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Allocation of chemical and structural defenses in the sponge

Melophlus sarasinorum

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Abstract

Sponges have evolved a variety of chemical and structural defense mechanisms to avoid predation. While chemical defense is well established in sponges, studies on structural defense are rare and with ambiguous results. We used field and laboratory experiments to investigate predation patterns and the anti-predatory defense mechanisms of the sponge *Melophlus sarasinorum*, a common inhabitant of Indo-Pacific coral reefs. Specifically, we aimed to investigate whether *M. sarasinorum* is chemically or structurally defended against predation and if the defenses are expressed differently in the ectosomal and choanosomal tissue of the sponge. Chemical defense was measured as feeding deterrence, structural defense as feeding deterrence and toughness. Our results demonstrated that chemical defense is evenly distributed throughout the sponge and works in conjunction with a structurally defended ectosome to further reduce predation levels. The choanosome of the sponge contained higher protein levels, but revealed no structural defense. We conclude that the equal distribution of chemical defenses throughout *M. sarasinorum* is in accordance with Optimal Defense Theory (ODT) in regards to fish predation, while structural defense supports ODT by being restricted to the surface layer which experiences the highest predation risks from mesograzers.

Keywords

Porifera; sponges; optimal defense theory; predator-prey interactions; chemical defense; structural defense; secondary metabolites; spicules; *Melophlus*

Introduction

Marine sponges are a major component of the benthic community on tropical coral reefs (Van Soest, 1994). One characteristic of coral reefs is high levels of predation which significantly influences the distribution and abundance of prey species (Hay, 1991). Sponges are part of predator-prey dynamics on coral reefs with several studies indicating that spongivory affects sponge community composition. For example, species which were abundant in mangrove habitats with low predator density were rare or absent in adjacent

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habitats with higher predation pressure (Wulff, 2000). Additionally, predation on sponge species transplanted from mangrove habitats was 19 times higher compared to sponges from the reef habitat (Dunlap and Pawlik, 1996). It would seem therefore, that sponge distribution is affected by spongivory and that sponges that occur on predator rich reefs have adapted to predation risk.

Similar to terrestrial plants, sponges have evolved a variety of defensive mechanisms to reduce predation, including chemical and structural defenses (Hill et al., 2005; Pawlik et al., 1995). While sponge extracts have yielded a vast array of secondary metabolites with multiple ecological functions (Faulkner, 2000; Paul et al., 2007; Pawlik et al., 1995), the most studied role of sponge chemical metabolites is anti-predatory defense (Burns et al., 2003; Paul, 1992; Pawlik, 1993; Thoms and Schupp, 2007). A comprehensive study by (Pawlik et al., 1995) assessing the chemical deterrence of 71 Caribbean sponges, found that 69% of sponge extracts were feeding deterrent. This trend is also supported by other studies from the Pacific, Mediterranean, Red Sea and Antarctica (Becerro et al., 2003; Burns et al., 2003; Peters et al., 2009).

Besides chemical defenses, sponges also harbor structural components such as spicules, sponging and chitin fibers (Brunner et al., 2009; Hooper and Van Soest, 2002b), which roles in sponge defenses have been more equivocal (Brunner et al., 2009; Chanas and Pawlik, 1996; Hooper and Van Soest, 2002a). While siliceous spicules have been shown to provide stability in sponge tissue (Koehl, 1982), their role as defenses against feeding is less clear. No evidence was found to support a deterrent effect for spicules from eight different sponge species from the Caribbean (Chanas and Pawlik, 1995) and intact sponge skeletons of two species did not reduce palatability (Chanas and Pawlik, 1996). However, Uriz et al. (1996) found physical defense in the sponge *Crambe crambe* and a deterrent role of sponge spicules has been demonstrated in four Caribbean and two Red Sea sponges (Burns and Ilan (2003)). Additionally, and in contrast to Chanas and Pawlik (1995), Jones et al. (2005) found that spicules from only three of seven tested Caribbean sponge species deterred feeding, while Hill et al. (2005) demonstrated deterrent effects of spicules in two temperate sponge species. Huang et al. (2008) found that spicules of the sponge *Amphimedon viridis* weakly deter feeding by the pinfish *Lagodon rhomboides*. It has been proposed that the size of the spicules is important with only those megascleres over 250µm deterring feeding (Burns and Ilan, 2003).

