



No accounting for taste: Palatability of variably defended Caribbean sponge species is unrelated to predator abundance



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ABSTRACT

The antipredatory chemical ecology of Caribbean reef sponges has been well studied, with two distinct ecological strategies described: chemically defended species use secondary metabolites to deter fish predators, while palatable species lack chemical defenses, but grow, reproduce, or recruit fast enough to persist despite predation. While most palatable sponge species are consistently undefended from fish predators in laboratory feeding assays, there are some species that exhibit individual inter- and intra-site variation ranging from palatable to defended. The objective of this study was to better document the extent of chemical defense variability for 7 of these species over a wider geographic range and to test the dual hypotheses that variably defended sponge species would be (1) more palatable, and (2) exhibit greater variability in chemical defenses from reefs without sponge predators than from reefs where predators are abundant. Laboratory feeding assays using the bluehead wrasse confirmed intraspecific variability of chemical defenses for *Clathria virgultosa*, *Cribrochalina vasculum*, *Desmapsamma anchorata*, *Dragnacidon reticulata*, *Iotrochota birotulata*, *Niphates digitalis*, and *Xestospongia muta* for sites across the Caribbean. Surprisingly, logistic regression models relating levels of sponge chemical defense to a site-specific index of spongivorous fish abundance offered little support for the hypothesis that predator abundance alters palatability for any of these variably defended sponge species. The presence of symbiotic zoanthids in the tissue of *I. birotulata* and *N. digitalis* did not alter palatability. While the selective effect of predation has segregated most species of Caribbean reef sponges into chemically defended and undefended categories, this study concludes that the situation is more complex for variably defended sponge species, and suggests that automimicry is a possible explanation for this alternative strategy.

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1. Introduction

Predation is an important process that mediates trait selection, causing particular traits to become more common through non-random selective mortality (Pettorelli et al., 2011; Relyea, 2002). Predation pressure drives prey species to develop diverse strategies of survival, ranging from behavioral responses (Lima and Dill, 1990) to physical (e.g. Gowda, 1996; Harvell and Fenical, 1989) and chemical defenses. Many sessile and soft-bodied organisms that are vulnerable to predation employ chemical defenses by maintaining high concentrations of deterrent secondary metabolites in their tissues (see reviews by Levin, 1976; Paul et al., 2007; Pawlik, 1993, 2012; Pohnert, 2004). The synthesis and storage chemical defenses require resources that could be used for other life processes such as reproduction or growth, and trade-offs should exist to offset the cost of defense (Lerdau and Gershenson, 1997; Pawlik, 2011, 2012). Trade-offs between growth and defense have been documented in terrestrial plants (Fine et al. 2006; Herms &

Mattson 1992) and coral reef sponges (Leong and Pawlik, 2010), and the selective pressure of predation is considered responsible for the divergence of these two strategies.

The results of the selective process of predation are particularly evident in the Caribbean sponge fauna. In an early survey of Caribbean sponges, Pawlik et al. (1995) found clear distinctions in chemical defense strategies: some species were defended against generalist predators and others were consistently undefended. Subsequent research demonstrated that Caribbean sponges exhibit resource trade-offs that reveal the cost of chemical defenses (Leong and Pawlik, 2010; Pawlik et al., 2008; Walters and Pawlik, 2005), and empirical tests supported a conceptual model of the effect of predation on the structure the sponge community on Caribbean reefs (Loh and Pawlik, 2014; Loh et al., 2015; Pawlik, 2011; Pawlik et al., 2013). The defense status of over 100 species of Caribbean sponges has been categorized (Loh and Pawlik, 2014). While the majority of species found on Caribbean reefs were either chemically defended or consistently palatable in laboratory feeding assays, a small subset of ~20 species exhibited intraspecific variability in chemical defenses, in that the results of laboratory fish-feeding assays conducted on individual sponges within or between sites

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ranged from palatable to defended (Loh and Pawlik, 2014). While some of these variably defended species are rare, two are among the 5 most common sponges on Caribbean reefs (*Xestospongia muta*, *Iotrochota birotulata*) and represent an important part of the benthic community (Loh and Pawlik, 2014).

In general, variability in phenotypes improves survivorship (Johnson et al., 2014) because some phenotypes will be more adaptive than others. However, the magnitude of phenotypic variability should conform to selective constraints; predator-mediated post-settlement mortality of marine invertebrates is a significant process (Gosselin and Qian, 1997), and among the sponge species that are variably defended it would be expected that only the individuals with more potent chemical defenses should survive post-settlement predation on reefs where predators are abundant (Loh and Pawlik, 2014). More in-depth previous studies confirmed that chemical defenses were variable for two species: *Xestospongia muta* (Chanas and Pawlik, 1997) and *Chondrilla caribensis* (Swearingen and Pawlik, 1998) with much greater replication than the original survey (Pawlik et al., 1995). Both of these studies demonstrated significant differences in chemical defense across sites, with crude extracts of tissue from specimens collected from Florida scoring as more palatable in laboratory fish-feeding assays than those from the Bahamas. This variation was attributed to differences in predator abundance among sites (Chanas and Pawlik, 1997).

