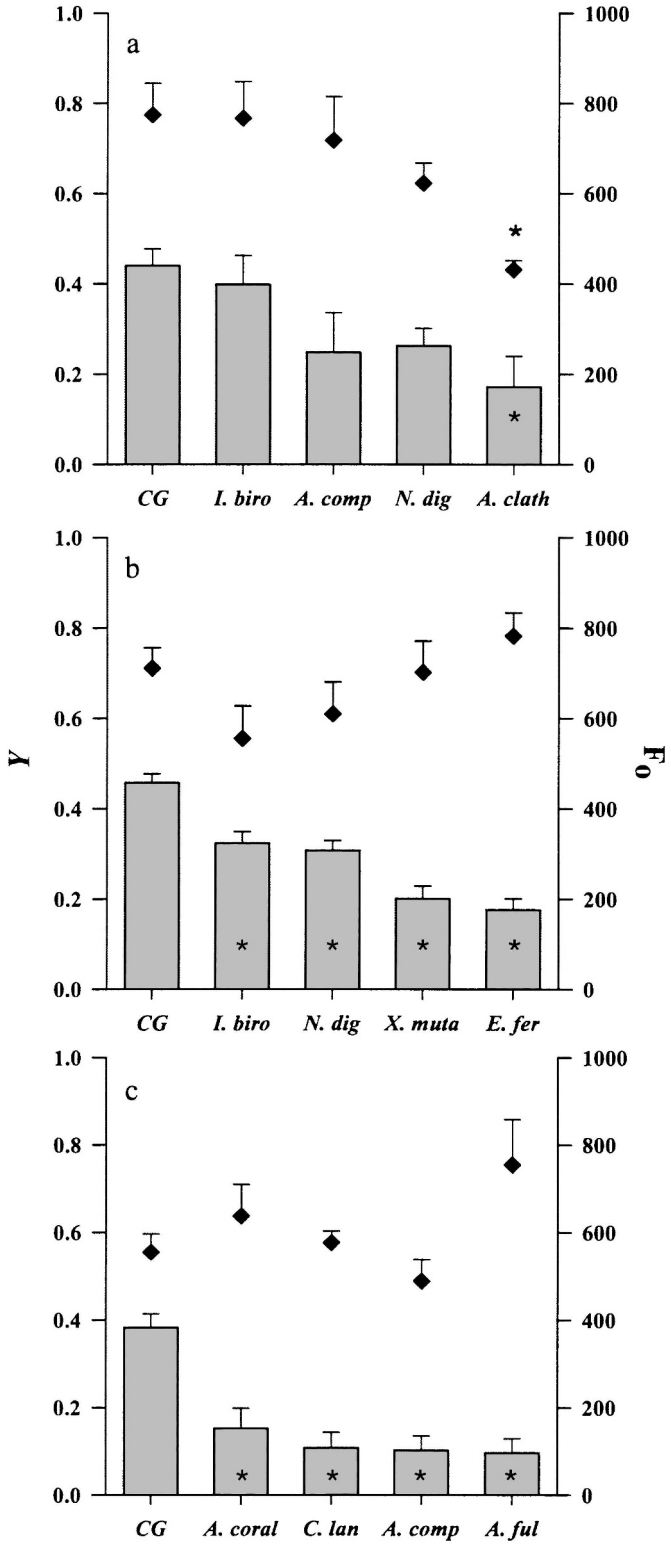

Fig. 1. In situ effects of polysaccharide gels containing natural concentrations of secondary metabolites from Caribbean coral reef sponges on the maximal photosynthetic quantum yield (bars, Y) and ground state fluorescence (diamonds, F_o) of symbiotic algae (zooxanthellae) in the reef coral *Diploria labyrinthiformis* after ~18-h exposure (mean ± 1 SE, $n = 5$ except *I. biro* in (a), $n = 4$). Asterisks indicate treatment means that are significantly different from the control mean (two-factor ANOVA $p < 0.05$, Bonferroni-adjusted multiple comparisons, $p < 0.0125$). Each graph represents different runs of the experiment in which each bar and diamond represents the mean of five replicates on different coral heads. (a) First experiment with Phytagel as gelling agent. (b) Second experiment with carrageenan as gelling agent. (c) Third experiment with carrageenan as gelling agent. Treatments are as follows: CG, control gel; *A. clath,* gel containing extract of *Agelas clathrodes; A. comp, Amphimedon compressa; A. coral, Aka coralliphagum; A. ful, Aplysina fulva; C. lan, Cliona langae; E. fer, Ectyoplasia ferox; I. biro, Iotrochota birotulata; N. dig, Niphates digitalis; X. muta, Xestospongia muta.*

a significant decrease in the photosynthetic potential of the zooxanthellae (Y) without any significant bleaching effect. The first two species in this category are important agents of coral reef bioerosion (Sullivan et al. 1983; Lopez-Victoria et al. 2006). We anticipate using the techniques described herein to isolate and identify the allelopathic metabolites present in different sponge species and to characterize their alternative effects on different species of corals.

Metabolites from one sponge species, *Iotrochota birotulata,* had the least effect on coral tissue relative to controls, with no significant effect in the first experiment (Fig. 1a), and the least significant effect on the photosynthetic potential in the second (Fig. 1b). Interestingly, this sponge species has been demonstrated to lack chemical defenses against predation and overgrowth in other studies (Pawlik et al. 1995; Engel and Pawlik 2000).

Although the overall trends were consistent, treatment differences between the first experiment and the second and third are likely due to differences in experimental protocol. Phytagel was used as the gelling agent for the first experiment, whereas carrageenan was used for both the second and third. Our past experience suggests that Phytagel releases metabolites at a slower rate than carrageenan (*see* release-rate data in Engel and Pawlik 2000); therefore, the significant effects of treatment gels on Y in the second and third experiment probably reflect the faster release of metabolites by carrageenan, resulting in a more intense effect over the same ~18-h experimental period. Despite the slower release rate of Phytagel, the extract of *Agelas clathrodes* had the most intense effect on F_o during the first experiment, providing further evidence of the potency of the metabolites from this sponge species.