Increased defensive efficiency in sponges comes not only from a combination of different defense mechanisms, but also from the non-random allocation of secondary metabolites within the individual. Optimal Defense Theory (ODT) states that defenses are expressed in a way that maximizes fitness and minimizes costs (Rhoades, 1979). One way of optimizing defenses is to concentrate it in tissues that have the highest chance of encountering predators (i.e. exposed body parts, or the ectosome) or which have the highest fitness value for the organism. Studies on intraspecimen variation of defenses in sponges are few, and focused mainly on the concentrations of secondary metabolites. For example, Becerro et al. (1998) and Schupp et al. (1999) found higher concentrations of defensive metabolites in more apical parts compared to basal parts of the sponge, while two other studies found no evidence in other sponge species that the ectosome from eight sponge species was better defended than the choanosome (Burns et al., 2003; Swearingen and Pawlik, 1998). Freeman and Gleason (2010) found one sponge species with higher concentrations of fish deterrent metabolites in the outermost layer, while two other species had higher concentrations in the deeper tissue layer. A study of 27 Antarctic sponges revealed that 68% contained deterrent secondary metabolites against starfish predators in their outermost layer, while only 62% of these defended species were also defended in their inner tissue (Peters et al., 2009). In this study we will be investigating intraspecimen variation in the structural defenses of the

sponge *Melophlus sarasinorum* Thiele, 1899, in combination with chemical defenses, in both ectosomal and choanosomal tissue. To our knowledge, this type of study has not previously been carried out.

The sponge *M. sarasinorum* is a common member of the benthic community around Guam and greater Micronesia (Hooper and Van Soest, 2002a). However, it does not occur on all reefs around Guam. While it is very abundant on reefs in Apra Harbor, it does not occur in the Tumon Bay Marine Preserve ca. 20 km north. Fishing is prohibited in the Marine Preserves and fish abundances are consequently higher than at sites outside the preserves (Porter et al., 2005). Furthermore, the sponge communities on the two reefs are very different as well. Apra Harbor is characterized by a high sponge diversity with more than 50 species (Schils et al., 2009), while Tumon Bay is coral-dominated with only few sponge species in low abundances (pers. obs.). However, it is unknown whether the low number of sponges in Tumon Bay is a consequence of higher predation rates due to higher fish abundance, or caused by other biotical and abiotical factors like competition, food resources, nutrients, wave action and currents, or substrate complexity.

Numerous secondary metabolites have previously been isolated from *M. sarasinorum* for pharmacological research (e.g. Aoki et al., 2000; Xu et al., 2006), but nothing is known about its general or chemical ecology. Consequently, the natural predators of *M. sarasinorum* are also unknown. In general, tropical sponges are preyed on by turtles, angelfishes, trunk fishes, filefishes and parrotfishes (Pawlik, 1998; Randall and Hartman, 1968; Wulff, 1994).

Unlike many sponges, which lack distinct tissues (Schupp et al., 1999), *M. sarasinorum* has a distinct differentiation between the ectosomal and choanosomal tissue (Fig. 1). The ectosome layer is 3-4 mm thick and appears to be much denser and harder than the underlying choanosome tissue. As stated above, studies on intraspecimen variation of defense in sponges are rare and so far focused on chemical defenses. The presence of a distinct ectosome and choanosome has only been described for few sponge species, e.g. *Anthosigmella varians* (Hill, 1999), or *Geodia* spp. (Hill and Hill, 2002), and provides a rare opportunity to assess whether chemical and structural defenses are allocated differently in the two tissues, and if this enhances defensive capabilities and reduces potential costs as predicted by ODT.

Hence the aims of this study were to investigate (1) whether predation affects the growth of *M. sarasinorum*, and if so, (2) whether predation drives the distinct ecological distribution of *M. sarasinorum* by preventing it from growing on predator-rich reefs. We also investigated whether (3) *M. sarasinorum* uses chemical and structural defenses to protect itself against predation and (4) whether the defenses are expressed differently in the ectosome and choanosome of the sponge.

