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SPONGE RESEARCH DEVELOPMENTS

Does concentrating chemical defenses within specific regions of marine sponges result in enhanced protection from predators?

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Abstract Chemical defenses are an effective mode of predator deterrence across benthic marine organisms, but their production may come with associated costs to the organism as limited resources are diverted away from primary processes like growth and reproduction. Organisms concentrating ecologically relevant levels of these defenses in tissues most at risk to predator attack may alleviate this cost while deterring predators. We addressed this hypothesis by investigating the deterrence of chemical extracts from the inner and outer regions of the sponges *Aplysina fulva*, *Ircinia felix*, and *I. campana* from a temperate hard-bottom reef in the South Atlantic Bight. Assays were conducted using natural fish assemblages and sea urchins. Although, *A. fulva* and *I. felix* have higher concentrations of defensive metabolites in the outer and inner regions, respectively, extracts from these regions did not display enhanced deterrency against fish or mobile invertebrate predators. Likewise, extracts from both regions of the sponge *Ircinia campana*, which has a uniform distribution of defensive chemicals throughout, did not differ in their ability to deter either group of predators. Since chemical defenses were effective deterrents at lower concentrations, secondary metabolite allocation patterns observed among these sponges are likely not driven by predation pressure from generalist fish and mobile invertebrate predators on these reefs. Alternatively, these patterns may be driven by other ecological stressors, another suite of predators, or may be more effective at deterring predators when combined with structural defenses.

Keywords Chemical defense · Temperate sponges · South Atlantic Bight · Feeding assays

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Introduction

Secondary metabolite production is a common method of defense against consumers in sessile terrestrial and marine organisms (Rhoades, 1979; Paul, 1992; Pawlik et al., 1995). While the effectiveness of these chemicals against predators is well-documented, their production may come with considerable metabolic cost to the prey (McKey, 1974, 1979; Rhoades, 1979). Organisms able to maintain defenses at the lowest, but still effective concentration or organisms that sequester these defenses in

particular regions of the body may thus be at a selective advantage. In plants, the Optimal Defense Theory (ODT) hypothesizes that the cost of chemical defenses can be reduced by concentrating defensive compounds in regions of the body with the highest fitness value or those that are most at risk to consumers (Rhoades, 1979). In recent years, the ODT has been tested more and more frequently on marine algae and soft-bodied invertebrates such as sponges (Hay, 1996) because, like plants, these marine organisms coexist with numerous species of potential consumers and lack active escape mechanisms.

Consistent with the ODT, some sponges show higher concentrations of deterrent compounds in the outer regions of the sponge, where the risk of predator attack may be greatest. For instance, brominated compounds are found in spherulous cells common to the ectosomal region of sponges in the genus *Aplysina* (Thompson et al., 1983; Turon et al., 2000), and toxic guanidine alkaloids accumulate in spherulous cells in *Crambe crambe*, leading to increased toxicity toward the periphery (Becerro et al., 1997). Likewise, *Rhopaloeides odorabile* has higher levels of diterpenes in the outer 2 cm (Thompson et al., 1987) of the sponge body and *Negombata magnifica* concentrates the metabolite latrunculin B toward its periphery (Gillor et al., 2000). While increased concentrations of these metabolites in the outer region imply a defensive role against fish and large mobile invertebrate predators, elevated levels of deterrent compounds toward the interior of other sponge species and high variation in chemical defense allocation within some genera imply that there may be multiple, complex forces driving the selection of chemical defense production and allocation within sponges (Freeman & Gleason, 2010; Sacristán-Soriano et al., 2011). In addition, since investigations on the effectiveness of allocating defensive chemistry to the outer region of a sponge have produced conflicting results, our understanding of the ecological significance of surface allocation on predator–prey interactions is lacking. For example, Furrow et al. (2003) reported that the Antarctic sponge *Latrunculia apicalis* sequesters defensive compounds in the outer 2 mm of its tissues and that chemical extracts from this region are significantly more deterrent against sea stars than extracts from the inner region. In contrast, while the sponge *Chondrilla nucula*, six species of Red Sea sponges, and *Melophlus sarasinorum* sequester defensive compounds toward their periphery, chemical extracts from this outer region do not display an enhanced ability to deter predators when compared to extracts from within the sponge (Swearingen & Pawlik, 1998; Burns et al., 2003; Rohde & Schupp, 2011).

