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## Surface sequestration of chemical feeding deterrents in the Antarctic sponge *Latrunculia apicalis* as an optimal defense against sea star spongivory

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**Abstract** *Latrunculia apicalis* is a spherically shaped demosponge that previous investigations have shown is rarely preyed upon by sea stars which are the dominant spongivores in antarctic benthic communities. Prior studies have also demonstrated that *L. apicalis* produces organic compounds that elicit a tube foot retraction response in the keystone spongivorous sea star *Perknaster fuscus* that can be used as a reliable assay for feeding deterrence. *L. apicalis* is known to contain discorhabdin alkaloids which serve, among other roles, as the source of its green coloration. To assess the defensive nature of the discorhabdin alkaloids toward *P. fuscus*, we have determined discorhabdin G concentrations in discrete sponge layers and evaluated those concentrations in the *P. fuscus* bioassay. In discorhabdin G-bearing sponges, we found a gradient of discorhabdin G that falls off rapidly toward the center of the sponge. On average, 52% of total discorhabdin G in a given sponge was found within 2 mm of the sponge surface. Tube foot retraction responses to extracts from the surface tissues (0–2 mm depth) of *L. apicalis* were compared to those of an inner layer (8–10 mm depth) and to a sample comprised of the same inner layer spiked with discorhabdin G at a concentration equivalent to that of the surface tissues. Tube foot retraction response times to extracts of the surface layers and the

spiked inner layers were not statistically different, but were significantly greater than responses to the unaltered inner layer and controls. These results support the predictions of the optimal defense theory as *L. apicalis* sequesters its defensive chemistry (discorhabdin G) in its most vulnerable surface tissues, where the likelihood of predation from sea stars is highest. As antarctic sponges are generally preyed upon by extraoral feeding sea stars rather than deeper biting predators such as fish, surface sequestration may be uniquely adaptive in sessile macroinvertebrates occurring in antarctic marine benthic environments.

### Introduction

Sessile or sluggish benthic marine invertebrates, like plants and other organisms lacking mobility, have a number of mechanisms to avoid or survive predation (Cronin 2001, Stachowicz 2001). Such defensive mechanisms span a wide range of methods broadly categorized as biological, physical and/or chemical. For example, biological defenses include ecological (e.g., niche selection), behavioral (e.g., nocturnal habits), or physiological (e.g., growth and/or reproductive rate optimization) adaptations, while physical means include elaboration of skeletal concretions (e.g., shells or spines) or inclusions (e.g., spicules). Chemical defenses are characterized by the biosynthesis or dietary sequestration of toxic, noxious or distasteful metabolites (Eisner and Meinwald 1995; McClintock and Baker 2001). Presumably, organisms must balance the energetic costs of defense against those of growth and reproduction (Coley et al. 1985; Cronin 2001).

Studies of the functional roles of defensive metabolites in marine invertebrates have received increasing attention (Paul 1992; Pawlik 1993; Hay 1996; Faulkner 2001; McClintock and Baker 2001). We have been interested in trophic interactions of Antarctic invertebrates which are mediated by secondary metabolites

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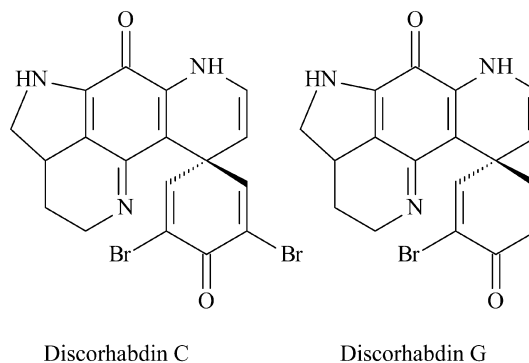
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(McClintock and Baker 1997; Amsler et al. 2001 a,b). Below the zone of ice scour at high latitudes, the shallow-water benthos is dominated by sponges (Dayton et al. 1974). Predation by the keystone spongivorous sea star *Perknaster fuscus* and other sea stars are the primary biological interactions structuring this system (Dayton et al. 1974; McClintock 1994). Because sponges are well known to produce diverse secondary metabolites (Faulkner 2001), often for the purpose of mediating trophic relationships (Paul 1992; Pawlik 1993; Hay 1996; McClintock and Baker 1997; Amsler et al. 2001a,b), we have investigated the impact of sponge natural product chemistry on aspects of feeding behavior in the sea star *P. fuscus* (McClintock et al. 1994, 2000; McClintock and Baker 1997; Amsler et al. 2000a).

