

Overfishing of sponge predators

Nanoparticle delivery of interfering RNA

Transcription-coupled DNA repair

Oxygen requirements of early animals

Ecotourism and poverty reduction





Cover image: Pictured is an orange sponge, *Mycale laevis*, growing over reef-building brain corals off Martinique, where fish trapping has removed most sponge-eating fishes from the ecosystem. Tse-Lynn Loh and Joseph R. Pawlik surveyed sponge and fish communities on Caribbean reefs with different levels of fishing intensity. In overfished areas, sponges normally eaten by fishes grew more abundantly than other sponges with chemical defenses against fish predation. The palatable sponges likely compete for space and resources with reef-building corals. See the article by Loh et al. on pages 4151–4156. Image courtesy of Joseph R. Pawlik.

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Chemical defenses and resource trade-offs structure sponge communities on Caribbean coral reefs

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Ecological studies have rarely been performed at the community level across a large biogeographic region. Sponges are now the primary habitat-forming organisms on Caribbean coral reefs. Recent species-level investigations have demonstrated that predatory fishes (angelfishes and some parrotfishes) differentially graze sponges that lack chemical defenses, while co-occurring, palatable species heal, grow, reproduce, or recruit at faster rates than defended species. Our prediction, based on resource allocation theory, was that predator removal would result in a greater proportion of palatable species in the sponge community on overfished reefs. We tested this prediction by performing surveys of sponge and fish community composition on reefs having different levels of fishing intensity across the Caribbean. A total of 109 sponge species was recorded from 69 sites, with the 10 most common species comprising 51.0% of sponge cover (3.6–7.7% per species). Nonmetric multidimensional scaling indicated that the species composition of sponge communities depended more on the abundance of sponge-eating fishes than geographic location. Across all sites, multiple-regression analyses revealed that spongivore abundance explained 32.8% of the variation in the proportion of palatable sponges, but when data were limited to geographically adjacent locations with strongly contrasting levels of fishing pressure (Cayman Islands and Jamaica; Curaçao, Bonaire, and Martinique), the adjusted R^2 values were much higher (76.5% and 94.6%, respectively). Overfishing of Caribbean coral reefs, particularly by fish trapping, removes sponge predators and is likely to result in greater competition for space between faster-growing palatable sponges and endangered reef-building corals.

chemical ecology | indirect effects | community structure | marine protected areas | trophic dynamics

Caribbean coral reef ecosystems are threatened by several decades of loss of reef-building corals (1). Sponges are now the dominant benthic animals on most reefs, and there is evidence that sponge abundance is increasing (2–5). Predation is important in this ecosystem; spongivores such as angelfishes and parrotfishes selectively feed on chemically undefended sponge species (6). Although sponges are generally thought to feed by capturing particles, they may also absorb dissolved organic material directly from the seawater they pump through their bodies (7, 8). This feeding versatility may help explain why the bottom-up effects of particulate food availability on sponge growth appear to be negligible, suggesting that sponge communities are primarily structured by top-down factors (9). Although many Caribbean sponge species are chemically defended by secondary metabolites that include alkaloids, terpenoids, and glycosides (10), other species are palatable to sponge predators (9, 11), revealing a resource trade-off between the production and maintenance of chemical defenses versus other life functions. Manipulative experiments have demonstrated that sponge species that are palatable to predators recruit more rapidly than defended sponges (12) and have faster rates of wound healing and tissue growth that act in opposition to grazing by predators (10, 13, 14). These traits allow palatable sponges to persist on reefs alongside chemically defended sponges, which produce secondary metabolites that deter predators, but grow, reproduce, or recruit more slowly than species that lack chemical defenses

(10). Hence, sponges on Caribbean coral reefs provide a relatively uncomplicated ecosystem to examine resource allocation theory because food web dynamics are simplified (primarily top-down control), consumption is limited to a few, large, generalist predatory species (little impact of insect-equivalent, often specialist, mesograzers), the impacts of extinction events or invasive species are minimal, and resource trade-offs are distinct (sponge chemical defense vs. growth or reproduction).

Based on the foregoing, a conceptual model was developed for sponge ecology on Caribbean coral reefs (10). If sponge predators were removed from the system, the higher rates of growth or reproduction of palatable sponge species should result in their greater proportional abundance within the sponge community. The coral reefs of the Caribbean provide a large-scale, long-term, manipulative experiment for testing this conceptual model because many reefs, particularly those off Jamaica, Martinique, and Panama, have been heavily overfished for decades with fish traps that indiscriminately remove not only the usual food-fish targets of groupers and snappers, but smaller fish species that include angelfishes and parrotfishes (15). In contrast, reefs such as those off Bonaire, the Cayman Islands, and parts of the Bahamas have long-established and well-enforced marine protected areas (MPAs) with little or no fishing allowed (15). The variability in fishing pressure and fish densities across the Caribbean provides a gradient against which sponge assemblages can be assessed. In this study, we surveyed sponge communities and spongivorous fish populations on overfished and less-fished Caribbean reefs and compared sponge assemblages across all survey sites and at geographically adjacent groups of sites that have strongly contrasting levels of fishing pressure.

Significance

Chemical defenses are known to protect some species from consumers, but it is often difficult to detect this advantage at the community or ecosystem levels because of the complexity of abiotic and biotic factors that influence species abundances. We surveyed the community of sponges and sponge predators (angelfishes and parrotfishes) on coral reefs across the Caribbean ranging from heavily overfished sites to protected marine reserves. High predator abundance correlated with high abundance of chemically defended sponge species, but reefs with few predators were dominated by undefended sponge species, which grow or reproduce faster than defended species. Overfishing may enhance competition between palatable sponge species and reef-building stony corals, further impeding the recovery of Caribbean coral reefs.