Intraspecific differences in chemical defenses have been observed in terrestrial plants, however much of the work reported in the terrestrial literature has aimed to test optimal defense theory with a focus on intra-individual variation as opposed to variation among individuals (e.g. Agrawal, 2011; McCall and Fordyce, 2010). Variation of chemical defenses among individuals has been documented in other benthic marine organisms including octocorals and seaweeds, and it has been suggested that patterns of variation may be driven by ecological factors such as competition and predation (Maida et al., 1993; Paul et al., 2001). For example, colonies of the soft coral *Sinularia flexibilis* were shown to vary in secondary metabolite profiles for antifouling and allelopathic compounds at reef sites on the Great Barrier Reef with different degrees of benthic competition (Maida et al., 1993). In another case, Paul and Fenical (1986) quantified variation of secondary metabolites among geographically distinct populations of five species of green algae and found different metabolites, generally higher concentrations of metabolites, and generally greater mixtures of metabolites in seaweeds collected on shallow reefs, where rates of herbivory were presumed to be greater than adjacent seagrass beds and deep reefs. In order to test the hypothesis that local differences in herbivory drive patterns of intraspecific variation of algal chemical defenses, Paul and Van Alstyne (1988) collected three species of the green alga *Halimeda* from reef slopes (where herbivorous fishes were reported to be abundant) and reef flats (where herbivores were rare) in Guam, then measured the major metabolites using thin layer chromatography and tested the crude extracts for deterrent activity in field assays. The results were mixed; differences were detected among geographically separate collection sites and also between reef slope and flat habitats, but differences were not consistently significant and low replication combined with unbalanced statistical design confounded the analysis (Paul and Van Alstyne, 1988). Nevertheless, Paul and Van Alstyne (1988) concluded that herbivory has been a strong selective force in the evolution of these algae and that chemical defenses were likely greater where the pressure of herbivory was greater.

The present study was predicated on the idea that predation may be responsible for patterns in the variability of chemical defenses within species of Caribbean sponges. Two predator-mediated processes, post-settlement mortality of palatable individuals and the induction of chemical defenses within individuals that experience episodes of predation, both could lead to patterns of variability in chemical defenses. In the former case, palatable individuals recruit onto the reef and survive at sites where predators have been removed; as palatable individuals become more common, the sponges on the reef will exhibit greater variation

in chemical defenses and appear, on average, to be more palatable than the same species at a site with abundant predators. In the case of induction, sponges cease to induce chemical defenses at locations where predators have been removed. Regardless of the mechanism, it was expected that predator removal would result in these variably defended sponge species exhibiting greater palatability and greater variability in chemical defense.

Previous studies have focused on metabolite concentrations in prey tissue. While it may be beneficial to directly measure metabolites, systems of defensive chemistry are often complicated, with complex mixtures of multiple bioactive metabolites and multiple roles for single metabolites (Kubaneck et al., 2002; Schmitt et al., 1995). Even if the deterrent metabolite or the concentration required to deter predators is known, some organisms contain far more of the deterrent metabolites than the amount necessary to deter feeding (e.g. Jones et al., 2005) and metabolite variation among individuals may not alter the response of the predator. The present study takes a functional approach by assessing the response of potential predators to the crude extract of a sponge. Therefore, the term *chemical defense* is used in a functional sense; “greater chemical defenses” does not necessarily indicate more metabolites, simply a greater deterrentcy in feeding assays. Reef sites across the Caribbean provide an opportunity to test the hypotheses outlined above because fish populations are well protected in some areas and elsewhere overfished through the use of fish-traps to the point of absence, and these differences have been stable for many decades (Loh and Pawlik, 2014; Loh et al., 2015). The goal of this study was to compare intraspecific chemical defenses of sponge species in the variable defense category for sponges exposed to different levels of sponge-eating fishes at sites across the Caribbean. It was expected that palatable individuals would be more abundant, and the sponges collected from a single site would exhibit greater variability of chemical defenses, at overfished sites.

2. Materials and methods

2.1. Sponge collection

Sponge samples were collected from the following locations: Jamaica, March 2012; Cayman Islands, June 2012; Mexican Yucatan, July 2012; Bahamas Islands, July 2013; Key Largo, Florida, USA, May 2012 and June 2013; Isla Colon, Bocas del Toro, Panama, December and January 2012–13 and 2013–14. Sponge tissue was non-fatally collected while SCUBA diving by cutting a small portion (<15 ml) of the sponge with a sharp knife and placing it in a sealable plastic bag. Approximately 10 individual sponges of each species were collected from each site. The 7 target sponge species were previously identified as variably defended (Pawlik et al., 1995): *Clathria virgulosa*, *Cribrochalina vasculum*, *Desmapsamma anchorata*, *Dragmacidon reticulata*, *Iotrochota birotulata*, *Niphates digitalis*, and *Xestospongia muta*.

