1 Formatted for the *Journal of Experimental Marine Biology and Ecology*

2 Revised: 29 August 2016

3

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

6

7

8 Micah Jaarsma Marty^a, James E. Blum^b, and Joseph R. Pawlik^{a,1}

9 ^aDepartment of Biology and Marine Biology and Center for Marine Science, University
10 of North Carolina Wilmington, 5600 Marvin K Moss Lane, Wilmington, North Carolina
11 28409, USA

12 ^bDepartment of Math and Statistics, University of North Carolina Wilmington, 601 S.
13 College Road, Wilmington, North Carolina 28403, USA

14

15 ¹Corresponding Author. Email: pawlikj@uncw.edu

16

1
2
3
4 **17 ABSTRACT:**
5

6
7 18 The antipredatory chemical ecology of Caribbean reef sponges has been well studied,
8
9 19 with two distinct ecological strategies described: chemically defended species use
10
11 20 secondary metabolites to deter fish predators, while palatable species lack chemical
12
13
14 21 defenses, but grow, reproduce, or recruit fast enough to persist despite predation. While
15
16 22 most palatable sponge species are consistently undefended from fish predators in
17
18 23 laboratory feeding assays, there are some species that exhibit individual inter- and intra-
19
20
21 24 site variation ranging from palatable to defended. The objective of this study was to
22
23 25 better document the extent of chemical defense variability for 7 of these species over a
24
25 26 wider geographic range and to test the dual hypotheses that variably defended sponge
26
27 27 species would be (1) more palatable, and (2) exhibit greater variability in chemical
28
29
30 28 defenses from reefs without sponge predators than from reefs where predators are
31
32 29 abundant. Laboratory feeding assays using the bluehead wrasse confirmed intraspecific
33
34 30 variability of chemical defenses for *Clathria virgultosa*, *Cribrochalina vasculum*,
35
36 31 *Desmapsamma anchorata*, *Dragmacidon reticulata*, *Iotrochota birotulata*, *Niphates*
37
38 32 *digitalis*, and *Xestospongia muta* for sites across the Caribbean. Surprisingly, logistic
39
40
41 33 regression models relating levels of sponge chemical defense to a site-specific index of
42
43 34 spongivorous fish abundance offered little support for the hypothesis that predator
44
45
46 35 abundance alters palatability for any of these variably defended sponge species. The
47
48 36 presence of symbiotic zoanthids in the tissue of *I. birotulata* and *N. digitalis* did not alter
49
50
51 37 palatability. While the selective effect of predation has segregated most species of
52
53 38 Caribbean reef sponges into chemically defended and undefended categories, this study
54
55
56 39 concludes that the situation is more complex for variably defended sponge species, and
57
58
59 40 suggests that automimicry is a possible explanation for this alternative strategy.
60
61
62
63
64
65

KEYWORDS: predation, chemical ecology, resource trade-offs, automimicry, coral reefs, Porifera.

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

1
2
3
4 65 defense (Lerdau and Gershenzon, 1997; Pawlik, 2011; 2012). Trade-offs between growth
5
6 66 and defense have been documented in terrestrial plants (Fine et al. 2006; Herms &
7
8 67 Mattson 1992) and coral reef sponges (Leong and Pawlik, 2010), and the selective
9
10 68 pressure of predation is considered responsible for the divergence of these two strategies.
11
12
13 69
14
15 70 The results of the selective process of predation are particularly evident in the Caribbean
16
17 71 sponge fauna. In an early survey of Caribbean sponges, Pawlik et al. (1995) found clear
18
19 72 distinctions in chemical defense strategies: some species were defended against generalist
20
21 73 predators and others were consistently undefended. Subsequent research demonstrated
22
23 74 that Caribbean sponges exhibit resource trade-offs that reveal the cost of chemical
24
25 75 defenses (Leong and Pawlik, 2010; Pawlik et al., 2008; Walters and Pawlik, 2005), and
26
27 76 empirical tests supported a conceptual model of the effect of predation on the structure
28
29 77 the sponge community on Caribbean reefs (Loh and Pawlik, 2014; Loh et al. 2015;
30
31 78 Pawlik, 2011; Pawlik et al., 2013). The defense status of over 100 species of Caribbean
32
33 79 sponges has been categorized (Loh and Pawlik, 2014). While the majority of species
34
35 80 found on Caribbean reefs were either chemically defended or consistently palatable in
36
37 81 laboratory feeding assays, a small subset of ~20 species exhibited intraspecific variability
38
39 82 in chemical defenses, in that the results of laboratory fish-feeding assays conducted on
40
41 83 individual sponges within or between sites ranged from palatable to defended (Loh and
42
43 84 Pawlik, 2014). While some of these variably defended species are rare, two are among
44
45 85 the 5 most common sponges on Caribbean reefs (*Xestospongia muta*, *Iotrochota*
46
47 86 *birotulata*) and represent an important part of the benthic community (Loh and Pawlik,
48
49 87 2014).
50
51
52
53
54
55
56
57
58
59
60 88
61
62
63
64
65