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Results

Sponge Community Across Caribbean Reefs. A total of 109 sponge species was recorded from surveys conducted at 69 sites (Fig. 1 and [Datasets S1](#) and [S2](#)). Sponge populations in the Caribbean were not dominated by any particular species, with the 10 most common sponges across all sites each comprising 3.6–7.7% of total sponge cover ([Table S1](#), sponge diversity by site in [Dataset S2](#)). Across all sites, 57.5% of sponges recorded were chemically defended, whereas 23.2% were consistently undefended and 19.4% were variably defended; hence, 42.5% were palatable to sponge predators. Of the 109 sponge species, 67 species were chemically defended, 26 species were consistently undefended, and 16 species were variably defended (42 species were palatable) ([Dataset S2](#)). The most common variably defended sponges were the giant barrel sponge *Xestospongia muta* and the green branched sponge *Iotrochota birotulata*. Sponges with unknown chemical defense strategies comprised less than 1% of the total sponge assemblage. An updated list of feeding assay results for 109 species of Caribbean sponges can be found in [Table S2](#), including categorization of chemically defended, consistently undefended, and variably defended species.

Relationship Between Sponge Community and Predator Abundance.

When the number of occurrences of chemically defended, consistently undefended, and variably defended sponges were pooled by location and the relative percentages determined, chemically defended sponges dominated sponge communities at locations with many spongivores (Fig. 2). For Curaçao and Bonaire especially, chemically defended sponges comprised >90% of sponge cover. Sponge assemblages were markedly different on overfished reefs, where >50% of the sponges surveyed were palatable at most locations. The difference in proportions of chemically defended vs. palatable sponges corresponded with spongivorous fish abundance at most locations, with more fishes recorded at locations that were reportedly protected from fishing (including MPAs) compared with locations classified as overfished (Fig. 2). The abundance and types of spongivores recorded at each site are listed in [Dataset S3](#). Images of angelfishes (*Pomacanthus arcuatus* and *Pomacanthus paru*) consuming the consistently undefended sponge species *Callispongia vaginalis* and

Callispongia armigera on Conch Reef, FL (site F1, [Dataset S1](#)), can be seen in [Movie S1](#).

Multiple-regression analysis revealed that the abundance of spongivorous fishes explained 32.8% of the variation in palatable sponge distribution across all sites ([Table 1](#), all sites). The relationship between spongivorous fishes and palatable sponges for all sites was also significant ($P < 0.001$). From the regression, the expected proportion of palatable sponges when no sponge-eating fishes were present is 0.52 ± 0.04 ($P < 0.001$), or about one-half of the sponge population. Two types of spongivorous fishes had significant impacts on the palatable sponge population ([Table 1](#), all sites). Across all sites, for each angelfish within a seawater volume of 2,000 m³ directly above the reef, the percentage of palatable sponges on the reef was reduced by 1.3% (0.013 ± 0.006 ; $P = 0.041$), and for each individual of *Sparisoma viride* (stoplight parrotfish), the percentage was reduced by 0.8% (0.008 ± 0.006 ; $P = 0.033$).

The effect of spongivorous fishes on the proportion of palatable sponges was more pronounced when regression analyses were repeated on subsets of sites that were geographically adjacent and had strongly contrasting impacts of fishing. For sites from the Cayman Islands and Jamaica, the multiple regression for palatable sponges was significant ($P = 0.025$), with an adjusted- R^2 value of 0.765 ([Table 1](#), sites off Cayman Islands and Jamaica). The analysis for sites from Bonaire, Curaçao, and Martinique was also significant ($P = 0.010$), with an adjusted- R^2 value of 0.946, indicating that spongivorous fish abundance explained almost all of the variation in the proportion of palatable sponges at these locations ([Table 1](#), sites off Bonaire, Curaçao, and Martinique). For both regressions, *Sparisoma aurofrenatum* (redband parrotfish) had a significant effect on sponge distributions ([Table 1](#), sites off Cayman Islands and Jamaica, and sites off Bonaire, Curaçao, and Martinique). For the analysis of sites from the Cayman Islands and Jamaica, the percentage reduction of palatable sponges within the survey area by each *S. aurofrenatum* was 2.3% (0.023 ± 0.007 ; $P = 0.022$), whereas for sites from Curaçao, Bonaire, and Martinique, the percentage was 1.2% (0.012 ± 0.002 ; $P = 0.015$). For the latter analysis, angelfishes also had a significant effect, with each



Fig. 1. Map of sites across the Caribbean where surveys were performed of sponge and fish community composition. Shading of site markers indicates abundance of sponge-eating fishes based on survey data using spongivore index (SI) ([Dataset S3](#)). Black, overfished ($SI < 10$); gray, intermediate ($11 \leq SI \leq 20$); white, protected ($SI \geq 21$). Overlapping markers have been displaced for clarity.