Freshly collected wet sponge tissue was placed in extraction medium or maintained frozen (–20 °C) until extraction. Epibionts were carefully excluded from extraction, except for *Parazoanthus* spp. on *I. birotulata* and *N. digitalis*, which were specifically noted when present in the sponge tissue prior to extraction and considered a factor in the analysis. Species identification followed the nomenclature of Zea et al. (2014); when necessary, Sven Zea (Universidad Nacional de Colombia, Santa Marta, Colombia) confirmed identification using microscopic examination of spicule mounts or tissue sections.

2.2. Chemical defense assays

The extraction and assay procedures were performed as described by Marty and Pawlik (2015). Each individual sponge was independently subjected to these procedures in order to provide replicates within each site. Briefly, fresh or frozen tissue was measured by volumetric displacement in solvent, then extracted twice: first in a solvent mixture of 1:1

dichloromethane and methanol (DCM:MeOH) and then in 100% MeOH. Solvents were removed using rotary evaporation and vacuum concentration at low heat (<40 °C). The two extracts were combined in a single vial and stored frozen (–20 °C) under N₂ gas until use in feeding assays. Extracts were reconstituted in a nutritionally appropriate food matrix of sodium alginate and squid mantle at the same volumetric concentration as the source sponge, then formed into hardened strands in calcium chloride and cut into 4 mm pellets. Control pellets were prepared in the same manner but without the addition of sponge extract. Pellets from each extract were presented in laboratory feeding assays to each of ten independent groups of yellow-phase bluehead wrasse, *Thalassoma bifasciatum*. Extract-treated pellets were considered rejected if sampled by the fish three times or sampled once and subsequently ignored. Significance of differences in consumption of treated versus control pellets was evaluated using a modified version of Fisher's exact test; extracts were scored as deterrent if six or fewer pellets were consumed (Marty and Pawlik, 2015). Intraspecific differences in chemical defense among sponge populations were evaluated with ANOVA and a Tukey HSD test. *Iotrochota birotulata* exhibited two distinct growth morphs depending on collection site; an erect, rope-like form that grows conspicuously up into the water column and a repent, low-lying form, the latter of which is clearly a result of grazing by fish predators (i.e., bite marks are evident). Differences in chemical defense between these two growth forms were evaluated using ANOVA ($\alpha = 0.05$).

2.3. Regression analyses

An abundance-based index of spongivore predation reported by Loh and Pawlik (2014) for each of the sites included in this analysis was paired with the feeding assay data as a predictor variable for regression analyses. The disparity in timing and location between sponge collections and fish surveys was minimal; in most cases, sponge collections for this study were performed simultaneously with the fish surveys reported in Loh and Pawlik (2014) at the same dive site. In one case, a collection in the Bahamas was performed ~100 km from the fish survey site, but the pairing of these sites was considered justifiable because they both experienced minimal fishing pressure and no qualitative differences were observed in the fish communities. Linear regression was used to compare the variability of defense to the spongivore index, with variability measured as the standard deviation around the mean number of pellets eaten for each population. The standard deviation of five populations of *X. muta* collected by Chanas and Pawlik (1997) were included to expand the taxonomic scope of linear regression analyses; both studies employed identical collection, extraction, and assay methodologies to those described above. Linear regression output was subjected to an F Test ($\alpha = 0.05$). Logistic regression models were fit to the data using the spongivore index as a predictor variable and the number of pellets eaten in the assay (out of 10) as the response. To contextualize the logistic regression model output, multicategory logistic regression models were fit to the data, again using the spongivore index as a predictor variable to determine the increase in spongivore index necessary to cause a sponge to shift to a lower defense category (defined as 0–0.4 = high defense; >0.4–<0.7 = intermediate defense; 0.7–1 = undefended). Additional predictive factors were modeled with logistic regression for *N. digitalis* (size class, color, and commensal zoanths) and *I. birotulata* (commensal zoanths and growth form) to determine whether these attributes varied with palatability.

3. Results

Crude organic extracts from all 7 sponge species exhibited both intra- and inter-site variation in their capacity to deter feeding by *Thalassoma bifasciatum* (Fig. 1). Specimens of *Niphates digitalis* from 14 sites throughout the region were tested and ANOVA detected significant differences in the chemical defenses among these sites (Fig. 2). Most sites contained some individuals that were chemically defended, but

samples of *N. digitalis* from four sites contained exclusively palatable individuals. Significant differences were also detected among sites for *Iotrochota birotulata*, however for this species some sites contained exclusively defended individuals and none of the sites contained exclusively palatable individuals (Fig. 2). A modified version of Fisher's exact test revealed that the mean number of pellets eaten differed significantly from controls for *I. birotulata* from all sites ($p < 0.05$), and therefore, this species belongs in the category of chemically defended sponges sensu Loh and Pawlik (2014). When segregated by morphology, a significant difference was detected between the repent individuals of *I. birotulata* and the erect individuals ($p < 0.0001$, ANOVA), with fewer pellets eaten for the repent individuals. At each site, individuals of *I. birotulata* represent only one of the two morphotypes (because this morphology is a consequence of fish grazing), so the analysis could not separate site differences from morphology. For both *N. digitalis* and *I. birotulata*, there was a gradient of increasing palatability: each site had ≥ 2 other sites from which it was not significantly different. For the remaining species, including *Cribrachalina vasculum* (Fig. 2), variability of chemical defense was observed within sites, although ANOVA did not detect significant differences among sites.