In addition to the differential effects sponge metabolites have on the coral-algal symbiosis, this is the first field demonstration of chemical warfare by sponges against corals in which other possible competitive mechanisms (e.g., smothering, mucus production, cellular digestion) have been decoupled from the allelopathic activities of sponge metabolites. Observations on Caribbean reefs indicate that most coral-sponge competitive interactions are static or slow-moving "standoffs" (Aerts 2000), but some sponge species have the ability to overgrow rapidly (Plucer-Rosario 1987), and others opportunistically dominate after coral bleaching events (Aronson et al. 2002). Although the mechanisms of release of allelopathic

metabolites by sponges is not known for most species, the method used in this study makes a more realistic attempt at simulating the overgrowth process (cf. Sullivan et al. 1983). Sponges may elaborate compounds on their surfaces at a more rapid rate than the diffusion rate of metabolites from gels, the rate may be slower, or sponges may not release metabolites at all. Kubanek et al. (2002) determined that surface concentrations of triterpene glycosides were sufficiently high to deter bacterial settlement and fouling of two sponge species, one of which, *Ectyoplasia ferox,* was tested in the present study and was strongly allelopathic (Fig. 1b).

Among Caribbean reef sponges, clear patterns are emerging of chemical defenses against predators, competitors, and pathogens (Pawlik 1997; Engel and Pawlik 2005; Kelly et al. 2005). With the present work, sponge allelopathy can be added to the list of important biotic determinants of coral cover, which include recruitment, predation by fish and invertebrates, and competition with macroalgae, and this further underscores the complexity of coral reef ecosystem function. These patterns provide a new perspective on ecosystem function that may have management implications. The establishment of reef areas protected from fishing and collecting may result in changes in the populations of sponge-eating turtles and fishes (primarily angelfishes and parrotfishes) that have cascading effects on the abundance of sponges that competitively dominate reef building corals through allelopathic interactions.

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