THE CHEMICAL ECOLOGY OF ANTARCTIC SPONGES

by

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A DISSERTATION
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Sponges were collected from shallow waters along the western Antarctic Peninsula near Palmer Station on Anvers Island. The majority (78%) of the sponges were found to have outer tissues defended against the omnivorous, Antarctic sea star Odontaster validus. Of the species that had outer tissues defended, 62% of them also had internal tissues that inhibited feeding by O. validus. Lipophilic or hydrophilic extracts coated on artificial food pellets were found to be unpalatable for all of the sponge species tested. These data provide evidence that defenses are common survival strategies in sessile macroinvertebrates from Antarctica but that the allocation of the defenses do not follow predictions made by the Optimal Defense Theory in an environment where sea stars drive the evolution of defenses. While it cannot be determined whether all of the unpalatability is due to secondary metabolites in these sponges, a large proportion of the defenses can be explained in those organisms that were analyzed.

The secondary metabolites present in the sponges did not inhibit the majority of bacteria isolated from the same system from which the sponges were collected. These chemical compounds did cause significant diatom mortality when presented to a diatom species collected from the same area (Syndroposis sp.). It appears that bacterial pathogens are not a substantial threat to sponge survival in these waters due to the sponges not producing compounds that would prevent bacterial growth in their presence.
However, fouling by diatoms does appear to be a threat which these sponges try to prevent.

No solid conclusions can be made concerning the ability of a sponge to have defenses induced by the constant presence of predators. The sponges are commonly found to have defensives present in all body tissues although some compounds have been seen to be isolated in external tissues. These sequestered compounds are not always feeding deterrents. There are many questions that remain relating to predator-prey relationships in coastal Antarctic Peninsula waters.

Keywords: Chemical ecology, Antarctica, Porifera, Antimicrobial, Optimal Defense Theory, Inducible defenses
DEDICATION

To my family who have supported me through this long journey and never stopped believing in me.
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<td>ACC</td>
<td>Antarctic Circumpolar Current</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<tr>
<td>°C</td>
<td>degrees Celsius</td>
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<td>CaCl₂</td>
<td>calcium chloride</td>
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<td>cm</td>
<td>centimeter</td>
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<td>CNBH</td>
<td>carbon:nutrient balance hypothesis</td>
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<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>IDM</td>
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<td>mm</td>
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<td>mya</td>
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<tr>
<td>n</td>
<td>number of replicates</td>
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<td>optimal defense theory</td>
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<td>OPP</td>
<td>Office of Polar Programs</td>
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<td>RAM</td>
<td>resource availability model</td>
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<td>rRNA</td>
<td>ribosomal ribonucleic acid</td>
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<td>second</td>
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<tr>
<td>SCUBA</td>
<td>self contained underwater breathing apparatus</td>
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<td>University of Alabama at Birmingham</td>
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INTRODUCTION

Chemical ecology is the study of the use and nature of chemicals utilized in interactions between organisms or between organisms and their environment (Smith & Smith 1998). These interactions can include chemical communication, environmental sensing, and chemical defenses (Amsler 2008). Chemical defenses can be useful in deterring predation, biofouling, colonization by pathogens, or preventing competitors from encroaching upon a substrate that is already occupied (McClintock & Baker 2001). The compounds that are responsible for these chemical defenses are often referred to as secondary metabolites (Whittaker & Feeny 1971).

The allocation of these secondary metabolites has been studied in both terrestrial and marine systems. Ecological theories have been formulated whereby based on assumptions as to fitness costs of these defenses, predictions can be made as to the type(s) of defenses that will be present and the locations of these defenses (Cronin 2001, Stamp 2003). The majority of these theories have been established to explain allocation of defenses in plants, but some of them can be just as useful when studying benthic, sessile invertebrates. Just like plants, these organisms cannot flee when being preyed upon and thus must adapt or face extinction.

The optimal defense theory has been proposed with the assumption that organisms have been able to maximize individual fitness through evolution and allocation of defenses (Rhoades 1979). In order for the theory to be plausible, the defenses must be
adaptive to the various situations (Stamp 2003). The production of these defenses also
means that metabolic energy that could otherwise be used for growth or reproduction has
been shunted from these other metabolic processes. Costs of defenses can be measured
in the amount of energy that is required to create the defense and then maintain it until
the time it is required. Consequently, the defenses are predicted to be present in direct
proportion to the risk of predation and indirectly proportional to the metabolic cost of the
defense (Rhoades 1979). Areas with a large number of predators are predicted to have
benthic invertebrates that have evolved more defenses than organisms in areas where
predation risk is less frequent. In this situation, these secondary metabolites are normally
larger molecules and effective against a broad suite of predators; however, they are only
effective when large amounts are consumed (Feeny 1976). Conversely, benthic
invertebrates that live in areas with less predation or are less apparent to predators are
predicted to have fewer defenses that are more potent in low doses to a smaller group of
specialist predators.

Other hypotheses dealing with optimal defensive strategies include individuals
allocating defenses in direct proportion to areas that are more valuable to survival with
more valuable portions of the individual getting a greater percentage of the defenses
(Rhoades 1979). Defenses are also predicted to be decreased in the absence of predators
and increased when they are present. A predatory event will then be able to induce
greater production of the defenses only when they are most beneficial to survival
(Rhoades 1979). Lastly, because defenses are created with metabolic energy that could
otherwise be used for other processes, stressed individuals will produce fewer defenses
than unstressed individuals (Rhoades 1979).
Ecological theories have also been proposed for plants that make predictions on defenses based on resource availability more so than risk of or actual predation (Stamp 2003). Because these were based on terrestrial plants and their access to light or nutrients, they have more limited relevance to benthic invertebrates. These are the growth-differentiation balance hypothesis [GDBH (Herms & Mattson 1992)], resource availability model [RAM (Coley et al. 1985)], and carbon:nutrient balance hypothesis [CNBH (Bryant et al. 1983)]. The GDBH incorporates ideas on relative importance of growth versus production of differentiated tissues and defenses (Stamp 2003). The RAM predicts different allocation strategies in slow-growing organisms versus fast-growing organisms on evolutionary timescales and also on the types of secondary metabolites that would be produced under different resource limitation scenarios (Cronin 2001). The CNBH makes similar resource limitation predictions on ecological timescales (Bryant et al. 1983).

One of the most diverse taxonomic groups of benthic, sessile invertebrates are the poriferans or sponges. Sponges are animals that attach to substrates in aquatic environments and filter bacteria and other organic particles out of the water column (Brusca & Brusca 2003). There are incurrent ostia that allow water to enter filtration chambers where choanocytes keep the water flowing and conduct the actual filtration of the water (Brusca & Brusca 2003). The water flows through the filtration chambers and out of the sponge through an osculum (Brusca & Brusca 2003). Sponges may only have one osculum or several oscula depending on the complexity of the individual’s morphology. Antarctic sponges are ecologically important organisms predominantly
found in deep water habitats with there being 352 sponge species identified around the
continent (McClintock et al. 2005).

Geologic events have caused the isolation of Antarctic waters from the other
oceans. Gondwanaland initiated its break up in the Cretaceous [140 mya (Tingey 1991)]
with Antarctica fully separating from South America by the late Oligocene [28 mya
(Kennett et al. 1997)]. Ultimately, this formed the Drake Passage between the two
continents and facilitated the initiation of the Antarctic Circumpolar Current (ACC), a
clockwise current that is the largest on earth. For at least the last 22 million years, the
waters around Antarctica have been essentially isolated by the ACC (Kennett 1977)
although some gene flow across it is possible (Belcher & Halanych 2005).

Anvers Island is positioned along the western side of the Antarctic Peninsula and
is the location of Palmer Station, a United States research station (64º 46.5’ S, 64º 03.3’ W).
The waters along the Antarctic Peninsula are characterized by abundant and diverse
faunal communities with studies having examined aspects of the biology and ecology of
crustaceans [amphipods (Huang et al. 2006), isopods (McClintock et al. 2003), krill
(Quetin et al. 2003)], echinoderms (Dearborn 1977, McClintock 1994, McClintock et al.
2003), fish (Daniels 1982, Iken et al. 1997, 1999), tunicates (McClintock et al. 2004),
mollusks (Iken 1999), octocorals (Orejas et al. 2002), and sponges (Konecki & Targett
1989).

Much of what is known about the community ecology of Antarctic benthic
macroinvertebrates comes from studies at McMurdo Station [77º 51’ S, 166º 39’ E
(Dayton et al. 1974, McClintock 1987)] which lies approximately 13° farther south than
Palmer Station. Sponges and other benthic macroinvertebrates of this region have been

Northern regions of the waters off the Antarctic Peninsula differ from other areas of Antarctica in that they lack extended periods of complete darkness or direct sunlight. Moreover, the shallow waters around Anvers Island and along the western Antarctic Peninsula lack anchor ice that is documented to have dramatic effects on the benthic ecosystem in more southerly regions by removing large areas of substrata (Dayton et al. 1969). At shallow depths (<35 m), macroalgae completely dominate the hard benthos in most areas (Amsler et al. 1995, Brouwer et al. 1995, Quartino et al. 2001) due to their ability to outcompete benthic macroinvertebrates. At greater depths, near the edge of the macroalgal zone and deeper, the benthic community shifts to one dominated by sessile macroinvertebrates.

Sponges often dominate the benthos of Antarctic coastal waters below shallower depths where macroalgae and anchor ice occur (Dayton et al. 1970, Dayton et al. 1974, Sarà et al. 1992, Teixido et al. 2002). The species richness of Antarctic sponges has recently been estimated to be between 300-352 species with 81% of these belonging to the Demospongiae and the majority exhibiting circumpolar distributions (reviewed by McClintock et al. 2005). High abundance and diversity of sponges are also true in warmer seas throughout the world with sponges often being one of the most abundant
sessile organisms on coral reefs (Wulff 2001). These sponges are often found to be unpalatable in tropical (Assmann et al. 2000, Kubanek et al. 2000) and temperate latitudes (Becerro et al. 2003). While McClintock et al. (1994, 2000) used a tube-foot retraction assay to indirectly evaluate the feeding deterrent properties of a suite of Antarctic sponges, no study has directly measured palatability in Antarctic sponges with an ecologically relevant predator.