1
2
3
4 89 In general, variability in phenotypes improves survivorship (Johnson et al., 2014) because
5
6
7 90 some phenotypes will be more adaptive than others. However, the magnitude of
8
9 91 phenotypic variability should conform to selective constraints; predator-mediated post-
10
11 92 settlement mortality of marine invertebrates is a significant process (Gosselin and Qian,
12
13 93 1997), and among the sponge species that are variably defended it would be expected that
14
15 94 only the individuals with more potent chemical defenses should survive post-settlement
16
17 95 predation on reefs where predators are abundant (Loh and Pawlik, 2014). More in-depth
18
19 96 previous studies confirmed that chemical defenses were variable for two species:
20
21 97 *Xestospongia muta* (Chanas and Pawlik, 1997) and *Chondrilla caribensis* (Swearingen
22
23 98 and Pawlik, 1998) with much greater replication than the original survey (Pawlik et al.
24
25 99 1995). Both of these studies demonstrated significant differences in chemical defense
26
27 100 across sites, with crude extracts of tissue from specimens collected from Florida scoring
28
29 101 as more palatable in laboratory fish-feeding assays than those from the Bahamas. This
30
31 102 variation was attributed to differences in predator abundance among sites (Chanas and
32
33 103 Pawlik, 1997).
34
35 104
36
37 105 Intraspecific differences in chemical defenses have been observed in terrestrial plants,
38
39 106 however much of the work reported in the terrestrial literature has aimed to test optimal
40
41 107 defense theory with a focus on intra-individual variation as opposed to variation among
42
43 108 individuals (e.g. Agrawal, 2011; McCall and Fordyce, 2010). Variation of chemical
44
45 109 defenses among individuals has been documented in other benthic marine organisms
46
47 110 including octocorals and seaweeds, and it has been suggested that patterns of variation
48
49 111 may be driven by ecological factors such as competition and predation (Maida
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4 112 et al., 1993; Paul et al., 2001). For example, colonies of the soft coral *Sinularia flexibilis*
5
6 113 were shown to vary in secondary metabolite profiles for antifouling and allelopathic
7
8
9 114 compounds at reef sites on the Great Barrier Reef with different degrees of benthic
10
11 115 competition (Maida et al., 1993). In another case, Paul and Fenical (1986) quantified
12
13
14 116 variation of secondary metabolites among geographically distinct populations of five
15
16 117 species of green algae and found different metabolites, generally higher concentrations of
17
18
19 118 metabolites, and generally greater mixtures of metabolites in seaweeds collected on
20
21 119 shallow reefs, where rates of herbivory were presumed to be greater than adjacent
22
23
24 120 seagrass beds and deep reefs. In order to test the hypothesis that local differences in
25
26 121 herbivory drive patterns of intraspecific variation of algal chemical defenses, Paul and
27
28
29 122 Van Alstyne (1988) collected three species of the green alga *Halimeda* from reef slopes
30
31 123 (where herbivorous fishes were reported to be abundant) and reef flats (where herbivores
32
33
34 124 were rare) in Guam, then measured the major metabolites using thin layer
35
36 125 chromatography and tested the crude extracts for deterrent activity in field assays. The
37
38 126 results were mixed; differences were detected among geographically separate collection
39
40
41 127 sites and also between reef slope and flat habitats, but differences were not consistently
42
43 128 significant and low replication combined with unbalanced statistical design confounded
44
45
46 129 the analysis (Paul and Van Alstyne, 1988). Nevertheless, Paul and Van Alstyne (1988)
47
48 130 concluded that herbivory has been a strong selective force in the evolution of these algae
49
50
51 131 and that chemical defenses were likely greater where the pressure of herbivory was
52
53 132 greater.
54
55 133
56
57
58 134 The present study was predicated on the idea that predation may be responsible for
59
60 135 patterns in the variability of chemical defenses within species of Caribbean sponges. Two
61
62
63
64
65

1
2
3
4 136 predator-mediated processes, post-settlement mortality of palatable individuals and the
5
6 137 induction of chemical defenses within individuals that experience episodes of predation,
7
8
9 138 both could lead to patterns of variability in chemical defenses. In the former case,
10
11 139 palatable individuals recruit onto the reef and survive at sites where predators have been
12
13 140 removed; as palatable individuals become more common, the sponges on the reef will
14
15 141 exhibit greater variation in chemical defenses and appear, on average, to be more
16
17 142 palatable than the same species at a site with abundant predators. In the case of induction,
18
19 143 sponges cease to induce chemical defenses at locations where predators have been
20
21 144 removed. Regardless of the mechanism, it was expected that predator removal would
22
23 145 result in these variably defended sponge species exhibiting greater palatability and
24
25 146 greater variability in chemical defense.
26
27
28
29
30 147 Previous studies have focused on metabolite concentrations in prey tissue. While it may
31
32 148 be beneficial to directly measure metabolites, systems of defensive chemistry are often
33
34 149 complicated, with complex mixtures of multiple bioactive metabolites and multiple roles
35
36 150 for single metabolites (Kubaneck et al., 2002; Schmitt et al., 1995). Even if the deterrent
37
38 151 metabolite or the concentration required to deter predators is known, some organisms
39
40 152 contain far more of the deterrent metabolites than the amount necessary to deter feeding
41
42 153 (*e.g.* Jones et al., 2005) and metabolite variation among individuals may not alter the
43
44 154 response of the predator. The present study takes a functional approach by assessing the
45
46 155 response of potential predators to the crude extract of a sponge. Therefore, the term
47
48 156 *chemical defense* is used in a functional sense; “greater chemical defenses” does not
49
50 157 necessarily indicate more metabolites, simply a greater detergency in feeding assays. Reef
51
52 158 sites across the Caribbean provide an opportunity to test the hypotheses outlined above
53
54 159 because fish populations are well protected in some areas and elsewhere overfished
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4 160 through the use of fish-traps to the point of absence, and these differences have been
5
6 161 stable for many decades (Loh and Pawlik 2014; Loh et al. 2015). The goal of this study
7
8
9 162 was to compare intraspecific chemical defenses of sponge species in the variable defense
10
11 163 category for sponges exposed to different levels of sponge-eating fishes at sites across the
12
13
14 164 Caribbean. It was expected that palatable individuals would be more abundant, and the
15
16 165 sponges collected from a single site would exhibit greater variability of chemical
17
18
19 166 defenses, at overfished sites.
20
21 167
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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 virgultosa*, *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