To evaluate the feeding deterrent properties of sponges we developed a behavioral bioassay which assesses the response of the chemosensory tube feet of spongivorous sea stars (Sloan 1980). While direct feeding assays, assessing acceptance or rejection of food pellets containing extracts of sessile or sluggish marine invertebrates, are possible with some omnivorous antarctic sea stars (e.g., *Odontaster validus*, McClintock and Baker, 1997; Avila et al. 2000), the keystone spongivore *P. fuscus* is not amenable to food pellet assays. Nonetheless, when presented extracts of sponges, *P. fuscus* tube feet display responses characteristic of feeding preferences observed in nature (Dayton et al. 1974). For example, the rapidly growing, space-dominating sponge *Mycale acerata* does not cause tube foot retractions, while the brightly colored *Kirkpatrickia variolosa* that is avoided by sea star predators elicits tube foot retractions (further species-specific examples given in Amsler et al. 2000a, 2001a). Although extracts from sponges, or other items *P. fuscus* finds palatable, initially cause the tube foot to retract into the ambulacral groove, followed several to 30 s later by the tube foot extending back to its original position and/or attaching to nearby substrate, extracts from sponges that *P. fuscus* finds unpalatable can cause the tube foot to remain retracted for up to 60 s or more. Consequently, this bioassay can be used to indicate sea star feeding preferences (see Amsler et al. 2000a, 2001a).

Among Antarctic sponges studied to date, chemical extracts from the dark green sponge *Latrunculia apicalis* Ridley and Dendy (family Latrunculiidae, order Hadrimerida), produce a behavioral response in *P. fuscus* which is indicative of feeding deterrence (McClintock et al. 1994, 2000). We have reported two discorhabdin alkaloids, discorhabdin C and G (Fig. 1) from *L. apicalis* (Yang et al. 1995), both of which were found to have cytotoxic and antimicrobial bioactivities. A distinct color gradient in sectioned fresh specimens of *L. apicalis* suggested a strong pigmentation gradient falling off towards the interior of the sponge. Previous investigations reported that antarctic sponges are subjected to intense surface feeding through the extraoral feeding of sea stars (Dayton et al. 1974), while lacking deeper biting fish predators common in tropical marine systems



**Fig. 1** Chemical structures of discorhabdin alkaloids from *Latrunculia apicalis* (Yang et al. 1995)

(McClintock and Baker 2001). We exploited a unique opportunity to assess whether surface sequestration of defensive chemistry occurs in the sponge *L. apicalis*.

## Materials and Methods

### Sponge and sea star collection

Individuals of the sponge *Latrunculia apicalis* were collected at depths of 35–40 m from several locations in McMurdo Sound, Antarctica (77°S, 164°E), including the continental coast at Granite Harbor and the coast of Ross Island, in the austral summers of 1996 and 1997. Intact sponges for chemical analysis were frozen immediately upon collection while taxonomic vouchers were photographed (in situ and in the laboratory) and preserved in 70% alcohol. Field observations revealed two distinct color morphs, a light and a dark green. In our collections, only one of the individuals belonged to the light green morph and was kept separate. Taxonomic identification of the sponges was carried out by Professor Robert van Soest, University of Amsterdam. The sea star *Perknaster fuscus* was collected either from nearby McMurdo Sound sites or from similar depth ranges in the Anvers Island archipelago (64°S, 64°W) on the Antarctic Peninsula. Sea stars were maintained in flowing sea water aquaria at ambient (-1.8 to 1.5°C for McMurdo, -1 to +1°C at Anvers Island) temperature when not being used in bioassays.

### Analytical determination of discorhabdin G

The alkaloid discorhabdin G was isolated from *L. apicalis* according to Yang et al. (1995) for use as a standard. Serial dilutions of 400, 300, 200, 100, 50, 25, and 5 µg discorhabdin G/100 µl methanol were then analyzed by high performance liquid chromatography (HPLC) using a Waters 401 HPLC system outfitted with a Waters 490E UV detector. The analytical method utilized two YMC AQ (250 mm × 10 mm, 5 µm) HPLC columns in series. In our solvent system of 49.9:49.9:0.2 MeOH/Water/TFA, discorhabdin G displayed a retention time of 25.5 min with no other UV absorptions (254 nm) closer than 3 min in either direction. Peak areas from triplicate analyses of UV response from each dilution were plotted versus mass of discorhabdin G injected to generate a calibration curve.

