



Mesofauna associated with the marine sponge *Amphimedon viridis*. Do its physical or chemical attributes provide a prospective refuge from fish predation?

J.P. Huang^a, J.B. McClintock^{a,*}, C.D. Amsler^a, Y.M. Huang^{a,b}

^a Department of Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294-1170, USA

^b Department of Marine Sports and Recreation, National Penghu University, Makung City, Penghu 880, Taiwan

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ABSTRACT

Marine sponges often harbor an abundance of associated organisms. We characterized mesofauna associated with the common tropical sponge *Amphimedon viridis*, and then tested whether physical (spicules) or chemical (lipophilic or hydrophilic extracts) properties of this sponge provide a prospective refuge for mesofauna from fish predation. Sponge analyses revealed a moderately diverse and numerically rich community of sponge-associated mesofauna comprised primarily of mesocrustaceans (82% of total fauna). Eighty-nine percent of these were amphipods, but smaller numbers of tanaids, decapods, and isopods also occurred. Quantitative sampling of outer surfaces and interstices of fifteen *A. viridis* yielded a total mean \pm 1 SD density of sponge-associated mesofauna of 53 ± 9.3 individuals per 100 cm³ wet sponge tissue. Among the numerically dominant amphipods, 65% occurred on outer sponge surfaces where they are most vulnerable to fish predators. We evaluated whether *A. viridis* provides a prospective refuge from predation by assessing the palatability of this sponge to the sympatric generalist pinfish *Lagodon rhomboides*. When presented small (2 mm) bite-size pieces of whole sponge tissue, similar in size to what fish might incidentally ingest should they attempt to consume sponge-mesofaunal associates, pinfish displayed strong feeding deterrence. Alginate food pellets containing tissue-level concentrations of sponge spicules caused a weak but significant deterrent response. In contrast, alginate pellets containing tissue-level concentrations of either lipophilic or hydrophilic extracts of *A. viridis* were highly deterrent to pinfish. Thus, chemical, and to a considerably lesser degree, physical defenses (spicules) may contribute to this sponge serving as a protective refuge for associated mesofauna.

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1. Introduction

Marine sponges provide habitats for a wide assortment of organisms. These include a diverse array of bacteria (e.g., cyanobacteria) algae, mesofauna (polychaetes, amphipods, isopods) and macro-invertebrates (brittlestars, other sponges, etc.) (Pawlik, 1982; Oshel and Steele, 1985; Rai, 1990; Frost et al., 1997; Althoff et al., 1998; Fredrich et al., 1999; Bieler, 2003; Thakur and Muller, 2005; Henkel and Pawlik, 2005). While some of these relationships are clearly symbiotic in nature (e.g. mutualistic cyanobacteria, Thakur and Muller, 2005), prospective factors selecting for less obligate sponge associations have received little attention.

One explanation for the association of mesofauna with marine sponges is that the defensive attributes of the sponge itself provides a prospective refuge from predation. This would be analogous to studies that have demonstrated that mesocrustaceans (amphipods, crabs, polychaetes, and gastropods) may associate with chemically defended macroalgae in order to decrease the likelihood of fish predation (Hay et al., 1990; Duffy and Hay, 1991; Riberio et al., 2003; Skilleter et al., 2005). Marine

sponges may likewise harbor chemicals that serve as feeding deterrents effective against fish and other predators (Pawlik et al., 1995; reviewed in Paul, 1992; Pawlik, 1993; McClintock and Baker 2001). Moreover, many sponges also possess skeletal elements including siliceous and calcium carbonate spicules that can be effective deterrents against fish predators (Randall and Hartman, 1968; Chanas and Pawlik, 1995).

The common tropical marine demosponge *Amphimedon viridis* (Order Haplosclerida; Family Niphathidae) occurs on shallow reefs and in seagrass beds and has a wide biogeographic distribution including the Gulf of Mexico, Atlantic, Caribbean, Indian Ocean, and regions of the Red Sea and Micronesia (Burns et al., 2003; Collin et al., 2005). This sponge's morphology ranges from massive (upright) to prostrate with 1–3 cm branches with small numbers of volcano shaped oscules. It is known to possess potent toxic compounds including a purified mixture of halitoxin and amphitoxin, both highly bioactive pyridinium alkaloids (Kelman et al., 2001). These compounds are likely to have fish antifeedant properties. Burns et al. (2003) demonstrated that the combined crude organic extracts of *A. viridis* are deterrent to two species of sympatric wrasse. They did not investigate the effects of spicules as potential deterrents.