1
2
3
4 191 The extraction and assay procedures were performed as described by Marty and Pawlik
5
6 192 (2015). Each individual sponge was independently subjected to these procedures in order
7
8
9 193 to provide replicates within each site. Briefly, fresh or frozen tissue was measured by
10
11 194 volumetric displacement in solvent, then extracted twice: first in a solvent mixture of 1:1
12
13 195 dichloromethane and methanol (DCM:MeOH) and then in 100% MeOH. Solvents were
14
15 196 removed using rotary evaporation and vacuum concentration at low heat (< 40 °C). The
16
17 197 two extracts were combined in a single vial and stored frozen (-20 °C) under N₂ gas until
18
19 198 use in feeding assays. Extracts were reconstituted in a nutritionally appropriate food
20
21 199 matrix of sodium alginate and squid mantle at the same volumetric concentration as the
22
23 200 source sponge, then formed into hardened strands in calcium chloride and cut into 4 mm
24
25 201 pellets. Control pellets were prepared in the same manner but without the addition of
26
27 202 sponge extract. Pellets from each extract were presented in laboratory feeding assays to
28
29 203 each of ten independent groups of yellow-phase bluehead wrasse, *Thalassoma*
30
31 204 *bifasciatum*. Extract-treated pellets were considered rejected if sampled by the fish three
32
33 205 times or sampled once and subsequently ignored. Significance of differences in
34
35 206 consumption of treated versus control pellets was evaluated using a modified version of
36
37 207 Fisher's exact test; extracts were scored as deterrent if six or fewer pellets were
38
39 208 consumed (Marty and Pawlik, 2015). Intraspecific differences in chemical defense among
40
41 209 sponge populations were evaluated with ANOVA and a Tukey HSD test. *Iotrochota*
42
43 210 *birotulata* exhibited two distinct growth morphs depending on collection site; an erect,
44
45 211 rope-like form that grows conspicuously up into the water column and a repent, low-lying
46
47 212 form, the latter of which is clearly a result of grazing by fish predators (i.e., bite marks
48
49 213 are evident). Differences in chemical defense between these two growth forms were
50
51 214 evaluated using ANOVA ($\alpha = 0.05$).
52
53
54
55
56
57
58
59
60
61
62
63
64
65

215

216 2.3 Regression Analyses

217 An abundance-based index of spongivore predation reported by Loh and Pawlik (2014)
218 for each of the sites included in this analysis was paired with the feeding assay data as a
219 predictor variable for regression analyses. The disparity in timing and location between
220 sponge collections and fish surveys was minimal; in most cases, sponge collections for
221 this study were performed simultaneously with the fish surveys reported in Loh and
222 Pawlik (2014) at the same dive site. In one case, a collection in the Bahamas was
223 performed ~100 km from the fish survey site, but the pairing of these sites was
224 considered justifiable because they both experienced minimal fishing pressure and no
225 qualitative differences were observed in the fish communities. Linear regression was used
226 to compare the variability of defense to the spongivore index, with variability measured
227 as the standard deviation around the mean number of pellets eaten for each population.
228 The standard deviation of five populations of *X. muta* collected by Chanas and Pawlik
229 (1997) were included to expand the taxonomic scope of linear regression analyses; both
230 studies employed identical collection, extraction, and assay methodologies to those
231 described above. Linear regression output was subjected to an F Test ($\alpha = 0.05$). Logistic
232 regression models were fit to the data using the spongivore index as a predictor variable
233 and the number of pellets eaten in the assay (out of 10) as the response. To contextualize
234 the logistic regression model output, multcategory logistic regression models were fit to
235 the data, again using the spongivore index as a predictor variable to determine the
236 increase in spongivore index necessary to cause a sponge to shift to a lower defense
237 category (defined as 0-0.4 = high defense; >0.4-<0.7 = intermediate defense; 0.7-1 =
238 undefended). Additional predictive factors were modeled with logistic regression for *N.*

239 *digitalis* (size class, color, and commensal zoanthids) and *I. birotulata* (commensal
240 zoanthids and growth form) to determine whether these attributes varied with palatability.