These subsequent parts of this dissertation expand the overall knowledge of the chemical ecology (mostly chemical defenses) of sponges from Antarctic peninsular waters. The overall palatability of these sponges was determined as well as the role that internal chemistry plays in any seen unpalatability. Using information previously reported concerning the benthic communities of Antarctica, predictions of the optimal defense theory were tested to determine if it could accurately be used to predict defensive allocation patterns in Antarctic sponges. Secondly, the antimicrobial activity of the secondary metabolites present in the sponges was determined against both photosynthetic fouling organisms and potentially pathogenic prokaryotes. Lastly, I attempted to determine the ability or lack thereof one particular sponge species, *Hymeniacidon fernandezi*, to produce increased defenses in the presence of predators.
PALATABILITY AND CHEMICAL DEFENSES OF SPONGES FROM THE WESTERN ANTARCTIC PENINSULA

by

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ABSTRACT

This study surveyed the palatability of all sponge species that could be collected in sufficient quantities in a shallow-water area along the western Antarctic Peninsula. Of 27 species assayed, 78% had outermost tissues that were significantly unpalatable to the sympatric, omnivorous sea star *Odontaster validus*. Of those species with unpalatable outer tissues, 62% had inner tissues that were also unpalatable to the sea stars. Sea stars often have been considered as the primary predators of sponges in other regions of Antarctica and their extra-oral mode of feeding threatens only the outermost sponge tissues. The observation that many of the sponges allocate defenses to inner tissues suggests the possibility that biting predators such as mesograzers which could access inner sponge layers may also be important in communities along the Antarctic Peninsula.

In feeding bioassays with extracts from 12 of the unpalatable species in artificial foods, either lipophilic or hydrophilic extracts were deterrent in each species. These data indicate an overall level of chemical defenses in these Antarctic sponges that is comparable to and slightly greater than a previous survey of tropical species.
Sponges are abundant and ecologically dominant members of the Antarctic benthos that occur across a broad range of depths and are often characterized by circumpolar distributions (McClintock et al. 2005). Being sessile, sponges are at a distinct disadvantage when compared to motile organisms in terms of their ability to seek refuge or move away from threats. Consequently, sponges and other groups of sessile marine invertebrates have evolved alternate modes of defense including the incorporation of physical defenses (Chanas & Pawlik 1995, Huang et al. 2008) and/or the production of defensive secondary metabolites (Pawlik et al. 1995, Assmann et al. 2000, Kubanek et al. 2000, McClintock & Baker 2001, Furrow et al. 2003).

Marine predator-prey relationships have been interpreted within the context of several key ecological theories (Cronin 2001, Amsler & Fairhead 2006). One of these is the optimal defense theory (ODT). The ODT considers variations in defenses within organisms and, assuming these defenses invoke some fitness cost to the organism, predicts when and where defenses will be allocated in lieu of internal competition for resources for growth and defense (Rhoades 1979). In an environment in which predation risk is absent or low, the ODT predicts there would be no benefit to producing defenses; however, in an environment in which predation risk is high, the cost of the defense could be outweighed by the benefit of being protected. If the probability of attack is high, the ODT predicts that defenses will be under strong positive selection. Conversely, if the
cost of producing the defense is greater than the benefit obtained, then the ODT predicts that selection will favor the production of fewer defenses. At the level of the individual, ODT predicts that defenses will be allocated in higher concentrations to the most vulnerable and/or valuable tissues. This will vary between prey depending on the type of predators influencing the evolution of defenses in the prey.

The shallow, nearshore waters of the Antarctic Peninsula are characterized by a rich and diverse benthos (Brand 1974, Barnes & Brockington 2003, Barnes 2005). The predominant predators of benthic marine sponges in this and other regions of Antarctica are sea stars (Dayton et al. 1974, McClintock et al. 2005), with an abundance of omnivorous sea star species that include sponges among a broad array of prey, as well as strictly spongivorous species (Dearborn 1977, McClintock 1994).

As sea stars are the dominant predators of Antarctic sponges they are likely the primary driving force in the evolution of anti-predator defenses. Accordingly, sponge defenses would be predicted to be differentially allocated within sponges such that they most effectively deter sea stars from feeding upon them. The mode of feeding of sea stars is unique in that each arm is equipped with rows of chemosensory tube feet that upon contact facilitate an evaluation of prey palatability (Sloan 1980). If a potential prey is subsequently deemed acceptable, sea stars then extrude their cardiac stomach directly against the surface of the prey to initiate extraoral digestion. This unique extraoral mode of feeding results in sea stars encountering only outer surfaces of prey (Dearborn 1977). As such, the most vulnerable region of sponges attacked by sea stars is the exterior surface. Allocation of defenses to inner sponge tissues would not be an efficient
utilization of resources, as sea stars encounter these regions of the sponge only after first digesting outer layers.

There are several different mechanisms by which sponges could be undesirable as food sources to predators. One possibility is structural defenses that preclude predators access to palatable regions. This might be achieved by concentrating large, rigid spicules near the sponge exterior, preventing predators from accessing the softer layers beneath the spicules (van Alstyne & Paul 1992). While this could be an effective mechanism to deter predators, it also would require additional energy and resources to produce large protective spicules, in addition to possibly reducing surface area available to choanocytes that actively filter water and provide nutrition to the sponge. In Gulf of Mexico and Caribbean Sea sponges, studies have found little or no evidence of spicules providing significant feeding deterrence (Chanas & Pawlik 1995, Huang et al. 2008). Both of these studies utilized biting fish as predators. However, a study of temperate sponges found that spicules can significantly deter feeding from a hermit crab predator (Hill et al. 2005). In Antarctica, where sea stars are the primary predators on sponges (Dayton et al. 1974), spicules appear to generally be an unlikely mechanism of defense. This may be related to the ability of sea stars to extrude their cardiac stomachs and therefore to potentially digest tissue around the spicules (Dearborn 1977).

Low nutritional benefit to predators (Duffy & Paul 1992, Bullard & Hay 2002) and/or the presence of chemical compounds (Assmann et al. 2000, Becerro et al. 2001, Mahon et al. 2003, Amsler et al. 2005) may also influence predatory preferences for particular prey. As sea stars have to invest time and energy reserves in order to digest their prey, it is possible that poor quality prey may not compensate for the investment in
digestion. This may drive selection for the exploitation of high quality prey. However, it is unlikely that this is the case in the antarctic benthos where sponges have relatively high nutritional levels (particularly soluble protein; McClintock 1987).

Defensive secondary metabolites in marine sponges contribute to prey unpalatability or may potentially inhibit digestive processes post-ingestion (Becerro et al. 1998). Chemical defenses are common in sessile marine organisms lacking external protective shells including algae (Steinberg 1985, Amsler 2008) and invertebrates (Pawlik et al. 1995, Kubanek et al. 2000, Iken et al. 2002, Becerro et al. 2003, Mahon et al. 2003), and sponges are perhaps one of the most well known examples of invertebrate phyla known to exploit such defenses (Wilson et al. 1999, Assmann et al. 2000, McClintock & Baker 2001, Burns et al. 2003). As sponges qualitatively and quantitatively dominate antarctic benthic communities below the algal zone (Barnes & Brockington 2003, Barnes 2005), an understanding of the factors that contribute to their defenses is particularly important for understanding the dynamics of these communities.

The goals of this study were to test three general predictions concerning the relationship between sponges and their sea star predators in shallow, antarctic coastal waters. These include (1) most sponges will have outer tissues that are unpalatable to sympatric sea stars because these tissues are continuously exposed to predation, (2) the inner tissues of sponges with unpalatable outer tissues will be palatable to sea stars. This is because sea stars have an extraoral mode of predation that should restrict their predation on sponges to the outer tissues alone and remove selection of allocation of defenses to internal tissue, and (3) secondary metabolite chemistry should have a
substantial role in the provision of defenses in sponges subjected primarily to sea star spongivory.
MATERIALS AND METHODS

Collections

Multiple individuals of each sponge species were collected by hand using SCUBA from subtidal waters from numerous locations within 3.5 km of Palmer Station, Anvers Island, Antarctica [64° 46.5’ S, 64° 03.3’ W; cf. Amsler et al. (1995) for map] during two successive field seasons (January-May, 2003 and February-June, 2004). Sponges ranging in mass from tens of grams to several kilograms were collected from hard bottom substrates at depths of 5-39 m. Macroalgae dominated many of the sponge collecting sites, although some had vertical cliffs and overhangs where macroalgae covered less than 100% of the benthos. These areas were where the majority of the sponges were found. In order to assess the percentage of palatable versus unpalatable species with an unbiased experimental design, every demosponge species that was encountered was collected for analysis. Sponges were returned immediately to the laboratory and sorted into distinct species. Voucher photographs and specimens are maintained at the University of South Florida.

Outer vs. Inner Fresh Tissue Bioassays

In order to test the palatability of the fresh outer sponge layers, a small (approximately ½ cm³) piece of sponge was excised using a single edge razor from the outer surface of each individual. Each sponge was then dissected to expose its
approximate center (for mounding species) and a similarly sized piece of tissue was excised from this central region. Each individual sponge was used as the source of only a single piece each of internal and external tissue for feeding assays. Encrusting species and tubular species had internal tissue taken from areas farthest from the exterior where predators would encounter them although some species were not amenable to separation of outer tissue from inner tissue due to the close proximity of both tissues. These sponge pieces were then presented to the sympatric omnivorous (including sponge prey) sea star *Odontaster validus* following the methods described in McClintock and Baker (1997).

Prior to feeding assays, *Odontaster validus* were placed in ambient flow-through seawater tanks for no less than 24 hours before being used in any feeding assay. The maintenance diet consisted of control artificial food pellets. When held in aquaria, *O. validus* move up the interior wall until they reach the surface and extend one or more arms along the air-water interface. This provided access to the oral side of arms whose chemosensory tube feet line the ambulacral groove. Excised sponge pieces from the exterior surface of sponges were presented to *O. validus* equidistant between the oral opening and the arm tip such that the outer surface of the sponge cube (pinacoderm) was in direct contact with the chemosensory tube feet. The behavioral feeding response of the sea star was then noted as an acceptance when the potential food item was carried to the oral opening and held there for extra-oral digestion. A rejection response was considered any response other than this acceptance behavior. The most common rejection behaviors observed included moving the potential food item out of the ambulacral groove and off the side of the arm, retracting the tube feet and letting the potential food item drift away, or moving the potential food item away from the mouth towards the arm tip and then
releasing it. Responses occurred within the first minute of presentation the majority of the time; however, items were left on the ambulacral groove were for 5 minutes before a rejection was noted.