One method to address the disparity across studies is to combine quantification of chemical defenses with tests of their ability to deter relevant consumers (as in Furrow et al., 2003; but not in Swearingen & Pawlik, 1998; Burns et al., 2003). Using this methodology, Schupp et al. (1999) confirmed that increased concentrations of defensive chemicals in regions of a sponge most vulnerable to attack by fishes made them deterrent. In contrast, Becerro et al. (1998) found that concentrations of major metabolites were higher in the tips than the base of the sponge *Cacospongia* sp., but extracts from the tips were consumed by fish just as readily as extracts from the base. Contrasting results from studies such as those described above highlight the need for further work to fully understand the relationship between allocation of chemical defenses within sponges and predator deterrence.

We have recently reported the presence of three distinct chemical defense allocation patterns in three sponge species that are common on temperate reefs of the North Atlantic Ocean off the coast of Georgia, USA (Freeman & Gleason, 2010). We used liquid chromatography-mass spectrometry (LC-MS) to identify a suite of brominated tyrosine derivatives isolated from the sponge *Aplysina fulva* (Pallas, 1766) and gas chromatography-mass spectrometry (GC-MS) to identify furanosesterterpene tetronic acids (FTAs) from the sponges *Ircinia felix* (Duchassaing & Michelotti, 1864), and *Ircinia campana* (Lamarck, 1814). Finally, we used high performance liquid chromatography (HPLC) to quantify the natural concentrations of these metabolites within the inner and outer regions of these sponges with the assumption that tissues toward the periphery are more vulnerable to consumption by fish and macroinvertebrate predators (Freeman & Gleason, 2010). While the ability of both brominated tyrosine derivatives and FTAs to deter predators has been reported previously (Ebel et al., 1997; Pawlik et al., 2002; Thoms et al., 2004), allocation patterns we

documented in these three sponge species were not consistent with predictions arising from the ODT. Specifically, higher concentrations of brominated tyrosine derivatives were measured in the outer regions of the branching sponge, *A. fulva*, as might be expected by the ODT, but levels of FTAs were greater in the inner regions of the amorphous massive sponge *I. felix* and were equal in inner and outer regions of the vase sponge *I. campana*. Thus, the study reported here was conducted to determine whether the higher levels of chemical defense observed within some of these sponges translates into enhanced predator deterrence. Based on the documented allocation patterns, we predicted that the higher levels of chemical defenses in the outer tissue regions of *A. fulva* and the inner regions of *I. felix* would result in enhanced deterrence, while the similar concentrations found in both regions of *I. campana* would deter predators equally.

Methods

Artificial food preparation

To determine if higher concentrations of defensive compounds within certain regions of sponges correspond with increased protection of those areas from predators, we compared the palatability of extracts from the outer 2 mm and the corresponding inner regions (hereafter referred to as outer and inner regions as in Freeman & Gleason, 2010) of a sponge to fish and urchin predators. Methods for the extraction of crude organic extracts from *A. fulva*, *I. felix* and *I. campana* and artificial food preparation followed those described by Becerro et al. (2003) and Ruzicka & Gleason (2008). We did not have enough sponge tissue to test the palatability of *I. felix* extracts against generalist reef fish, so only *A. fulva* and *I. campana* were used in this assay. All three species were used in feeding assays with the sea urchin *Arbacia punctulata*.

Sponges were collected from J-Y Reef (31°36.056N, 80°47.431W), a hard-bottom area in the South Atlantic Bight (SAB) characterized by sandstone and scallop shell ridges (Freeman et al., 2007; Freeman & Gleason, 2010). We collected a total of 24 individuals of *A. fulva*, 20 individuals of *I. felix*, and 22 individuals of *I. campana* from this site (Freeman & Gleason, 2010). Each of these individuals were separated into inner and outer regions and then utilized for HPLC quantification of metabolite concentrations, nutritional quality, and structural component analyses as reported in Freeman & Gleason (2010) and for feeding assays as discussed below. Discrepancies between the total number of sponges collected and those used in feeding assays were due to the fact that there was sometimes not enough tissue on the smaller samples for all analyses. In all cases though, at least 14 individuals of each sponge species were used to make food cubes for feeding assays. Chemical extracts from multiple sponges were never combined thus allowing for adequate replication in all feeding assays.