241 242 3. RESULTS

243 Crude organic extracts from all 7 sponge species exhibited both intra- and inter-site
244 variation in their capacity to deter feeding by *Thalassoma bifasciatum* (Figure 1).
245 Specimens of *Niphates digitalis* from 14 sites throughout the region were tested and
246 ANOVA detected significant differences in the chemical defenses among these sites
247 (Figure 2). Most sites contained some individuals that were chemically defended, but
248 samples of *N. digitalis* from four sites contained exclusively palatable individuals.
249 Significant differences were also detected among sites for *Iotrochota birotulata*, however
250 for this species some sites contained exclusively defended individuals and none of the
251 sites contained exclusively palatable individuals (Figure 2). A modified version of
252 Fisher's exact test revealed that the mean number of pellets eaten differed significantly
253 from controls for *I. birotulata* from all sites ($p < 0.05$), and therefore, this species belongs
254 in the category of chemically defended sponges *sensu* Loh & Pawlik (2014). When
255 segregated by morphology, a significant difference was detected between the repent
256 individuals of *I. birotulata* and the erect individuals ($p < 0.0001$, ANOVA), with fewer
257 pellets eaten for the repent individuals. At each site, individuals of *I. birotulata* represent
258 only one of the two morphotypes (because this morphology is a consequence of fish
259 grazing), so the analysis could not separate site differences from morphology. For both *N.*
260 *digitalis* and *I. birotulata*, there was a gradient of increasing palatability: each site had ≥ 2
261 other sites from which it was not significantly different. For the remaining species,
262 including *Cribrochalina vasculum* (Figure 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* ($R^2 = 0.197$, $p = 0.451$) (Figure 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 2). Additional predictive factors of size class, color, and the presence of commensal zoanthids were not significant in subsequent logistic regression models for *N. digitalis* ($p > 0.05$). Presence of commensal zoanthids 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 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

