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SPONGE COMMUNITY STRUCTURE AND ANTI-PREDATOR DEFENSES ON TEMPERATES REEFS OF THE SOUTH ATLANTIC BIGHT

by

RICHARD ROBERT RUZICKA III

Under the Direction of Daniel F. Gleason

ABSTRACT

The interaction between predation and anti-predator defenses of prey is important in shaping community structure in all ecosystems. This study examined the relationship between sponge predation and the distribution of sponge anti-predator defenses on temperate reefs in the South Atlantic Bight. Significant differences in the distribution of sponge species, sponge densities, and densities of sponge predators were documented across two adjacent reef habitats. Significant differences also occurred in the distribution of sponge chemical and structural defenses with chemical deterrence significantly greater in sponges associated with the habitat having higher predation intensity. Structural defenses, although effective in some instances, appear to be inadequate against spongivorous predators thereby restricting the distribution of sponge species lacking chemical defenses to habitats with lower predation intensity. These results, when compared to published data from tropical studies, also indicate that predation pressure and the production of anti-predator defenses may be inversely correlated with latitude.

INDEX WORDS: Community structure, Temperate reefs, Sponge, Spongivorous fish, Chemical defenses, Structural defenses, South Atlantic Bight

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by

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B.A., Hanover College, 1996

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

STATESBORO, GA

2005

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CHAPTER 1

SPONGE COMMUNITY STRUCTURE AND ANTI-PREDATOR DEFENSES ON TEMPERATES REEFS OF THE SOUTH ATLANTIC BIGHT

Introduction

Predation is an important source of mortality in marine ecosystems and plays a significant role in shaping community structure (Hixon 1997). A variety of morphological, physiological, and behavioral adaptations have evolved to deter predators in marine habitats (Vermeij 1978; Menge and Lubchencho 1981; Paul 1992; Pennings and Paul 1992). An effective anti-predator mechanism that is becoming better understood is secondary metabolites (Paul 1992; Bolser and Hay 1996; Pawlik 1997). A variety of species employ secondary metabolites as a chemical defense and their widespread use has been documented in tropical (Bolser and Hay 1996), temperate, (Wright et al. 1997; Becerro et al. 2003) and polar benthic communities (McClintock 1987). Studies involving marine algae, soft corals, tunicates and sponges have also demonstrated that physical defenses may work independently or in conjunction with chemical defenses to inhibit predation (Harvell et al. 1988; Pennings and Paul 1992; Schupp and Paul 1994; Uriz et al. 1996; Burns and Ilan 2003; Hill et al. 2005). Mineralized elements such as calcium carbonate, siliceous spicules, and organic, proteinaceous fibers may reduce predation by providing a protective outer membrane (Hill and Hill 2002), secondarily lowering the nutritional quality of the prey thereby reducing its dietary attractiveness to predators (Duffy and Paul 1992; Pennings et al. 1994; Chanas and Pawlik 1995), or by irritating the mouth, gut, and stomach of predators (Randall and Hartman 1968).

Sponges are widely distributed in tropical and temperate marine systems and are one of the major taxa, in terms of both biomass and species diversity, found in hard-bottom communities (Sara and Vacelet 1973). Despite the abundance of sponges at all latitudes, the bulk of our understanding of how predation regulates their distribution comes from studies conducted in the tropics. In tropical sponge communities, it has been suggested that the majority of undefended species inhabit environments such as seagrass beds and mangrove roots where predation pressure is minimal or absent. Meanwhile species that occupy coral reefs, where predation pressure is the most intense, possess chemical defenses that discourage predation (Pawlik 1998). This idea has been supported by studies that examined the deterrent properties of secondary metabolites from 71 Caribbean sponge species and through manipulative experiments that transplanted species from seagrass and mangrove habitats to coral reefs (Pawlik et al. 1995; Dunlap and Pawlik 1996; Pawlik 1998). As a result, it has been argued that sponges on tropical reefs produce strongly deterrent chemical compounds while species that yield palatable metabolites are limited to cryptic habitats on the reef or to relatively predator free environments such as mangroves and seagrasses (Pawlik 1997).

Although several studies have investigated how sponge distributional patterns on temperate reefs are controlled by abiotic processes (Roberts and Davis 1996; Bell and Barnes 2000b; Bell and Barnes 2000c), the influence of predation on sponge community structure at temperate latitudes is unknown. For a couple of reasons, temperate reefs located in the South Atlantic Bight (SAB) of the Western Atlantic provide an excellent opportunity to study the effects of predation on the distribution of sponges. First, annual water temperatures within this region drop below the threshold for the survival of

scleractinian corals resulting in sponge and tunicate dominated benthic communities. Second, SAB hard bottom areas consist of two distinct sponge communities immediately adjacent to one another. Natural reefs in the SAB are characterized by limestone ledges ("scarps") that provide one to several meters of vertical, rocky relief surrounded by extensive areas of shifting sand ("plateaus"). The vertical surfaces and first 1-2 m of the flat top of the scarp are typically densely colonized by many small, encrusting or large, amorphous sponge species (here after referred to as the "scarp" sponge community, Figure 1). After the first 1-2 m of the flat top, the reef immediately transitions into the sandy environment and is inhabited predominantly by arborescent, pedunculate, and digitate sponge species (here after referred to as "plateau" sponge community, Figure 1).

Unlike tropical coral reefs, where sponge communities are separated by lagoons or large distances, these two distinct sponge assemblages lay within a few meters of one another. Although spongivorous fishes appear to have the opportunity to forage on the plateau sponge community, they tend to remain within the vicinity of the scarp. As a result, predation intensity may be greatest along the scarp, and may contribute to shaping sponge community structure on temperate reefs. Thus, the goals of this study were to determine: (1) if scarp and plateau sponge communities do indeed differ, (2) if predation pressure is higher on the scarp than on the plateau, and (3) if both of the above are true, does the presence or absence of sponge anti-predator defenses help to explain the sponge community structure on SAB reefs.

Methods

Study Sites

Three sites in the SAB were used for this study: Gray's Reef National Marine Sanctuary (GRNMS) (31° 36.056 N, 80° 47.431 W), J Reef (31° 36.056 N, 80° 47.431 W), and R2 Tower live bottom (31° 24.305 N, 80° 35.490 W). Surveys for sponge and spongivorous fish species distribution and feeding assays to test sponge palatability were conducted at GRNMS and J Reef only. All sites are located within 20 km of each other and consist of either relic scallop shell or limestone ridges of minor to moderate relief (projecting 1-2 m above the bottom) surrounded by large plateau areas of shifting sediment. Water depth ranges from 18 to 30 m. Bottom water temperature ranges from 11ºC during winter to 26ºC in summer (Hunt 1974). The limestone ridges are colonized by a variety of epifaunal species, which together with the scarp form a three-dimensional habitat occupied by a diversity of invertebrate and small cryptic fish species. Preliminary surveys conducted during the summer of 2003 indicated that GRNMS, J Reef, and R2 Tower live bottom all harbor a similar benthic invertebrate fauna.

Sponge Community Structure and Predator Abundance

Surveys were conducted in the summer of 2003 and 2004 at GRNMS and J Reef to determine sponge distribution and abundance. Estimates of mean sponge species richness and densities of individual sponge species were assessed using 0.25 m^2 quadrats haphazardly placed alongside 25 m transects. To avoid over-estimating sponge abundance, amorphous and digitate species were counted as a single individual until clear spatial separation was observed between individuals. Scarp populations were quantified by transects run parallel and on top of the scarp. Transects on the plateau were laid

perpendicular to the scarp and started 2 m behind the elevated side of the scarp. A total of 104 quadrats were sampled from both GRNMS and J Reef; 52 quadrats each for the scarp and plateau habitat for both reefs.

Similarly, 2 transects of 50 m length were run both parallel and perpendicular to the scarp at GRNMS and J Reef to assess predator densities on and off the ledge. Predation on sponges is primarily attributed to a few specialist fishes although other species can include sponges in their diets (Randall and Hartman 1968; Wulff 1994; Meylan 1998). Spongivorous fishes, however, intentionally target sponges which comprise >70% of their diets (Randall and Hartman 1968). Divers swam a 30 minute timed transect and recorded all spongivorous fishes present within diver vision. Visual census is an efficient and reliable method of quantifying fish densities at GRNMS and J Reef because (1) the conspicuous anatomical features of spongivorous fishes make identification straightforward, and (2) water turbidity on these sites often limits side to side visibility to 10 m or less, ensuring that fish occurring far a field of the transect are not recorded.

Sponge Chemical and Structural Defenses

All sponges were collected from either GRNMS or J Reef by SCUBA divers with the exception of *Cliona celata*, which was collected at these two sites as well as the R2 Tower live bottom. Collections were made between May and December 2004. Samples of sponge tissue, up to 10 ml in volume, were removed with dive knives from larger sponges or by collecting whole sponges from the substrate. Sponge samples were placed individually into plastic bags, and stored on ice in coolers at the surface. Sponges were frozen at -80ºC upon returning to Georgia Southern University approximately four hours

after initial collection. A total of 20 species were collected: 11 species from the scarp habitat, 8 from the plateau habitat, and one species evenly distributed across both habitats. A minimum of 30 samples were collected for each species. When possible, sponges were identified on the basis of morphology or spicule and tissue preparations. Confirmation as to the identification of each species was substantiated by Dr. Rob Van Soest, University of Amsterdam.

Methods described by Pawlik et. al. (1995) and Becerro et al. (2003) were followed to isolate crude organic extracts and formulate foods for testing the palatability of sponge secondary metabolites to fishes. For each sample, approximately a 5 ml volume of sponge tissue was measured by displacement of water in a graduated cylinder. Samples were frozen at -80˚C, lyophilized, and weighed to the nearest mg on an electronic balance (APX-60, Denver Instruments, Denver, CO). Freeze-dried samples were crushed with mortar and pestle or cut with scissors into small pieces and extracted three times for 24 hours each in a 1:1 methanol:dichloromethane mixture. Samples remained at 4ºC during extractions. After the third extraction, all extracts were combined and filtered (P8 coarse filter paper, Fisher Scientific Company L.L.C., Pittsburgh, PA) to remove sponge debris. Excess solvent was removed by rotary evaporation (Brinkmann/Buchi Collegiate, Eppendorf, Germany) at low heat (~20ºC) until approximately 5 ml remained. The remaining 5 ml of solvent was transferred to a pre-weighed 20 ml scintillation vial and concentrated to dryness by vacuum evaporation (SC210A-115, Thermo Electron Corporation, Somerset, NJ). The vial containing extract was then reweighed to obtain a crude organic extract weight. The crude organic extract was stored at -80ºC until further use.

The concentrated crude organic extract for each 5 ml replicate was dissolved in 0.75 ml 100% methanol. Samples were sonicated and visually inspected to ensure the extract had dissolved into solution. Artificial food was created using a mixture of 7.5 g powdered squid mantle, 3.5 g Type I carageenan:agar (85:15), and 150 ml of distilled water. The nutritional value of sponge tissue is known to influence its dietary attractiveness to predators (Duffy and Paul 1992; Pennings et al. 1994); therefore, the amount of powdered squid mantle used in food preparation was based upon the mean protein concentration (~20.7 mg ml^{-1}) of 71 Caribbean sponge species surveyed by Pawlik et. al. (1995). In 25 ml batches, the carageenan:agar, squid mantle, and distilled water were thoroughly mixed and heated in a microwave until boiling. Immediately after heating, 4.25 ml of food mixture were poured into each vial containing the 0.75 ml methanol and sponge extract. This mixture was stirred and allowed to cool. When the mixture cooled, it formed a mold that was carefully removed from the vial and cut into 1 x 1 x 1 cm cubes for feeding assays.

To verify that this food preparation process retained the original weight of the extract, several cubes were lyophilized, extracted, filtered, and placed under vacuum to re-isolate the crude organic extract mass. This extract was weighed and compared to the initial weight of the extract isolated from fresh sponge tissue. No significant differences were found between the extract mass weight obtained from fresh sponge tissue and that re-extracted from food cubes ($t = 1.44$, $df = 46$, $p = 0.08$). Thus, the volume of crude organic extract in the carrageen-based food matched the naturally occurring extract concentration of the sponge.

For each replicate, a control cube was prepared by the same method, but with 0.75 ml of methanol only. When appropriate, food coloring was added to control cubes to match the natural color of the crude organic extract so predatory behavior of fishes would not be influenced by cube color.

Methods developed by Uriz et al. (1996) were used to prepare sponge samples for assays of structural defenses. For each sample, fresh sponge tissue containing a random mixture of inner and outer tissue layers was cut into 1 ml pieces. Thirty samples for each species were placed in a 500 ml beaker and extracted three times for 24 hours at 4ºC in 1:1 methanol:dichloromethane. During extractions, water mixed with methanol resulting in a methanol:water phase that separated from the dichloromethane phase. As a result, samples were shaken two or three times during each extraction to ensure that sponge tissue was exposed to both phases. Thin layer chromatography performed on a fourth extraction substantiated all compounds had been removed. After the third extraction, excess solvent was removed and samples were rinsed three times for one minute with distilled water. Samples were placed on cardboard drying racks to air-dry at room temperature overnight allowing any remaining solvent to evaporate. All sponge pieces were bagged and stored at -20ºC until further use.

For food preparation, the intact sponge tissue, with all chemical compounds removed, was thawed and rinsed with distilled water. Artificial food was created using a concoction of 2.5 g powdered squid mantle, 1.2 g type I carageenan:agar (85:15), and 50 ml of distilled water. The carageenan:agar powder, squid mantle, and distilled water were thoroughly mixed and heated in a microwave until boiling so the carageenan:agar would set. If necessary, food coloring was added to the mixture to match as closely as

possible, the natural color of the sponge. After heating, sponge pieces were added to the food mixture, stirred, and allowed to soak until the mixture was almost solid. Only when the food mixture was slightly viscous were the sponge pieces removed. This method allowed for absorption of artificial food into the sponge tissue and embedded the sponge inside a food cube. As a result, structural food cubes offered in my feeding assays were of high nutritional value because they contained natural levels of sponge protein in addition to the protein of the food cube recipe. Thus, deterrence by structural elements found in this study was assigned as a negative response by fishes to these defenses rather than a result of lowering the nutritional quality of the prey (Chanas and Pawlik 1995). Control cubes were prepared in the same manner but without the addition of sponge tissue.