For fish feeding assays, frozen sponge samples from single individuals were thawed and 5 ml of the outer and inner regions were measured by displacement of water. These samples were freeze dried and extracted three times in 10 ml of DCM:MeOH (Freeman & Gleason, 2010). The three extracts obtained from a single sponge were combined, filtered, and evaporated to dryness. The dried crude extract was weighed and then reconstituted in 0.50 ml of MeOH and sonicated to ensure complete dissolution. Artificial food was made in 150 ml batches from a mixture of 7.5 g powdered squid mantle, 2.975 g Type I Carrageenan, 0.525 g agar, and 150 ml distilled water. This food mixture resulted in artificial food approximating the average nutritional quality present in sponges (Chanas & Pawlik, 1995). Once heated, approximately 4.5 ml of food mixture was added to each 27 mm diameter × 58 mm height vial containing 0.50 ml of MeOH and dissolved sponge extract. The mixture was homogenized by stirring rapidly and then was allowed to cool. Once the artificial food cube had hardened, it was cut into 1 ml cubes for feeding assays. Control food cubes were prepared the same way, but with crude extract omitted. To ensure that selection of food cubes by predators was not influenced by color, food coloring was added to both control and treatment mixtures before heating until both appeared similar in color by visual comparison. For the urchin feeding assays, frozen sponge samples from all three species were thawed and processed in the same manner as in the fish trials, but 7.5 g of powdered algal disks were substituted for powdered squid mantle and only 3 ml of tissue from the outer

and inner regions of each replicate and 0.30 ml of MeOH were used. In both fish and urchin feeding assays, the deterrence of chemical defenses was tested at natural concentrations. This was accomplished by incorporating extracts from 5 and 3 ml of sponge tissue into 5 and 3 ml food cubes for the fish and urchin assays, respectively. Thus, chemicals extracted from the sponge were dissolved and incorporated into artificial foods that were at a similar volumetric concentration.

Feeding assays

Fish feeding assays carried out with extracts from *A. fulva* and *I. campana* were conducted in the field at J-Y Reef (Freeman & Gleason, 2010) in one dive per species. The assays began by releasing several control food cubes to stimulate feeding activity of natural assemblages of generalist reef fish including black seabass (*Centropristus striata*), spottail pinfish (*Diplodus holbrooki*), pinfish (*Lagodon rhomboides*), and the gray triggerfish (*Balistes capriscus*). Inner, outer, and control food cubes were then offered one or two at a time. Once the released food cubes had been consumed or rejected (typically <5–10 s), additional food cubes were offered. Since each food cube represents extract obtained from a single sponge, a total of 21 food cubes of each type (inner, outer, and control) were released for *I. campana* and 14 of each type were released for *A. fulva*. Food cubes were released haphazardly so that not more than four of the same types of cube were released in a row and the fish could not habituate to a pattern of cube release or become accustomed to one type of food. A food cube was considered unpalatable if it was rejected by fishes two or more times or if it sank to the bottom uneaten. In these assays, the dominant consumers of food cubes were the black seabass (*Centropristus striata*) and spottail pinfish (*Diplodus holbrooki*). These fish species were also the dominant consumers for chemical assays conducted by Ruzicka & Gleason (2009) off the Georgia coast, consuming 80% of the 570 food cubes fed to fish.

Feeding assays were also conducted for *A. fulva*, *I. felix*, and *I. campana* in the wet lab at Georgia Southern University using the urchin *Arbacia punctulata* as the predator. This is a prevalent echinoderm species and the most abundant urchin species at J-Y Reef and is a common invertebrate grazer on hard-bottom reefs off the coast of Georgia (Freeman & Gleason, pers. obs.). While we have not observed *A. punctulata* feeding on sponges at J-Y Reef, other investigators have reported that this species, as well as others in this genus are omnivorous scavengers preying on a wide variety of algae and benthic animals, including coral polyps and sponges (reviewed in Ridder & Lawrence, 1982). In addition, although we did not use strictly spongivorous predators in either set of feeding assays, the use of generalist predators in assays to determine the deterrency of sponge chemical defenses is widespread in the literature (Waddell & Pawlik, 2000a, b; Burns et al., 2003) and the utility of using generalist predators has been well-outlined by Pawlik et al. (1995) and Ruzicka & Gleason (2009).