Once a sea star either accepted or rejected the fresh sponge tissue, an artificial food pellet was presented to the sea star as a control. The control food consisted of a 2% alginate matrix infused with 5% (dry wt) lyophilized, powdered krill in sea water (McClintock et al. 2003, McClintock et al. 2004). The alginate and krill combination was gelatinized using 1 M cold CaCl₂ and pellets similar in size to the excised sponge pieces were presented to the sea star. Once the outer sponge tissue had been assayed, the tissue excised from the interior of the sponge was assayed in exactly the same manner. Sea star feeding bioassay sample sizes were up to 16 individual sponges of a given species but lower if lesser numbers of individuals of a given sponge species were collected. One species was assayed twice so as to include two morphotypes which were distinct in the field. Responses were similar independent of whether the item being assayed was fresh sponge tissue or artificial food pellet. Each food item was presented to a separate sea star and no sea star was used multiple times in fresh tissue or artificial food assays that would be statistically compared with one another.

Extract Bioassays

Thirteen of the sponge species that were found to be unpalatable as fresh tissue were used in extract bioassays following methods previously described in McClintock et al. (2003, 2004). Once the sponges had been dissected as described above, both inner and outer tissues were combined, weighed, frozen and lyophilized, and then re-weighed.
in preparation for chemical extraction. A lipophilic crude extract was prepared using 3 changes of 1:1 dichloromethane:methanol. Immediately following the lipophilic extraction, a hydrophilic crude extract was prepared from the previously extracted tissue using 3 changes of 1:1 methanol:water. Crude extracts were then dried under reduced pressure and weighed providing the yield of extract per mass of dry sponge.

The dried extracts were added to the control food noted in the previous section (5% dried krill in 2% alginate marine solution) in the following manner. The extracts were solubilized in a minimal amount of solvent before being added to the dried krill such that extract concentration on the krill equaled the extract concentration naturally found in the sponge on a wet weight basis. The krill coated with extract was dried under reduced pressure, added to a 2% alginate solution, and thoroughly mixed. 1 M cold CaCl₂ was added to gelatinize the mixture. Solvent controls containing the same volume of solvents used to solubilize the extract as well as the 5% krill and 2% alginate were also prepared. Artificial food pellets were cut into blocks (approximately ½ cm³) using a single edge razor.

Experimental and solvent control pellets were presented to sea stars as given above for fresh tissue feeding assays. Non-solvent treated control food pellets were prepared as above and used as satiation controls. Only feeding assays where the satiation control was accepted were included in statistical analysis. Once 12 replicates were successfully completed, the extract-containing pellets acceptance rate was compared to the solvent control pellets acceptance rate. For any given sponge, pellets containing lipophilic extracts were always tested in sequence first, and only if accepted were pellets containing hydrophilic extract tested.
**Statistical Analysis**

Fisher’s Exact Tests were performed using Vassar Stats (http://faculty.vassar.edu/lowry/VassarStats.html) to determine which outer and/or inner layers of sponges were rejected significantly more often than controls as well as which of those unpalatable sponges contained crude organic extracts that were unpalatable.
RESULTS

Twenty-seven sponge species were collected in sufficient numbers (n ≥ 3) for statistical analysis with one additional species having only one individual collected. The majority (18 species) were of the Order Poecilosclerida with there also being species of the Orders Hadromerida, Halichondrida (2 species), Haplosclerida (4 species), and Dendroceratida (Table 1). Individuals representing 1 unknown sponge species were also included. Taxonomic identification was not possible for this species due to the loss of voucher material.

Outer vs. Inner Fresh Tissue Bioassays

Of 27 sponge species that had fresh outer tissue presented to *Odontaster validus* in feeding bioassays, 21 (78%) were significantly rejected (P ≤ 0.05; Figure 1). In 13 of these 21 (62%), the result was highly significant (P ≤ 0.01). Two additional species (7%) displayed apparent trends towards unpalatability but the sample sizes were too small for these to be statistically significant. The Fisher’s Exact Test has little power when there are a small number of replicates. Two of the sponges were not amenable to separation of inner and outer layers. Of the remaining 25 sponge species that had inner tissues presented to *O. validus*, 14 of these (56%) had their inner tissue significantly rejected.

Of the 21 sponge species that were significantly rejected as outer tissue, 8 (38%) had inner tissues not significantly rejected by *Odontaster validus* (Table 1). One sponge
Phorbas areolatus had its inner tissue significantly rejected \( (p = 0.0238) \) while its outer tissue was not significantly rejected \( (p = 0.1032) \). This species only had 5 replicates tested and out of these 5 outer samples, 4 of them were rejected but with the small sample size, this was an insignificant result. Four species, including \( P. \) areolatus, had \( P \) values close to being significant with the inner layer almost being significant in these other three cases (Haliclona rudis, Isodictya antarctica, and Lissodendoryx ramilobosa) although the small number of replicates did not appear to be as great a factor to these three other cases \( (n \geq 7 \) in all cases).

**Extract Bioassays**

Given time constraints in the field, only 12 of the 21 sponge species that had fresh tissue rejected could be included in extract bioassays. The extracts for these 12 species were the first available and no selection was involved in determining which order to analyze the extracts. These 12 sponges consisted of 7 species that had both outer and inner tissues rejected, 4 that had outer but not inner tissues rejected, and one species \( (P. \) areolatus) that had inner but not outer tissue rejected. Either lipophilic or hydrophilic extracts of all 12 species were rejected in sea star feeding bioassays when compared to solvent controls (Figure 2). Lipophilic extracts were significantly rejected \( (p \leq 0.05) \) for 10 of the 12 species \( (83\%) \). Lipophilic extracts from the remaining two species \( (P. \) areolatus and Isodictya antarctica) were not significantly rejected \( (p = 0.0775 \) for both). Both of these species had their hydrophilic extracts subsequently assayed and both of these demonstrated highly significant \( (p \leq 0.01) \) levels of pellet rejection.
<table>
<thead>
<tr>
<th>Species</th>
<th>Order</th>
<th>Suborder</th>
<th>Family</th>
<th>Subfamily</th>
</tr>
</thead>
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<tr>
<td><em>Artemisina plumosa</em> lipocelia Hentschel, 1914</td>
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<td>Tetaniidae</td>
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FIG. 1. Results of bioassays offering outer and inner sponge tissues to sea stars. Numbers of replicates are shown above each set of columns with each species having equal outer and inner tissue replicates. *Haliclona* sp. and *Homaxinella balfourensis* only had outer tissue presented to sea stars. All species with 3 or more replicates were analyzed for statistical differences between acceptance of sponge tissue and control (Fisher’s exact test); *p ≤ 0.05, **p ≤ 0.01.*
FIG. 2. Results of bioassays offering artificial foods containing lipophilic or hydrophilic sponge extracts to sea stars (n = 12 for all species). Lipophilic extracts were always offered first and only if they were not significantly rejected were hydrophilic extracts offered. Asterisks indicate significant difference between extract and control (Fisher’s exact test); *p ≤ 0.05, **p ≤ 0.01
DISCUSSION

This survey examined the incidence of unpalatability among a broad suite of demosponges from the western Antarctic Peninsula to the ubiquitous, sympatric, omnivorous sea star, *Odontaster validus*. Although this study included only approximately 8% (30 of 352 species; McClintock et al. 2005) of the total demosponge fauna of Antarctica, it encompassed all of the shallow-water demosponges that could be collected in sufficient quantities for bioassays in the vicinity of Anvers Island along the central western Antarctic Peninsula.

In Antarctica, sea stars are the dominant predators of sponges and other benthic macroinvertebrates (Dayton et al. 1974, Dearborn 1977). *Odontaster validus* is one of the most abundant sea stars along the western Antarctic Peninsula (Stanwell-Smith & Clarke 1998, Peck et al. 2008) including the region immediately surrounding Palmer Station (authors’ personal observations). The vast majority (78%) of the sponges assayed in this study had outer tissues that were unpalatable to *O. validus* and additional species that could only be assayed with relatively small sample sizes displayed strong trends toward unpalatability. One mechanism by which sponges may render themselves unpalatable is through the production of physical defenses such as spicules. However, this prospective defense mechanism has been demonstrated variable results (Chanas & Pawlik 1995, Hill et al. 2005, Huang et al. 2008) and does not appear to be a likely method in an environment where sea stars are the dominant predators and should be able
to bridge such physical defenses during predation. Another mechanism that could
discourage predation is that sponges are poor quality prey that lack sufficient nutrients to
make them worth consuming (Duffy & Paul 1992, Bullard & Hay 2002). While no
nutritional value measurements of the sponges were made in our study, nutritional
compositions of sponges from McMurdo Sound, Antarctica (77°51’ S, 164°40’ E) have
been gathered previously (McClintock 1987). Five species examined in this previous
study from McMurdo Sound are also represented among the sponges in the present study.
All five had outer tissues rejected by O. validus and had total protein levels ranging from
28 – 56% dry weight. With such a wide range of protein values, a small sample size of
sponges, and distinct geographic differences between the two study regions, it is not
possible to rigorously evaluate whether there is a relationship between nutritional content
and palatability in these sponges. The protein levels reported in McClintock (1987) are
in the same range as those reported by Chanas and Pawlik (1995) for Caribbean sponges
although the data were reported differently (gravimetric vs volumetric) because the
ecologically relevant predators have different feeding methods. A similar analysis
examining the chemical defenses of a broad suite of marine macroalgae on the western
Antarctic Peninsula found no correlation between algal nutritional value (Peters et al.
2005) and palatability (Amsler et al. 2005).

Differences in methodologies between this study and previous studies make direct
comparisons problematic. Previous studies of defenses in multiple sponge species have
tested sponge spicules and extracts imbedded in food pellets (Chanas & Pawlik 1995,
Pawlik et al. 1995). As we employed fresh sponge tissues, this makes it difficult to make
direct comparisons with these earlier studies. However, despite our subsampling extracts
from only 12 of the 21 sponge species that were unpalatable, the fact that all 12 species displayed sea star deterrence in at least one extract (lipophilic or hydrophilic) supports our hypothesis that chemical defenses play a major role in determining patterns of sponge predation in Antarctica. With this information, we are able to compare the fresh outer tissue data to data reported from previous studies where only the chemical aspect of defense was analyzed.