Amphimedon viridis occurs in the shallow seagrass communities of Saint Joseph Bay, Florida. Here it is found to occur in sympatry with an abundant and diverse array of crustacean mesograzers (Huang, 2006).

* Corresponding author. Tel.: +1 205 975 2525.

E-mail address: mcclinto@uab.edu (J.B. McClintock).

Moreover, this shallow bay is home to a number of generalist fish that include mesocrustaceans in their diets, especially the common pinfish *Lagodon rhomboides* (Heck et al., 2000). Qualitative observations in Saint Joseph Bay indicate that mesocrustaceans (amphipods) removed by hand from *A. viridis* and released into the nearby water column are rapidly attacked and consumed by pinfish (Y.M. Huang and J.P. Huang, pers. obs.).

The objectives of the present study were to: 1) quantify the mesofauna associated with a sub-population of *Amphimedon viridis* in a shallow seagrass habitat of Saint Joseph Bay, Florida, 2) determine what proportion of the mesofauna occurred on the surfaces of sponges where they would be most vulnerable to fish predation, and 3) evaluate whether structural (spicule) or chemical (lipophilic or hydrophilic organic extracts) attributes of this sponge were deterrent to the pinfish *Lagodon rhomboides*.

2. Materials and methods

2.1. Sponge associated mesofauna

Fifteen individuals of *Amphimedon viridis* were collected from a population of sponges located in a shallow (1–2 m depth) seagrass habitat of Saint Joseph Bay, Florida (29°36.032' N; 85° 24.264' W) in April 2006. Zip-closure plastic bags were placed quickly over each sponge and each lifted free of the substrate and immediately sealed in the bags to ensure that sponge-associated mesofauna were retained. Sponges were then transported on ice to the University of Alabama at Birmingham where they were immediately weighed (wet wt) and their respective volumes determined by volumetric displacement of seawater in a graduated cylinder. For each sponge, the contents of the bag were gently washed into a glass jar. Any resultant mesofauna were considered sponge-surface associates, as were all mesofauna subsequently removed by forceps from the surface of each sponge under a dissecting microscope (Nikon Model 77041). Once the surface-associated mesofauna were completely removed, the sponge was meticulously dissected under a dissecting microscope, and all mesofauna present in the interstices of the sponge were collected. Mesofauna from the surface and interstices of each sponge were then separately preserved in 70% ethanol for enumeration and identification. As crustaceans dominated the mesofaunal samples, an emphasis was placed on their taxonomic identification. Vouchers of mesocrustaceans were shipped to Dr. Sara E. LeCroy, University of Southern Mississippi, Gulf Coast Research Laboratory, for taxonomic identification or verification. All other mesofauna were identified to the lowest taxon possible.

For the fifteen sponges sampled, the mean total number of mesofaunal sponge associates was determined, as were the mean percent compositions of each group of sponge-associated species. Species diversity (Shannon diversity index), species evenness (Shannon evenness index), and species richness (total numbers of associated faunal species per sponge) values for sponge-associates were also determined. The Shannon diversity index (Shannon and Weaver, 1947) was defined as $H' = \sum_{i=1}^s (p_i \ln p_i)$ where s is the number of species in the sample and p_i is the proportion of the organisms in the sample represented by the i th species. The Shannon evenness index was calculated as $E_H = H'/H'_{\max} = H'/\ln S$ where S is the number of species in the sample, H' is the Shannon Diversity Index, and H'_{\max} is the maximum value of H' . In all the above calculations, raw data were first standardized to number of mesofauna per 100 cm³ of wet sponge mass.