The experimental design for the urchin assays followed a method similar to that employed by Hay et al. (1994) using fiberglass window screening to hold the artificial food in place. Before adding artificial food to the sponge crude extract, a small ($\sim 7 \times 7$ mm) piece of fiberglass window screening (with 1×2 mm openings) was added to the vial containing the extract to act as webbing for attachment of the food cube. Approximately, 2.7 ml of artificial food was then added to the vial and stirred vigorously until the extract was thoroughly mixed within the food. Before allowing the artificial food to harden, the small piece of window screening was pushed to the bottom of the vial in the middle of the food cube. Once the food had hardened, the food cube was removed from the vial, blotted dry, and weighed.

For each replicate, urchins were given a choice of cubes containing compounds from inner and outer sponge tissues or a control cube containing no sponge extract. To do this, we attached pre-weighed food cubes to a large ($\sim 140 \times 130$ mm) piece of window screening by sewing the small piece of screen embedded in the food cube to the larger screen base with monofilament line. To keep food cubes equidistant from each other, we used an acrylic template containing three holes oriented in a triangle to position the cubes on the large screen base. This triangular arrangement ensured that when an urchin was placed in the center, its tube feet would be in contact with all three food types.

In preparation for the feeding assay, the large screen base containing all three food cubes was attached to the bottom of a feeding arena using

monofilament line. These arenas were 3.1 l Glad© plastic containers that had holes drilled in the sides, bottom, and lid to allow water flow. Stainless steel hex nuts were cable tied to the sides for negative buoyancy. The lids prevented the urchins from escaping during the assay period and also allowed us to conduct 20 assays at once by stacking arenas within the aquarium. In order to prevent positional effects, the orientation of the window screen within the feeding arena was adjusted haphazardly among replicates.

Arbacia punctulata were collected from J-Y Reef and transported to Georgia Southern University in aerated coolers. Urchins were held in 75.7 l aquaria containing filtered, artificial seawater at 30–32 ppt. Urchins were maintained on a diet of Wardley® algal disks until 48 h before their assay. All urchins used in the assay had a test diameter between 4 and 5.5 cm. An assay began when an urchin was placed in the center of the three food types, with as little stress due to exposure to air and handling as possible, and the arena was covered and placed on the bottom of the aquarium. A complete set of assays for a sponge species was completed in 1 day because these arenas could be stacked. Preliminary assays found that urchins allowed to feed for more than 8 h could consume all of at least one type of food, so all assays were stopped at 8 h. We found that urchins typically moved around on the window screen, trying at least two types of food, but usually fed extensively only on one. Urchins that did not feed at all ($\sim$10% of runs) provided no information on the relative deterrence of extracts and were not included in the statistical analysis. Urchins were only used once in these assays. At the completion of the assay, urchins were removed from the feeding arenas. Individual food cubes were removed from the window screening, blotted dry, and weighed to determine how much of each cube had been consumed.

Tests assessing weight loss and degradation of food cubes resulting from the 8 h exposure to water were conducted simultaneously with the urchin feeding assay by placing food cubes in feeding arenas lacking *A. punctulata*. In all instances, weight loss in these cubes was below 2% and there was no obvious degradation of secondary compounds due to water exposure. The integrity of the chemical defenses in artificial food cubes used in both fish and urchin assays was confirmed by re-extraction of leftover cubes and analysis by thin layer chromatography (TLC) and HPLC. In all instances, the compounds extracted from the sponges were present in the artificial food and had not undergone obvious degradation during the experimental procedure.