### Sponge analysis

Successive layers of sponge were removed from individual *L. apicalis* in 2-mm increments (±0.5 mm as judged by calipers) by shaving freeze-dried spherical specimens with a scalpel. Ten 2-mm

layers were removed from each sponge. Shavings from each discrete layer for a given individual were combined and extracted in methanol ( $3 \times 100$  ml, 24 h each) and the methanol extracts concentrated to give a crude methanol extract for each layer. A 30-mg aliquot from methanol extracts of each layer was analyzed for discorhabdin G content using the HPLC method described above. Detector response (area under the curve) was correlated to milligram discorhabdin G using the calibration curve.

Intraspecific differences in discorhabdin G concentration among the discorhabdin G-bearing sponges were derived by expressing alkaloid mass from each layer as a proportion of sponge material from which it was derived. Thus, discorhabdin G mass in the 30-mg aliquot ( $DRG_A$ , in milligrams, derived from the calibration curve) was expressed as an extract concentration ( $DRG_E = DRG_A/30$ , in mg discorhabdin G/mg extract). Total extract content of discorhabdin G ( $DRG_T$ , in milligrams) is then  $DRG_E$  times the extract mass. Summation of  $DRG_T$  for the ten layers provides individual sponge discorhabdin G content ( $DRG_S$ , in milligrams). This total mass value ( $DRG_S$ ) was divided by the dry weight of the sponge material (in grams) from which it was extracted, yielding discorhabdin concentration in parts per thousand (mg/g).

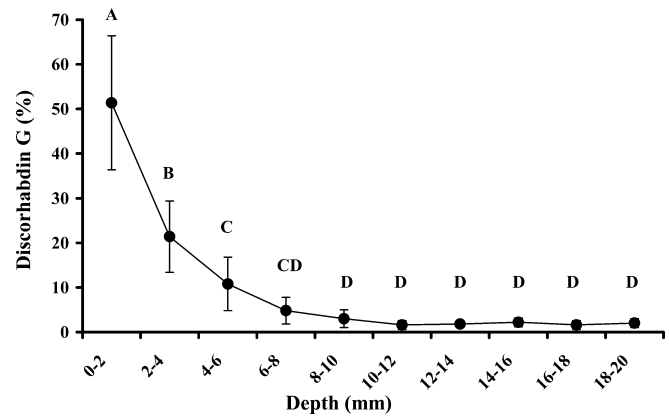
Within-individual distribution of discorhabdin G was derived by comparing the discorhabdin G content, as a percent of total sponge discorhabdin G, from each layer of the same sponge. These data are derived by expression of discorhabdin G mass ( $DRG_E$ ) as a proportion of total sponge discorhabdin G ( $DRG_S$ ). Intraspecific differences in within-individual discorhabdin G distribution were established by plotting individual *L. apicalis* discorhabdin G distributions as  $DRG_E/\text{dry weight of layer tissue mass}$ . The one light green morph of *L. apicalis* proved to lack discorhabdin G. Therefore, three additional light green specimens were subsequently collected, extracted, and analyzed for discorhabdin G (Yang et al. 1995). None of these individuals contained discorhabdin G. Therefore, the present study focuses on the dark green discorhabdin-G-bearing individuals.

## Bioassay

Details of the bioassay protocol have been published (McClintock et al. 1994, 2000). In the current study, individuals from a pool of ten *P. fuscus* were randomly selected, used for one treatment series, then returned to an isolated aquarium to keep them separate from unused sea stars. All ten sea stars were used with each treatment series and no individual sea star was used with more than one replicate of any individual treatment. A single treatment series entailed evaluation by one sea star of five different individual treatments. Each set of five treatments included: (1) a sponge outermost layer (0–2 mm) extract; (2) a sponge inner layer (8–10 mm) extract; (3) the same inner layer extract spiked with the natural concentration of discorhabdin G found in the outer layer; (4) a fish tissue-extract control and (5) a pure silicone grease control (McClintock et al. 1994). The order of presentation of treatments within an individual series (individual sea star) was randomized. Each treatment was adhered to individual glass rods with silicone grease and the rods were placed in the proximity of extended tube feet of an upturned *P. fuscus* in a finger bowl of ambient temperature sea water. The length of time that tube feet remained retracted was recorded, up to a maximum of 60 s. Given the high individual variation in discorhabdin G content (Fig. 2), we chose the lowest natural outer layer concentration (equivalent to 6 mg/g sponge) for spiking experiments.