All feeding assays were conducted at GRNMS or J reef. Food cubes were dispensed individually to natural assemblages of reef fish. Several control cubes were released first to initiate feeding activity. Control and test cubes were then offered in a random sequence so fish could not habituate to a systematic pattern of deterrent cube release. Divers recorded if the cube was accepted or rejected and recorded the species of predator responsible for consumption. A food cube was considered unpalatable if fishes rejected it three or more times or if it sank to the bottom uneaten. For both the chemical and structural assays, the most common generalist predatory reef fishes at GRNMS and J Reef were targeted. These fishes were black seabasses (*Centropristus striata*), tomtates (*Haemulon aurolineatum*), and spottail pinfish (*Diplodus holbrooki*). If chemical compounds or structural mechanisms produced by sponges are effective defenses, they should be aimed at generalist predators in particular because (1) they are less likely to

have evolved the abilities to circumvent specialized defenses, and (2) they represent the majority of predators on reef ecosystems (Pawlik et al. 1995; Becerro et al. 2003). Thus, the results of these assays should reflect a general pattern of predator deterrence occurring on SAB reefs. For all assays, a minimum of 30 control and sample cubes were offered for each sponge species.

Transplantation Experiments

To further investigate if there is a relationship between predation pressure, anti-predator defenses, and sponge community structure on SAB reefs, reciprocal transplant experiments were carried out between the scarp and plateau sponge communities. Twenty four bricks (20 x 10 x 6 cm) were deployed at J Reef: 12 each on the scarp and plateau. Six of the bricks in each habitat remained uncaged while 6 were enclosed in Vexar mesh (5 cm^2 opening) cages. Each brick contained predrilled holes in the top and bottom and was secured to the reef with a stainless steel rod. The rods were sunk into holes drilled into the substrate with a pneumatic drill (Chicago Pneumatic, CP785H, Rock Hill, SC) and secured with marine epoxy. Each pair of caged and uncaged bricks was placed within 1 m of each other and labeled with flagging tape.

Three scarp species, *Chondrilla nucula*, *Chondrosia collectrix*, and *Hyrtios violaceaus* were transplanted to the plateau, and 4 plateau species, *Axinella waltonsmithi*, *A. pomponiae*, *Desmapsamma anchorata*, and *Ptilocaulis walpersi* were moved to the scarp. Species used in these experiments were selected based on the results of feeding assays and their ability to tolerate subsampling (i.e. preliminary transplants revealed rapid mortality in transplanted *Ircinia felix* and *Ircinia campana* samples on caged bricks). Samples, 3-20 ml in size, depending on the species, were carefully removed from larger

colonies, or when appropriate, whole colonies were gently uplifted from the substrate to minimize tissue damage and exposure of inner tissue layers. Samples were placed in plastic bags underwater, brought to the surface, and immediately emptied into large coolers containing aerated seawater. At sea, the volume of each sample was measured to the nearest 0.5 ml by displacement of seawater in a 100 ml graduated cylinder and returned to the cooler containing the aerated seawater. Sponges for a single caged or uncaged replicate were measured, strung on monofilament line (9 kg test) ~2.5 cm apart, and placed into a labeled plastic bag containing aerated seawater. For example, a replicate for a caged scarp transplant would contain one sample each of *Axinella waltonsmithi*, *A. pomponiae*, *Desmapsamma anchorata*, and *Ptilocaulis walpersi*. A total of 6 replicates were prepared for each treatment for the scarp and the plateau transplants. Sponges were returned to the bottom and attached with the monofilament line to bricks in the appropriate habitat and treatment within 3 hours of initial collection. For caged bricks, cages were cable tied shut after the sponges were attached. After 9 days, sponges were collected, placed in plastic bags, and measured as described before. This experiment was repeated twice.

Statistical Analysis

Differences in sponge community structure were compared with a two-way ANOVA using reef (GRNMS or J Reef) and habitat (scarp or plateau) as factors for the following variables: mean sponge species richness, overall sponge density, and the density of individual sponge species if the species was recorded at 3 or more sites (ie. GRNMS-scarp, J Reef-scarp, GRNMS-plateau, J Reef-plateau). When an individual species was recorded at only two sites, a one-way ANOVA was used to compare the

density of that species. The density of spongivorous fishes between habitats was compared with a one-way ANOVA. Shannon-Weaver diversity (*H'*) and Pielou's evenness indices were calculated and compared across all 4 sites and Jaccard's coefficient of similarity for species presence/absence data was compared across reefs and between habitats (Sokal and Rohlf 1995).

An index of sponge chemical and structural deterrence was generated for each species by dividing the number of food cubes rejected by the total number offered. Mean chemical and structural deterrence for the scarp and plateau sponge community was calculated as the percentage of food cubes rejected for all species assayed in each community. To determine if mean chemical or structural deterrence differed within the scarp or plateau sponge community, the chemical and structural deterrence for each species associated with their respective community was compared with a Wilcoxon paired-sample test. To evaluate if overall deterrence varied across the scarp and plateau sponge communities, the chemical and structural deterrence for each species was combined, ranked, and compared with a Kruskal-Wallis test. *Cliona celata* was omitted from the community analyses because it was found to be in equal abundance in the scarp and plateau habitats.

To determine if predation occurred on sponges transplanted between the scarp and plateau habitats, differences in sponge volume were compared between caged and uncaged treatments for each species with a Wilcoxin paired-sample test.

Results

Sponge Community Structure and Predator Abundance

Mean sponge species richness and sponge density were similar for GRNMS and J Reef (Figures 2 & 3, Tables 1 & 2). Within reef, no significant differences in mean sponge species richness occurred between habitats (Figure 2, Table 1). All 4 sites (GRNMS-scarp, J Reef-scarp, GRNMS-plateau, J Reef-plateau) differed by <1 species m^{-2} (Figure 2). However, within reef sites, sponge density was significantly higher on the scarp than on the plateau (Figure 3, Table 2). The marked decrease in sponge abundance in the plateau community is due to the absence of *Chondrilla nucula, Chondrosia collectrix,* and *Scopalina ruetzleri.* Combined, these three species account for 75% of the sponges occurring on both GRNMS and J Reef scarps.

The scarp at both reefs is dominated by *Chondrilla nucula* with this species responsible for ≥64% of the sponges recorded. *Ircinia felix, Chondrosia collectrix, Scopalina ruetzleri,* and *Spirastrella sp*. were the four next most abundant species on the scarp at both reefs (Table 3). Together, these five species account for >88% of the sponges colonizing the scarp. In contrast, sponge populations on the plateau do not appear to be dominated by any single species or any small subset of the species present. The four most abundant plateau species were *Axinella waltonsmithi, Axinella bookhouti, Cinachyrella alloclada,* and *Axinyssa ambrosia* (Table 3), but combined, these species account for <35% of the sponges on the plateau.

Sponge diversity (*H'*) and evenness (J) reflect the similarities in sponge community structure across reefs but also highlight the differences between habitats within reef. Sponge diversity and evenness for J Reef and GRNMS are nearly identical

(Table 4), but the value of these indices is nearly double on the plateau as opposed to the scarp (Table 5). Again, these results are due to 5 sponge species accounting for >88% of the sponge populations on the scarp while the density of many species on the plateau is similar (Table 3).

Of the 32 species recorded from both sites, 31 are represented at J Reef and 28 at GRNMS (Table 4). Twenty-seven species were common to both reefs, 4 species were exclusive to J Reef, and 1 to GRNMS. Plateau sites had greater species richness than scarp locations (Table 5). Little overlap occurred in the distribution of sponge species between the scarp and plateau (Figure 4). Of the 32 species found at GRNMS and J Reef the density of 30 species was significantly higher in either the scarp or plateau habitat. In 17 of these 30 cases the species was exclusive to one habitat. Interestingly, these 30 species are evenly split between the two habitats: 15 species are significantly more abundant or solely occupy the scarp and likewise 15 for the plateau (Table 5).

The morphological characteristics of the sponges also vary with habitat. Fifteen of the 21 species recorded on the scarp at GRNMS and J Reef are amorphous or encrusting species (Tables 3 & 5). The majority of these species belong to the characteristically amorphous and encrusting sponge families of Geodidae, Chondrosiideae, Spongiidae, Thorectidea, Dysidea, and Aplysinellidae and are well represented on SAB scarps. *Aplysina fulva* and *Clathria prolifera*, however, were exceptions to the predominant morphotypes occurring on the scarp. *Aplysina fulva* is branching and *Clathria prolifera* is pedunculate, and both species were significantly more abundant on scarp (Table 3). Meanwhile, the majority of plateau species are arborescent (branching), pedunculate or digitate sponges. Fifteen of the 23 species present on the

plateau fell into one of these 3 categories (Tables 3 & 5). A boring species of *Cliona* is the only encrusting phenotype that was more abundant on the plateau than on the scarp (Table 3). *Cinachyrella alloclada* was not included in this analysis because it did not fit into any of the morphological classifications used here.

During routine dives I observed 5 species of spongivorous fishes at GRNMS or J Reef. They were *Holocanthus bermudensis* (blue angelfish), *Cantherhines macrocerus* (orange-spotted filefish), *Lactophrys quadricornis* (scrawled cowfish), *Pomacanthus arcuatus* (gray angelfish), *and Pomacanthus paru* (french angelfish). Of these 5 however, only 2 species, *H. bermudensis* and *C. macrocerus*, were common and recorded during surveys. The density and distribution of spongivorous fishes was similar at GRNMS and J Reef. Spongivorous fish were observed only on the scarp and 75% of the fishes recorded were *H. bermudensis* (Figure 5).

Tests for Sponge Chemical and Structural Defenses

Food cubes containing sponge chemical extracts varied widely in their ability to deter predation by fishes. Food cubes for any single sponge species assayed were neither consumed nor rejected 100% of the time. A wide range of deterrence was observed in the scarp community. *Aplysina fulva* was the most deterrent species with 91% of its food cubes rejected, while *Hyrtios violaceaus* showed the least deterrence with only 3% of its food cubes rejected (Figure 6). Deterrence was not as variable for the plateau species as food cubes for 5 of the 8 species including *Axinella pomponiae, Axinella bookhouti, Cinachyrella alloclada, Desmapsamma anchorata, and Ptilocaulis walpersi* were all rejected <25% of the time (Figure 6).

Deterrence of fish feeding by food cubes containing intact, extracted sponge tissue also varied widely among species. However, in contrast to the results for chemical defenses, structural deterrence by scarp sponge species was less variable than species assayed from the plateau. Food cubes were rejected by fishes from 25 to 75% of the time for 6 of the 8 plateau species, while deterrence of fishes occurred <25% of the time for 9 of the 11 scarp species (Figure 7).

Deterrence of fish feeding through chemical defenses was significantly higher than structural defenses for species from the scarp community (Figure 8). No significant difference in mode of deterrence was detected for sponges from the plateau (Figure 8). When the results of the chemical and structural assays are combined, no significant differences in overall deterrence occurred across communities (Figure 9).

The trends in habitat-specific patterns of defense are correlated with sponge morphology. The highest levels of chemical deterrence were observed in the aspiculate, amorphous and encrusting species colonizing the scarp. Combined, chemical food cubes from the 8 aspiculate species, *Hyrtios violaceaus, Chondrosia collectrix, Ircinia felix, Ircinia campana, Dysidea fragilis, Coscinoderma lanuga, Aplysina fulva,* and *Aiolochroia crassa,* deterred fishes 44.2% (± 10.8 SE) of the time. In contrast, the lowest level of deterrence was found in the arborescent and pedunculate sponges inhabiting the plateau. Food cubes from the 5 species in this cateogory, *Axinella bookhouti, Axinella pomponiae, Axinella waltonsmithi*, *Desmapsamma anchorata,* and *Ptilocaulis walpersi*, only discouraged feeding 21.4% (± 10.3 SE) of the time. A converse pattern was observed for structural deterrence. The 8 aspiculate species deterred predation only

12.2% (± 1.7 SE), whereas food cubes for the 5 arborescent and pedunculate species were deterrent 41.9% (± 8.6 SE) of the time.

Three species of fish were primarily responsible for the consumption of food cubes at GRNMS and J Reef. All three species, *Centropristus striata* (black seabass), *Haemulon aurolineatum* (tomtate grunt), and *Diplodus holbrooki* (spottail pinfish), represent the most abundant generalist fish predators encountered on SAB temperate reefs. *Centropristus striata* consumed the greatest percentage of food cubes and consumed chemical and structural cubes in equal proportions (Figure 10). *Haemulon aurolineatum* consumed a greater number of chemical food cubes than structural, while the reverse was true for *Diplodus holbrooki* (Figure 10).

Transplant Experiments

All sponges transplanted across habitats showed reductions in volume during the 9 day experiment (Figure 11). Reductions in volume were significantly larger for uncaged treatments for 3 of the 4 plateau species transplanted to the scarp. One scarp species transplanted to the plateau, *Hyrtios violaceaus*, showed significantly larger volume reductions in uncaged treatments (Figure 13).

Predation scars on the plateau species transplanted to the scarp were clearly evident, but across sponge species different types of predators appeared to be responsible. Invertebrate grazing scars were apparent on *Axinella waltonsmithi* while fish bites were almost exclusively observed on *Ptilocaulis walpersi*. *Desmapsamma anchorata* was preyed upon by both fishes and invertebrates. Interestingly, no predation scars were evident on *Hyrtios violaceaus*.