Methods

Field experiment

All *Melophlus sarasinorum* specimens were collected at Western Shoals in Apra Harbor on the west coast of Guam (13.44° N, 144.65° E) at depths between 10-15 m. 72 specimens were cut at the base from the substratum, put into individual 4-L plastic bags *in situ*, then deposited into a cooler filled with seawater for transportation to the University of Guam Marine Laboratory (UOGML). At the UOGML the sponges were placed in a 1500-L outdoor flow-through tank. The following day, the volume of each sponge was measured by seawater displacement in a graduated cylinder and each sponge received an individual aluminum tag for individual identification. The next day, sponges were allocated randomly

to two groups. One group was transported back to the collection site, Western Shoals, and the other group to the Marine Preserve Tumon Bay. At both sites, 32 cm long nails were pounded in the ground at 13 m depth and the sponges were attached to the nails using cable ties. On each site sponges were randomly distributed among three treatments (n=12): Treatment 1: Control - the sponges were left uncovered, allowing access by all potential predators; Treatment 2: Caged - plastic mesh cages (30 × 30 × 30 cm, 1 cm mesh size) were installed around the sponges to deny access to predators; Treatment 3: Cage Controls - cages with two adjacent sides removed allowing predator access, but imitating light and water flow regimes inside the fully-closed cages.

To identify predators on *M. sarasinorum*, we used a video camera to record fish activities around the uncaged control sponges. The camera was positioned on the ground 2 m away from control sponges by SCUBA divers. The divers left the site instantly after the recording was started. After 90 minutes the camera was retrieved again. The sponges were photographed before and after the video recordings to estimate the size of potential bite marks. The tapes were analyzed for the number of fish bites on the sponges. At both transplant sites, we recorded 90 minutes video per week, resulting in 540 minutes of video per site.

All sponges were collected after 6 weeks and their volumes remeasured. Proportional volume changes were calculated as $(\text{Volume (end)} / (\text{Volume (start)} / (\text{Volume (start)}))$. Volume differences were arcsin-transformed and tested for normality (Shapiro-Wilk-test) and homogeneity of variances (Levene's test). Differences in volume changes between treatments were analyzed by an ANOVA with site and caging treatment as fixed factors (SPSS 17).

Field surveys

To check whether mobile invertebrates like echinoderms or crustaceans are important predators of *M. sarasinorum* we conducted field surveys of all potential mobile invertebrate predators at Apra Harbor where specimens of *M. sarasinorum* were recorded. We monitored 72 transects (25 m × 1 m dimensions) during daylight hours (24 transects were laid at 1 m, 3 m and 10 m depths) and 21 transects (25 m × 2 m dimensions) at night time, to account for nocturnal predators (6 transects were laid at 1 m and 5 m and 9 transects at 10 m). Any mobile invertebrates in direct contact with *M. sarasinorum* were recorded (potentially feeding on the sponge).

To assess the present fish community at both sites, species composition and abundance at both sites were obtained from underwater visual census using snorkeling. Three 25 m transects were running parallel to the shore (Tumon Bay) or the reef edge (Western Shoals) at 8 – 11 m depth were surveyed with at least 10m between each transect. Fish species were counted along each transect in a 5 meters wide swath. Fish surveys were conducted three times at each of the caging experiment sites.

To normalize the data set which contains many zeroes, and to down weight the influence of the most abundant species on the analysis, the data was $\ln(x+1)$ transformed prior to analysis. The data was converted to a similarity matrix using the Bray-Curtis measure of similarity, and then analyzed using a cluster analysis and non-metric multidimensional scaling (PRIMER, v. 6). The species most responsible for the observed patterns were then identified using the SIMPER procedure (Clarke and Warwick, 2001).

Chemical defense of *M. sarasinorum*

To investigate if *M. sarasinorum* used chemical defenses against a fish predator the pufferfish *Canthigaster solandri* was used in laboratory feeding assays. *C. solandri* is very