## Statistical analyses

To determine whether significant differences occurred between discorhabdin G levels in different sponge layers, parametric one-way analysis of variance with a General Linear Models procedure using SPSS software (SPSS Inc., Chicago, Ill.) were performed. When significant differences were found, the specific differences



**Fig. 2** Concentration of discorhabdin G in layers of *L. apicalis* ( $n=5$ ) expressed as a percentage relative to total discorhabdin G determined for all layers. Mean  $\pm$  1 SD. Analysis of variance indicated significant differences between layers ( $F_{9,49} = 30.048$ ,  $P < 0.0005$ ). Post hoc analysis Ryan-Einot-Gabriel-Welsch (REGWQ,  $p < 0.05$ ) results indicating differences between means are represented by letters above markers. Means with different letters are significantly different

were identified with a Ryan-Einot-Gabriel-Welsch (REGWQ) post hoc test with level of significance set at  $P=0.05$ . In order to satisfy assumptions of equal variance, discorhabdin G concentration data were transformed by  $\log_{10}$  and percent total discorhabdin G data were transformed by  $\log_{10}[\text{arcsine}(\text{square root})]$ .

Statistical analyses to determine whether significant differences occurred between treatments in tube foot retraction experiments were performed by non-parametric one-way analysis of variance with a Friedman Test using SPSS software. When significant differences were found, the specific differences were identified using pair-wise Wilcoxon signed ranks tests (SPSS Software) followed by a sequential Dunn-Sidak method (Sokal and Rohlf 1994) to correct for type I error. The level of significance was set at  $P=0.05$  after error correction. The Friedman and Wilcoxon signed ranks tests are non-parametric tests for related samples. Because the same ten sea stars were used for all treatments within an experiment the samples were not independent, necessitating these tests.

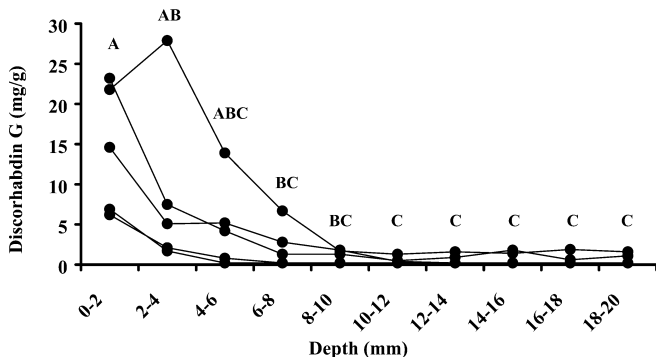
## Results

Taxonomic analysis of the dark green and light green color morphologies of *Latrunculia apicalis* supported their assignment to the same species, but noted distinct differences in spicule size and shape (van Soest, personal communication). As noted above, light green sponge morphs do not possess discorhabdin G. Nonetheless, in an earlier study we demonstrated that sea star tube foot retractions occur in response to lipophilic extracts of light green morphs of *L. apicalis* and are caused by discorhabdin C (Fig. 1; Yang et al. 1995). The present study focuses on chemical sequestration in the dark green discorhabdin-G-bearing sponge morph.

Sponge mass and volume were tracked in a linear fashion (Table 1;  $r^2 = 0.99$ ). However, discorhabdin G content, expressed either in absolute mass in an individual specimen or relative to sponge tissue mass, is highly variable among individuals and does not track either sponge mass or volume (Table 1).