2.2. Pinfish feeding bioassays

2.2.1. Fish maintenance

Twenty pinfish (*Lagodon rhomboides*) (mean length = 10 cm) were procured from the Gulf Specimen Marine Laboratories (Panacea, Florida) and maintained at 25 °C at densities of 2 fish per 34 L aquarium, each provisioned with artificial seawater (33 ppt) and a recirculating water pump with a charcoal filter system (Tetra Whisper®

Biobags). Fish were fed a standard maintenance diet consisting of 2 dry g commercial fish food flakes (Tetramarine) per fish per week prior to feeding experiments.

2.2.2. Whole sponge fish feeding assay

Ten additional individuals of the sponge *Amphimedon viridis* were collected from two shallow (1–2 m depth) seagrass habitats off Sugarloaf Key, Florida (24° 38.255' N; 81° 34.779' W and 24° 39.639' N; 81° 26.194' W) in October, 2006. These collection sites were employed because it was difficult to locate populations of *A. viridis* in Saint Joseph Bay during fall and winter months. Sponges were returned to the University of Alabama at Birmingham and frozen at –20 °C until their use in the fish feeding bioassays described below. As the sponges were thawed prior testing, it is possible there may have been some alteration of chemical activity. However, the likelihood of secondary metabolites losing their activity due to thawing is remote (B.J. Baker, pers. comm.). Moreover, even if there were a reduction in chemical deterrence this would mean our evaluation of deterrence is conservative.

In order to first evaluate whether pinfish reject whole sponge tissue, bite-size, spherical pieces (2 mm diameter) of whole sponge tissue were presented to an individual pinfish isolated in a 34 L aquarium. The outcome of each feeding trial was scored as either: 1) accepted (pinfish ingested and swallowed the item), or 2) rejected (pinfish mouthed the item and then spit it out). Immediately following the presentation of each piece of sponge tissue, each fish was presented a control food (similar sized piece of fresh squid mantle tissue). 13 replicate trials were conducted and no fish was tested more than once. A Fisher's Exact Test was used to determine significant differences between percent pinfish rejection of whole sponge tissues and control squid tissues.

2.2.3. Preparation of sponge spicules

In order to determine an ecologically relevant concentration of spicules to mix into feeding pellets, we first examined intraspecific variability in spicule content in *Amphimedon viridis*. For each of six sponges, a 10 cm³ piece of sponge tissue (determined by seawater volumetric displacement) was placed in a test tube and submerged in 20 ml of standard chlorine bleach (sodium hypochlorite, 5.25%). Once the bleach solution had stopped bubbling (approx 5 h), the suspension was centrifuged and the supernatant was discarded. This process was repeated two more times. Each spicule pellet was then washed 3 times with distilled water, transferred to a pre-weighed 25 ml scintillation vial, dried in a drying oven at 90 °C for 12 h. The natural concentration of spicules [small uniform shaped monoaxons (mean ± 1 SD length = 4.2 ± 0.2 μm; n = 6)] in each of the 6 sponge samples was calculated by determining the mass of the spicule pellet and using the known volume of the original sponge sample (10 cm³) the spicule content was standardized to spicules/cm³ sponge tissue. Spicule mass displayed little intraspecific variation, ranging from 42–55 mg/cm³ sponge (n = 6). Therefore, we used a mean ratio of 48 mg spicules/cm³ sponge tissue as a proxy to prepare alginate food pellets with spicules (see below). Once the supply of spicules from the above bleach extractions was exhausted, additional sponge material was subjected to the same protocols to provide additional spicules for pellet preparations.

2.2.4. Preparation of sponge extracts

10 individual *Amphimedon viridis* were weighed and lyophilized. Dry weights of each sponge were determined and sponges broken into small pieces (~1 cm³). Sponge pieces were combined for extraction, placed into a one L glass beaker and subjected to three 24 h exchanges of a 1:1 dichloromethane:methanol solution to yield a lipophilic extract. The extract was then filtered (Whatman Qualitative #1 filter paper), evaporated to dryness on a rotary evaporator, spun for 24 h on a speed vacuum, and weighed. A hydrophilic extract was prepared by subjecting extracted sponge material (see above) to three 24 h exchanges of a 1:1 methanol:water solution. The hydrophilic extract was then filtered, evaporated to dryness on a rotary evaporator, lyophilized for 24 h, and