1
2
3
4 312 spongivore index, derived from counts of sponge-eating fish abundance in a 2000 m³
5
6 313 parcel of water, has a region-wide mean and maximum of 28 and 85 units, respectively
7
8
9 314 (Loh and Pawlik, 2014). An increase in 150 units exceeds the range of sponge-eating fish
10
11 315 abundances that would be ecologically possible (i.e., insufficient food to support the
12
13
14 316 population, both parrotfishes and angelfishes are territorial), and this result confirms that
15
16 317 predator abundance does not appear to influence the magnitude of chemical defenses in
17
18
19 318 these variably defended sponge species
20
21 319
22
23 320 The assay results for *Niphates digitalis* provide a good example of intraspecific variation
24
25 321 in chemical defenses among the variably defended sponge species examined in this study:
26
27 322 for the 118 individuals sampled from 14 sites, levels of chemical defense were highly
28
29 323 variable, ranging from 1/10 pellets eaten to 10/10 pellets eaten. The linear regression
30
31 324 analysis indicated that intra-site variation did not correlate with the spongivore index.
32
33 325 Although the logistic regression model indicated that the probability of assay fish eating a
34
35 326 pellet decreased significantly with increasing spongivore index, the multcategory logistic
36
37 327 regression model showed that the magnitude of effect was not significant. The inclusion
38
39 328 of other predictive factors in logistic regression models did not affect these results,
40
41 329 suggesting that size class, color, and the presence of commensal zoanthids do not play a
42
43 330 role in palatability.
44
45 331
46
47
48 332 The results from 74 individuals of *Iotrochota birotulata* reported here provide an update
49
50 333 to the previous surveys of 7 individuals; the mean number of pellets eaten $\pm$ standard
51
52 334 error in this study was 3.68 ± 0.26 , compared to 7.14 ± 1.16 reported by Pawlik et al.
53
54 335 (1995). These results place *I. birotulata* in the category of a chemically defended sponge
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4 336 species (mean number of pellets eaten + standard error ≤ 6) as defined by Loh & Pawlik
5
6 337 (2014). Despite observing proportionally fewer palatable individuals in the present study,
7
8
9 338 many individuals were palatable or just below threshold levels of palatability (*e.g.*, ≥ 5
10
11 339 pellets were eaten for 38% of the extracts). These results are perhaps most interesting
12
13
14 340 because several field studies report that *I. birotulata* constitutes a significant food
15
16 341 resource for angelfishes (Feddern, 1968; Hourigan *et al.*, 1989; Randall & Hartman,
17
18 342 1968; Wulff, 1994). One angelfish in particular, *Holacanthus tricolor*, is thought to
19
20
21 343 specialize on *I. birotulata*: Randall & Hartman (1968) reported that *I. birotulata*
22
23 344 constituted 15.6% of total gut content by volume for *H. tricolor*, and Feddern (1968)
24
25 345 reported *I. birotulata* constituted 6.8% of total sponge in the gut by mass. In support of
26
27 346 these gut content results, Wulff (1994) observed *H. tricolor* to forage exclusively on this
28
29 347 sponge on reefs in San Blas, Panama. *Holacanthus tricolor* has even been kept alive for
30
31 348 several weeks in the laboratory fed only with *I. birotulata* (West, 1979). Other Caribbean
32
33 349 angelfishes from the genera *Holacanthus* and *Pomacanthus* are known to feed on *I.*
34
35 350 *birotulata* as well (Feddern, 1968; Hourigan *et al.*, 1989). Furthermore, *I. birotulata*
36
37 351 grows rapidly like undefended species (Leong and Pawlik, 2010; Pawlik *et al.*, 2013) and
38
39 352 its distribution resembles that of an undefended sponge; it is more abundant at sites with
40
41 353 few predators and less abundant at sites where predators are common. Feeding assays
42
43 354 conducted with the bluehead, *Thalassoma bifasciatum*, have provided an excellent proxy
44
45 355 for the responses of sponge-eating fishes; indeed, the crude extracts of sponge tissue that
46
47 356 were palatable to blueheads were from the same sponge species that were most common
48
49 357 in the guts of spongivores (Pawlik *et al.* 1995; Randall and Hartman, 1968). However, *I.*
50
51 358 *birotulata* appears to represent an exception to this generality. Angelfishes may have
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4 359 overcome the distastefulness of secondary metabolites present in the tissues of *I.*
5
6 360 *birotulata* that are usually effective against a generalist omnivore like the bluehead.
7
8
9 361
10
11 362 The variability of chemical defenses in *I. birotulata* was similar among sites and linear
12
13 363 regression analysis showed no relationship between variability and the spongivore index.
14
15 364 ANOVA detected significant differences in the number of pellets eaten among sites, but
16
17 365 the logistic regression model did not show a significant effect of the spongivore index on
18
19 366 the probability of fish eating a pellet in feeding assays. Significant differences were also
20
21 367 detected between growth forms: fewer pellets were eaten for the repent morph than the
22
23 368 erect morph. Bite marks are common on repent individuals, providing good evidence that
24
25 369 spongivorous fishes restrict *I. birotulata* to the repent growth form at some sites in the
26
27 370 way that *Haliclona caerulea* (Carballo *et al.*, 2006) and *Mycale laevis* (Loh & Pawlik,
28
29 371 2009) are affected by predatory fishes. A likely explanation for the enhanced deterrent
30
31 372 activity of repent individuals is that the grazing activity of fishes increases tissue density,
32
33 373 and therefore the volumetric concentration of tissue extract, an outcome that has been
34
35 374 discussed in the past (Pawlik, 2012). It has been proposed that *Parazoanthus swiftii*,
36
37 375 commensal to *I. birotulata*, confers a chemical defense to the sponge (West, 1976),
38
39 376 however the evidence for this notion was obtained using less ecologically relevant
40
41 377 methods (Pawlik, 2012). The results from the present study, consistent with Pawlik *et al.*
42
43 378 (1995), indicate that none of the commensal zoanthids (including *P. swiftii*) found on
44
45 379 these sponges enhance the deterrent properties the sponge extract.
46
47 380
48
49 381 The levels of chemical defense for the remaining species were largely consistent with
50
51 382 previous surveys. Replication was low for *Clathria virgultosa*, but only one extract
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4 383 exceeded the previous range of palatability. *Cribrochalina vasculum* was generally more
5
6 384 defended than previous observations, although all sites contained both palatable and
7
8
9 385 defended individuals. *Desmapsamma anchorata* exhibited a wider range than previous
10
11 386 observations, and exclusively palatable individuals were found at a site with a very low
12
13
14 387 spongivore index, helping to drive the model. In contrast to this result, *Dragmacidon*
15
16 388 *reticulata*, which exhibited a comparable range to previous observations, was highly
17
18
19 389 variable at the site with the maximum spongivore index value. Assay results for samples
20
21 390 of *X. muta* reported in this study from heavily overfished reefs in Bocas del Toro,
22
23
24 391 Panama, were similar to the values reported by Chanas and Pawlik (1997) at sites in
25
26 392 Florida and the Bahamas where spongivorous fishes are abundant. Although the original
27
28
29 393 assay data from Chanas and Pawlik (1997) could not be included in the logistic
30
31 394 regression models, data for *X. muta* qualitatively match the overall result that predator
32
33 395 abundance is unrelated to patterns of variability in prey chemical defenses for this sponge
34
35
36 396 species.
37
38 397
39
40 398 Caribbean sponge species use different strategies to survive on the reef: undefended
41
42
43 399 species grow, reproduce, or recruit fast enough to persist despite predation while
44
45
46 400 defended sponges use secondary metabolites to deter predators from feeding on them
47
48 401 (Pawlik, 2011). Does intraspecific variability of chemical defenses provide an alternative
49
50
51 402 strategy for survival? While some palatable individuals persist at sites with abundant
52
53 403 predators, it is surprising that this phenotype does not become more common in the
54
55 404 absence of predators because the release from predation should allow a phenotypic
56
57
58 405 change in the population (Magurran et al., 1992). The 7 variably defended species studied
59
60 406 herein cluster as a distinct group when compared to chemically defended and undefended
61
62
63
64
65

sponge species from the Caribbean (Figure 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 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 less than 10, while variably defended sponges represented less than 10% the sponge community at the majority of the sites with a pooled spongivore index greater than 30 (Loh and Pawlik, 2014). Therefore, populations of variably defended sponge species remain under the control of sponge-eating fishes.