**Table 1** Sponge and natural product characteristics for five *Latrunclia apicalis* specimens investigated in this study. Sponge volume determined by displacement of water in a graduated cylinder and discorhabdin G content (dry wt) based on the sum of concentrations determined for outermost ten layers (see Materials and Methods section for details). n/d No data

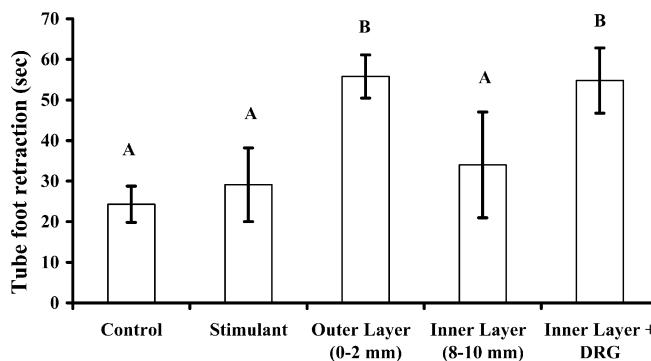
Sponge mass (g)	Sponge volume (cm <sup>3</sup> )	Discorhabdin G content (mg)	Discorhabdin G concentration (mg/g)
59.5	370	299.0	42.2
57	n/d	499.0	79.8
50	n/d	77.3	10.2
20.4	145	33.4	10.5
16.2	115	72.3	37.0



**Fig. 3** Concentration of discorhabdin G expressed in milligrams per gram of sponge (parts per thousand) for five individuals of *L. apicalis*. Analysis of variance indicated significant differences between layers ( $F_{9,49} = 5.747$ ,  $P < 0.0005$ ). Post hoc analysis (REGWQ,  $P < 0.05$ ) results indicating differences between means are represented by letters above markers. Means with different letters are significantly different

Analysis of variance revealed significant differences in discorhabdin G levels in the different layers of discorhabdin-G-containing sponges (Fig. 2). The outermost sponge layer (0–2 mm) contained significantly more discorhabdin G than any other layer (mean 52%, range 35–78%; Fig. 2). Discorhabdin G was differentially sequestered in the outermost layer with significant decreases in concentration between each of the first three 2-mm layers and a constant, very low concentration from the 8–10 mm layer inward (Fig. 2).

There was a four-fold variation in absolute discorhabdin G concentrations between individual sponges (Fig. 3). Concentrations in the outer layer ranged from 6 to 23 mg/g. However, the pattern for decreasing concentration towards the interior was consistent within all individual discorhabdin-G-containing sponges and the overall decrease was still significant even when concentrations were compared on an absolute basis (Fig. 3). With the exception of one specimen, concentrations fell off rapidly between layer 1 (0–2 mm) and layer 2 (2–4 mm), continuing a roughly exponential curve toward an asymptotic level below 3 mg/g. The single individual not following this pattern had a slightly higher concentration in the 2- to 4-mm layer than the outermost, 0- to



**Fig. 4** Tube foot retraction responses of *Perknaster fuscus*. Means  $\pm 1$  SE ( $n = 10$ ). Control group consisted of a glass rod coated with the silicone grease matrix used to coat extracts onto the rod. Stimulant group consisted of a methanol extract of freeze-dried Antarctic cod muscle (*Dissostichus mawsoni*) blended with silicone grease matrix (1:1 w/w). Remaining experimental groups consisted of extracts from the described layer blended with silicone grease matrix (1:1 w/w). Results of statistical pair-wise comparisons (Wilcoxon signed ranks test with sequential Dunn-Sidak correction) indicating differences between means are represented by letters above markers. Means with different letters are significantly different. DRG Discorhabdin G

2-mm layer but concentrations decreased from there inwards toward the interior as with the other sponges.

Analysis of variance (Friedman test) of results from the *P. fuscus* tube foot retraction bioassay (Fig. 4) indicated significant differences ( $P < 0.0005$ ) between treatments. Pair-wise comparisons (Wilcoxon signed ranks test with sequential Dunn-Sidak correction) revealed that the outermost layer (0–2 mm) extract and the inner layer (8–10 mm) extract spiked with 6 mg/g discorhabdin G elicited tube foot retraction responses that were not significantly different from each other but that both were significantly longer ( $P < 0.05$ ) than the responses to the inner layer extract alone or to the glass rod or feeding stimulant controls (Fig. 4). The response to the inner layer was not significantly different from either control (Fig. 4).