To examine the relationship between predator abundance and variability of chemical defenses among the sponges collected from each site, the standard deviation around the mean number of pellets eaten was regressed against the spongivore index. Despite a slight negative trend, variation did not correlate significantly with the spongivore index in linear regression analyses for all species together ($R^2 = 0.008$, $p = 0.765$) or for the following species run separately (for which there was sufficient replication): *I. birotulata* ($R^2 = 0.066$, $p = 0.718$); *N. digitalis* ($R^2 = 0.048$, $p = 0.563$); and *X. muta* ($R^2 = 0.197$, $p = 0.451$) (Fig. 3).

Logistic regression models provided conflicting results among sponge species; the probability that assay fish would eat a pellet decreased slightly with increasing spongivore index for all species together and for *Desmapsamma anchorata* and *N. digitalis* in species-specific analyses, however the probability of eating a pellet increased significantly for *Clathria virgultosa* (Table 1). Multicategory logistic regressions modeling the probability of an individual sponge shifting to a lower defense category with increasing spongivore index revealed no significant response from all species together or from each species analyzed separately (Table 1). Additional predictive factors of size class, color, and the presence of commensal zoanths were not significant in subsequent logistic regression models for *N. digitalis* ($p > 0.05$). Presence of commensal zoanths was also not significant for *N. digitalis* and *I. birotulata* together or for *I. birotulata* alone ($p > 0.05$).

4. Discussion

Previous surveys of the chemical defenses of Caribbean demosponges revealed that most species were either strongly chemically defended or consistently undefended, with only a few species exhibiting intraspecific variability in this trait (Loh and Pawlik, 2014; Pawlik et al., 1995). Closer examination of intraspecific variability in chemical defenses for *Xestospongia muta* and *Chondrilla caribensis* was the subject of subsequent research (Chanas and Pawlik, 1997; Swearingen and Pawlik, 1998). The present study aimed to test a hypothesis about the effects of predation on defense by comparing levels of chemical defense to the abundance of sponge-eating fishes at sites across the Caribbean for several species of variably defended sponges. It was expected that populations of variably defended sponge species would exhibit greater variability of chemical defenses, and possess a greater abundance of palatable individuals, at overfished sites when compared to sites with abundant predators. Linear regressions revealed no significant relationship between the standard deviation around the mean number of pellets eaten and the spongivore index. The logistic regression model for all species combined showed a significant negative effect of the spongivore index on the probability of assay fish eating a

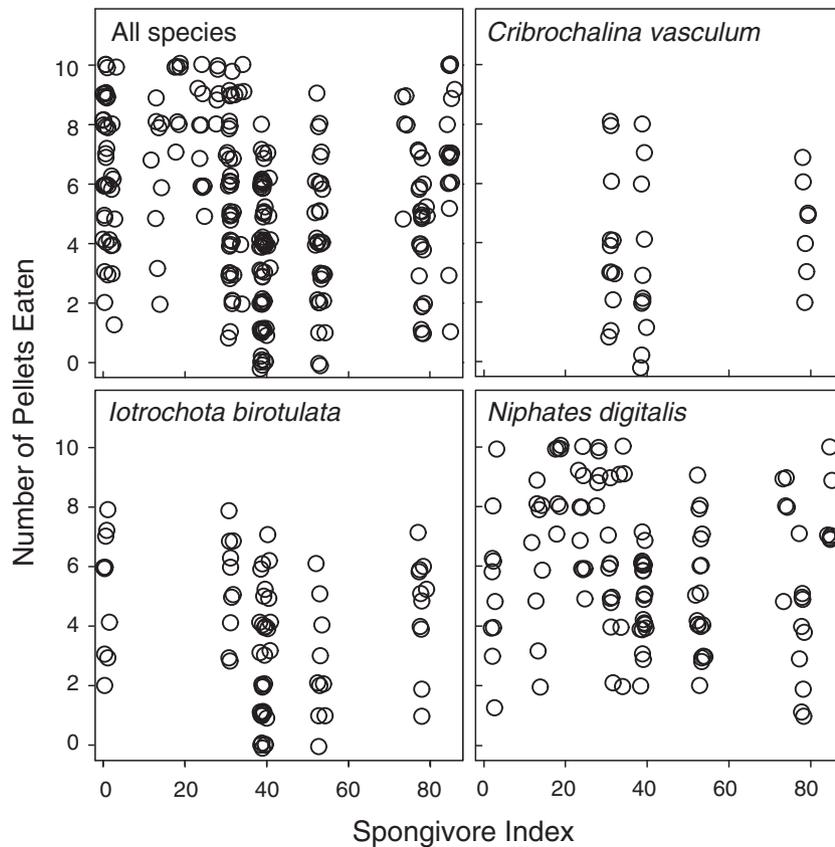


Fig. 1. Scatterplots relating fish feeding assay data with the spongivore index (Loh and Pawlik, 2014). The “All species” designation includes *Clathria virgultosa* (4 sites), *Cribrochalina vasculum* (3 sites), *Desmapsamma anchorata* (2 sites), *Drarmacidon reticulata* (1 site), *Iotrochota birotulata* (7 sites), *Niphates digitalis* (14 sites), and *Xestospongia muta* (1 site). Species for which the total number of individual sponges sampled was <30 are not shown in individual panels. Each data point was randomly skewed by ± 0.1 on the vertical axis and ± 0.5 on the horizontal axis to allow the visualization of identical observations.