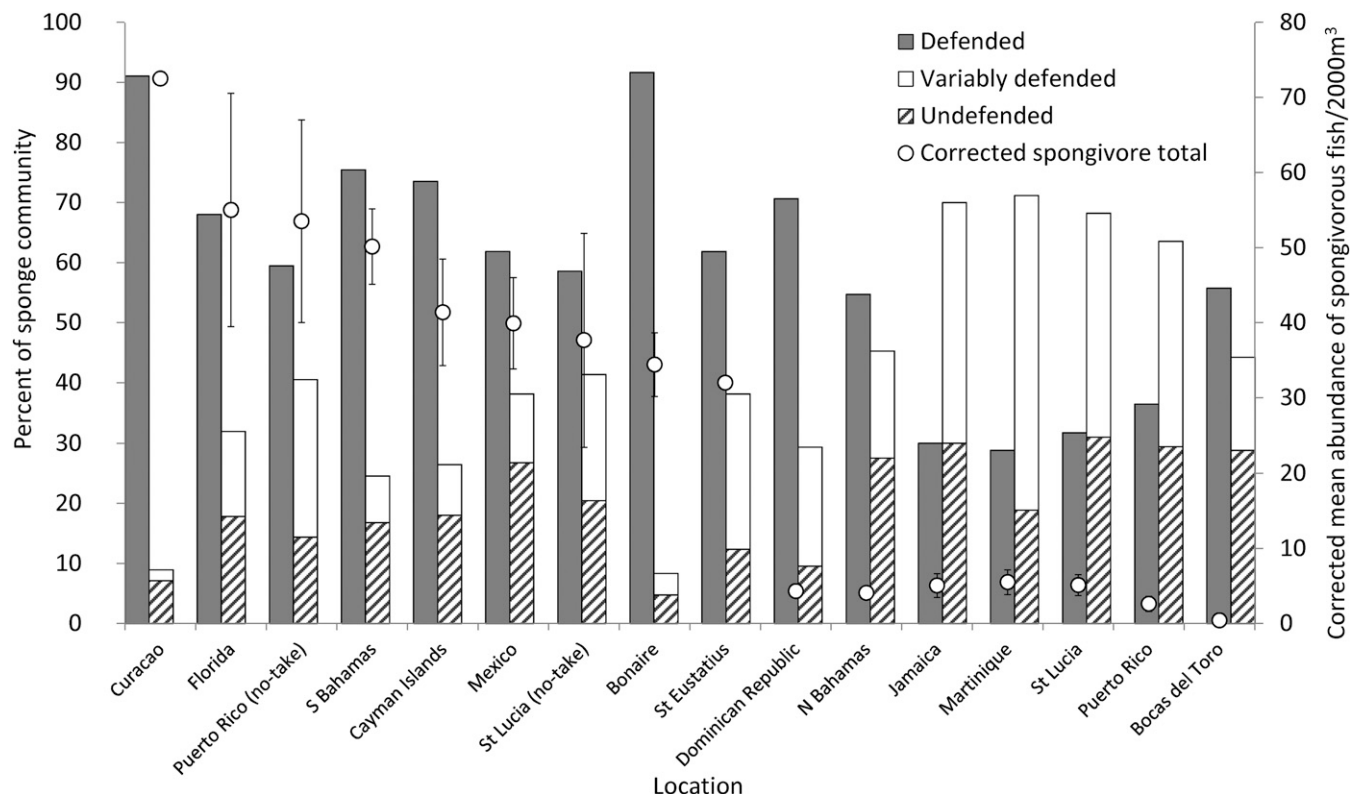


Table 1. Output of multiple-regression analyses with the proportion of palatable sponges per site as the response variable and the corrected abundance of spongivorous fishes within 2,000 m³ of seawater along the reef transect as factors

Multiple regression output	Coefficient	SE	P value
Palatable sponges, all sites			
Adjusted R ²	0.328		
Regression ANOVA	P < 0.001*		
Intercept*	0.523	0.035	<0.001
Filefishes	0.010	0.023	0.659
Trunkfishes	0.002	0.009	0.853
Redband parrotfish	−0.003	0.002	0.087
Redtail parrotfish	−0.004	0.008	0.642
Stoplight parrotfish*	−0.008	0.004	0.033
Angelfishes*	−0.013	0.006	0.041
Palatable sponges, Cayman Islands and Jamaica			
Adjusted R ²	0.765		
Regression ANOVA	P = 0.025*		
Intercept*	0.731	0.076	<0.001
Filefishes	−0.018	0.024	0.490
Trunkfishes	−0.196	0.103	0.114
Redband parrotfish*	−0.023	0.007	0.022
Redtail parrotfish	−0.031	0.016	0.099
Stoplight parrotfish	0.035	0.018	0.109
Angelfishes	−0.032	0.022	0.202
Palatable sponges, Bonaire, Curaçao, and Martinique			
Adjusted R ²	0.946		
Regression ANOVA	P = 0.010*		
Intercept*	0.728	0.067	0.002
Filefishes	0.019	0.041	0.664
Trunkfishes	−0.013	0.014	0.440
Redband parrotfish*	−0.012	0.002	0.015
Redtail parrotfish	0.088	0.039	0.112
Stoplight parrotfish	0.002	0.003	0.665
Angelfishes*	−0.086	0.015	0.010

Shown are separate analyses of all sites, sites off Cayman Islands and Jamaica, and sites off Bonaire, Curaçao, and Martinique.

*Denotes factors that are significant at $P < 0.05$.

undefended sponges or individuals grew in refuge locations, such as in close proximity to, or among, fire corals, gorgonians, chemically defended sponges (Movie S1), or between branches or plates of stony corals, with the last tactic particularly used by the common and chemically undefended sponge *Mycale laevis* (Table S1) (22). These direct observations of predation, or of undefended sponges in refuge locations, are not possible to represent in our data, which are limited to relative abundance as a proportion of benthic cover. From our analyses, spongivorous fish also had an effect on variably defended sponges, presumably on individuals within the population that were less well defended.