common around Guam, feeding on sponges, ascidians, other invertebrates and benthic algae (Schupp, pers. obs., Amesbury and Myers, 1982). Ten individuals (7.1 – 7.6 cm total length) were kept individually in 70 l flow-through tanks and fed on the days prior to the feeding assays to avoid the loss of preference patterns (Cronin and Hay, 1996). The feeding stations for the assays were made up as follows: Ten sponge individuals were collected from the field experiment site, and their volumes measured before being frozen, freeze-dried and ground to fine powder in a blender. The material was pooled and extracted exhaustively in methanol / ethyl acetate (1:1). The extract was filtered and the solvent removed under vacuum by rotary evaporation. The extract was then incorporated at natural volumetric concentration in an artificial diet, following a modified method by Hay et al. (1994) and Schupp et al. (1999). Commercial catfish food (Rangen Extruded 350, 2 g, ground to a powder), Agar (0.36 g) and distilled water (18 ml) were mixed, resulting in dry mass concentration comparable to the sponge, and heated in a microwave until boiling. After the agar had cooled down, the extract equivalent to 20 ml sponge tissue was dissolved in 2 ml MeOH, added to the catfish food and poured into a mold. Control food was treated in the same way, but only 2 ml solvent was added to the artificial diet. The mold was 2 mm thick with two 2.5×25 cm openings cut into it. One of the openings in the mold was filled with treated food, and the other with control food. A piece of window screen (mesh size 1 mm) was clamped between the mold and wax paper. When the agar cooled completely it was firmly attached to the screen. The screen was then cut perpendicular to the food strips, resulting in screens containing rectangles of one treated and one control food strip (2.5×2.0 cm). To determine the amount of control and treated food eaten, the squares in the window screen served as a grid, and the number of squares where the food had been completely removed were counted. Replicates with no feeding at all were disregarded (always ≤ 1). The food strips were checked periodically and pulled out when at least half of the total food mass was consumed, but not exceeding 3 hours. The results were analyzed using Wilcoxon's signed rank test.

Chemical defense of different sponge tissues

To test whether different tissues of *M. sarasinorum* exhibit different levels of predator deterrence, ten sponge individuals were freeze-dried and their ectosome tissue removed from the choanosome. *M. sarasinorum* possesses a very tough ectosome layer (ca. 3 mm thick) that can easily be separated from the inner choanosome. The separated ectosome and choanosome of the ten sponges were volumetrically measured, each combined, ground to a fine powder and extracted separately as described above. Two-choice feeding assays were conducted with ectosome and choanosome extracts as choices and compared to solvent controls. Differences in consumption rates were analyzed using Wilcoxon's signed rank test.

Field feeding assays

The field assays with generalist reef fishes were conducted following the method by Schupp and Paul (1994). Tissue of whole *M. sarasinorum*-specimen and separated ectosome and choanosome (80 ml each) were extracted as described above. The extracts were dissolved in 4 ml methanol and added to fish food containing 8 g catfish food, 1.5 g carrageenan and 76 ml water. Controls were prepared by adding the same amount of pure methanol instead of the extract. Dissolved extracts always were added to molten food, only after it had cooled down to 60°C in order to avoid compound degeneration due to heat. Mixtures were poured into 1 cm³ molds containing rubber o-rings. The resulting food cubes were attached with the o-rings and safety pins to ropes. Twenty pairs of ropes, with each rope holding either four treated or four control food cubes, were attached on the reef of Western Shoals, Apra Harbor, Guam. The fish community present was dominated by the damselfishes *Amblyglyphidodon curacao*, *Abudefduf sexfasciatus*, *A. vaigiensis*, the wrasses *Cheilinus fasciatus*, *Epibulus insidiator*, the surgeonfish *Naso vlamingii*, the parrotfish *Scarus*

sordidus, and the triggerfish *Balistapus undulates*. We collected the rope pairs when the fishes had removed half of the cubes per rope pair. Wilcoxon's signed-ranks test was used for paired comparisons to test for significant differences in the number of cubes eaten.

Structural defense

We used a slightly modified method of Chanas and Pawlik (1996) to assess if the ectosome of *M. sarasinorum* had a higher structural defense than the choanosome. Lyophilized ectosome and choanosome were extracted sequentially in methanol / ethyl acetate (1:1) for 12 hours and methanol for 4 hours. The sponge skeleton was air-dried for 24 hours, followed by soaking it in 0.5% sodium hypochloride for 5 minutes to remove all remaining cellular material, and rinsing repeatedly in distilled water before drying again.

Skeletons from ectosome and choanosome were cut into $3 \times 2 \times 2$ mm pieces and embedded in an alginate food (Chanas and Pawlik, 1996). In short, skeleton pieces were dipped sequentially into alginate-squid mixtures with increasing concentrations of alginic acid (0.5 - 3%) and then the pieces were hardened in 0.25 M calcium chloride. Control pellets were made of hardened alginate-squid mixtures without sponge skeleton. A control and treated pellet were offered to ten *C. solandri* that were kept individually in separate tanks. A second control pellet was offered subsequently to confirm that fish had not ceased feeding. A rejection was scored when both control pellets were eaten and the treated pellet was spat out at least three times. Three replicate assays were performed for each treatment with a two day interval between assays to avoid conditioning. To test whether the palatability of treated pellets was significantly reduced compared to control pellets, a Fisher exact test (one way) was used (Zar, 1984) and a treatment was regarded as deterrent when six or less treated pellets were eaten ($p < 0.043$, Chanas & Pawlik 1996).