treated pellet, however the magnitude of the effect was weak. The application of these data in the multcategory logistic regression model for all species demonstrated that no plausible change in predator abundance would be capable of significantly affecting the palatability for any of these sponge species. For example, to shift the odds ratio of an assay fish eating an extract-treated pellet from 7:3 to 4:6 (i.e., to shift the palatability of sponges at a given site from a mean of 7/10 pellets eaten to a mean of 4/10 pellets eaten), the predictive model requires an increase in the spongivore index of 150 units. The spongivore index, derived from counts of sponge-eating fish abundance in a 2000 m³ parcel of water, has a region-wide mean and maximum of 28 and 85 units, respectively (Loh and Pawlik, 2014). An increase in 150 units exceeds the range of sponge-eating fish abundances that would be ecologically possible (i.e., insufficient food to support the population, both parrotfishes and angelfishes are territorial), and this result confirms that predator abundance does not appear to influence the magnitude of chemical defenses in these variably defended sponge species.

The assay results for *Niphates digitalis* provide a good example of intraspecific variation in chemical defenses among the variably defended sponge species examined in this study: for the 118 individuals sampled from 14 sites, levels of chemical defense were highly variable, ranging from 1/10 pellets eaten to 10/10 pellets eaten. The linear regression analysis indicated that intra-site variation did not correlate with the spongivore index. Although the logistic regression model indicated that the probability of assay fish eating a pellet decreased significantly with increasing spongivore index, the multcategory logistic regression model showed that the magnitude of effect was not significant. The inclusion of other predictive factors in logistic regression models did not affect these results, suggesting that size class, color, and the presence of commensal zoanthids do not play a role in palatability.

The results from 74 individuals of *Iotrochota birotulata* reported here provide an update to the previous surveys of 7 individuals; the mean number of pellets eaten \pm standard error in this study was 3.68 ± 0.26 , compared to 7.14 ± 1.16 reported by Pawlik et al. (1995). These results place *I. birotulata* in the category of a chemically defended sponge species (mean number of pellets eaten + standard error ≤ 6) as defined by Loh and Pawlik (2014). Despite observing proportionally fewer palatable individuals in the present study, many individuals were palatable or just below threshold levels of palatability (e.g., ≥ 5 pellets were eaten for 38% of the extracts). These results are perhaps most interesting because several field studies report that *I. birotulata* constitutes a significant food resource for angelfishes (Feddern, 1968; Hourigan et al., 1989; Randall and Hartman, 1968; Wulff, 1994). One angelfish in particular, *Holacanthus tricolor*, is thought to specialize on *I. birotulata*: Randall and Hartman (1968) reported that *I. birotulata* constituted 15.6% of total gut content by volume for *H. tricolor*, and Feddern (1968) reported *I. birotulata* constituted 6.8% of total sponge in the gut by mass. In support of these gut content results, Wulff (1994) observed *H. tricolor* to forage exclusively on this sponge on reefs in San Blas, Panama. *Holacanthus tricolor* has even been kept alive for several weeks in the laboratory fed only with *I. birotulata* (West, 1976). Other Caribbean angelfishes from the genera *Holacanthus* and *Pomacanthus* are known to feed on *I. birotulata* as well (Feddern, 1968; Hourigan et al., 1989). Furthermore, *I. birotulata* grows rapidly like undefended species (Leong and Pawlik, 2010; Pawlik et al., 2013) and its distribution resembles that of an undefended sponge; it is more abundant at sites with few predators and less abundant at sites where predators are common. Feeding assays conducted with the bluehead, *Thalassoma bifasciatum*, have provided an excellent proxy for the responses of sponge-eating fishes; indeed, the crude extracts of sponge tissue that were palatable