Most of the sponges on the reefs we surveyed were protected from predators by secondary metabolites, with over 60% of species chemically defended and over 57% of the sponge community across all sites chemically defended. Because sponges of the genera *Aplysina* and *Aiolochoira* are the most abundant sponges among defended species (Table S1), brominated tyrosine-derived alkaloids are the most common defensive secondary metabolites among Caribbean reef sponges (23, 24). Furthermore, with *Aplysina cauliformis* representing 7.7% of the sponge community across all sites (Table S1), the metabolite fistularin 3 is likely the most abundant defensive metabolite among Caribbean sponges (Fig. 4). For tissue samples of *A. cauliformis* from the Florida Keys and Bahamas Islands, fistularin 3 occurred at 2.4 mg/mL of sponge volume (SD = 1.1, $n = 59$) and, unlike

other *Aplysina* species, had very low levels of other related metabolites in its tissues (24, 25). Other *Aplysina* species, including *Aplysina fulva*, which ranked sixth most abundant across all sites, also had fistularin 3 as a dominant chemical defense (24).

Variably defended sponge species made up the smallest category defined by our feeding assay data (16 of 109 species, or 14.7%; Dataset S2), and species within this category have been the subject of more extensive studies in the past. For example, intrapopulation variation in the chemical defenses of the giant barrel sponge *Xestospongia muta* is very high (26), with differential grazing by parrotfishes on some individuals within a population over others (27). Similarly, the chicken-liver sponge *Chondrilla caribensis* (formerly *Chondrilla nucula*) exhibited highly variable chemical defenses, and undefended individuals were rapidly eaten by angelfishes (28). Finally, individuals of *Iotrochota birotulata* were variably defended in our feeding assays (Table S2), yet this species is a preferred food item for the angelfish *Holacanthus tricolor* (29) and exhibited the rapid growth rates associated with other chemically undefended sponge species (9, 14). Although currently the subject of ongoing research, we suspect that variably defended sponge species recruit into the population with different levels of chemical defenses [a constitutive trait or consequence of different microbial symbionts rather than through induction of defense (28)] and that subsequent predation affects sponge survival. Moreover, we speculate that some undefended individuals may persist within a population despite predation due to a form of automimicry [defended and undefended individuals look alike to predators that learn to avoid chemical defenses using visual cues (30)]. Both *X. muta* and *I. birotulata* were among the 10 most abundant sponge species in our surveys (Table S1), and their common presence in the sponge community likely decreased the percentage of variation explained by the presence of spongivores on undefended sponge species in multiple-regression analyses. These variably defended species would be expected to increase in abundance in the absence of predation, but not necessarily decrease in abundance if predation pressure is high. Therefore, considering the confounding effects of variably defended species, the propensity of consistently undefended species to persist in refuge locations despite high predator abundances, and location-specific differences in fishing pressure, it is remarkable that

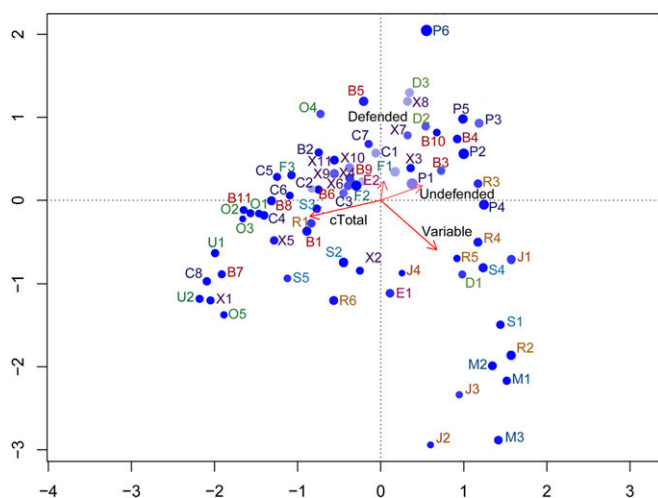


Fig. 3. Correspondence analysis of survey sites for occurrences of chemically defended, consistently undefended, and variably defended sponges, and corrected total abundance of sponge predators within 2,000 m³ of seawater directly above the reef. Prefixes of site names denote the following locations: B, Bahamas; C, Cayman Islands; D, Dominican Republic; E, St. Eustatius; F, Key Largo, FL; J, Jamaica; M, Martinique; O, Bonaire; P, Bocas del Toro, Panama; R, Puerto Rico; S, St. Lucia; U, Curaçao; X, Mexico.



All other analyses used data from all sponge species present at each site. The number of occurrences of chemically defended, consistently undefended, and variably defended sponges within the sponge assemblage were calculated for each site, and plotted in a CA with the corrected total number of sponge predators (both fishes and seastars) recorded in 2,000 m³ of seawater directly above the reef. Parrotfishes were significantly smaller in overfished areas (31), and to correct for fish size at overfished sites where fishes were small (<20 cm TL) and fish traps were observed, parrotfish abundance was divided by 10. This correction was considered highly conservative, as parrotfishes in these areas were often <10 cm in total length (TL) (the size that allowed them to escape fish traps). Examples of calculations from the literature include equivalent grazing estimates of one large (>25 cm TL) to 24 small (5–10 cm TL) parrotfishes (38), and one large (35 cm) to 75 small (15 cm) parrotfishes (39).