5. CONCLUSIONS:

1
2
3
4 430 This study found no evidence that variably defended sponge species from Caribbean
5
6 431 coral reefs are either (1) more palatable on average, or (2) more variable in chemical
7
8
9 432 defenses at sites with fewer predators. Although there was a slight effect of predator
10
11 433 abundance on the palatability of 2 of the 7 study species, the magnitude of the effect was
12
13
14 434 not ecologically significant for any species. Patterns of variability in chemical defenses
15
16 435 for these species cannot be accounted for by predator abundance. The persistence of
17
18
19 436 variability in chemical defenses for this small group of sponge species may be an
20
21 437 example of automimicry, with palatable mimics surviving among the chemically
22
23
24 438 defended models in a system where fully palatable sponge species represent an abundant
25
26 439 alternative food source.
27
28
29 440
30
31 441

32 33 442 **Acknowledgments** 34

35
36 443 This study was funded by grants to J.R.P. from the National Science Foundation,
37
38 444 Biological Oceanography Program (OCE-1029515, 1558580). Field assistance was
39
40
41 445 provided by Lindsey Deignan and John Hanmer. Laboratory assistance was provided by
42
43 446 Michael Wooster, Kyle Rice, Deanna Hardesty and Aaron Cooke. Sven Zea helped
44
45 447 confirm the identity of sponge specimens. Patrick Erwin provided constructive feedback
46
47
48 448 on a draft of the manuscript. Field surveys and non-fatal collection of sponge tissue
49
50
51 449 samples were conducted with permission at all locations: FKNMS-2009-126 in the
52
53 450 Florida Keys, National Commission on Aquaculture and Fisheries (Comisión Nacional de
54
55 451 Acuacultura y Pesca) Permit DAPA/2/06504/110612/1608 in the Yucatan (Mexico),
56
57
58 452 Department of Marine Resources Permit MAF/LIA/22 (Bahamas Islands), and
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

453 unnumbered permits or research contracts from the Cayman Islands, Jamaica and
454 Panama.
455

REFERENCES

- Agrawal, A.A., 2011. Current trends in the evolutionary ecology of plant defence: Plant defence theory. *Functional Ecology* 25, 420–432. doi:10.1111/j.1365-2435.2010.01796.x
- Bone, E.K., Keough, M.J., 2005. Responses to damage in an arborescent bryozoan: Effects of injury location. *Journal of Experimental Marine Biology and Ecology* 324, 127–140. doi:10.1016/j.jembe.2005.04.008
- Carballo, J.L., Avila, E., Enriquez, S., Camacho, L., 2006. Phenotypic plasticity in a mutualistic association between the sponge *Haliclona caerulea* and the calcareous macroalga *Jania adherens* induced by transplanting experiments. I: morphological responses of the sponge. *Marine Biology* 148, 467–478.
- Chanas, B., Pawlik, J.R., 1997. Variability in the chemical defense of the Caribbean reef sponge *Xestospongia muta*. Presented at the Proceedings of the 8th International Coral Reef Symposium, pp. 1363–1368.
- Cronin, G., 2001. Resource allocation in seaweeds and marine invertebrates: chemical defense patterns in relation to defense theories, in: McClintock, J.B., Baker, A.C. (Eds.), *Marine Chemical Ecology*. CRC Press, Boca Raton, pp. 325–353.
- Fedderm, H.A., 1968. Systematics and Ecology of Western Atlantic Angelfishes, Family Chaetodontidae, with an Analysis of Hybridization in *Holacanthus*. University of Miami.
- Gosselin, L.A., Qian, P.Y., 1997. Juvenile mortality in benthic marine invertebrates. *Mar Ecol Prog Ser* 146, 265–282. doi:10.3354/meps146265
- Gowda, J.H., 1996. Spines of *Acacia tortilis*: What Do They Defend and How? *Oikos* 77, 279–284. doi:10.2307/3546066
- Harvell, C.D., Fenical, W., 1989. Chemical and structural defenses of Caribbean gorgonians (*Pseudopterogorgia* spp.): Intracolony localization of defense. *Limnology and oceanography* 34, 382–389.
- Hourigan, T.F., Stanton, F.G., Motta, P.J., Kelley, C.D., Carlson, B., 1989. The feeding ecology of three species of Caribbean angelfishes (family Pomacanthidae). *Environmental Biology of Fishes* 24, 105–116.
- Johnson, D.W., Grorud-Colvert, K., Sponaugle, S., Semmens, B.X., 2014. Phenotypic variation and selective mortality as major drivers of recruitment variability in fishes. *Ecol Lett* 17, 743–755. doi:10.1111/ele.12273
- Jones, A.C., Blum, J.E., Pawlik, J.R., 2005. Testing for defensive synergy in Caribbean sponges: Bad taste or glass spicules? *Journal of Experimental Marine Biology and Ecology* 322, 67–81. doi:10.1016/j.jembe.2005.02.009