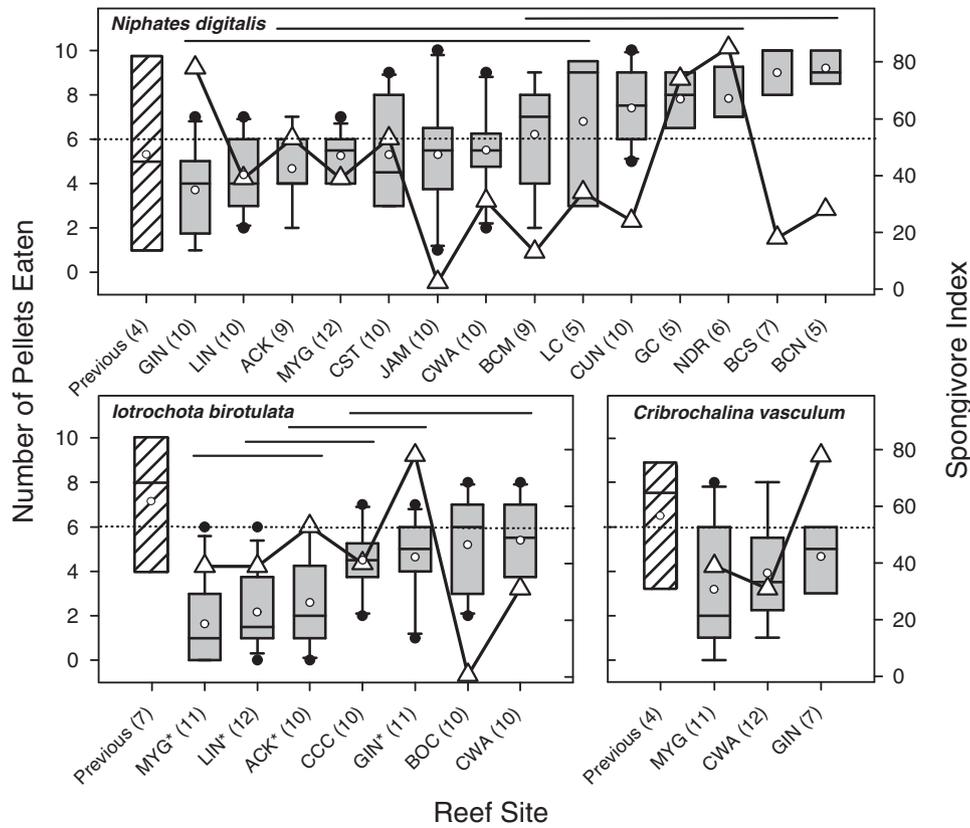


Fig. 2. A comparison of fish feeding assay data for extracts of variably defended sponge species and the abundance of sponge-eating fishes at reef sites around the Caribbean. The left axis is the scale for box and whisker plots of the number of food pellets eaten in laboratory assays. The spongivore index (Loh and Pawlik, 2014) is represented by white triangles with the scale on the right axis. Categories on the horizontal axis begin with the previous assay results compiled by Loh and Pawlik (2014) and continue with data from this study in order of increasing palatability (GIN = Great Inagua, Bahamas; LIN = Little Inagua, Bahamas; ACK = Acklins Island, Bahamas; MYG = Mayaguana, Bahamas; CST = Castle Island, Bahamas; CCC = Conception, Bahamas; JAM = Columbus Park, Jamaica; CWA = Conch Wall, Florida; NDR = North Dry Rocks, Florida; CUN = Cancun, Mexico; BCN = Banco Chinchorro North, Mexico; BCM = Banco Chinchorro Middle, Mexico; BCS = Banco Chinchorro South, Mexico; GC = Seaview Reef, Grand Cayman; LC = Sailfin, Little Cayman; BOC = Bocas del Toro, Panama). Replicate sponge samples appear in parentheses after the site name. Mean values for the number of pellets eaten indicated by white dots inside each boxplot. Black dots indicate outliers. The dashed line indicates the threshold value separating palatable from deterrent extracts (≤ 6 pellets eaten), as determined by a modified version of Fisher’s Exact test ($p < 0.05$; Marty and Pawlik, 2015). Horizontal lines above the boxplots indicate sites for which the distributions were not significantly different (ANOVA, Tukey HSD). Note: In the panel for *I. birotulata*, an asterisk following the site name on the horizontal axis denotes sites where *I. birotulata* exhibits a repent morphology; the absence of an asterisk indicates the erect growth form.

to blueheads were from the same sponge species that were most common in the guts of spongivores (Pawlik et al., 1995; Randall and Hartman, 1968). However, *I. birotulata* appears to represent an exception

to this generality. Angelfishes may have overcome the distastefulness of secondary metabolites present in the tissues of *I. birotulata* that are usually effective against a generalist omnivore like the bluehead.