## Discussion

Concentrations of discorhabdin G were highly variable within and among individuals of the Antarctic sponge *Latrunclia apicalis*. Several theories have sought to explain such patterns of secondary metabolite distribution. Among these theories are those that describe patterns of distributions within individual organisms (Loomis 1953; Rhodes 1979; Herms and Mattson 1992) and those addressing patterns among individuals of the same species (Rhodes 1979; Bryant et al. 1983). The variation between absolute discorhabdin G concentrations we observed in the outer layer of the five individual sponges might be explained by the inducible defense model (IDM; Karban and Myers 1989; Harvell 1990). The IDM predicts that defense production should be directly correlated with risk of attack. Without knowing

the predation history of the individual sponges we can only speculate on the possibility of differential predation levels resulting in differential levels of discorhabdin G induction. Nonetheless, temporal patterns of sea star predation on antarctic sponges, which can occur on the order of months (Dayton et al. 1974), are likely to favor the evolution of induced defenses (Amsler 2001).

Most resource allocation models that address patterns of secondary metabolite distributions are based on observations made in terrestrial plant systems (Cronin 2001). The most comprehensive of the resource allocation models is the optimal defense theory (ODT; Rhoades 1979), which examines within-organism variations in defensive chemistry in the context of the competing relationship between growth and the production of chemical defenses. The model assumes that there is some energetic (and/or other) cost to the production of defensive compounds. ODT predicts that defenses should be directly correlated with risk of attack and inversely correlated with the cost of a particular defense. Furthermore, the theory predicts that within an organism, defenses should be differentially allocated to those tissues or structures most valuable in terms of fitness and that there should be a correlation between energetic investment and defense in specific tissues. ODT was developed in the context of the common and often marked differences observed in defensive compound allocations to various organs and tissues in higher plants (McKey 1974, 1979; Denno and McClure 1983). Ecological parallels between plants and sessile or sluggish marine invertebrates have often been noted and these parallels extend to chemical defense mechanisms (e.g., Hay 1996).

Several studies with tropical marine sponges have demonstrated natural product allocation patterns characteristic of the ODT, although few have documented the ecological relevance. For example, Thompson et al. (1983) found aerothionin and homoaerothionin in spherulous cells (spherule-bearing cells that line the aquiferous conducts and are generally more common in the ectosomal surface or subsurface sponge regions; Uriz et al. 1996; Bourny-Esnault and Rützler 1997) of the sponge *Aplysina fistularis* where their antipredatory role may be enhanced (Thompson 1985). Similarly, Turon et al. (2000) using X-ray microanalysis and cryofixation techniques found brominated compounds localized within both spherulous cells and sponge fibers. The sponge *Crambe crambe* also concentrates its bioactive metabolites (guanidine alkaloids) in spherulous cells (Becerro et al. 1997). The sesquiterpene avarol may be localized in choanocytes of the sponge *Dysidea avara* (Müller et al. 1986), although Uriz et al. (1996) point out that cell types may have been incorrectly identified in this study. Spirodysin is present in the archaeocytes and choanocytes in *D. herbacea* (Flowers et al. 1998). Terpenes in the sponge *D. fragilis* have been located in what these investigators term "inter-cellular vesicles" (Marin et al. 1998); however it is not clear whether such vesicular structures occur in sponges and could be artifacts of

histology. Despite the suite of studies cited above, none of these investigations have examined ecologically relevant concentrations of compounds against relevant predators. While not an examination of surface sequestration of defensive chemistry, Schupp et al. (1999) did find antipredatory compounds localized in the stalk of the "lollipop sponge" *Oceanapia* sp., suggesting the gamete-bearing spherical mass rests atop a chemically defended stalk.

Antarctica is unique in that marine invertebrates in general and sea stars in particular are the major predators on benthic animals (Dayton 1972, 1990; Dayton et al. 1974). Sponges often dominate the benthic fauna (Dayton et al. 1970; Barthel and Gutt 1992; Barnes 1995; Sáiz-Salinas et al. 1997) and sea stars are keystone predators controlling sponge populations (Dayton 1972, 1990; Dayton et al. 1974; Dearborn 1977). Sea stars are markedly different from other, biting predators such as fish because they typically feed by extruding their cardiac stomach against their prey for external digestion (Hyman 1955). This feeding behavior should be a particularly strong selective force for surface sequestration of chemical defenses, especially in Antarctica where sea stars are the major sponge predator and deeper biting spongivores are uncommon (Dayton et al. 1974; McClintock 1994). We have demonstrated that *L. apicalis* also sequesters defensive secondary metabolites in areas that are particularly vulnerable to predators as would be predicted by ODT. Furthermore, we have demonstrated that the surface layer where the metabolite (discorhabdin G) is sequestered is significantly defended from sea star predators while the inner layers are not. However, if discorhabdin G is added to extracts of the inner layers at the minimum levels detected in outer layers, these "spiked" inner layers are then equally well defended against the predator. This demonstrates that the differential distribution of this compound is sufficient to explain the differential response of the sea star predator to inner and outer layer extracts. In sequestering discorhabdin G in this way, *L. apicalis* can conserve resources which would have gone into provisioning the entire sponge volume with equal concentrations of the defensive chemistry.