To investigate the relationship between the abundance of spongiivorous fishes and sponge community structure, multiple-regression analyses were performed separately for the proportion of consistently undefended sponges and the proportion of palatable species (consistently undefended and variably defended species) within the sponge community at each site as the response variables, and the following sponge predator groups as factors: angelfishes (*Holacanthus* and *Pomacanthus* spp.), redband parrotfish (*Sparisoma aurofrenatum*), stoplight parrotfish (*S. viride*), redtail parrotfish (*Sparisoma chrysotermum*), trunkfish (*Lactophrys* and *Acanthostracion* spp.), and filefish (*Cantherhines* spp.). Numbers of parrotfishes were corrected for body size as previously described. Multiple regressions were also conducted on subsets of data from latitudinally similar regions with contrasting and long-term levels of fishing pressure (subset 1: Cayman Islands

and Jamaica; subset 2: Bonaire, Curaçao, and Martinique). All multivariate analyses were carried out in the program R using the *vegan* and *ca* packages (40, 41).

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Supporting Information

Loh and Pawlik 10.1073/pnas.1321626111

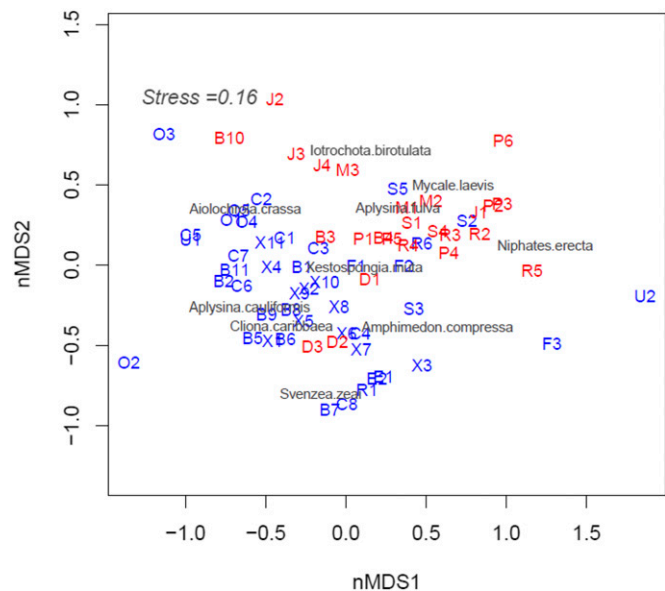


Fig. S1. Two-dimensional nonmetric multidimensional scaling (nMDS) ordination of survey sites by occurrences of the 10 most common sponge species (stress = 0.16). Sites designated as overfished are in red, and sites that were less fished are in blue, based on data in ref. 1. Prefixes of site names denote the following locations: B, Bahamas; C, Cayman Islands; D, Dominican Republic; E, St. Eustatius; F, Key Largo, FL; J, Jamaica; M, Martinique; O, Bonaire; P, Bocas del Toro, Panama; R, Puerto Rico; S, St. Lucia; U, Curaçao; X, Mexico.

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Table S1. The 10 most common sponge species from combined data for 69 surveys conducted on coral reefs across the Caribbean

Species	Order	% of sponge assemblage	Chemical defense
<i>Aplysina cauliformis</i>	Verongida	7.7	Defended
<i>Xestospongia muta</i>	Haplosclerida	6.2	Variable
<i>Niphates erecta</i>	Haplosclerida	5.8	Undefended
<i>Amphimedon compressa</i>	Haplosclerida	5.7	Defended
<i>Iotrochota birotulata</i>	Poecilosclerida	5.3	Variable
<i>Aplysina fulva</i>	Verongida	4.4	Defended
<i>Mycale laevis</i>	Poecilosclerida	4.3	Undefended
<i>Cliona caribbaea</i>	Hadromerida	4.1	Undefended
<i>Svenzea zeai</i>	Halichondrida	3.8	Defended
<i>Aiolochroia crassa</i>	Verongida	3.6	Defended

Table S2. Caribbean sponge species categorized as chemically defended (D), consistently undefended (U), or variably defended (V)