Previous studies have proposed a latitudinal gradient of chemical defenses in marine invertebrates with these defenses being more prevalent in low latitude, tropical environments, as opposed to higher latitudes where the incidence of fish predators preying upon their tissues is certainly diminished (Bakus & Green 1974, Ruzicka & Gleason 2008). If this were the case, then it would be expected that a smaller percentage of marine invertebrates in Antarctica would invest in chemical defenses, or secondary metabolite defenses might be expected to be weak. However, the percentage of Antarctic peninsular sponges in our survey that were defended against sea stars (outer tissues - 78%) is slightly higher than a sponge survey conducted in the Caribbean Sea where 69% of the species were found to be chemically defended against fish (Pawlik et al. 1995). We are aware of no comparable surveys conducted in temperate latitudes or other locations. However, the fact that there are similar levels of defenses in sponges from Antarctica and tropical waters indicates that at present there is no evidence of a latitudinal gradient for sponge chemical defenses. Becerro et al. (2003) came to the same conclusion using different methodologies comparing congeners from temperate and tropical waters.
We know of no other studies in temperate, tropical or polar latitudes that have examined differing levels of palatability between fresh tissues taken from the outer versus the inner central tissues of sponges; however, one previous study conducted with the sponge *Latrunculia apicalis* from McMurdo Sound, Antarctica did detect a strong gradient of chemical defenses, with greater levels in the outermost tissue and then a sharp decline moving deeper into the sponge (Furrow et al. 2003). Extracts prepared from different layers of benthic macroinvertebrates from warmer waters have yielded conflicting results. In one study, differences between tissue layers in their palatability were not detected (Burns et al. 2003), while in other studies body tissues first encountered by predators were found to be regions of increased defenses (Avila & Paul 1997, Schupp et al. 1999). In all of these studies, the predominant predators on the sponges were fish, which are capable of biting through outer tissues and thus feeding on both inner and outer tissues.

Our analysis of the patterns of palatability between outer and inner sponge tissues facilitates a test of the predictions of the ODT. As sea stars feed via extraoral digestion on the surfaces of their prey, this limits their encounter to the outer surfaces of sponges. Thus, defenses in sponges with sea star predators would be predicted (in accordance with the ODT) to be strongest in association with outer surfaces. We found that inner sponge tissues were palatable while outer tissues were unpalatable in 8 of the 21 species examined. Therefore, 38% of the species examined met the predictions of the ODT. However, the other 13 sponge species (62%) did not adhere to the predictions of the ODT, and have inner tissues that are defended even though they appear to be at little risk of attack by sea star predators. While 38% of species following the ODT prediction is
not trivial, that the other 62% are not following the ODT predictions suggest that there
might be a problem with the theory or with its underlying assumptions as applied to this
predator-prey system.

A possible explanation for the lack of some sponge species meeting the
predictions of the ODT is that, unlike previous studies of sponge communities in
McMurdo Sound (Dayton et al. 1969, Dayton et al. 1974, Dayton & Oliver 1977), the
marine communities that characterize the western Antarctic Peninsula are exposed to
considerable densities of biting and/or burrowing sponge predators that are able to
penetrate external defenses by burrowing or biting through defended outer tissues or
exploiting oscula or ostia for access to feed on internal palatable tissues. One possibility
is mesograzers, particularly amphipods, feeding on the sponges. Amphipods are a
remarkably abundant component of shallow water communities on the Antarctic
Peninsula including Anvers Island and its environs (Iken et al. 1997, Graeve et al. 2001,
Huang et al. 2007). In preliminary quantitative observations, we have found that
amphipods are common sponge-associates and sometimes have sponge spicules in their
guts (M. Amsler, unpublished observations). The majority of these amphipod-sponge
associations appear to occur within internal tissues, but it remains unknown how they
gain access to internal regions. One possibility is that amphipods enter the large oscula
some of the sponges possess thereby bypassing the defenses in the external tissues.
Conversely, if the amphipods are not driving production the of the internal defenses,
these might be evolutionary relics from ancestral sponges which existed in seas where
larger, biting predators that would have been able to access internal tissues were more
common.
Our present study has demonstrated that many sponges (43% of 30 species examined) have evolved both external and internal defenses that may deter burrowing mesograzer predators such as amphipods. In summary, if sponges and other sessile invertebrates in benthic communities along the Antarctic Peninsula are subject to a mesograzer-dominated environment that includes sponge predators, then there may be selection to allocate chemical defenses to both external and internal tissues.
ACKNOWLEDGEMENTS

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LITERATURE CITED


Brand TE (1974) Trophic interactions and community ecology of the shallow-water marine benthos along the Antarctic Peninsula. Ph.D., University of California, Davis


POTENTIAL CHEMICAL DEFENSES OF ANTARCTIC SPONGES TO SYMPATRIC MICROORGANISMS

by

KEVIN J. PETERS, CHARLES D. AMSLER, JAMES B. MCCLINTOCK, AND BILL J. BAKER

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Format adapted for dissertation
INTRODUCTION

Poriferans are basal metazoans found in most aquatic systems and compose a large percentage of the benthic biomass in these environments (Barnes & Bell 2002, Bell & Smith 2004, McClintock et al. 2005, Schlacher et al. 2007). The aquatic environments these animals live in and feed upon have variable amounts of water-borne microorganisms and the sponges themselves often have microorganisms at levels which are orders of magnitude greater than found in the water alone (Webster & Hill 2001, Taylor et al. 2007).

Coastal Antarctic waters are similar to warmer waters with prokaryotic communities present in the water column (Delong et al. 1994, Murray et al. 1998) and microalgal blooms occurring seasonally (Cerrano et al. 2004). Furthermore, Antarctic sponges are known to harbor various microorganisms within their tissues [e.g. archaea, bacteria, diatoms, dinoflagellates (Cerrano et al. 2000, Regoli et al. 2004, Webster et al. 2004)] while feeding on others (Thurber 2007). Many of these organisms are not known sponge affiliates but instead are associated with Antarctic seawater and sea ice.

Densities of bacteria residing in sponges vary, with some studies finding up to 40-60% of a sponge’s biomass being comprised of bacteria (Wilkinson 1978). Speculations as to the roles of these bacteria are numerous although definitive results are lacking. Possibly the most studied of these symbiotic relationships is those between sponges and cyanobacteria in oligotrophic environments where sponges with cyanobacterial
endobionts are abundant and often larger than sponges without cyanobacteria in their tissues (Wilkinson 1983, Thacker & Starnes 2003, Steindler et al. 2005, Erwin & Thacker 2007). Many of the sponge symbionts are regarded as sponge-specific because they are not found in detectable levels in the water column but are present in significant densities in the sponges (Hentschel et al. 2002, Hill et al. 2006).

Secondary metabolites from sponges have been found to deter predation (Pawlik et al. 1995, Wilson et al. 1999, Burns et al. 2003, Peters et al. in press) but it may be that these sponge-specific bacteria are the sources of the chemical defenses (Bewley et al. 1996, Thakur et al. 2003). Most studies are unable to differentiate between sponge-derived metabolites and microbial-derived ones, so it is possible that many of the secondary metabolites previously reported from sponges are of microbial origin. From an ecological standpoint, this is not of great importance unless the endobionts are able to freely leave the host. Conversely, sponge diseases have been linked to microbial “infections” (Webster 2007 and references therein), but only one study thus far has found a causative agent, which was an alpha-proteobacterium infecting sponges on the Great Barrier Reef (Webster et al. 2002). Possible infections have been observed in the Ross Sea, Antarctica, as well with several sponge species having discoloration and putrefaction of the tissues although no causative agent has yet been identified (Webster 2007). The efficacy of the sponge secondary metabolites is potentially just as important against pathogens and/or fouling organisms (e.g. microalgae, spores from macroalgae, larvae of other benthic invertebrates, etc.) in many environments (Kelly et al. 2003, Dobretsov et al. 2004).
Although the various sponge-microbe symbioses have been thoroughly documented, there are many more microorganisms that have no relationship with sponges while inhabiting the same environment. In Antarctica, sponges are often seen with epizoic diatoms (Gaino et al. 1994, authors’ personal observations, Amsler et al. 2000) and have been found to have parasitic diatoms within their tissues (Bavestrello et al. 2000). However, many of the sponges also appear to have no epizoic diatoms even though they are in close proximity to sponges with diatom coverage. Amsler et al. (2000) found several sponge species' extracts from McMurdo Sound, Antarctica (77°51’ S, 164°40’ E) caused diatom mortality but it has yet to be determined if sponges along the western Antarctic Peninsula exhibit similar defenses against sympatric diatoms.

The aims of this project are to assess the antimicrobial activity of both lipophilic and hydrophilic crude extracts from demosponges collected along the western Antarctic Peninsula against (1) bacterial strains isolated from the water-column and sympatric invertebrates and (2) epiphytic diatoms isolated from a sympatric alga.
MATERIALS AND METHODS

Sponges were collected by hand using SCUBA from subtidal waters near Palmer Station, Anvers Island, Antarctica [64°46.5’ S, 64°03.3’ W; cf. Amsler et al. (1995) for map] during two successive field seasons (January-May, 2003 and February-June, 2004). Sponges were collected from hard bottom substrates at depths from 5 to 39 m. Once collected, sponges were returned immediately to the station, separated into distinct species, and then frozen. Voucher photographs and specimens are maintained at the University of South Florida.

Sponges were then lyophilized and a wet weight to dry weight ratio was obtained for each species. A lipophilic crude extract of the dried sponge was prepared using 3 changes of 1:1 dichloromethane:methanol. Immediately following the lipophilic extraction, a hydrophilic crude extract was prepared using 3 changes of 1:1 methanol:water. Crude extracts were then combined, dried under reduced pressure, and weighed providing the yield of extract per mass of dried sponge.

Antibacterial Assay

Sponges and other invertebrates were collected in order to obtain bacteria. These organisms were collected and kept submersed in seawater during transport to the research station. Organisms were then taken directly from the seawater and portions were excised with a sterile scalpel and transferred into both 100% Difco marine broth 2216 (Difco
Laboratories; Sparks, MD, USA) and 50% glycerol in marine broth using aseptic
 technique. These samples were then frozen at no greater than -70°C and shipped back to
 the United States.