The toughness of ectosome and choanosome tissues of *M. sarasinorum* was assessed as follows; ten sponges were collected from the field experiment site and ectosome and choanosome separated using a scalpel. Three pieces (40×20 mm and 3 mm thick) of ectosome and choanosome were cut out from each sponge. An insect pin was fixed under a plastic cup. The sponge tissue was clamped below the needle between two horizontal PVC plates (10×4 cm). A 4 mm-diameter hole was drilled through the center of both plates to guide the needle. Water was slowly added to the cup until the needle penetrated the sponge tissue completely. The weight of the cup including the water was weighed to determine the force (Newton (N)) required to pierce the tissue. The three tissue pieces from ectosome and choanosome were analyzed separately and the mean was used for statistical analyses. A pair-wise comparison was performed using a paired t-test.

Analysis of structural material and nutritional quality

To quantify structural components and ash content, we used inner and outer pieces (5 g dryweight) of ten sponges, isolated the skeleton as described previously, dried and weighed them (structural material). The tissues were then placed into a muffle furnace at 450°C for 48 h to obtain the mass of ash. Fiber content was calculated as percentage of fiber mass (obtained by subtracting ash mass from total structural mass) per dry mass. Ash content was calculated as percentage of ash mass per dry mass (Becerro et al. 1998). Protein content of ectosome and choanosome was determined by using a Bradford assay (NaOH-soluble protein, Bradford 1976). Freeze-dried tissues of ten sponges were extracted in 1 N NaOH and protein content was analyzed using the Quick Start Bradford Protein Assay (BioRad, Hercules, USA) with bovine serum albumin as a standard. Concentrations were calculated as mg protein per g dry weight of the sponge tissue. Differences in structural components and protein content between ectosome and choanosome were analyzed with paired t-tests.

Results

Field experiment

Sponges lost 2.5 - 5% of their volumes over the course of the experiment (Fig. 2). Neither site, nor the caging treatment had a significant effect on the volume change of the sponges (2-way ANOVA, $p=0.85$ and $p=0.49$, resp.). Video and photo analyses of the sponges showed no incidence of predation. The only fish species that was recorded picking on *M. sarasinorum* was the surgeonfish *Ctenochaetus striatus*. But this species is a herbivore, which feeds on microalgae and its mouth is designed to scoop or strain-out microalgae and biofilm from the substrate (Myers, 1999). This makes penetrating bites through a thick skinned sponge like *M. sarasinorum* very unlikely and photo analyses showed no bite marks on the sponges.

Field surveys

A total of 213 individuals of *M. sarasinorum* were observed across all transects (0.07 Ind./m²). Day time surveys of potential predator groups found densities of (0.13 Ind./m²) for echinoderms, (0.06 Ind./m²) for gastropods and (0.02 Ind./m²) for crustaceans. Night time surveys revealed predator densities of (0.38 Ind./m²) for gastropods, (0.31 Ind./m²) for echinoderms and (0.49 Ind./m²) for crustaceans. No echinoderms or gastropods were observed in direct contact with *M. sarasinorum* and only 24 small hermit crabs (body size < 1 cm) and 3 small shrimps (body size 1-3 cm) were observed on *M. sarasinorum* during night surveys.

Fish surveys

Western Shoals and Tumon Bay revealed very different fish communities. The average similarity between the sites was 24%. While the fish community at Western Shoals was dominated by Damselfish species (50% contribution), the most abundant fishes at Tumon Bay were wrasses (27%), followed by Damselfish (18%) and parrotfish (15%). Species that belong to taxonomic groups known to feed on sponges (*Zanclus cornutus*, angelfish (Pomacanthidae), pufferfish (Tetraodontidae), triggerfish (Balistidae), parrotfish (Scaridae), some wrasses (*Thalassoma* spp.) were much more abundant on transects in Tumon Bay (on average 29.1 ± 3.7 SD individuals per transect) compared to Western Shoals (10.5 ± 1.2 SD individuals per transect).