Discussion

Sponges are important components of marine benthic communities spanning tropical, temperate, and polar habitats (Dayton et al. 1974; Targett and Schmahl 1984; Alvarez et al. 1990; Bell and Barnes 2000b). Until this study, abiotic processes like sedimentation, current regimes, and periodic disturbances from storms were considered the major factors influencing sponge distributional patterns on temperate reefs (Roberts and Davis 1996; Bell and Barnes 2000b; Bell and Barnes 2000a). Results presented here demonstrate that predation and the ability of sponges to resist predation may also contribute to sponge community structure on temperate reefs. These findings are in agreement with studies performed in the tropics demonstrating that sponges lacking chemical defenses may be restricted to environments where there is a relative paucity of predators, while chemically defended species can persist in habitats where predation is intense (Pawlik et al. 1995; Pawlik 1998). The results of this study also show that sponges may be structurally defended, but these defenses may not be adequate in areas with high spongivore predation pressure. The fact that a large majority of the sponge species tested here discouraged predation by generalist predators further demonstrates that sponges are well defended irrespective of geographic location (McClintock 1987; Pawlik et al. 1995; Becerro et al. 2003).

The data presented here confirms that sponge populations on SAB reefs are divided into two distinct communities. The distribution and density of sponge species were significantly different across the scarp and plateau communities (Table 5). A disjunct distribution of spongivorous fishes was also observed between the two habitats with a significantly higher abundance of predators occurring on the scarp as opposed to

the plateau (Figure 5). Deterrence of predation by chemical defenses was found to be more common in sponges on the scarp than on the plateau (Figure 8). While a positive correlation between the presence of chemical defenses and the abundance of fish predators on SAB reefs clearly exists, it should be pointed out that other factors may account for the high levels of chemical defenses observed in scarp sponges.

Secondary metabolites in sponges have multiple ecological functions. Besides anti-predator properties, secondary metabolites are effective anti-viral and anti-fouling agents and may impede the settlement of competing species (Uriz et al. 1992; Kubanek et al. 2002). Much like coral reefs, space on SAB scarps is limited and competition between sessile organisms intense. Sponges can enhance their competitive ability through allelopathy or by increasing their toxicity (Becerro et al. 1995; Thacker et al. 1998). Greater predation intensity on the scarp may also result in a greater frequency of attack. Sponges primarily suffer partial rather than complete predation when inflicted by spongivorous fishes often leaving the animal with exposed wounds (Wulff 1994). Secondary metabolites have anti-pathogen properties (Kubanek et al. 2002) and if sponges are producing these compounds to neutralize infection after attack (Walker et al. 1985), the correlation between predation intensity and the frequency of attack on scarp sponges may indirectly stimulate the production of these disease-resistant compounds. In contrast, competition between species is lower on the plateau because sedimentation most likely restricts the recruitment of many sessile species that are otherwise common on nearby rocky substrates (Zea 1993) and the susceptibility to attack is lower due to the absence of many predators on the plateau.

The results from this study suggest that natural sponge tissue with the crude organic extracts removed can deter predation by generalist fishes (Figure 7). The highest levels of structural deterrence were observed in the plateau sponge community (Figure 8) with the branching and arborescent species deterring predators 42% of the time. It has been suggested that these growth forms are well suited to tolerate sediment laden environments because their morphologies resist sediment accumulation that could lead to suffocation (Trammer 1983; Bell and Smith 2004). Species possessing this phenotype often require spicules to stabilize their erect growth forms (Koehl 1982). All of the arborescent and pedunculate species tested in this survey contained aggregations of spicules peripherally arranged throughout their tissue (Wells et al. 1960; Wiedenmayer 1977; Alvarez et al. 1998). As a result, spicules may not only provide structural support, but may aid in deterring generalist predators. Indeed, this idea has been proposed before. The physical defense theory holds that skeletal elements not only stabilize the structure of an organism, but may also serve as an exaptation providing a functional defense for the species (Gould and Vrba 1982; Jones et al. 2005).

The importance of physical defenses has been demonstrated in terrestrial and marine plants (Coley and Aide 1991; Pennings and Paul 1992; Hay et al. 1994) and parallels have been drawn between herbivory and grazing on sponges (Burns and Ilan 2003). Both plants and sponges are abundant, diverse, conspicuous, lack behavioral defense, and use structural elements such as lignified and proteinaceous fibers or mineralized compounds such as calcium carbonate or siliceous spicules to deter predation (Coley and Aide 1991; Hay et al. 1994; Burns and Ilan 2003; Hill et al. 2005). In addition, structural elements are often poor in nutritional quality and difficult to digest

and may thus inadvertently reduce the dietary attractiveness of the prey to predators (Duffy and Paul 1992; Chanas and Pawlik 1995).

Previous studies have produced contradictory results that structural elements in sponges can provide an effective defense against predators (Chanas and Pawlik 1995; Burns and Ilan 2003; Hill et al. 2005). Earlier studies, however, investigated the palatability of spicules in isolation or in unnatural orientations that disrupted the integrity of the sponge tissue (Chanas and Pawlik 1995; Burns and Ilan 2003; Hill et al. 2005). Two inherent problems arise from these earlier methodologies. First, spicules in their natural form are often concentrated in a particular region of the sponge skeleton in a specific orientation. This organization is disrupted when the skeleton is disassociated and reinserted into food cubes (Burns and Ilan 2003). Second, additional structural elements such as the outer cortex of the sponge and the fibrous network of spongin are eliminated and provide an incomplete test of sponge structural elements.

The integrity of the sponge tissue used in feeding assays for this study was maintained because the preparation of sponge structural elements in food cubes did not disassociate or remove parts of the sponge skeleton. Therefore, I feel this study provided a comprehensive test of sponge structural defenses against generalist predators. The evidence that spicules enhanced deterrence was supported by the high palatability of the 8 aspiculate species. The deterrence of fishes by food cubes formed from these 8 species, *Hyrtios violaceaus, Chondrosia collectrix, Ircinia felix, I. campana, Dysidea fragilis, Coscinoderma lanuga, Aplysina fulva,* and *Aiolochroia crassa,* were all below 12% (Figure 7).

As demonstrated in the feeding assays, no species, in either chemical or structural assays was consumed or rejected 100% of the time. Several factors may account for this variability. First, the predation history of the individuals sampled for each sponge species was unknown. It has been demonstrated that after simulated attack a sponge can increase both the production of secondary metabolites and spicules (Walker et al. 1985; Hill and Hill 2002). An induced response such as this would contribute to population-level variability in anti-predator defenses. Second, the inability of sponge defenses to deter fish predators 100% of the time may also be a product of the predators used in this study. Predators can exhibit species specific responses to prey defenses (Pennings et al. 1994). In my study, *Haemulon aurolineatum* consumed more chemical than structural food cubes while the opposite was true for *Diplodus holbrooki* (Figure 10). Fish assemblages on SAB reefs varied across reefs, feeding trials, and diving days. This variation in predator diversity and feeding behavior may explain why food cubes for any single sponge species were neither wholly consumed nor rejected. Interestingly, this result points out the importance of having sponge chemical and structural defenses that are effective against a diversity of generalist predators (Pawlik et al. 1995; Becerro et al. 2003).

At first, it would appear from the results of the feeding assays that the distribution of sponge anti-predator defenses is not correlated with predation intensity on SAB reefs (Figure 9). The transplant experiments however, provide evidence that predation on sponges does occur in both habitats and that predation is more intense on the scarp than on the plateau (Figure 11). In addition to spongivorous fishes, invertebrates preyed upon the plateau species transplanted to the scarp. Predation by invertebrates was

substantiated when one replicate set of transplanted plateau species was recovered from a crevice within the scarp rather than from its brick. When removed from the crevice, only the central core of the sponge *Desmapsamma anchorata* remained while all periphery tissue had been consumed. This type of reduction occurred often for individuals of *D. anchorata* and is highly indicative of crab predation. Although invertebrate predators were not quantified across habitats, it is likely that predators such as crabs, starfish, and sea urchins are more abundant along the scarp because of the greater topographic complexity of this habitat. Unfortunately, whether fishes or invertebrates preyed upon *Hyrtios violaceaus* (transplanted from the scarp to the plateau) could not be determined because replicates for *H. violaceau*s never suffered from partial predation. When recollected, individuals of *H. violaceaus* were either entirely absent or matched the initial volume of the individual before transplantation.

The higher levels of sponge predation by both fishes and invertebrates may warrant the greater employment of chemical defenses by scarp sponges (Figure 8). Spongivorous fishes and echinoids can tolerate the consumption of spicules (Randall and Hartman 1968; Birenheide et al. 1993; Wulff 1994; Pawlik 1998). Similarly, crabs may be less deterred by spicules because they can avoid spicule consumption through their feeding behavior (Waddell and Pawlik 2000a) or tolerate them when combined with chemically attractive prey (Hill et al. 2005). Indeed, it appears that invertebrates, in general, are less deterred by spicules than generalist reef fishes (Waddell and Pawlik 2000b; Waddell and Pawlik 2000a; Burns and Ilan 2003; Jones et al. 2005) but are equally so, or more readily deterred by chemical extracts than fishes (Waddell and Pawlik 2000a; Burns et al. 2003). In my study, generalist fishes reacted negatively to

structurally intact sponge tissue with the chemical compounds removed. Therefore, sponge structural defenses may deter many reef fishes occurring on the plateau, but may not be adequate against predators inhabiting the scarp that have evolved the ability to either tolerate or remove the defensive potential of structural components. Thus, chemical defenses may be a necessity for sponges to exist on the scarp.

What was not considered in this study was the synergistic effect of chemical and structural defenses on predators. Most studies that have examined sponge community structure in relation to predation and anti-predator defenses have done so by testing chemical and structural defenses in isolation rather than in combination. Evidence has been provided from several sponge species that chemical and structural defenses may interact to enhance deterrence against predators and that the effectiveness of these defenses may be underestimated when tested separately (Burns and Ilan 2003; Hill et al. 2005; Jones et al. 2005). No sponge species in my study was completely undefended, either chemically or structurally, from predators. This finding allows for the possibility of synergy in terms of defensive mechanisms and I would recommend that future studies use food cubes containing both structural and chemical elements to further advance our understanding of the relationship between anti-predator defenses and sponge community structure.

In three important ways, the results of this study expand our understanding of how predation and anti-predator defenses may influence sponge community structure. First, contrary to what has been thought (Chanas and Pawlik 1995; Chanas and Pawlik 1996), structurally intact sponge tissue is an effective defense against generalist reef fishes. Although the primary role of spicules is to stabilize the skeleton of a sponge (Koehl

1982), they may also be an exaptation for defense against generalist reef fishes. Second, predation by invertebrates may influence sponge distributional patterns more than previously thought. Predation on sponges on tropical reefs has primarily been attributed to spongivorous fishes (Dunlap and Pawlik 1996; Pawlik 1998) and turtles (Meylan 1998) even though evidence has suggested that invertebrates can restrict the distribution of some sponge species in arctic (Dayton et al. 1974) and seagrass benthic communities (Wulff 1995). The results of my study indicate that both spongivorous fishes and invertebrates can regulate the distribution of sponges. Lastly, my findings agree with those reported from tropical systems that chemical defenses in sponges may be more important in deterring predators than structural mechanisms in habitats with high predation intensity (Pawlik et al. 1995; Waddell and Pawlik 2000a). Thus, chemically undefended species may be restricted to habitats where predation intensity is lower even when those habitats are found in close proximity.

Table 1. Two-way ANOVA for mean species richness of sponges at two reefs (GRNMS and Reef) and two habitats (scarp and plateau) within each reef.

Source	SS	d.f.	*F*	*P*
Reef, R	0.58	1	0.17	0.67
Habitat, H	4.04	1	1.198	0.27
R x H	1.73	1	0.514	0.47
Within	688.01	204		
Total	694.37	207		

Table 2. Two-way ANOVA for mean density of sponges at two reefs (GRNMS and Reef) and two habitats (scarp and plateau) within each reef.

Source	SS	d.f.	*F*	*P*
Reef, R	81.25	1	1.17	0.28
Habitat, H	19578.48	1	282.49	<0.001
R x H	3.76	1	0.05	0.81
Within	14138.42	204		
Total	33801.92	207		

Table 3. Summary of distributional data, morphological classification, and skeletal structure for sponges found at GRNMS and J Reef. Habitat listed refers to the reef zone in which that sponge species is significantly more abundant or solely occurs: Sc = Scarp, Pl = Plateau, and ND = no difference in density between habitats. Density of individual species equals mean number of individuals ± SD recorded at the 2 scarp sites (GRNMS-scarp, J Reef-scarp) and 2 plateau sites (GRNMS-plateau, J Reef plateau). *N* = 104 quadrats of 0.25 m^2. Morphological classifications for sponges based upon descriptions by Esnault and Rutzler (1997): A = Amorphous/Massive, E = Encrusting, B = Branching, P = Pedunculate, D = Digitate, and N/C = not classified. For skeletal structure A = Aspiculate and S = Spiculate. Species listed by habitat and in order of highest to lowest density within habitat.