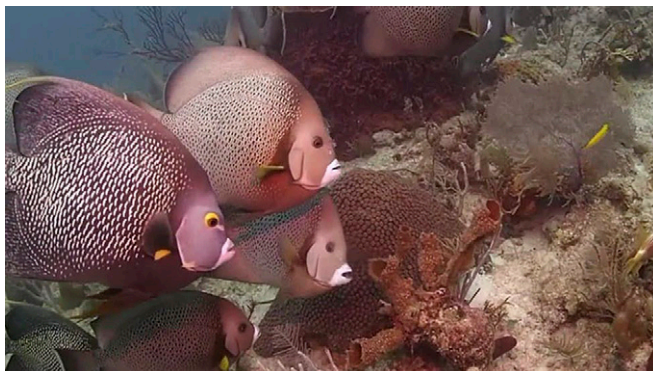
Species (former synonym)	Chemical defense, mean \pm SE	Replicates	Treatment pellets eaten/10	Reference
<i>Agelas clathrodes</i>	D, 0 \pm 0	5	0, 0, 0, 0, 0	Ref. 1
<i>Agelas conifera</i>	D, 0.20 \pm 0.20	5	0, 0, 1, 0, 0	Ref. 1
<i>Agelas dispar</i>	D, 0.33 \pm 0.33	3	0, 1, 0	Ref. 1
<i>Agelas inequalis</i>	D, 0 \pm 0	3	0, 0, 0	Ref. 1
<i>Agelas sceptrum</i>	D, 0 \pm 0	3	0, 0, 0	Ref. 1
<i>Agelas sventres</i>	D, 1	1	1	This study
<i>Agelas wiedenmayeri</i>	D, 0 \pm 0	3	0, 0, 0	Ref. 1
<i>Aiolochoia crassa</i> (<i>Pseudoceratina crassa</i>)	D, 0.50 \pm 0.50	4	2, 0, 0, 0	Ref. 1
<i>Amphimedon compressa</i>	D, 0.45 \pm 0.25	11	0, 0, 0, 0, 0, 0, 0, 2, 2, 1	Ref. 1
<i>Amphimedon complanata</i>	D, 2.50 \pm 0.50	2	2, 3	This study
<i>Amphimedon erina</i>	D, 0.67 \pm 0.33	3	0, 1, 1	Ref. 1
<i>Aplysina archeri</i>	D, 0.33 \pm 0.33	3	1, 0, 0	Ref. 1
<i>Aplysina cauliformis</i>	D, 0.20 \pm 0.20	5	0, 0, 0, 0, 1	Ref. 1
<i>Aplysina fistularis</i>	D, 0.67 \pm 0.33	3	0, 1, 1	Ref. 1
<i>Aplysina fulva</i>	D, 0 \pm 0	4	0, 0, 0, 0	Ref. 1
<i>Aplysina lacunosa</i>	D, 0.33 \pm 0.33	3	0, 0, 1	Ref. 1
<i>Axinella corrugata</i> (<i>Teichaxinella morchellum</i>)	D, 0 \pm 0	3	0, 0, 0	Ref. 1
<i>Bienma</i> sp.	D, 5.00 \pm 0.58	3	4, 6, 5	This study
<i>Biemna tubulata</i>	U, 8.17 \pm 1.28	6	2, 10, 9, 8, 10, 10	Ref. 1; this study
<i>Callyspongia fallax</i>	U, 9.33 \pm 0.67	3	10, 8, 10	Ref. 1
<i>Callyspongia plicifera</i>	U, 7.67 \pm 1.38	6	1, 8, 8, 9, 10, 10	Ref. 1
<i>Callyspongia tenerima</i>	U, 9.00 \pm 1.00	2	8, 10	This study
<i>Callyspongia vaginalis</i>	U, 9.25 \pm 0.37	8	7, 9, 9, 9, 10, 10, 10, 10	Ref. 1
<i>Chondrilla caribensis</i> (<i>C. nucula</i>)	V	247	(See refs.)	Refs. 1 and 2
<i>Chondrosia collectrix</i>	U, 8.00 \pm 0	3	8, 8, 8	Ref. 1
<i>Chondrosia reniformis</i>	U, 9.33 \pm 0.67	3	10, 8, 10	Ref. 1
<i>Cinanchyrella kukenthalli</i> (<i>C. alloclada</i>)	V, 6.57 \pm 0.72	7	7, 5, 8, 4, 5, 9, 8	Ref. 1
<i>Clathria schoenus</i>	U, 7	1	7	This study
<i>Clathria venosa</i>	D, 3.67 \pm 2.03	3	7, 4, 0	This study
<i>Clathria virgulitosa</i> (<i>Ptilocaulis spiculifera</i>)	D, 0.88 \pm 0.52	8	4, 1, 2, 0, 0, 0, 0, 0	Ref. 1; this study
<i>Cliona delitrix</i>	U, 7.67 \pm 1.20	3	7, 10, 6	This study
<i>Cliona varians</i> (<i>Anthosigmella varians</i>)	U, 7.00 \pm 0.68	6	6, 6, 9, 9, 7, 5	Ref. 1; this study
<i>Cribochalina vasculum</i>	V, 6.50 \pm 1.55	4	2, 7, 8, 9	Ref. 1
<i>Desmapsamma anchorata</i> (<i>Holopsamma helwigi</i>)	V, 7.00 \pm 1.00	3	8, 8, 5	Ref. 1
<i>Dictyonella funicularis</i>	V, 5.33 \pm 1.45	3	5, 3, 8	This study
<i>Diplastrella megastellata</i>	D, 0 \pm 0	3	0, 0, 0	Ref. 1
<i>Drumacidon reticulata</i> (<i>Pseudaxinella lunaecharta</i>)	V, 3.67 \pm 2.73	3	0, 2, 9	Ref. 1
<i>Dysidea etheria</i>	D, 2.33 \pm 0.88	3	1, 4, 2	Ref. 1
<i>Dysidea janiae</i>	U, 8.00 \pm 1.00	3	9, 9, 6	Ref. 1
<i>Ectyoplasia ferox</i>	D, 0.17 \pm 0.17	6	0, 0, 0, 0, 0, 1	Ref. 1
<i>Erylus formosus</i>	D, 0.33 \pm 0.33	3	0, 1, 0	Ref. 1
<i>Geodia gibberosa</i>	U, 9.33 \pm 0.33	3	9, 9, 10	Ref. 1
<i>Geodia neptuni</i>	U, 9.67 \pm 0.33	3	10, 9, 10	Ref. 1
<i>Halichondria melanodocia</i>	D, 1.25 \pm 0.63	4	0, 1, 1, 3	Ref. 1
<i>Haliclona hogarthi</i>	V, 7.00 \pm 1.53	3	10, 5, 6	Ref. 1
<i>Haliclona vansoesti</i>	U, 8.00 \pm 1.00	3	7, 7, 10	This study
<i>Haliclona vermeuleni</i>	U, 8.00 \pm 1.00	3	7, 10, 7	This study
<i>Haliclona walentinae</i>	D, 1.00 \pm 0.58	3	0, 2, 1	This study
<i>Halisarca caerulea</i>	V, 6.00 \pm 3.00	2	9, 3	This study
<i>Hippospongia lachne</i>	U, 7.33 \pm 1.20	3	5, 8, 9	Ref. 1
<i>Hyrtios cavernosus</i>	D, 0	1	0	This study
<i>Igernella notabilis</i>	V, 7.33 \pm 2.67	3	2, 10, 10	This study
<i>Iotrochota arenosa</i>	V, 6.25 \pm 1.80	4	1, 8, 9, 7	This study
<i>Iotrochota birotulata</i>	V, 7.14 \pm 1.16	7	3, 4, 5, 8, 10, 10, 10	Ref. 1
<i>Ircinia campana</i>	D, 2.0 \pm 1.0	3	1, 2, 3	Ref. 3
<i>Ircinia strobilina</i>	D, 0 \pm 0	4	0, 0, 0, 0	Ref. 1
<i>Lissodendoryx colombiensis</i>	U, 8.33 \pm 0.67	3	9, 7, 9	This study
<i>Lissodendoryx isodictyalis</i>	D, 3.00 \pm 3.00	3	9, 0, 0	Ref. 1