 Frozen tissue samples from the various invertebrates were thawed, emulsified,
 and incubated on marine agar. Individual bacterial colonies were isolated and grown on
 Difco marine agar 2216 (Difco Laboratories) at 4°C. Isolates were identified by
 sequencing their 16S rRNA gene using an ABI Prism® 3100 DNA Sequencer (AME
 Bioscience; Toroed, Norway). These bacteria were found to be capable of growth at both
 25°C and 4°C. Marine broth was then inoculated with pure cultures and incubated for
 approximately one day at room temperature on a shaker until growth was visible (slight
 turbidity). Once growth was visible, the bacterial cultures were transferred to a shaker at
 4°C until the bacteria were in stationary phase. 40µl of bacterial culture was spread
 evenly onto marine agar plates and allowed to soak in for 5 min before any of the extracts
 were added.

 Extracts were resuspended in either methanol (lipophilic extracts) or 1:1
 methanol:water (hydrophilic extracts) at 1 ml per 1 g wet tissue originally extracted and 1
 ml per 3 g wet tissue originally extracted. Paper antimicrobial assay disks, 6 mm
diameter (BBL Microbiology Systems 31039; Cockeysville, MD, USA), were prepared
 by adding 20 µl (10 µl per side) of these extract solutions or of solvent only to the disks.
 This volume has been previously reported as virtually saturating the filter disc (Mahon et
 al. 2003). The two extract concentrations added to the disks approximate the normal
 tissue concentration and three times the normal tissue concentration on both wet weight
 and volumetric bases. Once the filter discs had dried, they were placed onto the
inoculated marine agar plates. These plates were then placed at 4°C for several days until bacterial growth was visible and zones of inhibition could be measured. Antimicrobial activity was defined as larger zones of inhibition present around extract containing disks when compared to solvent control disks. Each condition was repeated two times for a total of three replicates per extract concentration.

*Antifouling Assay*

In order to present extracts at concentrations approximating those found in the outermost layers where diatoms would be present, 2 cm x 2 cm surface squares of dried sponges were “shaved” down to a depth of approximately 1 mm and then weighed, approximating the dry weight of the outermost 1 mm of a 4 cm² sponge surface. For those sponges where physical tissue was no longer available, an average of the weights from the other sponges was used (mean = 0.11g, range = 0.07 – 0.17 g). It was then possible to calculate an estimated yield of extract per surface area of the sponge on a wet weight basis assuming that extracts are evenly distributed throughout the sponge.

Diatom bioassays used *Syndroposis* sp., a chain-forming pennate diatom previously isolated near Palmer Station from the intertidal green alga *Cladophora repens*, maintained in f/2 media (McLachlan 1973) at the University of Alabama at Birmingham. In previous publications (McClintock et al. 2004, Amsler et al. 2005) this strain has been referred to as Pal D1.2.

Experiments were conducted in Falcon® 96-well tissue culture plates (Becton Dickinson, Franklin Lakes, NJ, USA). The yield of extract per surface area of the sponge was then used to determine the amount of extract needed for each 6 mm diameter well to
approximate the natural concentration found on the same area of sponge surface. This natural concentration as well as 30% and three times the natural concentration were determined and used in bioassays. The extracts were solubilized in the solvents they were produced with (lipophilic extract in 1:1 dichloromethane:methanol; hydrophilic extract in 1:1 methanol:water) and transferred to the wells. In order to coat extract only on the bottom surface of the well, the solubilized extracts were transferred in aliquots that just covered the surface of the wells. The plates with extract were then dried under reduced pressure and subsequent aliquots were added until the appropriate amount of crude extract coated the surface of each well. Solvent control wells were also made with the same protocol but only using the solvents. Each experimental and control treatment had three replicates.

The plate was then chilled and 40-µl of f/2 media was added to each well followed by 40-µl of concentrated diatoms in f/2 media. The plates were then incubated at 1.5°C (±0.5°C) for three days with a 12:12 light:dark photoperiod at an irradiance of 25 µmol photons m⁻² s⁻¹. Thereafter, the cells were stained with fluorescein diacetate and Evans blue as described by Amsler et al. (2000). The stained cells were observed at 400x magnification under epifluorescence and brightfield illumination on a compound microscope. Living cells appeared green under blue epi-illumination due to the fluorescein diacetate and dead cells appeared blue under brightfield illumination due to the Evans blue. A minimum of 100 cells haphazardly chosen were counted per replicate and scored as live or dead for use in percentage-dead calculations.

For statistical analyses, the percentage of dead diatoms was arcsine (square-root) transformed. These data were then subjected to Levene’s Test for homogeneity of
variances using SAS software (SAS Institute Inc., Cary, NC, USA) and all conditions with variance were found to be not-significantly different. The data were then compared statistically by analysis of variance and Tukey’s HSD post hoc test using SAS software.
RESULTS

Antibacterial Assay

Twenty-five sponge species had lipophilic and hydrophilic extracts assayed at two concentrations (1X and 3X natural concentration) against 16 strains of Gamma Proteobacteria, one Flavobacterium, and three unidentified species of bacteria isolated from seawater and invertebrates along the western Antarctic Peninsula. The 16 Gamma Proteobacteria consisted of five bacterial species with multiple strains of several species (Table 2). The majority of the bacterial isolates had no growth inhibition with only one isolate of Alteromonas elyakovii (P37) found to consistently have any growth inhibition due to sponge extracts and several other isolates had sporadic growth inhibition [another strain of A. elyakovii (P26), 2 strains of Psychrobacter fozii (P22 and P34), and an unidentified species (P68); (Table 3)]. A. elyakovii and P. fozii are both Gram negative and found in antarctic marine habitats. Of the species that had sporadic growth inhibition, all had no inhibition of growth by the solvent control discs. When inhibition was observed for the experimental conditions, it was only a “halo” of inhibition less than one millimeter around the filter discs. The A. elyakovii strain with regular growth inhibition was also inhibited minimally by the solvent controls discs. Every sponge tested against this strain had at least one replicate of either extract inhibit growth more than the control. However, in two sponge species (Mycale acerata and Isodictya aff.
cactoides) the natural concentration of neither the lipophilic nor hydrophilic extract inhibited growth of the most susceptible strain of *A. elyakovii*.

**Antifouling Assay**

Twenty-five sponge species had both lipophilic and hydrophilic extracts assayed at three concentrations (0.3X, 1X, and 3X natural concentration) against the chain-forming pennate diatom *Syndroposis* sp. Every sponge’s lipophilic extract at three times the natural concentration resulted in significantly greater (p < 0.05) numbers of dead diatoms when compared to solvent controls (Figure 3). At the estimated natural concentration of lipophilic extract, only one sponge species (*Artemisina plumosa*) did not have significantly more dead cells compared to the solvent controls. In fact, 9 of the 25 species had 100% mortality of diatoms at the estimated natural concentration. Even at 30% the estimated natural concentration of lipophilic extract, 15 of the 25 sponge species (60%) had significantly more dead diatoms compared to the solvent controls.

Results from hydrophilic extract assays were less extreme than the lipophilic extract results. Six of the 25 hydrophilic extracts were not significantly rejected at 3 times the estimated natural concentration (Figure 4). At the estimated natural concentration, 15 of the 25 sponges had significantly greater diatom mortality when compared to the solvent controls. At 30% of the estimated natural concentration, 6 of the sponge species’ hydrophilic extracts resulted in significant diatom death.
Table 2. Bacterial species that sponge extracts were tested against.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of isolates used</th>
<th>Strain (if known)</th>
<th>Order</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alteromonas elyakovii</td>
<td>6</td>
<td></td>
<td>Gammaproteobacteria</td>
</tr>
<tr>
<td>Halomonas sp</td>
<td>1</td>
<td>ARCTIC P-3</td>
<td>Gammaproteobacteria</td>
</tr>
<tr>
<td>Psychrobacter fozii</td>
<td>5</td>
<td></td>
<td>Gammaproteobacteria</td>
</tr>
<tr>
<td>Psychrobacter glacincola</td>
<td>2</td>
<td>ANT 9253</td>
<td>Gammaproteobacteria</td>
</tr>
<tr>
<td>Shewanella frigidimarina</td>
<td>2</td>
<td></td>
<td>Gammaproteobacteria</td>
</tr>
<tr>
<td>Uncultured Flavobacterium</td>
<td>1</td>
<td>SIC.ARTCIC.156</td>
<td>Flavobacteria</td>
</tr>
<tr>
<td>Undescribed sp. P58</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undescribed sp. P67</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undescribed sp. P68</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Antimicrobial activity of sponge extracts (H = hydrophilic, L = lipophilic) expressed as differences in the size of the zones of inhibition compared to solvent control discs with the mean (and range) of three replicates given. Only bacterial isolates that had growth inhibition due to sponge extracts are listed below (15 isolates had no growth inhibition). 0 = equal to control, 1 = <1mm more than control, 2 = 1-2mm more than control, 3 = 2-3mm more than control

<table>
<thead>
<tr>
<th>Sponge species</th>
<th>Concentration of extract</th>
<th>Alteromonas eyskernii P26</th>
<th>Alteromonas eyskernii P37</th>
<th>Psychrobacter foetidus P22</th>
<th>Psychrobacter foetidus P34</th>
<th>Undescribed sp. P68</th>
</tr>
</thead>
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<tr>
<td>Artemisia pluma</td>
<td>1x</td>
<td>0.33(0-1)</td>
<td>0.67(0-1)</td>
<td>0.67(0-1)</td>
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<td>1(0-2)</td>
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<td>Calioryx sp.</td>
<td>3x</td>
<td>0.33(0-1)</td>
<td>1.33(1-2)</td>
<td>1(1-1)</td>
<td>1(1-1)</td>
<td>1(0-2)</td>
</tr>
<tr>
<td>Clathria fabellata</td>
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<td>0.33(0-1)</td>
<td>0.33(0-1)</td>
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<td>Clathria sp.</td>
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<td>0.33(0-1)</td>
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<td>0.33(0-1)</td>
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<td>1.33(1-2)</td>
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<tr>
<td>Hymeniacidon aff. gausiana</td>
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<td>1.33(1-2)</td>
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<tr>
<td>Hymeniacidon forquata</td>
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<td>Iophon unicorne</td>
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<td>1.33(1-2)</td>
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<tr>
<td>Isodictya aff. cactoides</td>
<td>1x</td>
<td>1(1-1)</td>
<td>1(1-1)</td>
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<tr>
<td>Isodictya antarctica</td>
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<td>1.33(1-2)</td>
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<td>Kirkpatrickia variolosa</td>
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<td>1.33(1-2)</td>
<td>1.33(1-2)</td>
<td>1.33(1-2)</td>
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<td>Latruncula apicalis</td>
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<td>Lissodendoryx ramiolobosa</td>
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<td>Sphaerocystis antarctica</td>
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<td>Tedania charcoti</td>
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FIG. 3. Percent dead diatoms using three concentrations of lipophilic crude extracts. Statistics performed in each species independently with each condition compared to solvent controls using one-way ANOVA followed by Tukey’s HSD post hoc test. Bars with different letters are significantly different from one another ($P \leq 0.05$). *Haliclona scotti* had no variance between replicates but differences were assumed due to all experimental treatments resulting in 100% mortality and all control treatments resulting in 0% mortality.
FIG. 4. Percent dead diatoms using three concentrations of hydrophilic crude extracts. Statistics performed in each species independently with each condition compared to solvent controls using one-way ANOVA followed by Tukey’s HSD post hoc test. Bars with different letters are significantly different from one another (P ≤ 0.05).
DISCUSSION