- 1
2
3
4 492 Kubanek, J., Whalen, K., Engel, S., Kelly, S., Henkel, T., Fenical, W., Pawlik, J., 2002.
5 493 Multiple defensive roles for triterpene glycosides from two Caribbean sponges.
6 494 Oecologia 131, 125–136. doi:10.1007/s00442-001-0853-9
7
8
9 495 Leong, W., Pawlik, J., 2010. Evidence of a resource trade-off between growth and
10 496 chemical defenses among Caribbean coral reef sponges. Marine Ecology Progress
11 497 Series 406, 71–78. doi:10.3354/meps08541
12
13
14 498 Lerda, M., Gershenzon, J., 1997. Allocation Theory and Chemical Defense, in: Bazzaz,
15 499 F.A., Grace, J. (Eds.), Plant Resource Allocation, Physiological Ecology Series.
16 500 Academic Press, San Diego, pp. 265–277.
17
18 501 Levin, D.A., 1976. The Chemical Defenses of Plants to Pathogens and Herbivores.
19 502 Annual Review of Ecology and Systematics 7, 121–159.
20
21
22 503 Lima, S.L., Dill, L.M., 1990. Behavioral decisions made under the risk of predation: a
23 504 review and prospectus. Canadian Journal of Zoology 68, 619–640.
24 505 doi:10.1139/z90-092
25
26 506 Loh, T.-L., Pawlik, J.R., 2009. Bitten down to size: Fish predation determines growth
27 507 form of the Caribbean coral reef sponge *Mycale laevis*. Journal of Experimental
28 508 Marine Biology and Ecology 374, 45–50. doi:10.1016/j.jembe.2009.04.007
29
30
31 509 Loh, T.-L., Pawlik, J.R., 2014. Chemical defenses and resource trade-offs structure
32 510 sponge communities on Caribbean coral reefs. Proceedings of the National
33 511 Academy of Sciences 201321626.
34
35
36 512 Loh, T.-L., McMurray, S.E., Henkel, T.P., Vicente, J., Pawlik, J.R., 2015. Indirect effects
37 513 of overfishing on Caribbean reefs: sponges overgrow reef-building corals. PeerJ
38 514 3, e901. doi:10.7717/peerj.901
39
40
41 515 Magurran, A.E., Seghers, B.H., Carvalho, G.R., Shaw, P.W., 1992. Behavioural
42 516 Consequences of an Artificial Introduction of Guppies (*Poecilia reticulata*) in N.
43 517 Trinidad: Evidence for the Evolution of Anti-Predator Behaviour in the Wild.
44 518 Proceedings: Biological Sciences 248, 117–122.
45
46 519 Maida, M., Carroll, A.R., Coll, J.C., 1993. Variability of terpene content in the soft coral
47 520 *Sinularia flexibilis* (Coelenterata: Octocorallia), and its ecological implications.
48 521 Journal of Chemical Ecology 19, 2285–2296. doi:10.1007/BF00979664
49
50
51 522 Marty, M.J., Pawlik, J.R., 2015. A fish-feeding laboratory bioassay to assess the
52 523 antipredatory activity of secondary metabolites from the tissues of marine
53 524 organisms. Journal for Visualized Experiments 95, e52429 DOI:10.3791/52429
54
55
56 525 McCall, A.C., Fordyce, J.A., 2010. Can optimal defence theory be used to predict the
57 526 distribution of plant chemical defences?: Predicting the distribution of plant
58 527 chemical defences. Journal of Ecology 98, 985–992. doi:10.1111/j.1365-
59 528 2745.2010.01693.x
60
61
62
63
64
65

- 1
2
3
4 529 O'Neal, W., Pawlik, J.R., 2002. A reappraisal of the chemical and physical defenses of
5 530 Caribbean gorgonian corals against predatory fishes. Marine Ecology Progress
6 531 Series 240, 117–126.
7
8
9 532 Paul, V.J., Arthur, K.E., Ritson-Williams, R., Ross, C., Sharp, K., 2007. Chemical
10 533 Defenses: From Compounds to Communities. Biological Bulletin 213, 226–251.
11
12 534 Paul, V.J., Cruz-Rivera, E., Thacker, R.W., 2001. Chemical mediation of macroalgal-
13 535 herbivore interactions: ecological and evolutionary perspectives, in: McClintock,
14 536 J.B., Baker, A.C. (Eds.), Marine Chemical Ecology. CRC Press, Boca Raton, pp.
15 537 227–265.
16
17
18 538 Paul, V.J., Fenical, W., 1986. Chemical defense in tropical green algae, order
19 539 Caulerpaceles. Mar. Ecol. Prog. Ser 34, 157–169.
20
21
22 540 Paul, V.J., Van Alstyne, K.L., 1988. Chemical defense and chemical variation in some
23 541 tropical Pacific species of *Halimeda* (Halimedaceae; Chlorophyta). Coral Reefs 6,
24 542 263–269. doi:10.1007/BF00302022
25
26 543 Pawlik, J.R., 1993. Marine invertebrate chemical defenses. Chemical Reviews 93, 1911–
27 544 1922. doi:10.1021/cr00021a012
28
29
30 545 Pawlik, J.R., 2011. The Chemical Ecology of Sponges on Caribbean Reefs: Natural
31 546 Products Shape Natural Systems. BioScience 61, 888–898.
32 547 doi:10.1525/bio.2011.61.11.8
33
34
35 548 Pawlik, J.R., 2012. Antipredatory Defensive Roles of Natural Products from Marine
36 549 Invertebrates, in: Fattorusso, E., Gerwick, W.H., Tagliatella-Scafati, O. (Eds.),
37 550 Handbook of Marine Natural Products. Springer Netherlands, Dordrecht, pp. 677–
38 551 710.
39
40
41 552 Pawlik, J.R., Chanas, B., Toonen, R.J., Fenical, W., 1995. Defenses of Caribbean
42 553 sponges against predatory reef fish. I. Chemical deterrence. Marine ecology
43 554 progress series. Oldendorf 127, 183–194.
44
45
46 555 Pawlik, J.R., Henkel, T., McMurray, S., López-Legentil, S., Loh, T.-L., Rohde, S., 2008.
47 556 Patterns of sponge recruitment and growth on a shipwreck corroborate chemical
48 557 defense resource trade-off. Marine Ecology Progress Series 368, 137–143.
49 558 doi:10.3354/meps07615
50
51
52 559 Pawlik, J.R., Loh, T.-L., McMurray, S.E., Finelli, C.M., 2013. Sponge Communities on
53 560 Caribbean Coral Reefs Are Structured by Factors That Are Top-Down, Not
54 561 Bottom-Up. PLoS ONE 8, e62573. doi:10.1371/journal.pone.0062573
55
56 562 Pettorelli, N., Coulson, T., Durant, S.M., Gaillard, J.-M., 2011. Predation, individual
57 563 variability and vertebrate population dynamics. Oecologia 167, 305–314.
58 564 doi:10.1007/s00442-011-2069-y
59
60
61 565 Pohnert, G., 2004. Chemical Defense Strategies of Marine Organisms, in: Schulz, S.