Table S2. Cont.

Species (former synonym)	Chemical defense, mean \pm SE	Replicates	Treatment pellets eaten/10	Reference
<i>Lissodendoryx sigmata</i>	D, 2.50 \pm 0.50	2	3, 2	Ref. 1
<i>Microcionia ?prolifera</i>	U, 9.33 \pm 0.67	3	10, 10, 8	This study
<i>Monanchora arbuscula</i> (<i>Rhaphidophilus venosus</i>)	D, 0.75 \pm 0.41	8	0, 0, 0, 0, 2, 1, 3, 0	Ref. 1; this study
<i>Mycale laevis</i>	U, 8.40 \pm 0.60	5	9, 10, 9, 7, 7	Ref. 1
<i>Mycale laxissima</i>	D, 0.75 \pm 0.48	4	0, 0, 2, 1	Ref. 1
<i>Myrmekioderma gyroderma</i> (<i>M. styx</i>)	D, 2.00 \pm 0.58	3	1, 3, 2	Ref. 1
<i>Neofibularia nolitangere</i>	D, 2.83 \pm 0.95	6	6, 5, 3, 2, 1, 0	Ref. 1
<i>Neopetrosia carbonaria</i>	D, 3.67 \pm 0.33	3	3, 4, 4	This study
<i>Neopetrosia proxima</i>	U, 9.00 \pm 0.58	3	8, 9, 10	This study
<i>Neopetrosia rosariensis</i>	U, 6.67 \pm 0.33	3	7, 7, 6	This study
<i>Neopetrosia subtriangularis</i>	U, 7.50 \pm 0.50	2	7, 8	This study
<i>Niphates digitalis</i>	V, 7.00 \pm 2.46	4	1, 1, 9, 10	Ref. 1
<i>Niphates erecta</i>	U, 8.71 \pm 0.52	7	7, 7, 8, 9, 10, 10, 10	Ref. 1
<i>Oceanapia bartschi</i>	D, 2.67 \pm 2.19	3	0, 1, 7	This study
<i>Pandaros acanthifolium</i>	D, 0.67 \pm 0.67	3	0, 2, 0	Ref. 1
<i>Phorbas amaranthus</i>	D, 0.75 \pm 0.75	4	0, 0, 3, 0	Ref. 1
<i>Placospongia intermedia</i>	V, 7.67 \pm 1.86	3	9, 10, 4	This study
<i>Placospongia melobesioides</i>	U, 8.67 \pm 0.33	3	8, 9, 9	Ref. 1
<i>Plakinastrella onkodes</i>	D, 2.67 \pm 1.76	3	2, 0, 6	This study
<i>Plakortis angulospiculatus</i>	D, 0	1	0	Ref. 1
<i>Plakortis halichondroides</i>	D, 1.57 \pm 0.65	7	0, 0, 1, 1, 2, 2, 5	Ref. 1
<i>Plakortis lita</i>	D, 0 \pm 0	3	0, 0, 0	Ref. 1
<i>Polymastia tenax</i>	V, 5.67 \pm 1.20	3	5, 4, 8	This study
<i>Prosuberities laughlini</i>	D, 0.50 \pm 0.50	2	0, 1	This study
<i>Pseudocorticium</i> sp.	D, 0	1	0	This study
<i>Ptilocaulis walpersi</i>	D, 2.00 \pm 1.14	5	0, 0, 1, 3, 6	Ref. 1
<i>Rhaphidophilus juniperinus</i>	V, 4.50 \pm 2.32	4	1, 0, 8, 9	Ref. 1
<i>Scopalina ruetzleri</i> (<i>Ulosa ruetzleri</i>)	D, 2.75 \pm 1.18	4	1, 6, 3, 1	Ref. 1
<i>Siphonodictyon coralliphagum</i>	D, 0.33 \pm 0.33	3	0, 1, 0	Ref. 1
<i>Smenospongia aurea</i>	D, 0.25 \pm 0.25	4	1, 0, 0, 0	Ref. 1
<i>Smenospongia conulosa</i>	D, 0.67 \pm 0.33	3	0, 1, 1	This study
<i>Spirastrella coccinea</i>	D, 3.50 \pm 0.50	2	3, 4	Ref. 1
<i>Spirastrella hartmani</i>	U, 10	1	10	This study
<i>Spongia obliqua</i>	U, 8.33 \pm 0.88	3	10, 8, 7	Ref. 1
<i>Spheciospongia vesparium</i> (<i>S. othella</i>)	V, 6.70 \pm 1.16	10	0, 1, 5, 7, 7, 8, 9, 10, 10, 10	Ref. 1
<i>Stelletta kallitetilla</i>	V, 6.67 \pm 0.67	3	8, 6, 6	Ref. 1
<i>Strongylacidon griseum</i>	U, 8.50 \pm 1.50	2	10, 7	This study
<i>Strongylacidon</i> sp.	D, 1.00 \pm 0.44	7	2, 0, 0, 1, 0, 1, 3	Ref. 1; this study
<i>Svenzea christinae</i>	D, 0 \pm 0	3	0, 0, 0	This study
<i>Svenzea flava</i>	V, 4.33 \pm 2.60	3	4, 0, 9	This study
<i>Svenzea</i> sp.	D, 0	1	0	This study
<i>Svenzea tubulosa</i>	D, 0 \pm 0	2	0, 0	This study
<i>Svenzea zeai</i> (<i>Calyx podotypa</i>)	D, 4.67 \pm 1.20	3	4, 3, 7	Ref. 1
<i>Tedania ignis</i>	V, 6.00 \pm 1.53	3	5, 4, 9	Ref. 1
<i>Tethya actinia</i>	D, 0.33 \pm 0.33	3	0, 1, 0	Ref. 1
<i>Topsentia ophiraphidites</i>	U, 8.67 \pm 0.67	3	8, 8, 10	This study
<i>Verongula gigantea</i>	D, 0.25 \pm 0.25	4	0, 0, 0, 1	Ref. 1
<i>Verongula rigida</i>	D, 0 \pm 0	3	0, 0, 0	Ref. 1
<i>Verongula reiswigi</i>	D, 2.00 \pm 0.71	4	3, 0, 3, 2	This study
<i>Xestospongia bocatorensis</i>	U, 8.67 \pm 0.33	3	9, 8, 9	This study
<i>Xestospongia muta</i>	V	60	(See refs.)	Refs. 1 and 4