Out of 20 bacterial isolates, only 5 had any growth inhibition when presented with extracts from 25 different sponge species with a single strain being responsible for the vast majority of the activity. These bacteria were isolated from the ambient environment of the sponges. It was presumed that some of the bacteria would be food sources for sponges, others might be endosymbionts, while still others might be pathogens as these are common sponge-bacteria relationships detailed previously. The lack of bacterial growth inhibition by the sponge extracts suggests that bacterial pathogens may not be a serious threat to Antarctic sponges. Many of these sponges have previously been found to contain chemicals that caused weak to moderate antibacterial activity when presented to non-native bacteria (McClintock & Gauthier 1992). The current study sites display no visible evidence of diseased sponges, but this is not uncommon as necrotic tissue is often fragile and easily broken away from the main sponge assemblage (Webster 2007).

Studies from other areas of the world have found variable anti-bacterial activity when investigating sponge-associated secondary metabolites. They have ranged from sponge-associated compounds having no effect on bacterial settlement and growth (Dobretsov et al. 2004) to significant inhibition of bacterial attachment (Thakur & Anil 2000, Kelly et al. 2003). This gives more evidence that relationships between sponges and water-borne prokaryotes vary depending on the system studied.
The possibility that bacteria are not a significant threat to Antarctic sponges provides another difference between Antarctic waters and warmer waters where bacteria have been linked to sponge disease (Cervino et al. 2006) and are the presumable causative agent in many other cases of sponge mortality (Webster et al. 2002). However, there is evidence that some Antarctic sponges are susceptible to infection by as of yet unidentified agents. Many of the sponges that are found to be diseased have been previously tested for the presence of chemical defenses and have shown variable degrees of defenses. North Atlantic, Mediterranean, and Caribbean sponges that have displayed mortality due to suspected bacterial pathogens have had chemicals that deter predation and settlement (Waddell & Pawlik 2000, Thoms et al. 2004, Toth & Lindeborg 2008). Determining whether or not tissue necrosis is due to bacterial pathogens or some other causative agent is difficult as diseases can be present in underlying tissues before ever becoming externally visible as well as the surrounding water being laden with potential bacteria. The only confirmed bacterial pathogen of sponges is a novel alpha-proteobacterium (Webster et al. 2002) and others linked to sponge necrosis are closely related to terrestrial pathogens incorporated in pesticides and transmitted to the water through runoff (Cervino et al. 2006). These bacterial pathogens are used because of their effectiveness at killing arthropods and other terrestrial organisms that are closely related to many marine organisms as well. A mutation in these bacteria or gene transfer to marine bacteria could make the marine bacteria just as virulent to a new suite of hosts. It is possible that the marine sponges are able to tolerate minor infections by marine bacteria, but when the bacteria take on some terrestrial characteristics, they are unable to
adapt. Minor genetic differences between marine bacterial strains can have profound implications on sponge mortality if they are resistant to defenses already present.

Of the 20 bacterial strains used in this study, six were genetically different isolates of the same bacterial “species.” These six isolates had variable results ranging from no inhibition in four isolates, minimal inhibition in one, and the majority of the inhibition observed in the study occurring in the final isolate. This variability within closely related organisms provides evidence that minor genetic differences can have severe ecological implications.

Several of the sponges in the current study are known to harbor microorganisms within their tissues (Webster et al. 2004) and if these bacteria are endosymbionts of the sponges, then it is possible that some of the compounds that constitute the extracts gathered from the sponges are made by the bacteria within the sponge tissues. It is not possible to differentiate between sponge-derived secondary metabolites and microbial-derived secondary metabolites in this current study. The lack of bacterial growth inhibition may be due to the isolated microbes being endosymbionts within the sponges and possibly providing the host sponges with nutrients and/or defenses against potential predators, pathogens, or foulers. This has been found to be a common relationship seen in other parts of the world (Althoff et al. 1998, Hentschel et al. 2001, Webster & Hill 2001, Hill et al. 2006, Lee et al. 2006).

The 20 microbial isolates cultured in this study may not be the major components of the invertebrate associated microbial flora in this system. It is often found that although there are large microbial communities within sponges (Webster & Hill 2001, Taylor et al. 2007), many of the organisms are unable to be cultured outside the sponge
using modern culturing techniques. These 20 culturable strains provide a small window into the relationships between just a few of the Antarctic coastal marine organisms.

The antifouling activity of the sponge extracts to the diatoms was much greater than the weak bacterial growth inhibition. This suggests that diatoms may be more detrimental to Antarctic sponge survival as the sponges have defenses present that can counter their effects. Whereas bacteria are able to flow through a sponge’s filter system without causing major blockages, diatoms are often too large to flow smoothly through this system. In addition, previous studies have found diatoms to parasitize and be overall detrimental to Antarctic sponges (Bavestrello et al. 2000, Cerrano et al. 2000). So, reducing the ability of the diatoms to grow in contact or close proximity to sponges would be of great value to sponge survival.

The diatom strain utilized for this study (*Syndroposis* sp.) was isolated from the same community as the sponges in this study. In a previous study, this diatom species often displayed an “all or none” reaction to chemicals presented to it with there either being 100% mortality or close to no mortality signifying that this species has a threshold it can survive within (Amsler et al. 2005). Chain-forming diatoms like the one used in this study commonly forms chains that can be several hundred cells long (authors’ personal observations) and one of these chains settling on a sponge’s surface would have the potential to impede the sponge’s regular filtration system. Individual diatoms in great enough densities could also result in the same problems and the ability of a sponge to produce chemicals that would kill diatoms could be a necessary product in this system where seasonal microalgal blooms occur regularly. Seventeen of the 25 sponge species resulted in significant diatom mortality at 30% the estimated natural concentration using
either the hydrophilic or lipophilic extracts. When the estimated natural concentration was used, every sponge species had one of the extracts result in significant diatom mortality. It is apparent that the diatoms are unable to survive in the presence of the chemicals produced by the sponge or the sponge-associated microorganisms.

These findings are not uncommon as sponges often produce compounds that deter foulers and or competitors (Dobretsov et al. 2004, Fusetani 2004, Sjogren et al. 2004). Whether the sponges presently studied are able to incorporate the dead diatoms into their tissues as a food source is unknown but other studies have found diatom frustules within the tissues of Antarctic sponges at some times during the year (Cerrano et al. 2004, Regoli et al. 2004), indicating that sponges may feed on diatoms seasonally.

It is now known that the majority of the sponges studied from the western side of the Antarctic Peninsula contain chemicals that inhibit feeding by benthic predators (Peters et al., in press), inhibit fouling by epibiotic diatoms, and have no inhibitory effect against a suite of sympatric bacteria. These results provide insight into the Antarctic benthic community where predators and foulers are apparently a greater threat to benthic, sessile invertebrates than bacterial pathogens.
ACKNOWLEDGEMENTS

We thank Lukasz Kwapisz for help in antibacterial assays, Ted Hadfield and Susan Ditty for sequencing the bacteria, and Philip Bucolo for diatom identification. This work would not have been possible without logistical support in Antarctica provided by the employees and subcontractors of Raytheon Polar Services Company. This research was facilitated by National Science Foundation awards to C.D.A. and J.B.M. (OPP-0125181 and OPP-0442769) and to B.J.B. (OPP-0125152 and OPP-0442857).
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Bewley CA, Holland ND, Faulkner DJ (1996) Two classes of metabolites from Theonella swinhoei are localized in distinct populations of bacterial symbionts. Experientia 52:716-722


CAN FREQUENT CONTACT WITH SEA STARS INDUCE DEFENSES IN
*HYMENIACIDON FERNANDEZI* (PORIFERA, DEMOSPONGIAE)?

by

KEVIN J. PETERS, CHARLES D. AMSLER, JAMES B. MCCLINTOCK,
AND BILL J. BAKER
INTRODUCTION

Every living organism has a limited amount of resources from which it must allocate to various metabolic processes. Hypotheses have been proposed whereby increasing the amount of resources allocated to one metabolic function there must be a “cost” elsewhere in the organism with another metabolic process having less of the needed resource (Cronin 2001). One metabolic process that needs varying levels of resources is resistance to predation.

The presence of defenses in terrestrial and aquatic organisms which deter predation from potential consumers has been known for many years (Dethier 1954, Ehrlich & Raven 1964, Pasteels et al. 1983, Duffy & Paul 1992, Pawlik et al. 1995, Tollrian 1995, Amsler et al. 2005). Many defenses are constitutive which means they are present all of the time while the production of others may be induced when required. Constitutive defenses would be selected for in an environment where predation risk is high; comparatively, induced defenses may be selected for in environments with an intermediate risk of predation or areas where predation is variable both spatially and temporally. Organisms with a low risk of predation are less likely to produce defenses but rather to allocate all of their available resources to other metabolic functions.

Predators and their modes of predation can also influence defensive strategies. A predator that completely consumes an individual over a short amount of time will require
a different defensive strategy compared to a predator that feeds in small quantities over extended time periods (Karban & Myers 1989).