Chemical deterrence

Field feeding assays: The crude extract of *M. sarasinorum* was strongly deterrent to generalist reef fishes (Fig. 3). While the control food was readily eaten, the food containing extracts of whole *M. sarasinorum* specimen, ectosomal tissue and choanosomal tissue were scarcely consumed (Wilcoxon signed-ranks test, $p<0.0001$, Fig. 3)

Laboratory feeding assays showed that the crude extracts of whole *M. sarasinorum* individuals incorporated into agar-based food reduced feeding of the pufferfish *C. solandri* by over 60% compared to solvent controls (Wilcoxon's signed rank test, $p<0.001$, Fig. 4). Separate extracts of ecto- and choanosome also proved to be deterrent compared to solvent controls (57% or 68%, resp., Wilcoxon's signed rank test, $p=0.01$, Fig 5), but showed no difference in deterrence when compared with each other (Wilcoxon's signed rank test, $p=0.28$, Fig 5).

Structural defense

The spiculated skeleton from the ectosome of *M. sarasinorum* highly deterred feeding of *C. solandri* when incorporated into alginate food (Fisher exact test, $p<0.05$, Fig. 6). The

choanosome had no such effect (Fisher exact test, $p > 0.05$, Fig. 6). Tissue toughness, measured as puncture-resistance, was 7-fold higher in the ectosome ($6.94 \text{ Newton (N)} \pm 0.69 \text{ SD}$) compared to the choanosome ($0.69 \text{ N} \pm 0.11 \text{ SD}$) of *M. sarasinorum* (t-test, $p < 0.0001$).

Structural material and nutritional quality

The analyses of the structural components of ectosome and choanosome revealed significant differences in structural material, fiber content and ash content (t-tests, $p < 0.05$, Fig. 7). While the total structural material and ash content were higher in the ectosome, the fiber content was higher in the choanosome. Quantification of the protein content revealed a significantly greater amount of soluble protein in the choanosome (10.6 mg/ml , $\pm 1.5 \text{ SD}$) than in the ectosome (5.6 mg/ml , $\pm 2.1 \text{ SD}$; t-test, $p < 0.001$).

Discussion

Predation had no effect on *Melophlus sarasinorum* in our field experiment. There was no difference in volume loss between caged and uncaged sponges. This was true even in the marine preserve where fish abundance was much higher than at Western Shoals. Both, the feeding assays with the generalist reef fishes and with the pufferfish *C. solandri* demonstrated that *M. sarasinorum* is chemically defended. Food pellets containing crude extract highly deterred feeding by generalist reef fishes in the field and the pufferfish *C. solandri* in the lab compared to the controls. The analysis of the structural components in ectosome and choanosome confirmed the visual observations that the ectosome of *Melophlus* is much tougher than the underlying choanosome. Moreover, ectosome and choanosome not only proved to be different in their structural composition, but revealed different effects on the feeding behavior of *C. solandri*. Food pellets that contained the spiculated skeleton of the ectosome were highly deterrent, while the food pellets containing the choanosome skeleton had no deterrent effect.

In the marine preserve Tumon Bay the sponge community only consists of very few species (mainly *Stylissa massa*) in low abundances. Our study indicates that predation is not the main cause for the absence of sponges, especially *M. sarasinorum*. It is also unlikely that the prevalent conditions at Tumon Bay during the experiment prohibit the survival of *M. sarasinorum*, since the volume changes of caged individuals were similar at both sites. *M. sarasinorum* lost slightly volume during the experiment regardless of treatments. Since predation cannot be responsible for this volume loss, we think that this loss is caused by the fixation method, since the cable ties compressed the sponge tissue to some extent and had to be retightened over the course of the experiment. This probably caused the slight volume loss that was consistent over all treatments. Both, field feeding assays with the natural fish community and lab feeding assays with the pufferfish *C. solandri* demonstrated that *M. sarasinorum* is chemically defended. Food pellets containing crude extract highly deterred feeding by reef fishes and the generalist pufferfish *C. solandri* compared to controls. The congruent results from the field feeding assays and the pufferfish assays corroborate the use of *C. solandri* as bioassay organism for feeding preferences of benthic reef fishes. The feeding assays with extracts from ectosome and choanosome revealed that both extracts deterred feeding, indicating that the level of defense against fish predators was comparable in both tissues. However, HPLC analyses showed distinct differences in the chemical profiles of ectosome and choanosome extracts (Rohde, unpublished data), and it remains to be seen whether the defensive metabolites in ectosome and choanosome are identical, or if different compounds lead to similar deterrent levels in the different tissues. It has been shown for other marine benthic groups like algae or mollusks that defenses can be allocated to tissues which encounter the highest risk of predation (Avila and Paul, 1997; Hay and Fenical, 1988). In the sponges *Cacospongia* sp., and *Oceanapia* sp., tissue from apical parts