Kubaneck et al. (2002) documented differential distributions of defensive secondary metabolites in outer compared to inner layers of the tropical sponges *Ectoplasia ferox* and *Erylus formosus*. The concentration of a mixture of defensive triterpene glycosides in *Ectoplasia ferox* was approximately twice as high in the outer 2 mm layer of this sponge as in either the 2-6 or 6-14 mm deep layers and is suggested to deter very shallow-biting predators. In contrast, the concentration of the defensive triterpene glycoside formoside in *Erylus formosus* was only about one-third as high in the outer 1 mm layer as in 3 interior layers (1-3, 3-7, and 7-14 mm depths), where the concentration was uniform. Becerro et al. (1998) found concentrations of crude organic extracts containing defensive metabolites were higher at the base and in the outer tissues versus the inner core of the branching

tropical sponge *Cacospongia* sp., yet even the lowest concentration was effective against fish predators. Sweringen and Pawlik (1998) investigated the relative feeding deterrence towards predatory fishes of crude extracts of the outer 2 mm layer compared to all layers inwards of that in the tropical sponge *Chondrilla nucula* and observed no differences in defensive properties between the layers. The lack of differential deterrence in *C. nucula* and *Cacospongia* sp., lack of differential surface sequestration in *Erylus formosus*, and the much smaller differences between surface and inner layers observed in *Ectoplasia ferox* when compared to our data for *L. apicalis* are likely the result of a much greater importance of large biting predators that could easily penetrate outer sponge layers in tropical ecosystems compared to Antarctica.

The allocation of the bioactive compound discorhabdin G to the outermost layers of *L. apicalis* may serve additional roles beyond the prevention of sea star predation including both the inhibition of surface fouling and mediation of allelochemical interactions. Seasonal blooms of benthic epizooic diatoms infest Antarctic sponges potentially clogging pores and impeding efficient filter feeding (Moeller 1998). While secondary metabolites from *L. apicalis* have not been examined to date for their potential antifoulant activity, polar and non-polar organic extracts of seven of eight common Antarctic marine sponges have been shown to display bioactivity against sympatric benthic diatoms (Amsler et al. 2000 b). This suggests that antifoulant chemical defenses may be common among Antarctic sponges. That *L. apicalis* collected in the field are rarely fouled (Amsler, Baker, McClintock, personal observation) further suggests that this sponge may possess chemical antifoulants. The possibility that discorhabdin G or other potential defensive metabolites serve as allelochemicals may be less likely because this sponge is not an encrusting species. Nonetheless, while most Antarctic sponges have very low growth rates, there are several species including the common *Mycale acerata* and *Homaxinella balfourensis* that are capable of rapid growth and overgrowth of non-encrusting neighboring sponges and other sessile invertebrates (Dayton et al. 1974). Coupled with intense competition for space likely resulting from a very high percent bottom cover (Dayton et al. 1974), there is a reasonable likelihood that allelochemical interactions may yet be elucidated in antarctic sponges.

In summary, to date no studies have found such a striking localization as that demonstrated by the manifold increases in defensive metabolites in outer layers of the antarctic sponge *L. apicalis*. Sponge metabolite sequestration studies have been concentrated in tropical areas where fishes, whose bites penetrate well below the sponge surface, are the dominant sponge predators. Localization of defensive chemistry primarily to the outermost layers in antarctic marine sponges could be highly adaptive because of the ubiquity of sea star sponge predators that feed by extrusion of the cardiac

stomach. Additional studies broadening our examination of the surface sequestration of defensive metabolites in antarctic sponges are needed to further substantiate this hypothesis.

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