Various resource allocation models have been proposed where, depending on abiotic and biotic factors, organisms are predicted to allocate their resources in various manners. One of these models is the optimal defense theory (ODT) which predicts that organisms preferentially defend areas deemed more valuable to future survival or more vulnerable to predation (Rhoades 1979). The Induced Defense Model [IDM (Karban & Myers 1989, Harvell 1990)] continues with the ODT assumption that defenses are costly and would therefore be best produced only when needed. An induced defense is one not present constitutively but is initiated after the onset of a predatory event. When the prey organism detects predation, it will allocate energy that would otherwise be used for growth, reproduction, resource acquisition, or maintenance toward the production of defenses. This would only be an effective strategy if the compound(s) are produced and translocated in time to have an impact on consumption by the predator (Hay 1996, Amsler & Fairhead 2006).

The mode of predation is important because not every predatory event can be deterred with this defensive strategy. For inducible defenses to be of use, both temporal and spatial scales of predation are important (Amsler & Fairhead 2006). A predator that takes a large bite out of an item and then moves on or which can completely consume the prey rapidly will not be deterred by induced defenses. Mesograzers that cause only partial damage over short intervals and feed on the same item for extended periods of time would be effectively deterred by inducible defenses if the prey item is able to
produce and/or to transport the compounds to the damaged area in a short amount of time (Hay 1996).

In many environments, predators are not limited to feeding on a single item for an extended period of time, but in Antarctica, where sea stars are the predominant predators on sponges (Dayton et al. 1974), an induced defense is a likely strategy for some species. A sea star uses its chemosensory tube feet located on the oral surface of its arms to determine potential food sources. It then must move its oral opening over the food source at which time it extends its cardiac stomach and begins extraoral digestion of the food source (Hyman 1955). Once a sea star starts to feed on an item, it can remain there feeding on the same food source for an extended period of time (Dayton et al. 1974). Sea stars are abundant, but often not to the extent that every sponge has a sea star in contact with it at all times, so the risk of predation does not appear to be constantly high (authors’ personal observations). These observations of Antarctic organisms provide evidence that there might be a substantial number of Antarctic sponge species that have inducible defenses only produced when they sense sea star predation.

The sponge species used to test the IDM in this system is *Hymeniacidon fernandezii* Thiele, 1905. This species was chosen because it is relatively common in the study area and, in a previous study, marked differences in levels of a presumed defensive compound, suberitenone A, were found in different individuals (Bill Baker personal communication). The external layers of the sponges always had more suberitenone A present when compared to internal layers although the absolute amount of the compound varied between individuals. When this purified compound was presented to *Perknaster fuscus* using a tube foot retraction bioassay (McClintock et al. 2000), it resulted in the
retraction of their chemosensory tube feet (unpublished data). Varying abundances of sea stars have also been observed at different collection sites (authors’ personal observation) and these differing levels of compounds in different sponge individuals might be due to uneven levels of predation at the various collection sites.

This study will test whether the IDM is a relevant predictor of defenses in the Antarctic where sponge predators feed for extended periods of time on a defined area of an individual. By using a sponge species that is known to have variable amounts of defensive compounds present, the response by individuals in a high predator environment will be compared to the responses of individuals in environments with no predators. It is hypothesized that defenses will be induced or amplified in *H. fernandezi* when placed in environments with the constant presence of predators compared to the environments with no predators present based on previous observations where individuals had varying levels of defenses present.
MATERIALS AND METHODS

Sponges and sea stars were collected by hand using SCUBA from the shallow coastal waters (5 – 39 m) near Palmer Station located on Anvers Island off the western side of the Antarctic Peninsula (see Amsler et al. 1995 for map). Eighteen *Hymeniacidon fernandezi* were collected and kept submersed from the collection site to the laboratory where they were placed into separate buckets in ambient flow-through seawater tanks. The two experimental conditions as well as the control buckets had a range of sponge sizes as there were not 18 identically sized sponges collected. Each bucket had its own water source and the sponges were kept in this environment for several days in order to acclimate to the artificial environment.

Two sea star species, one a known omnivore (*Odontaster validus* Koehler, 1906) and the other a potential spongivore (*Perknaster aurorae* (Koehler, 1920)), were collected from the same general area as the sponges (vouchers of all organisms are maintained at the University of Alabama at Birmingham). Thirty-six *O. validus* and six *P. aurorae* were allowed to acclimate in seawater tanks with no food for several days. At the beginning of the experiment, a similar size range of six *O. validus* were added to each of six buckets with a *H. fernandezi*. As *P. aurorae* is a larger sea star, only one individual was added to each of six other buckets with a *H. fernandezi*. The six remaining buckets were maintained with a single sponge but no sea star predators.
Every day the buckets were observed and the number of sea stars in contact with the sponge was noted for each bucket. Any sea stars that were at the top of the buckets were removed from the wall and placed onto the sponge below in order to have the sponge sense a predator as often as possible. These actions were repeated everyday for 100 days. On day 65, the buckets were wiped clean of fouling diatoms so that the sea stars did not have another potential food source available.

At the end of the experiment, the sponges were removed, weighed, and used in feeding bioassays. Sponge tissue (approximately ½ cm³) was excised using a single edge razor from both the surface and the approximate center of each individual sponge and presented to *O. validus* not used in the artificial environments. Sponge pieces taken from the surface of the sponge were presented to the sea star such that the outer surface of the sponge was in contact with the sea star's tube feet. Acceptance and rejection were compared to an artificial, palatable control food item consisting of 5% dried, powdered krill in a 2% alginate matrix. Multiple replicates (n ≥ 8) of each sponge region were presented to individual sea stars with no sea star being used more than once.

The radius and weight of each sea star in a bucket were measured and representative samples were preserved. The sponges were frozen at -80°C and subsequently lyophilized. The dried sponges were cut into thin layers (approximately 1 mm wide) starting with the surface and moving inward to the center of the sponge. The outer and innermost layers of dried sponge were then extracted in three changes of 1:1 dichloromethane:methanol to obtain a lipophilic crude extract. The sponge tissue was subsequently extracted in three changes of 1:1 methanol:water to obtain a hydrophilic crude extract. These extracts were dried under reduced pressure resulting in a yield of
dried extract per weight of dried sponge. Using the wet weight to dry weight conversion recorded during lyophilization, the amount of extract per gram of wet sponge tissue was calculated. This concentration was the natural concentration of the extract in the sponge tissue.

The extracts were then added to a known palatable food item (lyophilized krill powder) at a concentration equal to the natural concentration in the sponge on a wet weight basis. This krill coated with sponge extract was then added to 2% alginate in seawater matrix and allowed to gelatinize with the addition of 1 M chilled calcium chloride. Solvent control food pellets were also prepared. Satiation controls consisting of 5% powdered krill in a 2% alginate matrix were utilized as well and only replicates where the satiation controls were accepted were included in statistical analyses. Rejection rates between extract-laden pellets and solvent control pellets were compared statistically. Due to the natural concentration of the extract always being rejected, 10% and/or 30% of the natural concentration were presented to the sea stars as well. The lipophilic extract was the only one presented to the sea stars during feeding assays. The natural concentration of both inner and outer layers was assayed for three of the sponges from each condition.

Statistical analysis used a Fisher Exact Test (VassarStats, www.faculty.vassar.edu/lowry/VassarStats.html) to determine palatability in all feeding assays. Sponge tissue was compared against control pellets in fresh tissue analyses while experimental pellets were compared with solvent control pellets when using extracts.
RESULTS

Over the duration of the 100 days the experiment was conducted, there were more interactions between *Odontaster validus* and *Hymeniacidon fernandezi* when compared to the *Perknaster aurorae* with the sponges. Observations were recorded at least once a day and on only three occasions were any of the *P. aurorae* in contact with a *H. fernandezi*. Conversely, the majority of the checks resulted in observations of multiple buckets with *O. validus* in contact with *H. fernandezi* (Table 4).

At the completion of the experiment, the sponges that were in buckets with *P. aurorae* had a mean weight of 251.5 g with a range of 101 – 353 g. The sponges in the buckets with *O. validus* had a mean weight of 257.3 g and a range of 128 – 522 g. The control sponges in buckets with no sea stars had a mean weight of 163.0 g and a range of 53 – 267 g. There were no initial weights taken of the sponges, and therefore, no conclusions can be made as to weight gain or loss during the experiment. However, no visible increase or decrease in sponge size was apparent.

The sea stars likewise did not appear to increase or decrease in size during the experiment. At the completion of the experiment, the mean *P. aurorae* radius (oral opening to tip of one arm) was 10.9 cm with a range of 9.5 – 12.0 cm. The average weight of these six asteroids was 264.1 g with a range of 161.6 – 349.9 g. The mean *O. validus* radius and weight were 4.6 cm and 22.8 g respectively. The radii ranged from 3.5 – 5.7 cm and the weights from 10.0 – 34.6 g.
Feeding bioassays performed using both inner and outer fresh sponge tissue at the completion of the experiment resulted in all sponge tissue being significantly rejected ($P \leq 0.05$) compared to the control food pellet (Figure 5). Not a single sponge piece taken from the exterior of *H. fernandezi* was accepted by *O. validus*. Both experimental conditions and control sponges were rejected similarly.

After extraction of the inner and outer sponge tissue layers, the natural concentration of the lipophilic crude extract on a wet weight basis was significantly rejected ($P \leq 0.05$) when added to the same artificial food used in the control pellets (Figure 6). A 10% dilution of the lipophilic extract from one sponge was readily accepted. Three of the nine sponges had a 30% dilution of the outermost layer's lipophilic extract significantly rejected by *O. validus*. When a 30% dilution of the innermost layer's lipophilic extract was presented to *O. validus*, two of the nine sponges were significantly rejected. One of the *H. fernandezi* that was in an enclosure with six *O. validus* (O1) had its 30% outermost dilution rejected but the innermost one was not significantly rejected.
Table 4. Number of days at least one sea star was in contact with a *Hymeniacidon fernandezi*. Numbers under sea stars indicate individual buckets with a single sponge in each one. There were six *Odontaster validus* in each bucket and only one *Perknaster aurorae*.