To test for significant differences in consumption of different food cube types by fish (expressed as % of cubes consumed), we carried out multiple 2×2 contingency tables with χ^2 analysis. For each species, the following comparisons were made: inner vs. control, outer vs. control, and inner vs. outer. For the urchin feeding assays, we analyzed square root arcsine transformed data (expressed as % of weight loss of each food type) with a repeated measures ANOVA. A repeated measures analysis was used because all three food cubes were exposed to one urchin simultaneously and were therefore not independent.

Results

Fish that consumed experimental food cubes were primarily the black seabass (*Centropristus striata*) and spottail pinfish (*Diplodus holbrooki*), with occasional consumption by the gray triggerfish (*Balistes capriscus*) and pinfish (*Lagodon rhomboides*). In both *A. fulva* and *I. campana*, 100% of the control food cubes were consumed while treatment cubes were consumed less frequently (*A. fulva*: Pearson's test: $X^2 = 6.222$, $P < 0.05$ and $X^2 = 10.435$, $P < 0.01$ for inner vs. control and outer vs. control, respectively; *I. campana*: Pearson's test: $X^2 = 4.909$, $P < 0.05$ and $X^2 = 4.190$, $P < 0.05$ for inner vs. control and outer vs. control, respectively) (Fig. 1). The frequency at which the two types of treatment food cubes were consumed, however, was not significantly different in either sponge species (Pearson's test: $X^2 = 0.735$, $P > 0.30$ and $X^2 = 0.094$, $P > 0.70$ for *A. fulva* and *I. campana*, respectively) (Fig. 1).

In laboratory feeding assays with the urchin, *A. punctulata*, control food cubes lost more weight than both treatment cubes in all sponge species, but this trend was significant in *A. fulva* ($F_{2,32} = 13.96$, $P < 0.001$ for inner vs. control and $F_{2,32} = 8.246$, $P < 0.01$ for outer vs. control) and not significant for either of the *Ircinia* spp. ($P > 0.05$). There was no significant difference ($P > 0.05$) in the percent change in weight between the two treatment cubes in any of the sponge species (Fig. 2).

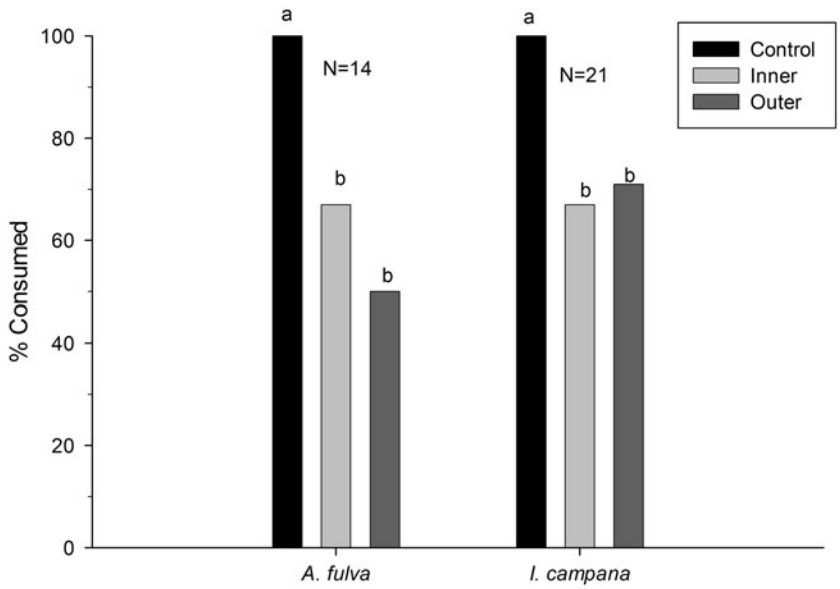

Fig. 1 Consumption of food cubes by a natural assemblage of temperate reef fish. Cubes containing crude extract from both the inner and outer regions of these sponges as well as control cubes possessing no extract were offered. Percentages are based on the number of cubes consumed out of the number indicated at the top of the bars. Data were analyzed using 2 × 2 contingency tables. Letters a and b indicate significant differences between treatment foods (outer vs. inner) or between a treatment food and control