Sponge Species	Habitat	Scarp Density	Plateau Density	Morphotype	Skeleton
Chondrilla nucula	Sc	17.5 ± 9.5		E	S
Ircinia felix	Sc	2.4 ± 1.5	0.3 ± 0.6	A	A
Chondrosia collectrix	Sc	1.9 ± 3.1		E	A
Scopalina ruetzleri	Sc	1.4 ± 1.6		E	S
Spirastrella sp.	Sc	1.4 ± 1.8	<0.1 ± 0.1	E	S
Aplysina fulva	Sc	0.7 ± 1.2	0.1 ± 0.7	B	A
Ircinia campana	Sc	0.5 ± 0.8	<0.1 ± 0.1	A	A
Hyrtios violaceaus	Sc	0.5 ± 0.9	<0.1 ± 0.1	A	A
Clathria prolifera	Sc	0.4 ± 0.7	<0.1 ± 0.1	P	S
Dysidea fragilis	Sc	0.2 ± 0.7		A	A
Coscinoderma lanuga	Sc	0.2 ± 0.6		A	A
Geodia gibberosa	Sc	<0.1 ± 0.1		E	S
Aiolochroia crassa	Sc	<0.1 ± 0.1		A	A
Myriastra sp.	Sc	<0.1 ± 0.1		E	S
Smenospongia cerebriformis	ND	0.1 ± 0.4	<0.1 ± 0.1	A	A
Cliona celata	ND	<0.1 ± 0.1	<0.1 ± 0.1	E	S
Aplysilla longispina	ND	<0.1 ± 0.1	<0.1 ± 0.1	E	A
Axinella waltonsmithi	Pl	<0.1 ± 0.1	1.0 ± 1.1	P	S
Cinachyrella alloclada	Pl	<0.1 ± 0.1	0.9 ± 1.3	N/C	S
Axinella bookhouti	Pl	<0.1 ± 0.1	0.6 ± 0.9	P	S
Dark finger sp.	Pl		0.5 ± 0.9	D	S
Unidentified sponge	Pl	<0.1 ± 0.1	0.5 ± 0.8	N/C	N/C
Axynissa ambrosia	Pl		0.5 ± 0.8	D	S
Higginsia strigilata	Pl		0.4 ± 0.7	P	S
Raspailia sp.	Pl		0.4 ± 0.7	D	S
Axinella pomponaie	Pl	<0.1 ± 0.1	0.3 ± 0.6	B	S
Desmapsamma anchorata	Pl	<0.1 ± 0.1	0.3 ± 0.5	B	S
Clathria carteri	Pl		0.2 ± 0.5	P	S
Cliona sp.	Pl		0.2 ± 0.8	E	S
Ciocalypta gibbsi	Pl		0.2 ± 0.5	D	S
Lissodendoryx stigmata	Pl		0.1 ± 0.4	A	S
Ptilocaulis walpersi	Pl	<0.1 ± 0.2	0.1 ± 0.3	B	S

Table 4. Sponge species richness, Shannon-Weaver diversity (*H'*), Pielou's evenness (J), Jaccard's similarity coefficient (*Sj*) for GRNMS and J Reef. Values are listed individually by reef and totals pooled for both reefs. N = 104 quadrats of 0.25 m^2 for GRNMS and J Reef sponge populations.

Classification	GRNMS	J Reef	GRNMS & J Reef
Scarp Species	16	19	21
Plateau Species	19	22	24
Total Species	28	31	32
Diversity (*H'*)	2.08	2.31	2.13
Evenness (J)	0.61	0.67	0.65
Reef Similarity (*Sj*)			0.87
Habitat Similarity within reef (*Sj*)	0.53	0.62	0.61

Table 5. Sponge species richness, species distribution, Shannon-Weaver diversity (*H'*), Pielou's evenness (J), and Jaccard's similarity coefficient (*Sj*) for the scarp and plateau habitats at GRNMS and J Reef. *N* = 104 quadrats of 0.25 m^2 for scarp and plateau sponge populations.

Classification	Scarp	Plateau
Total Species	21	24
Number of sponge species that were significantly more abundant or solely occurred in this habitat	15	15
Number of amorphous and encrusting species	15	8
Number of arborescent, pedunculate, and digitate species	6	15
Diversity (*H'*)	1.40	2.75
Evenness (J)	0.49	0.91
Across Reef Similarity (*Sj*)	0.76	0.72

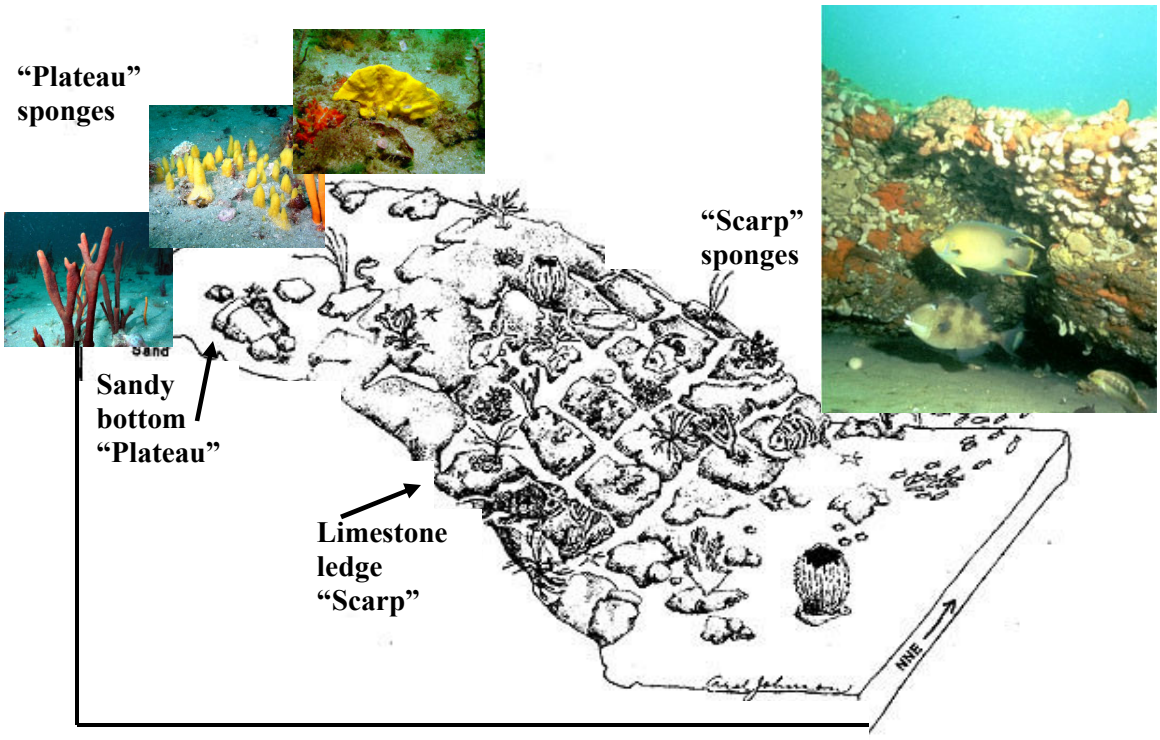

Figure 1. Diagram of a representative South Atlantic Bight temperate reef. Photographs demonstrate the contrasts in sponge morphology that occurs across habitats. Schematic representation of SAB reef by Barans and Henry, 1984. Photograph of scarp sponges from Greg McFall.

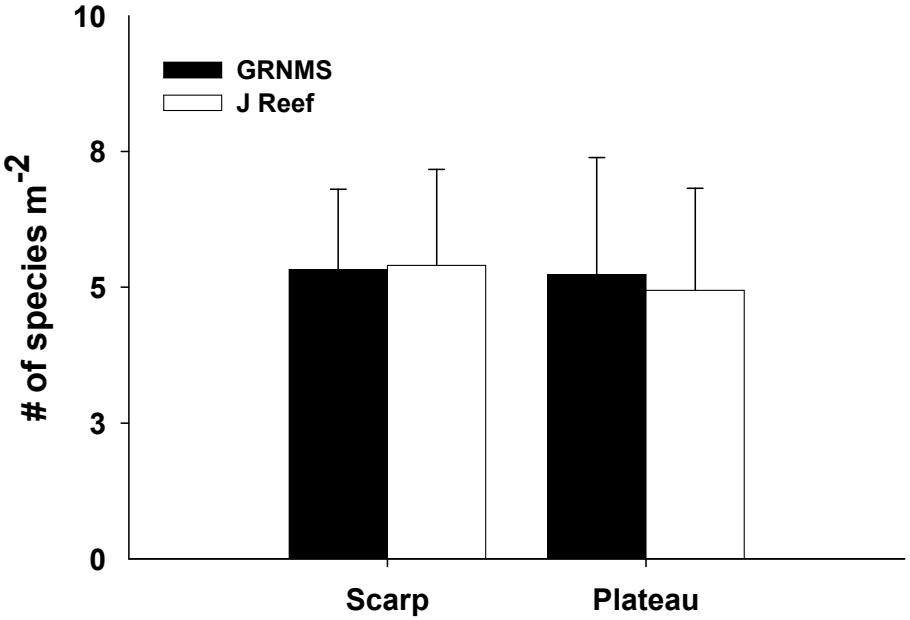

Figure 2. Mean sponge species richness (number of species $0.25m^{-2}$ ± SD) of sponge populations on the scarp versus the plateau at GRNMS and J Reef. N = 52 quadrats of 0.25 m^2 for GRNMS-scarp, J Reef-scarp, GRNMS-plateau, and J Reef-plateau. Significant differences for mean sponge species richness between reefs (GRNMS and J Reef) and habitats (scarp and plateau) are listed in Table 1.

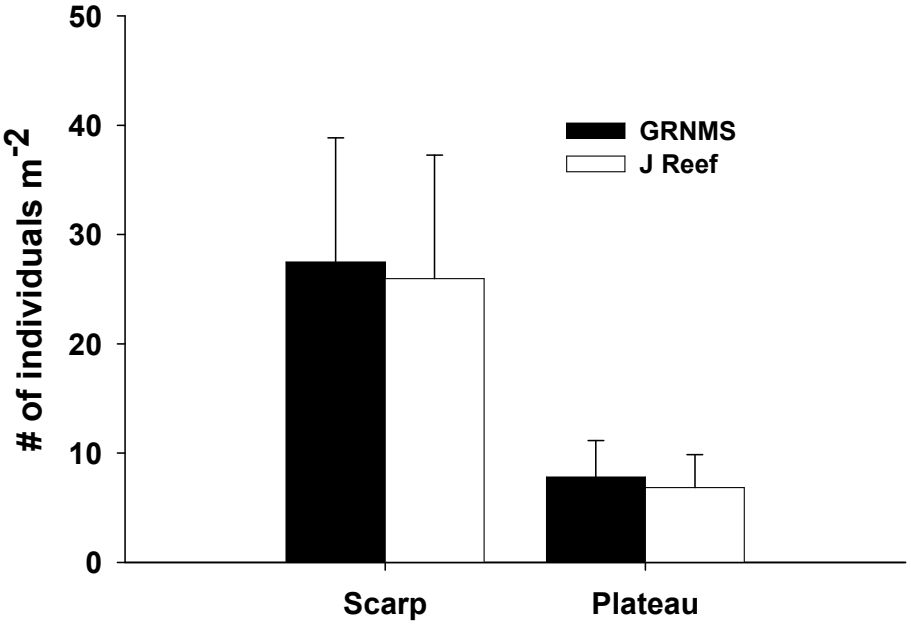

Figure 3. Mean sponge density (no. of individuals $0.25m^{-2}$ ± SD) of sponge populations on the scarp and plateau habitats at GRNMS and J Reef. N = 52 quadrats of 0.25 m^2 for GRNMS-scarp, J Reef-scarp, GRNMS-plateau, and J Reef-plateau. Significant differences for mean sponge density between reefs (GRNMS and J Reef) and habitats (scarp and plateau) are listed in Table 2.

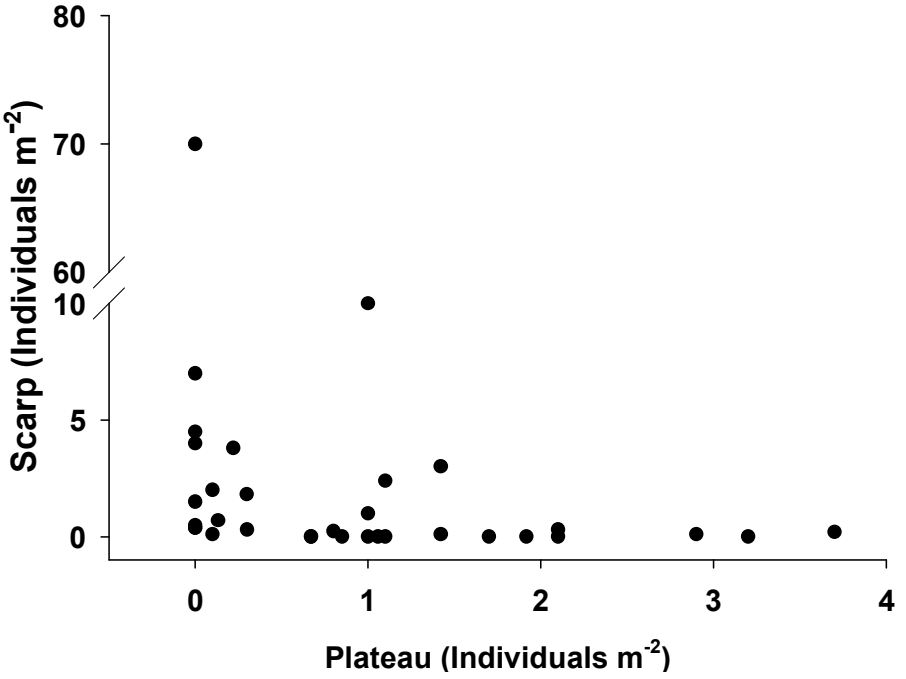

Figure 4. Distribution of the 32 sponge species recorded at GRNMS and J Reef. Each point represents the mean number of individuals m^{-2} for each species. The density of each sponge species on the scarp is plotted against the density for that species on the plateau. N = 104 quadrats of 0.25 m^2 for both species density on the scarp and for the plateau.

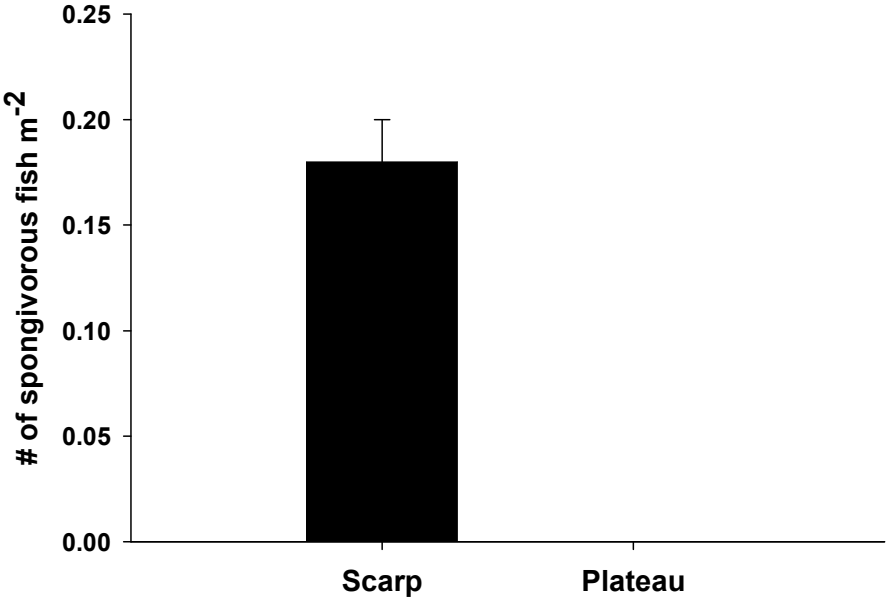

Figure 5. Mean density (± SE) of spongivorous fish populations on the scarp and plateau habitats from GRNMS and J Reef. $N = 4$, 50 m transects for each habitat.