<table>
<thead>
<tr>
<th>Odontaster validus</th>
<th>Number of days observed at least one sea star in contact with sponge</th>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
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<table>
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<tr>
<th>Perknaster aurorae</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
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<tr>
<td>2</td>
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<tr>
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<tr>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
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</table>
FIG. 5. Percent acceptance of feeding bioassays using fresh outer and inner sponge tissue fed to the sea star *Odontaster validus* upon completion of the experiment. All sponge tissue was significantly* (*p*≤0.05) rejected compared to control food item. Each alphanumeric label corresponds to an individual *Hymeniacidon fernandezi*. C1-C6 refer to control sponges with no predators present. O1-O6 refer to sponges with 6 *O. validus* in each enclosure. P1-P6 refer to sponges with one *Perknaster aurorae* in each enclosure.
FIG. 6. Percent acceptance of lipophilic extract concentrations coated on a palatable food item by the sea star *Odontaster validus*. Significant feeding deterrence (*p*≤0.05) compared to controls is noted by *. Each alpha-numeric label corresponds to an individual *Hymeniacidon fernandezi*. C1-C3 refer to control sponges with no predators present. O1, O3, and O6 refer to sponges with 6 *O. validus* in each enclosure. P2, P3, and P5 refer to sponges with one *Perknaster aurorae* in each enclosure.
DISCUSSION

Previously, varying levels of a compound that was deterrent to a predatory sea star were found in tissues of *Hymeniacidon fernandezi* with the greatest amount always found in the outermost layer of tissue (Baker unpublished data). The different locations sponges were collected from had differing levels of predators present and it was assumed that the different absolute levels and areas this compound was found in were due to increased predatory signals causing the sponge to create more of the defensive compound in the outermost layers which were susceptible to sea star predation. The compound, suberitenone A, while it is a sponge deterrent causing *Perknaster fuscus* sea stars to retract their tube feet in its presence, it does not deter *Odontaster validus* from feeding on a food item with the compound in it (unpublished data). The latter observation was not made until after this experiment was completed.

It is not possible to determine if defenses were induced or amplified in this experiment due to all of the sponges being significantly rejected. This sponge has previously been found to be unpalatable as inner and outer fresh tissue as well as the lipophilic extract being a feeding deterrent to *O. validus* (Peters et al. in press).

Antarctica lacks biting sponge predators that would feed on a short time scale before moving onto another food source (McClintock et al. 2005, Aronson et al. 2007). This and the fact that sponges are able to survive partial predation made this the ideal environment to look for inducible defenses. The sea stars were chosen as predators
because they are both the dominant predator in the system and feed for extended periods of time on single prey items (Dayton et al. 1974). If the sponge were able to produce increased defenses and translocate them to an attacked area, sea stars would be the best predators to use in order to elicit this response.

Even if the sea stars were not readily feeding on the sponges, they were in contact with the sponges every day of the experiment giving the sponges in this artificial environment a sense that predation risk and predator density were high. It was noted that the *Perknaster aurorae* did not stay in contact with the sponges as frequently as *O. validus*. *Perknaster fuscus* is a spongivorous sea star commonly seen residing on sponges and it was thought that *P. aurorae* was also spongivorous or at least omnivorous. If this sea star does feed on sponges, then its complete lack of apparent feeding on *H. fernandezi* lends support to this sponge having anti-feedant defenses. This sea star’s chemosensory tube feet may have been able to sense these defenses and consequently the sea star moved on in search of a potentially better food source.

Although there were *O. validus* in contact with the sponges during the majority of the observations, there were always more sea stars that had moved off the sponges and traveled up the vertical sides of the enclosure as if seeking out an alternative food source. This range of activity of the *O. validus* makes it hard to ascertain their affinity to the sponges. By having six sea stars in each enclosure, the probability of having a sea star in contact with the sponge was much greater than the enclosures with *P. aurorae* where only one sea star was included. *O. validus* is the most numerous predatory sea star observed in the collection areas and it has been found to find some sponges palatable previously (Peters et al. in press).
The compound suberitenone A, previously isolated from *H. fernandezi* and found to be a sea star deterrent was to be quantified in the various tissue layers collected. However, separate feeding experiments with the compound and a closely related one, suberitenone B, both individually and combined, did not result in feeding deterrence (unpublished data). Suberitenone A has previously been found to be concentrated close to the surface of individual *H. fernandezi* (Baker unpublished data) and it was presumed to deter predation. As of yet, the actual compound(s) in the sponge that produce(s) feeding deterrence is/are unknown. The sponge layers are currently stored at UAB awaiting chemical analysis when the antifeedant compound(s) has/have been identified.

Knowing that suberitenone A is concentrated near the surface of the sponge might mean that it is used by the sponge in a capacity other than as a feeding deterrent. Diatoms bloom in the austral summer and settle on any available substrate whether it is living or inanimate. These settling diatoms have been found to settle on and even parasitize sponges (Bavestrello et al. 2000). *H. fernandezi* has been found to have a lipophilic extract that causes significant diatom mortality when tested against the sympatric diatom *Syndroposis* sp. (Peters et al. in prep). While suberitenone A deters sea stars, it may be more useful as an antifoulant preventing diatoms from colonizing the surface of the sponge. This would support the increased concentration of suberitenone A in the exterior only because diatoms are unable to settle on the interior surfaces.

Although no conclusions concerning induced or amplified defenses of *H. fernandezi* in an environment with a high risk of predation can be made based on the results obtained from this experiment, the sponges appeared to survive the experimental conditions very well. A recent study has found sponges from the same area that are
palatable to sea stars as fresh tissue (Peters et al. in press). If these sponges are used in a similar experiment, perhaps the results would be in line with predictions of the IDM. However, it is also possible that these sponges rely on other strategies for survival. These are but a few of the many questions yet to be asked concerning relationships between Antarctic predators and prey.
ACKNOWLEDGEMENTS

Special thanks go to Margaret O. Amsler for her help in sponge dissections, Rob van Soest for sponge identification, and Raytheon Polar Services Company for logistical support in the field. This research was facilitated by National Science Foundation awards to C.D.A. and J.B.M. (OPP-0125181) and to B.J.B. (OPP-0125152).
LITERATURE CITED


CONCLUSION

During our analysis of the overall palatability of the Demospongiae from the western Antarctic Peninsula, we found that the majority of demosponges collected in the shallow coastal waters of the western Antarctic Peninsula were unpalatable to the omnivorous sea star *Odontaster validus*. Of the 27 sponge species collected, 21 (78%) of them had outer tissues that were defended against predation. With sea stars thought to drive the evolution of chemical defenses in this system, it was hypothesized that the sponges would maintain their harshest defenses along their surface and would not defend internal tissues as heavily. Upon conducting feeding bioassays with internal tissues of the sponges whose external tissues were found to be unpalatable, 8 of the 21 (38%) had internal tissues not defended as was hypothesized. However, the other 62% of the species do have internal tissues that are readily defended against predatory events that are not possible by sea star predators. It is now thought that mezograzer crustaceans are able to gain access to internal tissues through prominent oscula and bypass the external defenses. With there being multiple predators possibly driving the evolution of defenses in these sponges, definitive predictions as to where defenses may be found are more difficult to make.

Although defenses can be in many different forms, it was hypothesized that the most important facet of defenses in these sponges was internal chemistry in the form of secondary metabolites. Although not all of the sponges that had fresh sponge tissue
found to be unpalatable could be extracted and analyzed for the presence of defensive secondary metabolites, all of the species that were tested had at least one of their crude extracts significantly deter feeding by the same omnivorous sea star *O. validus*. While this does not let us determine the total level of chemical defenses present in sponges from the western Antarctic Peninsula, it does provide us with the understanding that secondary metabolites are just as important in coastal Antarctic waters as they are in waters from many other parts of the world.

The role of the secondary metabolites against potential harmful microorganisms was also analyzed and differing conclusions were realized. Bacteria isolated from the water column and internal tissues of organisms near the sponges were mostly resistant to the secondary metabolites isolated from the sponges. This leads us to believe that although our sample size was not very large (20 bacterial isolates), bacterial pathogens do not appear to be a prevalent problem for the sponges in this system. However, fouling microorganisms like diatoms appear to be a much more serious threat to sponge survival as crude extracts (especially lipophilic) from all of the sponges caused significant diatom mortality. These diatoms are numerous at certain times of the year and the ability of a sponge to prevent surface colonization by these organisms could be crucial for survival of the sponge.

Lastly, previous experiments had resulted in preliminary data where a deterrent compound was concentrated in surface tissues of the sponge *Hymeniacidon fernandezi*. This led us to believe that we would be able to possibly induce the production of the deterrent compound in individuals that were at high risk of predation and possibly see a decrease in defenses in sponges that had no risk of predation. We found that defensive
compounds are present in *H. fernandezi* even after 100 days of no contact with predators and no increased level of defenses could be ascertained in the individuals that had a high risk of predation.

Overall, a better understanding of the biology and ecology of sponges from the western Antarctic Peninsula was garnered through this study with the knowledge that secondary metabolites have a vital role in the relationships between these invertebrates and other organisms they encounter.
GENERAL LIST OF REFERENCES


Brouwer PEM, Geilen EFM, Gremmen NJM, Vanlent F (1995) Biomass, cover and zonation pattern of sublittoral macroalgae at Signy Island, South Orkney Islands, Antarctica. Botanica Marina 38:259-270


APPENDIX

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL FORM

Note: IACUC approval for this study was required only for the research conducted in 2004
NOTICE OF APPROVAL

DATE: March 2, 2004

TO: Charles D. Amsler, Ph.D.
    CH 378
    FAX: 205-975-6087

FROM: Suzanne M. Michalek, Ph.D., Vice Chair
      Institutional Animal Care and Use Committee

SUBJECT: Title: Collaborative Research: Chemical Ecology of Shallow-Water Marine
         Macroalgae and Invertebrates on the Antarctic Peninsula
         Sponsor: National Science Foundation
         Animal Project Number: U4020/119

On February 25, 2004, the University of Alabama at Birmingham Institutional Animal Care and
Use Committee (IACUC) reviewed the animal use proposed in the above referenced application.
It approved the use of the following species and numbers of animals:

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<th>Species</th>
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<td>30</td>
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<tr>
<td>Invertebrates</td>
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Animal use is scheduled for review one year from February 2004. Approval from the IACUC
must be obtained before implementing any changes or modifications in the approved animal use.

Please keep this record for your files, and forward the attached letter to the appropriate
granting agency.

Refer to Animal Protocol Number (APN) U4020/119 when ordering animals or in any
correspondence with the IACUC or Animal Resources Program (ARP) offices regarding this
study. If you have concerns or questions regarding this notice, please call the IACUC office at
934-7692.

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205.934.7692 • Fax 205.934.1188
iacuc@uab.edu
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