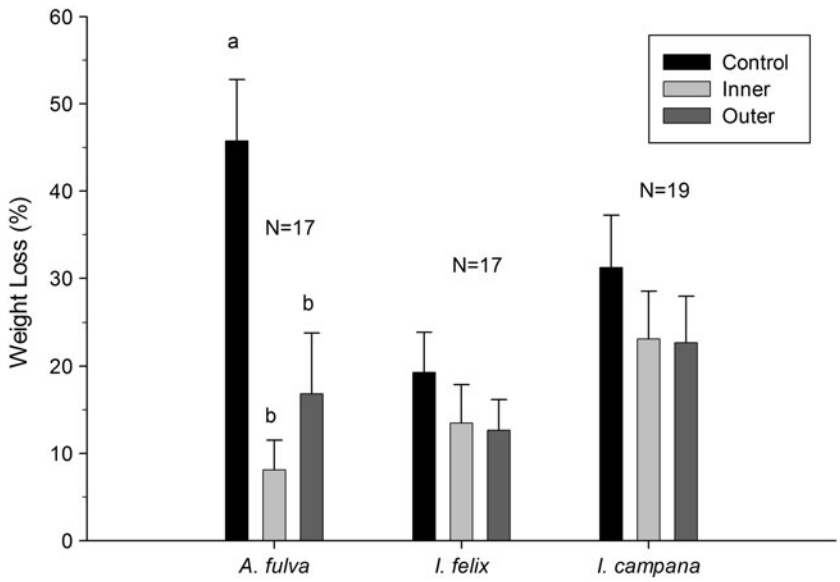

Fig. 2 Percent weight loss (±SE) due to feeding by the urchin *Arbacia punctulata* on food cubes created from chemical extracts of three sponge species. Extracts were obtained from inner and outer tissue regions of each sponge. Controls represent food cubes containing no chemical extract. Data were analyzed using a repeated measures ANOVA followed by a least squares comparison. Letters a and b indicate significant differences between treatment foods (outer vs. inner) or between a treatment food and control

Discussion

There is a paucity of experimental evidence supporting the contention that allocating chemical defenses to regions of the body most vulnerable to attack is ecologically beneficial to marine sponges (Becerro et al., 1997, 1998; Schupp et al., 1999; Burns et al., 2003; Furrow et al., 2003). Although such a strategy might confer protection at lower metabolic cost to the sponge, the concentrations of defensive metabolites throughout the sponge body are rarely measured (Swearingen & Pawlik, 1998; Burns et al., 2003), or the ecological benefit of increased concentrations of chemical defenses is not experimentally verified (Freeman & Gleason, 2010). In addition, investigations approaching this topic on both fronts have produced conflicting results, thus making generalization difficult (Becerro et al., 1997, 1998; Schupp et al., 1999; Furrow et al., 2003). In an initial survey of the defensive compounds in the sponges *A. fulva*, *I. felix*, and *I. campana* (Nuñez et al., 2008; Freeman & Gleason, 2010), we confirmed the presence of

secondary metabolites that are known to deter predators (Ebel et al., 1997; Waddell & Pawlik, 2000a, b; Pawlik et al., 2002; Thoms et al., 2004) and showed that the concentrations of these compounds differ between the inner and outer tissue regions in two of these species, *A. fulva* and *I. felix*, (Freeman & Gleason, 2010). Interestingly, the distribution of chemical defenses within these three species was not consistent with predictions of the ODT that costly defenses should be concentrated in regions of highest fitness value or regions that are most likely to encounter predators (McKey, 1974, 1979; Rhoades, 1979). Feeding assays conducted here provide support for this conclusion by showing that extracts obtained from sponge regions possessing higher concentrations of secondary metabolites (i.e., inner tissue regions of *I. felix* and outer regions of *A. fulva*) did not display enhanced deterrency to generalist temperate reef fish and one species of sea urchin. Thus, we conclude that differences in secondary metabolite allocation patterns observed among *A. fulva*, *I. felix*, and *I. campana* on temperate reefs of the southeastern US may not be driven by predation pressure from fish and urchins and that these known deterrent compounds may be allocated to different regions of these sponges to mediate other interactions besides predation.