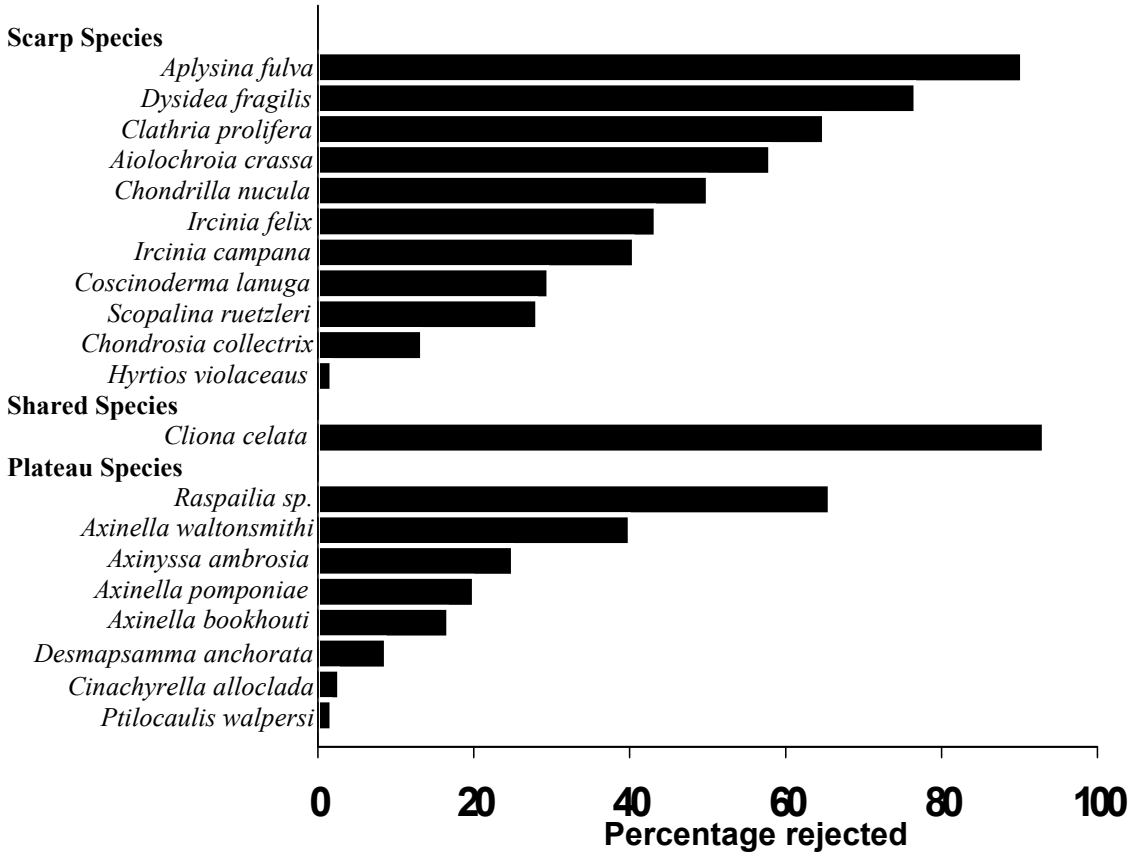

Figure 6. Deterrence of reef fishes at GRNMS and J Reef by food cubes containing the chemical extracts of sponges. Percentage rejected is based upon the number of food cubes going uneaten out of a minimum of 30 offered. Fish, in all cases, consumed all 30 control cubes. Species grouped by habitat and listed in order of highest to lowest deterrence.

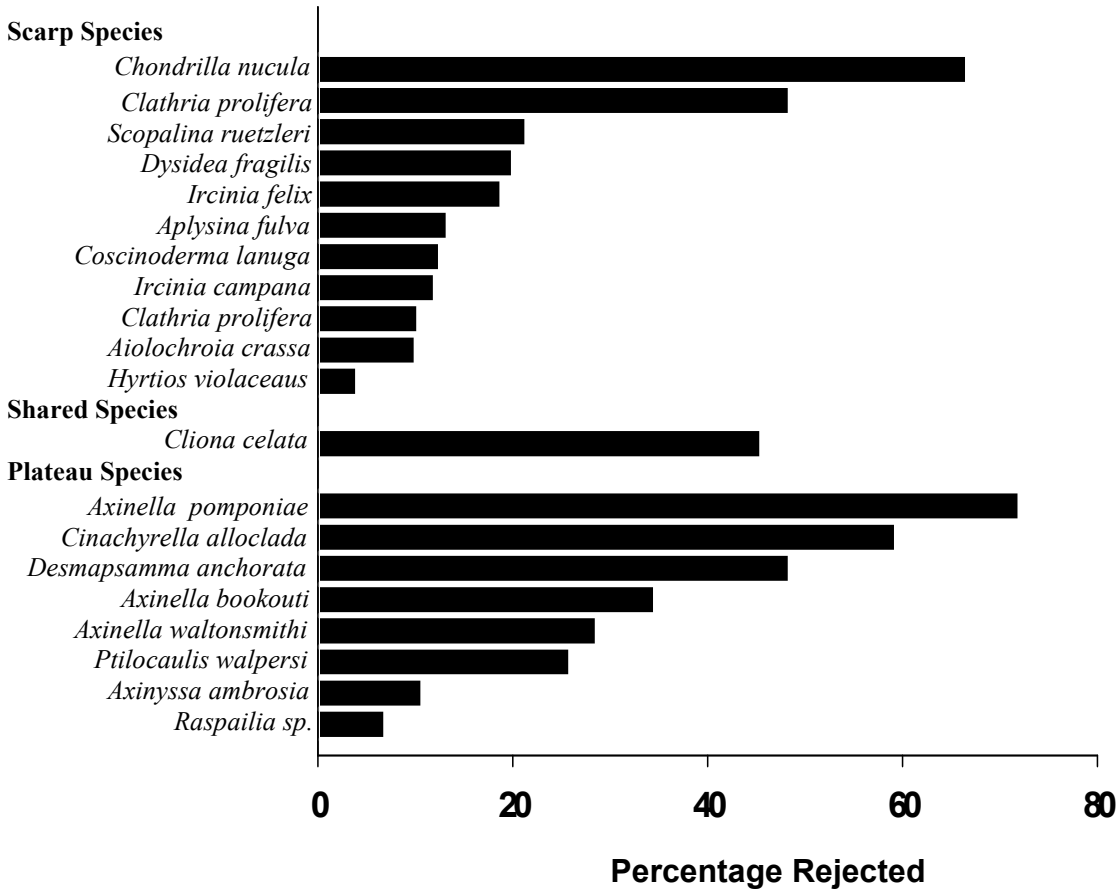

Figure 7. Deterrence of reef fishes at GRNMS and J Reef by food cubes containing whole sponge tissue with the secondary metabolites removed. Percentage rejected is based upon the number of food cubes going uneaten out of a minimum of 30 offered. Fish, in all cases, consumed all 30 control cubes. Species grouped by habitat and listed in order of highest to lowest deterrence.

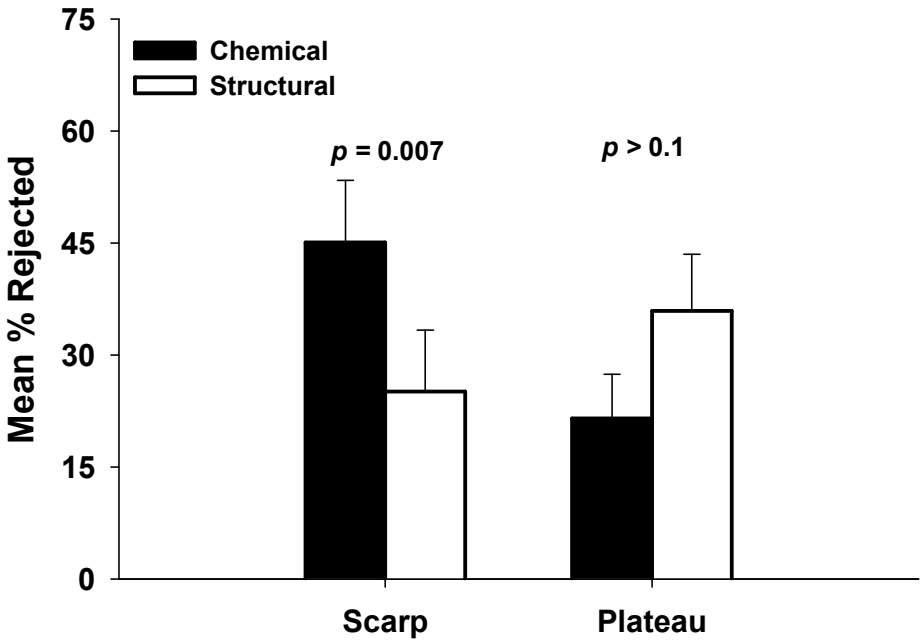

Figure 8. Mean deterrence (± SE) of reef fishes by food cubes containing the chemical extracts of sponges or whole, chemically extracted sponge tissue. Mean deterrence is based upon the percentage of food cubes rejected across species tested in chemical or structural assays. Significant differences in deterrence between assays were tested with a Wilcoxon paired-sample test. The number of sponge species assayed was 11 for the scarp and 8 for the plateau. *Cliona celata* was excluded from the analysis because it was equally abundant in both communities.

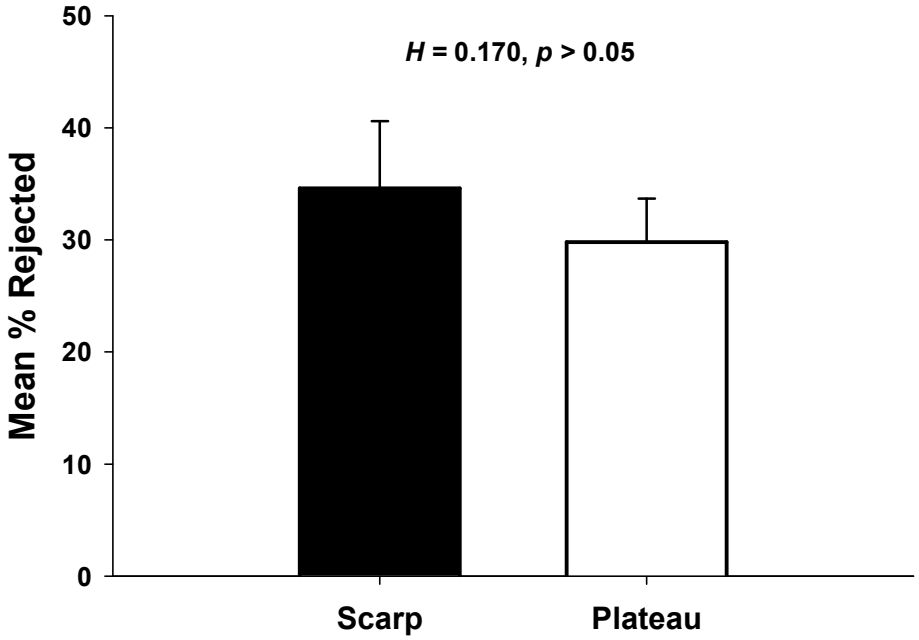

Figure 9. Combined mean deterrence (± SE) of reef fishes by food cubes containing the chemical extracts of sponges and whole, chemically extracted sponge tissue. Mean deterrence is based upon the percentage of total food cubes rejected for each sponge species tested in both assays. Significant differences in mean deterrence across the scarp and plateau sponge communities were tested with a Kruskal-Wallis test. The number of sponge species assayed was 11 for the scarp and 8 for the plateau. *Cliona celata* was excluded from the analysis because it was equally abundant in both communities.

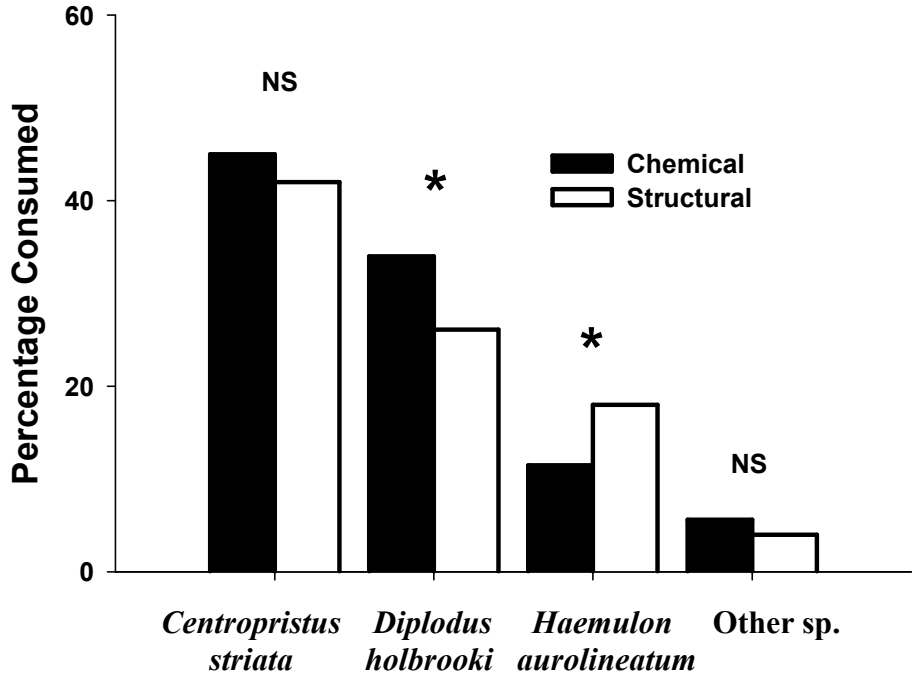

Figure 10. Consumption by fishes of food cubes containing the crude organic extracts of sponges or whole sponge tissue with secondary metabolites removed. Percent consumption equals the number of food cubes consumed by each fish species divided by the total number of food cubes consumed. A total of 475 crude organic extract cubes were consumed and 386 structural food cubes were consumed. Significance based on chi-square analysis comparing the total number of food cubes consumed in either assay: NS, non significant; *, $P < 0.05$.