- (Ed.), The Chemistry of Pheromones and Other Semiochemicals I, Topics in Current Chemistry. Springer Berlin Heidelberg, pp. 179–219.
- Randall, J.E., Hartman, W.D., 1968. Sponge-feeding fishes of the West Indies. Marine Biol. 1, 216–225. doi:10.1007/BF00347115
- Relyea, R.A., 2002. The Many Faces of Predation: How Induction, Selection, and Thinning Combine to Alter Prey Phenotypes. Ecology 83, 1953–1964. doi:10.2307/3071778
- Ruxton, G.D., Speed, M.P., 2006. How can automimicry persist when predators can preferentially consume undefended mimics? Proceedings of the Royal Society B 273, 373–378. doi:10.1098/rspb.2005.3238
- Schmitt, T.M., Hay, M.E., Lindquist, N., 1995. Constraints on Chemically Mediated Coevolution: Multiple Functions for Seaweed Secondary Metabolites. Ecology 76, 107–123. doi:10.2307/1940635
- Schulte, B.A., Bakus, G.J., 1992. Predation Deterrence in Marine Sponges: Laboratory Versus Field Studies. Bulletin of Marine Science 50, 205–211.
- Swearingen III, D.C., Pawlik, J.R., 1998. Variability in the chemical defense of the sponge *Chondrilla nucula* against predatory reef fishes. Marine Biology 131, 619–627.
- Van Alstyne, K., Dethier, M., Duggins, D., 2001. Spatial patterns in macroalgal chemical defenses, in: McClintock, J.B., Baker, B.J. (Eds.), Marine Chemical Ecology. pp. 301–324.
- Vuorisalo, T., Tuomi, J., 1986. Unitary and Modular Organisms: Criteria for Ecological Division. Oikos 47, 382–385. doi:10.2307/3565452
- Walters, K.D., Pawlik, J.R., 2005. Is there a trade-off between wound-healing and chemical defenses among Caribbean reef sponges? Integrative and Comparative Biology 45, 352–358.
- West, D.A., 1976. Aposematic Coloration and Mutualism in Sponge-Dwelling Tropical Zoanthids, in: Mackie, G.O. (Ed.), Coelenterate Ecology and Behavior. Springer US, pp. 443–452.
- Wulff, J.L., 1994. Sponge feeding by Caribbean angelfishes, trunkfishes, and filefishes, in: Sponges in Time and Space. Presented at the 4th International Porifera Congress, A A Balkema Publishers, Rotterdam; Brookfield Vt., pp. 265–271.
- Zea, S., Henkel, T.P., and Pawlik, J.R. 2014. The Sponge Guide: a picture guide to Caribbean sponges. 3rd Edition. Available online at www.spongeguide.org. Accessed on: 2015-05-01.

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 multcategory 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
Multcategory 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 $p < 0.05$

Figure legends

Figure 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), *Dragmacidon 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.

Figure 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.

Figure 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 virgultosa* (1 site), *Cribrochalina vasculum* (3 sites), *Desmapsamma anchorata* (2 sites), *Dragmacidon reticulata* (1 site), *Iotrochota birotulata* (7 sites), *Niphates 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

1
2
3
4 665 subjected to an F Test ($\alpha = 0.05$) to compute p values: for All species, $R^2 = 0.0083$ and p
5 666 = 0.7647; for *N. digitalis*, $R^2 = 0.0479$ and $p = 0.5625$.
6 667

7
8
9 668 Figure 4. Combined results from laboratory feeding assays with *Thalassoma bifasciatum*
10 669 reported by Loh and Pawlik (2014) and this study. The variably defended sponge species
11 670 from this study (plotted in grey) are presented alongside the two most common defended
12 671 (plotted in black) and undefended (plotted in white) sponge species in the Caribbean. The
13 672 number of replicate sponge individuals appears in parentheses after the species name.
14 673 Mean number of pellets eaten is denoted by white dots inside each boxplot. Black dots
15 674 indicate outliers. The dashed line indicates that an extract is considered deterrent if ≤ 6
16 675 treatment pellets are eaten, as determined by a modified version of Fisher's Exact test (p
17 676 < 0.05 ; Marty and Pawlik, 2015). Previous assay results compiled in Loh and Pawlik
18 677 (2014) are included with the new data from this study for the variably defended sponge
19 678 species.
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Figure 1

FIGURES

Figure 2

Figure 3

Figure 4