yield higher concentrations of secondary metabolites than their basal parts (Becerro et al., 1998; Schupp et al., 1999) and the sponge *Aplysina fulva* revealed higher concentrations of defensive metabolites on the outermost tissue (Freeman and Gleason, 2010). A recent study on sponges from Antarctica demonstrated that 42% of the sponge species that proved to be unpalatable were only chemically defended in the outermost layer, not in the choanosome (Peters et al., 2009). However, other studies could not detect differences in chemical defense between sponge surface and inner tissue (Burns et al., 2003; Swearingen and Pawlik, 1998, this study).

The percentages of all analyzed components, structural material, fiber content, ash content and protein content differed between ectosome and choanosome. Total structural material was much higher (over 70% of dry mass) in the ectosome than in the choanosome (56% of dry mass) and differed in composition between the two tissue types. The major component in the choanosome was organic fibers (42%), whereas in the ectosome it was ash (66%), which was composed of mainly silica spicules. These data are in line with earlier studies on sponge tissue composition (McClintock, 1987). The ectosome of *M. sarasinorum* is characterized by “a dense felt work of large oxeas, which are placed tangential to the surface at the sponge periphery” (Uriz, 2002). This arrangement of megascleres forms a tough protective outer coat which exhibited a 7-fold greater resistance against pin penetration than the choanosome. While our pin penetration method to measure toughness was not intended to mimic natural predation, puncture resistance is nevertheless often used as a measure of toughness (e.g. Duffy and Hay, 1991; Wakefield and Murray, 1998), and is appropriate to demonstrate basic morphological tissue characteristics. The development of the ectosome as a protective coat has been described for two other sponge species. Hill (1999) found that the choanosome of *Anthosigmella varians* is rapidly eaten by spongivores when the ectosome is removed. He suggested that the spicule- and spongin-rich ectosome of an antipredatory defense. Further, Hill and Hill (2002) found that spicule concentrations in the ectosome increased in response to simulated attacks, indicating an induced defense. However, their studies did not include assays to identify chemical or structural defensive properties.

Besides acting as a morphological defense, a highly resistant ectosome could also serve as an “exoskeleton” protecting the sponge from surface grazer like surgeonfishes or abiotical disturbances like storms.

Feeding assays confirmed the tough ectosomal tissue acts as an effective defensive layer against predation by omnivorous fish like *C. solandri*. While earlier studies found no indication that spicules serve as defensive agents in sponges (Chanas and Pawlik, 1995;1996), this view was challenged when further species were examined. Ferguson and Davis (2008) found that spicules from four of five temperate reef sponges reduced feeding. Also, two studies on Caribbean and Red Sea sponges demonstrated defensive effects of spicules (Burns and Ilan, 2003; Jones et al., 2005) with Burns and Ilan (2003) hypothesizing that only megascleres > 250µm deter feeding. The megascleres of *Melophlus* can reach up to 1.33 mm length (Thiele, 1899) thereby supporting this hypothesis. In the choanosome, these oxea were present, but not abundant and the spicule content was much lower than in the ectosome, diminishing the ability to deter feeding. At the same time, the protein content was much higher in the choanosome, making it more nutritious and more valuable for potential predators. Jones et al. (2005) proposed that structural defense in sponges may be an example of ‘addition exaptation’, i.e. where new functions become added to a trait with an initial functional advantage. Their case study with *X. muta* may support this theory, since spicules in *X. muta* are rather uniformly distributed in the sponge body, which suggests mainly a function of structural support than a defensive purpose. In contrast, the high concentration of spicules in the ectosome in *M. sarasinorum* strongly suggests that this spicule arrangement has evolved as a structurally defensive trait, rather than serving only structural support.