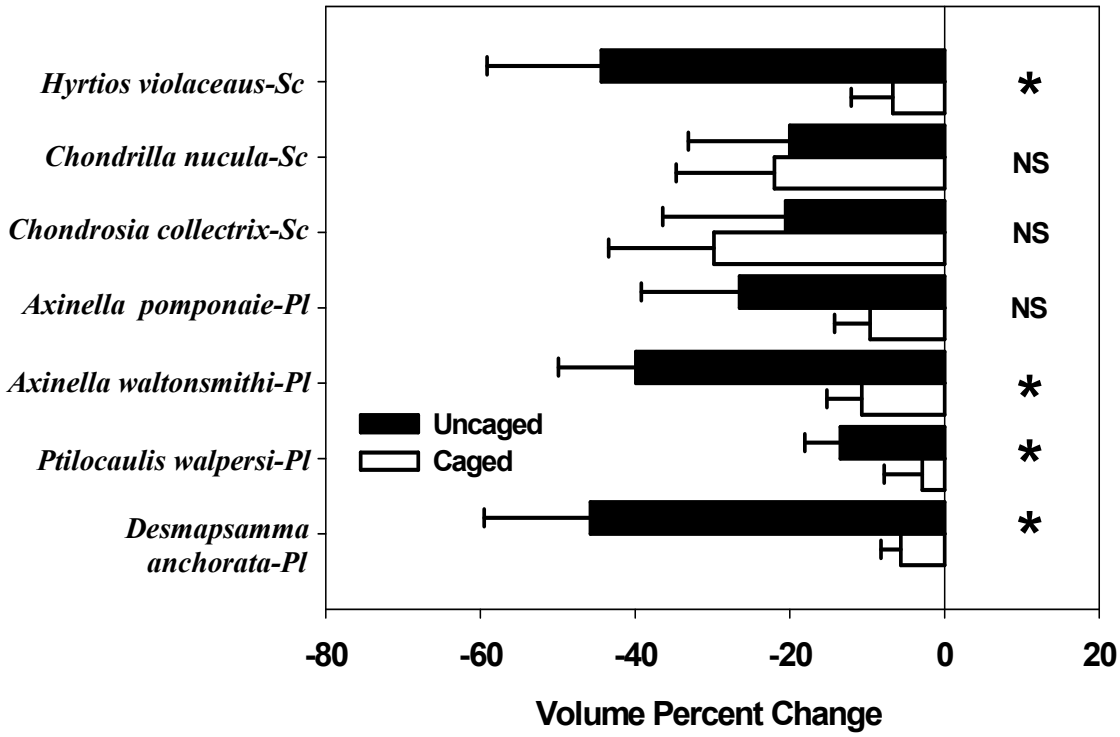

Figure 11. Mean percent change (± SE) in volume after nine days for three scarp species transplanted to plateau sponge communities and four plateau species transplanted to scarp habitats. $N = 11$ for scarp species and $N = 12$ for plateau species. Sc = scarp species, Pl = plateau species. Sample means were compared using a Wilcoxon paired-sample test: NS, non significant, *, $P < 0.05$.

CHAPTER 2

LATITUDINAL DIFFERENCES IN PREDATION PRESSURE AND ANTI-PREDATOR DEFENSES: AN EXAMPLE FROM SPONGE COMMUNITIES.

Introduction

A commonly accepted tenet of biogeography is that the intensity of both herbivory and predation are inversely proportional to latitude (Pianka 1966; Vermeij 1978; Bertness et al. 1981; Menge and Lubchencho 1981; Coley and Aide 1991; Pennings et al. 2001). This biogeographic pattern has been attributed to the greater diversity of consumers present at lower latitudes, although evidence supporting this hypothesis is mostly anecdotal (Vermeij 1978; Bertness et al. 1981; Pennings et al. 2001).

Concordant with the concept that the intensity of both herbivory and predation are inversely correlated with latitude is the notion that tropical prey species are better defended from predators than those in temperate regions (Bakus and Green 1974; Vermeij 1978; Bolser and Hay 1996; Pennings et al. 2001). Direct support for this idea is rare, but has been provided recently from a study investigating latitudinal differences in the palatability of 10 conspecific salt marsh plants ranging from the subtropics to high latitude temperates (Pennings et al. 2001). These investigators demonstrated that a diversity of herbivores showed a general preference for northern salt marsh plants as opposed to southern salt marsh plants. Their results are consistent with previous studies that documented a similar, inverse relationship in plant palatability for more distantly related species (ie., same genus, family, or order) tested from tropical and temperate plant communities (Coley and Aide 1991; Bolser and Hay 1996). The evidence supporting this broad biogeographic pattern in plant defenses at the species level is significant because

exceptions have been documented in a few orders of plants and marine algae (van Alstyne and Paul 1990; Targett et al. 1992; Swihart et al. 1994; Bolser and Hay 1996).

To date, the majority of studies investigating latitudinal variation in predator-prey interactions have been confined to herbivory on terrestrial plants, marine algae, and seagrasses (van Alstyne and Paul 1990; Coley and Aide 1991; Pennings and Paul 1992; Schupp and Paul 1994; Meekan and Choat 1997). Only a few studies have investigated the biogeographic relationship between the intensity of carnivory and the presence of prey defenses (Bakus and Green 1974; Vermeij 1978; Bertness et al. 1981; Menge and Lubchencho 1981). Although results of these studies also suggest a decrease in predation pressure and the prevalence of prey defenses with increased latitude, none of the above cited comparisons have quantified predation intensity nor tested the palatability of conspecific prey across regions. In fact, the only study making within species comparisons of prey palatability against carnivores across a latitudinal gradient suggests that prey species from temperate regions may be as well defended as those from the tropics (Becerro et al. 2003). The paucity of information in this area as well as the contradictory results obtained for those studies that have been completed, highlights the need for additional comparisons to determine how the intensity of carnivory and the incidence of prey defenses correspond with latitude.

Sponges are important contributors to marine benthic communities at all latitudes (Dayton et al. 1974; Diaz et al. 1990; Bell and Barnes 2000b) and many parallels have been drawn between predation on sponges and herbivory. For example, both plants and sponges are abundant, diverse, and often conspicuous. Both lack behavioral defenses, but possess chemical compounds that deter consumers (Levin and York 1978; McClintock

1987; Coley and Aide 1991; Schulte and Bakus 1992; Pawlik et al. 1995; Becerro et al. 2003). To date, there is no evidence supporting the hypothesis that temperate sponges are subjected to more intense predation than tropical species because predation pressure has never been quantified outside of the tropics. Likewise, no comparisons have been conducted within species to determine if an inverse relationship between sponge chemical defenses and latitude exists.

Temperate reefs in the South Atlantic Bight (SAB) of the United States provide an excellent opportunity to examine the relationship between predation pressure and chemical defenses in sponge species that have been studied in tropical coral reef systems. Several tropical Atlantic and Caribbean sponge species and spongivorous fish predators occur on SAB temperate reefs (Chapter 1). Therefore, by using sponge species that co-occur on SAB and tropical Atlantic/Caribbean reefs, the goals of this study were to: (1) quantify the abundance of sponges and spongivorous fish predators on temperate reefs of the SAB, (2) test the palatability of sponge secondary metabolites to generalist fishes of SAB reefs, and (3) compare statistically the results obtained in this temperate system with similar studies conducted on the same sponge species in the tropics. Thus, this study provides an initial assessment of how predation pressure on sponges and sponge chemical deterrence may vary with latitude.

Methods

Study Sites

Two reefs in the South Atlantic Bight, Gray's Reef National Marine Sanctuary (GRNMS; 31° 36.056 N, 80° 47.431 W) and J Reef (31° 36.056 N, 80° 47.431 W), were used for this study. Both reefs are ledges, typical of the temperate Western Atlantic

continental shelf and provide between 1-2 m of vertical relief along a narrow ridge. On the elevated side of the ledge the hard substrate becomes flat and quickly transitions into an extensive area of soft substrata due to a shifting layer of sediment 1-5 cm thick. SAB reefs are colonized by a variety of epifaunal species, which together with the substrate form a three-dimensional habitat occupied by a diversity of species of invertebrates and small cryptic fishes. GRNMS and J Reef are separated by 15 km and have similar depth profiles averaging 18 to 20 m. Although water temperatures reach 26°C during summer, many tropical species cannot survive the winter temperature of 11°C (Hunt 1974).

Sponge and Predator Abundance

Surveys were completed in the summer of 2003 and 2004 at GRNMS and J Reef to assess sponge and spongivorous fish species richness and abundance. To provide the best estimate of sponge and fish populations on these reefs, 25 and 50 m transects were run parallel and perpendicular to the ledge. For sponge distributional data, I recorded the total number of individuals for each species present in 0.25 m^2 quadrats haphazardly placed alongside the 25 m long transect. A total of 104, 0.25 m^2 quadrats were sampled at both GRNMS and J Reef: 52 quadrats for the sponge population on top of the ledge and 52 for the adjacent sponge community on the soft sediment habitat. Spongivorous fish populations were assessed with a 50 m swim transect in which divers recorded all spongivorous fishes present along the transect for 30 min. Visual census is an efficient and reliable method of quantifying fish densities at GRNMS and J Reef because the conspicuous anatomical features of spongivorous fishes make identification straightforward, and water turbidity on these sites often limits side to side visibility to 10 m or less, ensuring that fish occurring far a field of the transect are not recorded.

To determine if sponge community structure and predator abundance is similar between GRNMS and J Reef, mean sponge species richness, sponge density, and spongivorous fish density were compared between reefs with a student's t test. If no significant differences occurred between reefs, the data were pooled so that mean values for SAB reefs could be compared to results for mean sponge species richness and sponge and spongivorous fish densities published from tropical studies.

Palatability of Sponge Crude Organic Extracts

Over twenty species of sponges are known to be shared between the tropical and temperate Western Atlantic (Wells et al. 1960; van Soest 1984; Alvarez et al. 1998). Nine of these species, *Chondrilla nucula, Chondrosia collectrix, Cinachyrella alloclada, Ircinia felix, Aplysina fulva, Aiolocroa crassa, Ptilocaulis walpersi, Scopalina ruetzleri,* and *Clathria prolifera* were found at GRNMS and J Reef. The palatability of these species to generalist fish predators was assessed. *Clathria prolifera* was included in the analysis because *Rhaphidolphus juniperinus* is considered a junior synonym of *C. prolifera* (van Soest, pers. comm.) and has been used in similar Caribbean surveys.

Collections were made May through December 2004. Samples of sponge tissue, ≤10 ml in volume, were obtained by either subsampling large sponges or removing whole sponges from the substrate. In either case, samples were placed individually into plastic bags and stored on ice in coolers at the surface. Sponges were frozen at -80ºC upon returning to the lab, approximately 3 to 4 hours after initial collection. A minimum of 30 samples were collected for each species. Sponges were identified on the basis of morphology or spicule and tissue preparations. Identifications were confirmed by Dr. Rob van Soest, University of Amsterdam.

Methods described by Pawlik et. al. (1995) and Becerro et. al. (2003) were followed to isolate crude organic extracts and formulate foods for testing the palatability of sponge secondary metabolites to fishes. For each sample, approximately 5 ml volumes of sponge tissue were measured by displacement of water in a graduated cylinder. Samples were frozen at -80˚C, lyophilized, and weighed to the nearest mg on an analytical balance (model APX-60, Denver Instruments, Denver, CO). Freeze dried samples were crushed into small pieces and extracted three times at 4ºC for 24 hours by immersing the sample in a 1:1 methanol:dichloromethane (MeOH:DCM) mixture. All extracts were combined and passed through filter paper (P8 coarse, Fisher Scientific Company L.L.C., Pittsburgh, PA) to remove sponge debris. Excess solvent was removed by rotary evaporation (Brinkmann/Buchi Collegiate, Eppendorf, Germany) at low heat (<30ºC) until approximately 5 ml remained. The remaining 5 ml of solvent was transferred to a pre-weighed 20 ml scintillation vial and concentrated to dryness by vacuum evaporation (model SC210A-115, Thermo Electron Corporation, Somerset, NJ). The dried extract was stored at -80ºC until further use.

Concentrated crude organic extracts obtained from each sponge were dissolved in 0.75 ml of 100% methanol. Samples were sonicated and visually inspected to ensure the extract had dissolved into solution. Artificial food was created using a mixture of 7.5 g powdered squid mantle, 3.5 g Type I carageenan:agar (85:15), and 150 ml of distilled water. The amount of powdered squid mantle used in food preparation was based upon the mean protein concentration (~20.7 mg ml^{-1}) of 71 Caribbean sponge species surveyed by Pawlik et. al. (1995) and thus matched the amount of squid mantle used in Pawlik's tropical feeding assays. In 25 ml batches, the carageenan:agar, squid mantle, and

distilled water were thoroughly mixed and heated in a microwave to the boiling point. Immediately after heating, 4.25 ml of food mixture was poured into each scintillation vial containing the 0.75 ml methanol and extract. This mixture was stirred and allowed to cool forming a food mold. When cooled, the mold was carefully removed from the vial and cut into 1 x 1 x 1 cm cubes for feeding assays.