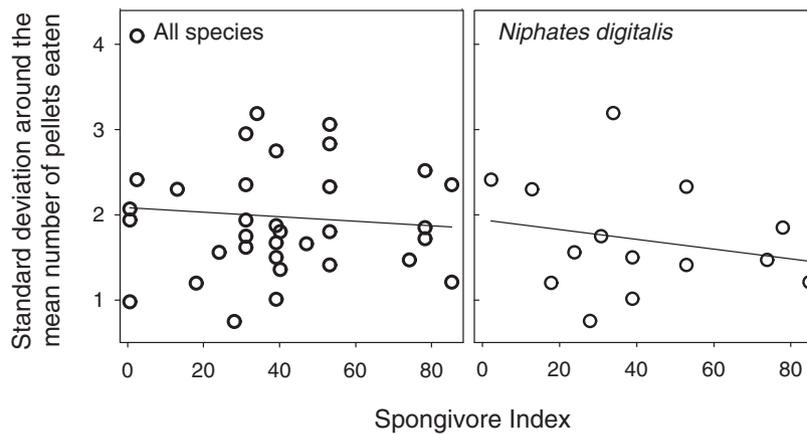


Fig. 3. Linear regression analyses. Each point represents a single site, with the standard deviation around the mean number of pellets eaten in feeding assays plotted against the spongivore index (Loh and Pawlik, 2014). The “All species” designation includes *Clathria virgulosa* (1 site), *Cribrrochalina vasculum* (3 sites), *Desmampsamma anchorata* (2 sites), *Dragnacidon reticulata* (1 site), *Iotrochota birotulata* (7 sites), *Niphatess digitalis* (14 sites), and *Xestospongia muta* (6 sites). Previously published data for *X. muta* from 5 sites were included (Chanas and Pawlik, 1997). Analysis by individual species was only conducted for species that had been sampled at ≥ 6 sites and only the two analyses with the highest replication are shown here. Regression output was subjected to an F Test ($\alpha = 0.05$) to compute p values: for All species, $R^2 = 0.0083$ and $p = 0.7647$; for *N. digitalis*, $R^2 = 0.0479$ and $p = 0.5625$.

Table 1

Output of logistic regression analyses with the spongivore index as the predictor variable. Probabilities were modeled using data from fish feeding assays. The spongivore index was generated from fish surveys by Loh and Pawlik (2014). The “All species” designation includes *Clathria virgultosa* (4 sites), *Cribrochalina vasculum* (3 sites), *Desmapsamma anchorata* (2 sites), *Dragmacidon reticulata* (1 site), *Iotrochota birotulata* (7 sites), *Niphates digitalis* (14 sites), and *Xestospongia muta* (1 site). Analysis by individual species was not conducted for species that were sampled at <2 sites. Defense categories for the multicategory regression analyses are defined by the proportion of pellets eaten in fish assays: 0–0.4 = high defense; >0.4–<0.7 = intermediate defense; 0.7–1 = undefended.

Logistic regression model	Estimate	p value
Probability of assay fish eating a pellet		
All species*	−0.0075	<0.0001
<i>Clathria virgultosa</i> *	0.0250	0.0415
<i>Cribrochalina vasculum</i>	0.0073	0.4098
<i>Desmapsamma anchorata</i> *	−0.0457	0.0004
<i>Iotrochota birotulata</i>	−0.0058	0.1061
<i>Niphates digitalis</i> *	−0.0061	0.0161
Multicategory logistic regression model	Estimate	p value
Probability of an individual sponge shifting defense category		
All species	0.0029	0.5112
<i>Clathria virgultosa</i>	−0.0129	0.6741
<i>Cribrochalina vasculum</i>	−0.0383	0.1554
<i>Desmapsamma anchorata</i>	−0.0409	0.3714
<i>Iotrochota birotulata</i>	−0.0018	0.8691
<i>Niphates digitalis</i>	0.0088	0.2161

* Denotes groups for which the spongivore index is significant at $p < 0.05$.

The variability of chemical defenses in *I. birotulata* was similar among sites and linear regression analysis showed no relationship between variability and the spongivore index. ANOVA detected significant differences in the number of pellets eaten among sites, but the logistic regression model did not show a significant effect of the spongivore index on the probability of fish eating a pellet in feeding assays. Significant differences were also detected between growth forms: fewer pellets were eaten for the repent morph than the erect morph. Bite marks are common on repent individuals, providing good evidence that spongivorous fishes restrict *I. birotulata* to the repent growth form at some sites in the way that *Haliclona caerulea* (Carballo et al., 2006) and *Mycale laevis* (Loh and Pawlik, 2009) are affected by predatory fishes. A likely explanation for the enhanced deterrent activity of repent individuals is that the grazing activity of fishes increases tissue density, and therefore the volumetric concentration of tissue extract, an outcome that has been discussed in the past (Pawlik, 2012). It has been proposed that *Parazoanthus swiftii*, commensal to *I. birotulata*, confers a chemical defense to the sponge (West, 1976), however the evidence for this notion was obtained using less ecologically relevant methods (Pawlik, 2012). The results from the present study, consistent with Pawlik et al. (1995), indicate that none of the commensal zoanthids (including *P. swiftii*) found on these sponges enhance the deterrent properties the sponge extract.

The levels of chemical defense for the remaining species were largely consistent with previous surveys. Replication was low for *Clathria virgultosa*, but only one extract exceeded the previous range of palatability. *Cribrochalina vasculum* was generally more defended than previous observations, although all sites contained both palatable and defended individuals. *Desmapsamma anchorata* exhibited a wider range than previous observations, and exclusively palatable individuals were found at a site with a very low spongivore index, helping to drive the model. In contrast to this result, *Dragmacidon reticulata*, which exhibited a comparable range to previous observations, was highly variable at the site with the maximum spongivore index value. Assay results for samples of *X. muta* reported in this study from heavily overfished reefs in Bocas del Toro, Panama, were similar to the values reported by Chanas and Pawlik (1997) at sites in Florida and the Bahamas where spongivorous fishes are abundant. Although the original

assay data from Chanas and Pawlik (1997) could not be included in the logistic regression models, data for *X. muta* qualitatively match the overall result that predator abundance is unrelated to patterns of variability in prey chemical defenses for this sponge species.