Our field surveys excluded echinoderms as major predators of *M. sarasinorum*. No sea stars, which are the main consumer of sponges in Antarctic waters (Dayton et al., 1974; McClintock et al., 2005), were found in contact with the sponge. The only invertebrates found on *Melophlus* were little hermit crabs and shrimps. Peter et al. (2009) hypothesized that mesograzers like amphipods feed on sponges in Antarctic waters. Our results do not indicate feeding of these small crustaceans, but these mesograzers and the surgeonfish *C. striatus*, which were apparently grazing on the surface of *M. sarasinorum* were the only mobile animals found in contact with the sponge. One can hypothesize that grazing of these or similar small mesograzers or grazing by detritus or biofilm feeding fishes such as the surgeonfish *Ctenochaetus* may have been the selecting force for the evolution of a structurally defended outer layer, since they may lack the ability to penetrate the tough ectosome of *Melophlus*. The allocation of structural defense supports ODT by being restricted to the surface layer that experiences the highest predation risk. We propose that the ectosome serves as a defensive coat against mesograzers feeding, or detritivores or biofilm feeding fishes, shielding the less protected, but more nutritious choanosome. Excluding sea stars and mesoherbivores as main predators, fishes seem to be the most likely predators. Trunk fishes or parrotfishes have been shown to feed on sponges in the Caribbean (Pawlik, 1998; Randall and Hartman, 1968; Wulff, 1994), and have the ability to penetrate even coral skeletons with their jaws (Bellwood and Choat, 1990). A structural defense would be ineffective against these predators and sponges would need to rely on their chemical defenses. The homogenous allocation of chemical defenses in *Melophlus* meets the prediction of the ODT, since fish bites will remove not only the ectosome, but also part of the choanosome. Thus it seems that by combining morphological and chemical defenses *Melophlus* can minimize predation, which was corroborated by the lack of fish feeding during our video observations.

In conclusion, our study shows that predation had no effect on the growth and distribution of *M. sarasinorum*, that the sponge is well defended against predation and that the allocation of secondary metabolites as well as structural defenses in *Melophlus* is in accordance with the ODT. This is the first study to demonstrate that structural defenses can be allocated to distinct parts of a sponge to increase defensive efficiency. Even though *M. sarasinorum* is a rather rare example, with a distinct ectosome and choanosome, this study nevertheless demonstrates that some sponges have evolved discrete tissues to adapt to different ecological pressures. These results support recent findings where sponges are often chemically defended in the first place, but also contain structural components which effectively increase anti-predatory defenses and can be an adaptation to predators that feed mainly on sponge surfaces.

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Figure 1. Intact specimen of *M. sarasinorum* (a) and a specimen in sagittal section (b) showing different texture of exosome and choanosome.

Figure 2.

Mean proportional volume change of *M. sarasinorum* in the field experiment. Sponges were exposed at two sites, Western Shoals and the marine preserve Tumon Bay, for six weeks to natural reef predators (uncaged), or protected from predation by cages (caged). Cage controls served as controls for cage artifacts. Data show the mean $\pm$ 1 SD (n=12).

Figure 3.

Results of three field feeding assays testing (a) food treated with Crude extract of whole specimen of *M. sarasinorum* against untreated controls, (b) food treated with crude extract of the ectosome against untreated controls and food treated with crude extract of the choanosome against untreated controls. In each assay, 20 pairs of ropes (each rope holding four food pieces) were offered to generalist reef fish. * indicate a significant difference in the pairwise analyses ($p < 0.001$, Wilcoxon's signed rank test).

Figure 4. Effect of crude extract of *M. sarasinorum* on predation by *C. solandri* (mean + 1 SD, n=12). Food pellets contained organic extracts at natural volumetric concentrations or solvent only. P-values indicate the result of the pair wise analysis (Wilcoxon's signed rank test).

Figure 5.

Effect of crude extracts from ectosome and choanosome from *M. sarasinorum* on consumption by *C. solandri* (mean + 1 SD, n=12). Food pellets contained organic extracts at natural volumetric concentrations or solvent only. P-values indicate the result of the pair wise analyses (Wilcoxon's signed rank test).

Figure 6.

Feeding assay of the spiculated skeleton from ectosome and choanosome of *M. sarasinorum* in prepared food. Three replicate assays were performed for each treatment. Mean number of pellets eaten + 1 SD is shown. Both control pellets were eaten in all assays. For any individual assay, an extract was considered deterrent if the number of pellets eaten was ≤ 6 ($p \leq 0.043$, Fisher's exact test, 1-tailed) as indicated by the dotted line on the figure.

Figure 7. Distribution of structural components in ectosome and choanosome tissue of *M. sarasinorum*. Bars represent the means + 1SD (n=10). P-values indicate the result of pair wise analyses (paired t-test).