For each replicate, control cubes were prepared by the same method, but with 0.75 ml of methanol only. When appropriate, food coloring was added to control cubes to match the color of the food cube containing the crude extract so predatory behavior of fishes would not be influenced by cube color. Control cubes were readily consumed in all feeding assays.

Feeding assays were conducted in situ at GRNMS and J reef. Food cubes were dispensed individually to natural assemblages of generalist reef fish predators. The benefit of using generalist predators is that they provide a reliable estimate of the effectiveness of these defenses against a diversity of predators (Pawlik et al. 1995; Becerro et al. 2003). In addition, the feeding behavior of fishes used in these assays was appropriate for the study because they habitually "mouth" or "taste" their prey before consuming it. Even in instances when fishes attempted to ingest the entire food cube, they would often regurgitate it whole providing another opportunity for a different species of predator to consume it.

Several control cubes were released first to initiate feeding activity and then control and test cubes were offered in a random sequence so fish could not habituate to a systematic pattern of deterrent cube release. Divers recorded if the cube was consumed or rejected. A food cube was considered unpalatable if fishes rejected it three or more

times or if it sank to the bottom. Assays targeted the most common generalist and opportunistic reef fishes at GRNMS and J Reef, including seabasses (*Centropristus striata*), tomtate grunts (*Haemulon aurolineatum*), and spottail pinfish (*Diplodus holbrooki*). For all sponge species assayed, a minimum of 30 samples were tested. In this study, an index of chemical deterrence for each sponge species was created by dividing the total number of food cubes consumed by the total number of food cubes offered.

Statistical Comparisons with Published Results from the Tropics

sponge and predator abundance

To determine if sponge species richness, mean species richness (number of species m^{-2}), and density (number of individuals m^{-2}) differ between temperate and tropical reefs, the results of this study were compared to a survey completed by Schmahl (1990) in the Florida Keys. Likewise, results for spongivorous fish species richness and density obtained here were compared with data from the Florida Keys (Hill 1998). In both surveys, the density of sponges and spongivorous fishes was enumerated as the number of individuals per m^2. Schmahl (1995) used 1 m^2 quadrats to quantify sponge populations in the tropics so I pooled the data obtained from every 4 consecutive 0.25 m^2 quadrats to standardize my sponge distributional data to number per m^2. Differences in mean sponge species richness and sponge and spongivorous fish density between the Florida Keys and SAB were compared with a one way ANOVA.

palatability of sponge crude organic extracts

To determine if palatability of sponges differs with latitude, the levels of consumption obtained in this study were compared to published data from feeding assays

conducted in the tropics with the bluehead wrasse, *Thalassoma bifasciatum* (Pawlik et al. 1995). Although palatability of tropical sponge extracts was assessed in aquaria rather than in the field, both studies tested the deterrence of sponge extracts to generalist predators. If secondary metabolites produced by sponges are effective anti-predator defenses, they should be aimed at generalist predators in particular because (1) they are less likely to have evolved mechanisms to circumvent specialized chemical defenses, and (2) they represent the majority of predators on reef ecosystems (Pawlik et al. 1995; Becerro et al. 2003). The total number of food cubes consumed for each sponge species was compared across latitude with a chi-square test using William's continuity correction (Sokal and Rohlf 1995). To determine if overall chemical deterrence varied between these tropical and temperate sponge populations, the mean consumption of food cubes was calculated across all 9 species assayed and overall deterrence evaluated with a Wilcoxon signed-ranks test (Sokal and Rohlf 1995).

Results

Sponge and Predator Abundance

grnms and j reef

A total of 32 sponge species were recorded at GRNMS and J Reef. Of these, 27 were present at both sites while four species, *Aiolochroia crassa, Myriastra sp., Aplysilla longispina,* and *Cliona celata*, were only recorded at J Reef and one species, *Geodia gibberosa*, was exclusive to GRNMS. Two species of spongivorous fishes, *Holacanthus bermudensis* and *Cantherhines macrocerus*, were recorded at both reefs. No significant differences in mean sponge species richness, sponge density, and spongivorous fish density were detected between GRNMS and J Reef (Table 6). Therefore, data from these

2 sites were pooled and the means for SAB reefs compared to published results from tropical studies (Schmahl 1990; Hill 1998).

statistical comparisons with published results from the tropics

Sponge species richness was lower on the temperate Atlantic reefs, but the density of both sponge species and individuals was significantly higher than on the tropical reefs (Table 7). The greater abundance of sponges on SAB reefs is largely explained by three encrusting species, *Chondrilla nucula, Chondrosia collectrix,* and *Scopalina ruetzleri*, common at both latitudes. Collectively, they account for >60% of the sponge population on SAB reefs with a mean density of 42.5 (± 44.5 SD) individuals m^{-2} (Chapter 1). On Florida Keys reefs, each of these species have a density of <1 m^{-2} (Schmahl 1990).

Both spongivorous fish species richness and density were significantly lower on temperate as opposed to tropical reefs (Table 7). Although other species, such as *Pomacanthus paru* and *Pomacanthus arcuatus*, have been observed on SAB reefs, their occurrence is rare and they were not recorded during my surveys.

Palatability of Sponge Crude Organic Extracts

grnms and j reef

Chemical extracts obtained from all 9 species assayed in this study deterred generalist reef fishes to some degree, however, effectiveness was highly variable across species. *Aplysina fulva* was the most deterrent while *Cinachyrella alloclada* and *Ptilocaulis walpersi* were the least deterrent. Predator deterrence in *Chondrilla nucula* was highly variable with exactly 50% of its food cubes consumed.

statistical comparisons with published results from the tropics

Of the 9 species tested, 5 (*Ptilocaulis walpersi, Scopalina ruetzleri, Cinachyrella alloclada, Ircinia felix,* and *Aiolocroa crassa*) were significantly less deterrent to generalist fish predators if they were from temperate as opposed to tropical reefs (Figure 12). No significant differences in palatability were detected for *Chondrilla nucula, Chondrosia collectrix, Clathria prolifera,* and *Aplysina fulva* (Figure 12). Pooling the data for food cube consumption across the 9 species assayed showed that the mean deterrence of fishes by chemical extracts was significantly lower (Wilcoxon signed-ranks test, $t_s = 1$, $p = <0.0039$) for temperate as compared to tropical sponges (Figure 13).

Discussion

Few studies have been conducted to test the hypothesis that (1) there is an inverse relationship between predation pressure and latitude and (2) that lower predation pressure at higher latitudes corresponds with a reduction in prey anti-predator defenses (Bertness et al. 1981; Coley and Aide 1991; Pennings et al. 2001). In sponges, the idea that tropical species are better defended chemically than their temperate counterparts was first proposed over 30 years ago (Bakus and Green 1974), but subsequent studies have demonstrated that secondary chemistry is common and important in sponges at all latitudes (McClintock 1987; Pawlik 1997; Becerro et al. 2003; Burns et al. 2003). Data presented here, combined with published results from tropical studies, support the hypothesis that predation pressure on sponges is lower at higher latitude and that this reduction corresponds with a decreased investment into prey chemical defenses.

Sponge and Predator Abundance

The significantly higher density of sponges and lower density of spongivorous fishes on two temperate Atlantic reefs of the SAB suggests that there may be lower predation pressure on sponges on temperate as opposed to tropical reefs (Table 7). The hypothesis that predation pressure is reduced on temperate reefs is further supported by the sponge community structure. For example, sponge populations on SAB reefs are conspicuously dominated by the encrusting species, *Chondrilla nucula* (Chapter 1). Considerable evidence suggests that *C. nucula* is a favored prey species of angelfishes, turtles, and other spongivorous fishes (Randall and Hartman 1968; Meylan 1998; Pawlik 1998), yet this is the most abundant species occurring on SAB reefs accounting for ~50% of the sponge assemblage (Chapter 1). In comparison, on tropical Atlantic reefs, the abundance of this species is low with a density <1 individual m^{-2} (Schmahl 1990). It has been demonstrated that in the absence of spongivorous predators, *C. nucula* can rapidly overgrow coral reefs and become a top spatial competitor (Hill 1998). No differences in the ability of *C. nucula* to chemically deter fish predators were observed across temperate and tropical studies (Figure 12), thus the differences in density of *C. nucula* between high and low latitude probably reflect lower predation pressure.

The lower diversity of spongivorous fishes on SAB reefs (Table 7) may equate to a lower frequency of attack for some sponge species. Spongivorous fish species exhibit feeding preferences for the prey species they consume (Randall and Hartman 1968; Wulff 1994). Only two species, *Holocanthus bermudensis,* and *Cantherhines macrocerus,* were recorded during my transects on two temperate reefs. In the survey completed by Hill

(1998) he recorded a total of five angelfish species. Thus, predation pressure on some sponge species may be alleviated on the temperate reefs studied here.

Palatability of Sponge Crude Organic Extracts

Although temperate sponges showed lower chemical deterrence of fish predators overall (Figure 13), some species (*Chondrilla nucula, Chondrosia collectrix, Clathria prolifera,* and *Aplysina fulva*) showed the same level of deterrence as they did in tropical assays (Figure 12). Although the explanation for these differential responses across species is unclear, secondary metabolites in sponges serve other ecological functions in addition to predator deterrence. Secondary metabolites produced by sponges are effective anti-viral and anti-fouling agents and impede the settlement of spatial competitors (Kubanek et al. 2002). Sponge populations on tropical and temperate reefs may be subjected to differing levels of spatial competition, temperature, water turbidity, nutrient availability, disease, and UV exposure, all of which may alter the production of secondary chemistry (Becerro et al. 1995; Turon et al. 1996; Targett and Arnold 1998). As a result, factors unrelated to predation may exert an influence on sponge secondary chemistry. If sponge populations on tropical and temperate Atlantic reefs are recruiting from genetically distinct pools, they may have evolved independently under these varying selective pressures (Targett and Arnold 1998; Becerro et al. 2003; Burns et al. 2003). However, if sponge populations in the tropical and temperate Atlantic are in panmixia then differences in sponge chemical deterrence may be in response to differing post-settlement cues or environmental stresses.

It has been suggested that the production of these chemical compounds is metabolically expensive (Paul 1992). Although rare in marine systems, the induction of

chemical defenses is known for some seaweeds (Harvell 1990; Cronin and Hay 1996). A high degree of variability in the production of chemical defenses has been exhibited by sponges (Swearingen and Pawlik 1998; Assmann et al. 2000), but whether these defenses are inducible or constitutive remains unknown. *Aplysina fistularis* has been shown to exude up to 100x the active metabolites after simulated attack (Walker et al. 1985), but it is unclear if this is in response to predation or to prevent infection of the wound. If production of these compounds represents an inducible defense, and sponges on temperate reefs are subjected to a lower frequency of attack than those in the tropics, this may account for the lower chemical deterrence in temperate sponge.

Interestingly, my results contrast with findings reported by Becerro et al. (2003). They conducted a total of 44 tropical-temperate comparisons by testing food cubes from 11 pairs of distantly related sponges (i.e. from the same genus, family or order; 1 tropical Indo-Pacific and 1 temperate Mediterranean species for each pair) to 4 different groups of generalist reef fishes. They detected no significant differences in overall chemical deterrence between tropical and temperate sponges and even found temperate species to be significantly more deterrent in 23% of their comparisons. In contrast, I found no instances where temperate sponges were significantly more deterrent than their tropical conspecifics (Figure 12). The reasons for these discrepancies in deterrence are unclear, but may illustrate the difference of working with distantly related species as opposed to conspecifics. Interestingly, Becerro et. al. (2003) did find a significantly higher concentration of crude organic extract in the tropical sponge population. Differences in the amount of crude organic extract were not compared in this study.

Both the study by Pawlik et al. (1995) and my study, tested chemical extract palatability against generalist reef fishes that are abundant in their respective locations, but only one species was used in the tropical feeding assays while a diversity of predators was used in the temperate study. It has been demonstrated that overall patterns of generalist fish behavior are similar between tropical and temperate reefs (Becerro et al. 2003; Burns et al. 2003), but species specific responses to anti-predator mechanisms can also occur (Pennings et al. 1994; Waddell and Pawlik 2000b). The results presented here for the tropical-temperate comparison would be misleading if the generalist predator used in tropical assays, *Thalassoma bifasciatum,* is less tolerant to sponge chemical extracts than the generalist predators used in the temperate assays. This situation would result in chemical deterrence for tropical sponges being overestimated. This scenario is unlikely, however, because it is believed that tropical predators have evolved a greater tolerance to anti-predator mechanisms than their temperate counterparts (Cronin et al. 1997). If the production of chemical defenses in conspecific sponges is equivalent across tropical and temperate reefs it would be anticipated that tropical predators would consume more food cubes than their temperate counterparts. This did not occur and suggests that differences in chemical production, rather than variability in predator response, is the most parsimonious explanation for the differences in predator deterrence observed at the two latitudes.

It should also be noted that predation by invertebrates is a significant source of sponge mortality (Dayton et al. 1974; Wulff 1995; Wright et al. 1997) and a shift from fish to invertebrate predation on sponges may occur at higher latitude (Dayton et al. 1974; McClintock 1987). Invertebrate predation was not quantified in this survey, but

may be of greater significance on temperate SAB reefs. If the sponge species used in this study are producing compounds targeted for predators different from those included in my feeding assays (i.e. invertebrates), the observed differences in sponge palatability between the tropical sites and the two SAB reefs may not necessarily represent lower deterrence, but adjustments to a different suite of predators.

Conclusions

This analysis supports the hypothesis that predation pressure on sponges by fishes is lower at higher latitude and that this reduced predation intensity corresponds with a decrease in chemical defenses that deter these predators. These findings agree with those conducted on gastropods, decapods, and insects indicating predation pressure by carnivores is reduced (Jeanne 1979; Bertness et al. 1981; Heck and Wilson 1987) and a concomitant reduction in prey anti-predator defenses occurs at higher latitude (Vermeij 1978). Not only does my study agree with these earlier findings, but extends them by demonstrating a quantitative reduction in the density of carnivores on temperate as opposed to tropical reefs and by providing experimental evidence that this difference coincides with a decrease in chemical defenses for prey species shared across these locations. Furthermore, my results corroborate those conducted with plants and marine alga (Targett et al. 1992; Bolser and Hay 1996) showing that differences in chemical deterrence across latitudes do not occur in all prey species. For some species of plants, algae, and sponges, it appears that factors unrelated to predation, such as competition (Thacker et al. 1998) and pathogen resistance (Kubanek et al. 2002), may be a proximate cause of these defenses. However, the relationship between carnivore predation pressure, prey defenses, and latitude documented in this study, combined with those obtained from

plant-herbivore systems, support a broad geographic pattern of declining predation pressure and prey chemical defenses with increased latitude.