Caribbean sponge species use different strategies to survive on the reef: undefended species grow, reproduce, or recruit fast enough to persist despite predation while defended sponges use secondary metabolites to deter predators from feeding on them (Pawlik, 2011). Does intraspecific variability of chemical defenses provide an alternative strategy for survival? While some palatable individuals persist at sites with abundant predators, it is surprising that this phenotype does not become more common in the absence of predators because the release from predation should allow a phenotypic change in the population (Magurran et al., 1992). The 7 variably defended species studied herein cluster as a distinct group when compared to chemically defended and undefended sponge species from the Caribbean (Fig. 4). Perhaps variability of chemical defense represents an alternative adaptive strategy to avoid predation. If a sponge species varies in palatability from one individual to the next on a contiguous reef tract, foraging reef fishes would incur a cost (increased handling time) as they cautiously sample each colony and reject the defended individuals. This could be described as an example of automimicry, whereby defended model individuals cannot be visually distinguished from the undefended mimics (Ruxton and Speed, 2006). Automimicry may be possible in this system because of the abundance of alternative palatable sponge species on which sponge-eating fishes can focus their attention. The adaptive success of automimicry could explain high levels of intra-site variability for these sponge species and the observed absence of a relationship between palatability and predator abundance. However, if it is adaptive for a sponge species to exhibit variable defenses within a population, it is not a comprehensive solution to avoiding predation. Loh and Pawlik (2014) observed that variably defended sponges were proportionally more

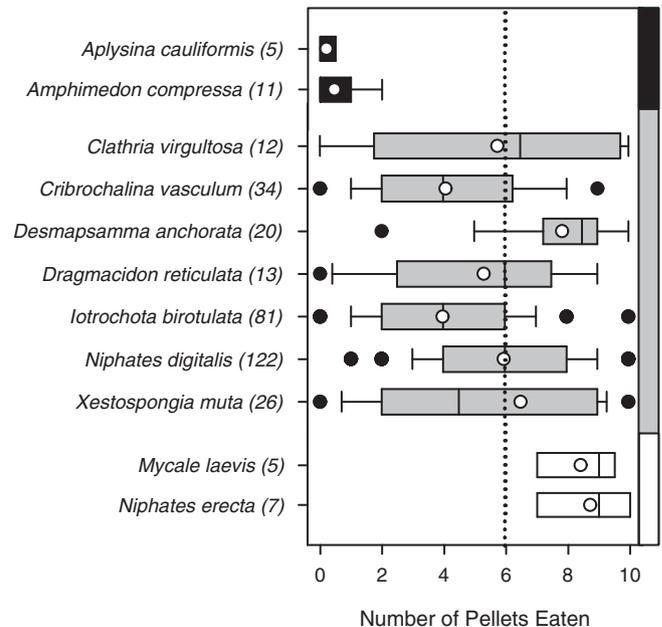


Fig. 4. Combined results from laboratory feeding assays with *Thalassoma bifasciatum* reported by Loh and Pawlik (2014) and this study. The variably defended sponge species from this study (plotted in grey) are presented alongside the two most common defended (plotted in black) and undefended (plotted in white) sponge species in the Caribbean. The number of replicate sponge individuals appears in parentheses after the species name. Mean number of pellets eaten is denoted by white dots inside each boxplot. Black dots indicate outliers. The dashed line indicates that an extract is considered deterrent if ≤ 6 treatment pellets are eaten, as determined by a modified version of Fisher's Exact test ($p < 0.05$; Marty and Pawlik, 2015). Previous assay results compiled in Loh and Pawlik (2014) are included with the new data from this study for the variably defended sponge species.

abundant at reef sites where predators had been removed than the same suite of species on reefs with abundant predators. For example, variably defended sponges represented over 50% of the sponge community on reefs in Martinique where the pooled spongivore index was <10, while variably defended sponges represented <10% the sponge community at the majority of the sites with a pooled spongivore index >30 (Loh and Pawlik, 2014). Therefore, populations of variably defended sponge species remain under the control of sponge-eating fishes.

5. Conclusions

This study found no evidence that variably defended sponge species from Caribbean coral reefs are either (1) more palatable on average, or (2) more variable in chemical defenses at sites with fewer predators. Although there was a slight effect of predator abundance on the palatability of 2 of the 7 study species, the magnitude of the effect was not ecologically significant for any species. Patterns of variability in chemical defenses for these species cannot be accounted for by predator abundance. The persistence of variability in chemical defenses for this small group of sponge species may be an example of automimicry, with palatable mimics surviving among the chemically defended models in a system where fully palatable sponge species represent an abundant alternative food source.

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