The feeding trials conducted here do corroborate previous findings that extracts from these sponge species deter predators because treatment food cubes were consumed less often than controls (Pawlik et al., 1995, 2002; Ebel et al., 1997; Thoms et al., 2004, 2006). The lack of evidence for a positive relationship between compound concentrations and predator deterrence may be attributable to at least three factors. First, defensive compounds may serve multiple functions and higher concentrations of these compounds may actually represent an adaptation to defend against competitors, reduce fouling, or inhibit microbial infection (Uriz et al., 1992; Becerro et al., 1997; Kubanek et al., 2002). Second, because multiple factors typically play a role in prey deterrence, testing chemical defenses in isolation may not sufficiently address the suite of defenses, such as structural components or variability in nutritional quality, encountered by predators (Hay et al., 1994; Hay, 1996). The three aspiculate species investigated here show within individual heterogeneity in the levels of structural components (i.e., spongin fibers) and nutritional quality (protein and carbohydrate) so this hypothesis warrants further investigation (Freeman & Gleason, 2010). Finally, if a threshold concentration for predator deterrence exists and the lower metabolite concentration (from the inner region of *A. fulva* and the outer region of *I. felix*) is above the threshold, then higher concentrations in adjacent regions would not confer increased deterrence. This hypothesis might also be relevant here because metabolite concentrations in the inner and outer regions of these two sponges differed by only 0.06% (*I. felix*) and approximately 4% of sponge dry weight (*A. fulva*) (Freeman & Gleason, 2010).

It should also be pointed out that different suites of chemical defenses are not necessarily functionally equivalent when it comes to deterring predators (Hay et al., 1988; Pennings & Paul, 1992). The FTAs and brominated tyrosine derivatives identified here are known to be deterrent compounds, but it is likely that they differ in their effectiveness against specific predators. This is supported in the current study, where FTA extracts from *I. campana* were only deterrent to fish predators whereas brominated tyrosine derivative extracts from *A. fulva* were deterrent to both fish and urchins. It is also possible that chemical variability within these sponges may be driven by other predators or even that the full suite of possible predators of these three species at J-Y Reef has yet to be adequately enumerated. For instance, a recent study by Sacristán-Soriano et al. (2011) suggests that higher levels of one compound in the outer region of the sponge *Aplysina aerophoba* may defend this sponge from generalist predators, while higher concentrations of other metabolites toward the interior of the sponge may act to defend the sponge from specialist predators. Thus, extending our methods to other predators or testing the effects of specific compounds against various predators may have yielded different results. Future assays should test the ability of individual compounds, at natural concentrations, to deter an array of potential predators including generalist and specialist fish and invertebrate predators and small, cryptic invertebrates (Hay et al., 1988). In addition, since chemical defenses may act synergistically to deter predators in some sponges (Hill et al., 2005) and sponges display some ability to deter predators using both chemical and physical defenses (Ruzicka & Gleason, 2009), future assays should include, at the very least,

the interactive effects of chemical and structural defenses to more accurately identify the effectiveness of variation in anti-predatory defenses within sponges.

In conclusion, while we have documented previously significant within-sponge variation in the distribution of defensive chemicals in two out of three temperate sponge species of the North Atlantic (Freeman & Gleason, 2010), this variation is not consistent with allocation models like the ODT that predict at risk regions should be more heavily defended from predators. Rather, results reported here suggest increased concentrations of chemical defenses do little to provide enhanced protection from damage by large biting or mobile invertebrate generalist predators. However, the possibility that concentrating chemical defenses within certain regions of these sponges is due to predation pressure from consumers not investigated here must be acknowledged. For instance, specialist spongivores like angelfish (*Holacanthus* sp. and *Pomacanthus* sp.), trunkfish (*Acanthostracion* sp.), and filefish (*Cantherhines macrocerus*) are present at J-Y and surrounding reefs and may exert strong selective pressure for differential within-sponge allocation of chemical defenses. In addition, other ecological stresses including competition with other reef organisms, risks of bacterial infection, or potential for biofouling might select for the allocation of defenses to certain regions or throughout sponge bodies. Given that we are just beginning to understand how sponges use secondary metabolites to mediate biotic interactions (Becerro, 2008), future research should extend beyond predator–prey interactions and address the other potential roles these compounds play in sponge defense and, ultimately, sponge community structure (Ruzicka & Gleason, 2009).

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