Table 6. Total sponge species richness, mean (± SD) sponge species richness, mean (± SD) sponge density, and spongivorous fish species richness and mean (± SE) density on two SAB reefs. Differences between sites in sponge mean species richness and spongivorous fish density were compared using a student's t test. For sponge distributional data $N = 104$, 0.25 m^2 quadrats for each site. For spongivorous fish data, $N = 4$, 50 m long transects for each site.

		Sponge		Spongivorous Fish	
Location	**Species richness**	**Species m^{-2}**	**Individuals m^{-2}**	**Species richness**	**Individuals m^{-2}**
GRNMS	28	10.0. ± 1.4	73.2 ± 45.0	2	0.14 ± 0.04
J Reef	31	9.3 ± 1.5	69.6 ± 48.1	2	0.20 ± 0.08
Significance	-	$p = 0.10$	$p = 0.46$	-	$p = 0.62$
t	-	1.642	0.737	-	0.525

Table 7. Sponge species richness, mean (± SD) sponge species richness, mean (± SD) sponge density, and spongivorous fish species richness and mean (± SE) density on tropical and temperate Atlantic reefs. Tropical Atlantic data for sponge composition is modified from Schmahl (1990) and for spongivorous fishes from Hill (1998). Data for sponge distributions and spongivorous fish densities only include surveys completed on reefs at 13-18 m depth because this depth is comparable for South Atlantic Bight reefs. Differences between mean sponge species richness, sponge density, and spongivorous fish density were compared using one way ANOVA. For sponge distributional data N = 160, 1 m^2 quadrats for tropical and N = 208, 0.25 m^2 quadrats (52 m^2) for temperate reefs. For spongivorous fish data, N = 26 transects for tropical and N = 8, 50 m long transects for temperate surveys.

		Sponge		Spongivorous Fish	
Location	**Species richness**	**Species m^{-2}**	**Individuals m^{-2}**	**Species richness**	**Individuals m^{-2}**
Tropical	84	8.0 ± 3.2	12.8 ± 6.35	5	0.29 ± 0.05
Temperate	32	9.7 ± 1.5	71.4 ± 46.9	2	0.18 ± 0.02
Significance	-	$p = 0.004$	$p < 0.001$	-	$p < 0.001$
F	-	8.598	75.902	-	17.696

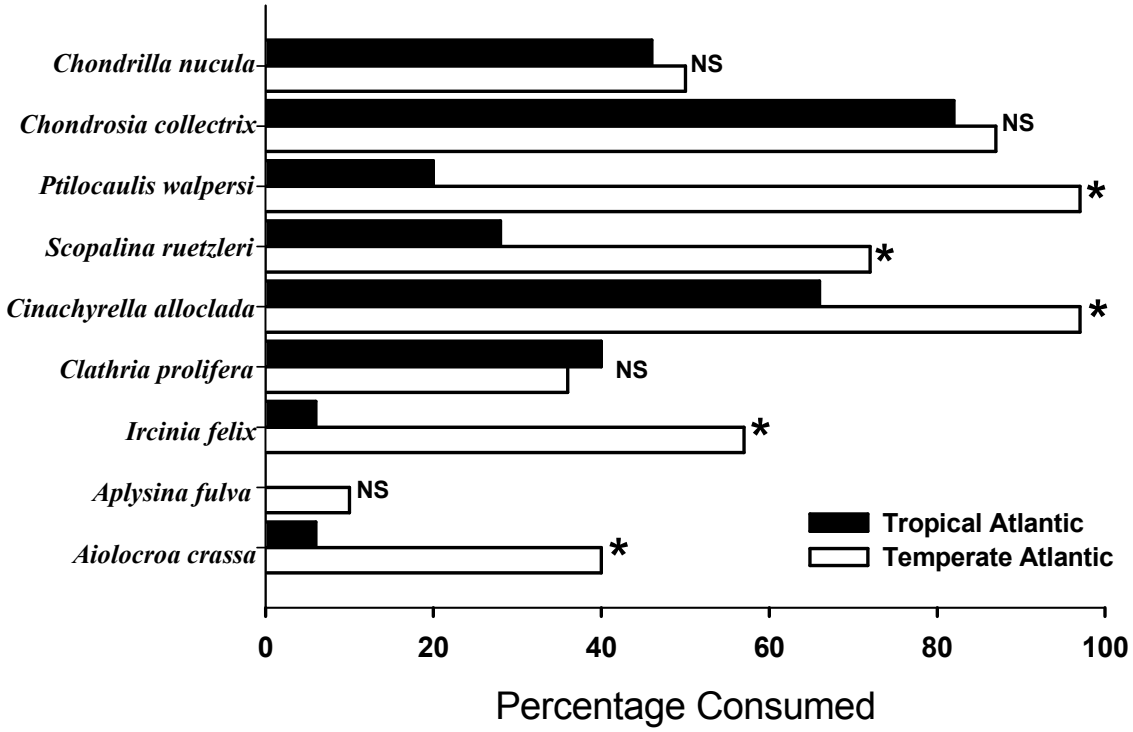

Figure 12. Consumption of food cubes by reef fishes containing the crude organic extracts of 9 sponge species shared between the tropical and temperate Atlantic. Tropical assays used *Thalassoma bifasciatum* while temperate assays used natural assemblages of reef fish on SAB reefs. Significant differences between tropical and temperate palatability were determined with a chi-square analysis (NS = not significant; * = $x^2 \geq 3.85$, $p < 0.05$). Data for tropical Atlantic chemical deterrence modified from Pawlik et. al (1995). In all cases, $N \geq 30$ replicates for both tropical and temperate assays.

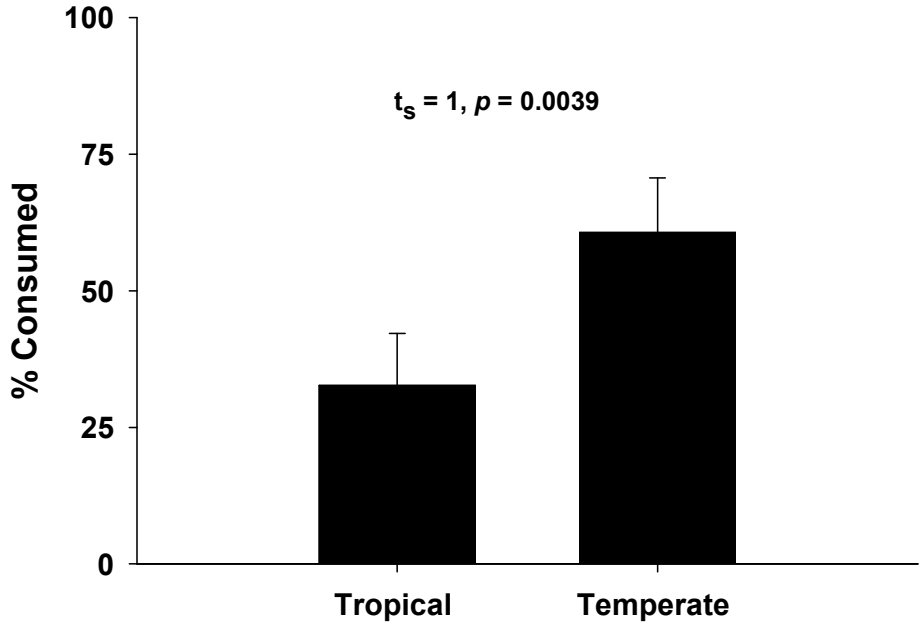

Figure 13. Mean deterrence (± SE) of generalist reef fishes by food cubes containing the crude organic extracts of 9 conspecific sponges from tropical and temperate Atlantic reefs. Percent consumed is based on the mean level of deterrence for all 9 species tested from either location. The difference in mean consumption was compared with a Wilcoxon signed-ranks test.

Chapter 3

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APPENDICES

A. Mean density (no. of individuals 0.25 m^{-2} ± SD) of all sponge species recorded at GRNMS and J Reef. Density for all four sites is listed. Differences in densities between reefs, GRNMS and J Reef, and between habitat, scarp and plateau, were compared with a one-way ANOVA for species recorded at 2 sites or with a two-way ANOVA for species found at 3 or more sites. For all two-way ANOVAs no significant interactions occurred between Reef and Habitat, except for *Clathria prolifera* and *Ptilocaulis walpersi*. Significant differences between reefs or habitats are listed. $N = 52$ for all 4 sites.

Sponge Species	GRNMS Scarp	J Reef Scarp	GRNMS Plateau	J Reef Plateau	Difference between Reefs	Difference between Habitats
Chondrilla nucula	18.1 ± 9.9	16.9 ± 8.9			NS	
Ircinia felix	2.7 ± 1.6	2.2 ± 1.4	0.5 ± 0.7	0.2 ± 0.6	NS	$P = 0.001$
Chondrosia collectrix	1.3 ± 2.3	2.6 ± 3.7			NS	
Scopalina ruetzleri	1.0 ± 2.5	1.8 ± 1.8			NS	
Spirastrella sp.	1.7 ± 2.2	0.8 ± 1.1		0.1 ± 0.3	$P = 0.001$	$P = 0.001$
Hyrtios violaceaus	0.6 ± 1.1	0.4 ± 0.8	0.02 ± 0.1		NS	$P = 0.001$
Ircinia campana	0.4 ± 0.7	0.6 ± 0.9	0.1 ± 0.3	0.05 ± 0.2	NS	$P = 0.001$
Aplysina fulva	0.4 ± 0.9	0.8 ± 1.3		0.2 ± 0.9	$P = 0.001$	$P = 0.001$
Clathria prolifera	0.7 ±0.9	0.1 ± 0.3	0.2 ± 0.5		$P = 0.001$	$P = 0.001$
Dysidea fragilis	0.4 ± 1.0	0.03 ± 0.2			$P = 0.001$	
Smenospongia cerebriformis	0.2 ± 0.5	0.1 ± 0.3	0.2 ± 0.1	0.04 ± 0.2	NS	NS
Coscinoderma lanuga	0.2 ± 0.7	0.1 ± 0.4			NS	
Aplysilla longispina		0.03 ± 0.1		0.02 ± 0.1	NS	NS
Geodia gibberosa	0.03 ± 0.2					
Aiolochroia crassa		0.03 ± 0.1				
Myriastra sp.		0.1 ± 0.3				
Cliona celata		0.1 ± 0.2		0.1 ± 0.2		NS
Axinella waltonsmithi		0.1 ± 0.3	1.2 ± 1.2	0.9 ± 0.9	NS	$P = 0.001$
Axinella bookhouti			0.6 ± 0.9	0.7 ± 0.8	NS	
Cinachyrella alloclada	0.05 ± 0.3		0.8 ± 1.2	1.0 ± 1.1	NS	$P = 0.001$
Raspailia sp.			0.4 ± 0.8	0.4 ± 0.7	NS	
Ciocalypta gibbsi			0.2 ± 0.5	0.2 ± 0.5	NS	
Axynissa ambrosia			0.5 ± 0.7	0.5 ± 0.8	NS	
Dark finger sp.			0.6 ± 1.2	0.3 ± 0.6	NS	
Unidentified sponge			0.6 ± 1.0	0.3 ± 0.6	NS	
Higginsia strigilata			0.5 ± 0.8	0.3 ± 0.6	NS	
Clathria carteri			0.2 ± 0.4	0.3 ± 0.6	NS	
Axinella pomponaie	0.03 ± 0.1		0.4 ± 0.7	0.2 ± 0.4	NS	$P = 0.001$
Ptilocaulis walpersi	0.1 ± 0.3	0.03 ± 0.1		0.2 ± 0.4	NS	$P = 0.05$
Desmapsamma anchorata		0.1 ± 0.1	0.4 ± 0.5	0.3 ± 0.5	NS	$P = 0.001$
Cliona sp.			0.4 ± 0.9	0.1 ± 0.5	NS	
Lissodendoryx sigmata			0.2 ± 0.4	0.1 ± 0.3	NS	

B. Mean percentage and volume of crude organic extract produced by each sponge species tested in chemical assays. $N \geq 30$, 5 ml samples for each species. N/A = freeze-dried data not available.

Sponge Species	Sponge freeze-dried weight (g)	Extract (g)	Extract as % of dry mass	Extract volume (mg extract/ml of sponge)
Chondrilla nucula	1.22	0.17	14.19	34.63
Ircinia felix	1.27	0.10	7.80	19.56
Chondrosia collectrix	1.21	0.08	6.96	16.85
Scopalina ruetzleri	1.22	0.23	11.95	46.92
Hyrtios violaceaus	1.29	0.14	10.42	28.30
Ircinia campana	N/A	0.10	N/A	19.21
Aplysina fulva	1.01	0.19	19.39	38.10
Clathria prolifera	0.85	0.08	9.17	15.68
Dysidea fragilis	1.47	0.08	5.86	16.40
Coscinoderma lanuga	1.16	0.05	4.01	9.28
Aiolochroia crassa	1.26	0.12	9.69	24.40
Cliona celata	2.83	0.34	13.18	67.97
Axinella waltonsmithi	1.02	0.17	17.26	33.64
Axinella bookhouti	0.89	0.16	18.13	31.98
Cinachyrella alloclada	1.15	0.14	12.53	28.79
Raspailia sp.	N/A	0.17	N/A	34.92
Axynissa ambrosia	2.16	0.15	8.55	30.53
Axinella pomponaie	0.93	0.18	18.89	36.33
Ptilocaulis walpersi	0.76	0.16	23.10	32.60
Desmapsamma anchorata	0.88	0.23	25.89	45.15