Defense categories based on the results of standardized laboratory feeding assays using the bluehead wrasse (*Thalassoma bifasciatum*) (1). For each replicate assay, a control (solvent-treated) pellet was offered to a set of three fish, followed by a pellet treated with the organic extract of the sponge sample at a volumetrically equivalent concentration as the sponge tissue itself (treatment pellet), followed by a control pellet to confirm that fish were still feeding. The assay was only considered valid if both control pellets were eaten, and was repeated until 10 independent groups of fish were assayed. Replicate assays were performed with extracts from sponge tissue sampled from geographically distant locations (>1 km). For each sponge species, replicate data consisted of the number of treatment pellets eaten in each assay, ranging from 0 to 10. A modified version of Fisher's exact test, in which the marginal totals for control and treatment pellets were fixed, treating them both as random samples, provided a probability value of 0.057 when seven pellets were eaten; hence, any replicate sponge extract was considered deterrent if six or fewer pellets were eaten, and palatable if seven or more pellets were eaten. To determine the relative palatability of each species, the mean number of pellets eaten and SE were calculated across all samples. The species was chemically defended (D) if $\text{mean} + \text{SE} \leq 6$, consistently undefended (U) if $\text{mean} - \text{SE} > 6$, and variably defended (V) if $\text{mean} - \text{SE} \leq 6 < \text{mean} + \text{SE}$.

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2. Swearingen DC, Pawlik JR (1998) Variability in the chemical defense of the sponge *Chondrilla nucula* against predatory reef fish. *Mar Biol* 131:619–627.
3. Pawlik JR, McFall G, Zea S (2002) Does the odor from sponges of the genus *Ircinia* protect them from fish predators? *J Chem Ecol* 28(6):1103–1115.
4. Chanas B, Pawlik JR, Lessios HA, Macintyre IG, eds (1997) Variability in the chemical defense of the Caribbean reef sponge *Xestospongia muta*. *Proceedings of the 8th International Coral Reef Symposium, Panama, June 24–29, 1996*, eds Lessios HA, Macintyre IG (Smithsonian Tropical Research Institute, Balboa, Panama), pp 1363–1367.



Movie S1. Predation on the gray tube sponge *Callyspongia vaginalis* and the gray branching sponge *Callyspongia armigera* by Gray and French Angelfishes (*Pomacanthus arcuatus* and *Pomacanthus paru*). Note in the second movie sequence that the gray branching sponge is intertwined in fire coral, where it likely gains a refuge by association. The movie was captured on July 1, 2013, Conch Reef, Pinnacle, offshore of Key Largo, FL, at 20-m depth. The movie is by J.R.P.

[Movie S1](#)

Other Supporting Information Files

[Dataset S1 \(XLSX\)](#)

[Dataset S2 \(XLSX\)](#)

[Dataset S3 \(XLSX\)](#)