Table 1Mean \pm 1 SD abundances of mesofauna associated with individuals of the demosponge *Amphimedon viridis* in seagrass habitats of Saint Joseph Bay, Florida in April 2006

	Amphipods				Isopod	Tanaid	Decapod	Polychaetes		Total Individuals	
	<i>Laticorophium baconi</i>	<i>Elasmopus levis</i>	<i>Erichthonius brasiliensis</i>	<i>Bemlos setosus</i>	<i>Paracerceis caudata</i>	<i>Hargeria</i> or <i>Leptochelia</i>	<i>Libinia dubia</i>	Unknown Errant Polychaete	Unknown Serpulid		<i>Diopatra cuprea</i>
Mean \pm SD	26 \pm 26	10 \pm 14	2 \pm 4	0.7 \pm 3	0.8 \pm 2	2 \pm 2	2 \pm 2	4 \pm 2	3 \pm 4	0.6 \pm 2	53 \pm 36
Range	0–76	0–49	0–13	0–11	0–5	0–5	0–5	0–8	0–15	0–7	14–121
# of sponges containing > 0 individual	11	9	5	1	4	8	10	14	10	2	

Abundances have been standardized to numbers of sponge-associates per 100 cm³ wet sponge mass. A total of fifteen sponges were sampled.

weighed. Extracts were stored at -20°C until use in pellet bioassays. The concentration of the lipophilic and hydrophilic extract in *A. viridis* was calculated by dividing the dry wt of each crude extract by the wet wt of the sponge material extracted. This ratio was used to prepare alginate food pellets (see below) containing natural concentrations of each sponge extract.

2.2.5. Preparation of alginate food pellets containing spicules or extracts

Alginate food pellets containing spicules or organic extracts were prepared using a modification of the procedures given in Burns et al. (2003). Control alginate food pellets were prepared by combining 0.3 dry g of alginic acid with 0.5 g freeze-dried, powdered, squid mantle tissue as a feeding stimulant and adding distilled water to bring the final volume to 10 ml. This solution was then taken up in a 3 ml syringe, the tip submerged into a 0.25 M solution of calcium chloride, and ejected to form a string of hardened alginate. The string was then cut with a razor into small pellets each measuring 3 mm in length. Experimental food pellets containing either spicules or extracts were prepared using the same procedure as above, but with the addition of natural concentrations of either sponge spicules or extracts.

2.2.6. Fish feeding assays with alginate food pellets containing spicules

In each feeding trial an individual fish was first isolated in a 34 L aquarium. Each pinfish was then presented a control food pellet followed by a spicule food pellet. If the experimental pellet was rejected this was followed by another control food pellet that served as a satiation control. If this pellet was rejected then the trial was not included in data analysis.

13 replicate trials were conducted and no fish was tested more than once. A Fisher's exact test was used to determine significant differences between percent pinfish rejection of food pellets with and without spicules.

2.2.7. Fish feeding assays with alginate food pellets containing organic extracts

In order to evaluate whether the solvents used in the process of preparing experimental pellets containing organic sponge extracts might have altered the feeding stimulants present in the squid control pellets, we compared the feeding deterrence of control food pellets with solvent food pellets to pinfish. Solvent pellets were prepared by making pellets containing freeze-dried squid mantle tissue powder that had been treated with the same amount and type of solvents used in preparation of either the lipophilic or hydrophilic extracts.

Table 2Numbers and percentages of total amphipods found occurring on the surfaces versus the interiors of ten individuals of the sponge *Amphimedon viridis*

	Sponge interstices	Sponge surfaces	Total
Range (#)	6–33	13–70	19–103
Mean \pm SD (%)	35 \pm 7%	65 \pm 7%	

Data are presented as total sponge associates per 100 cm³ wet sponge weight and only for those individual sponges with > 10 amphipods (as initial raw data).

Individual pinfish were first presented a control food pellet followed by a solvent food pellet.

To test the deterrent properties of sponge extracts, pinfish were first presented a control food pellet followed immediately by an experimental food pellet containing either lipophilic or hydrophilic sponge extract. If the experimental pellet was rejected then a satiation control pellet was presented to pinfish. If pinfish rejected the satiation control then this trial was not included in the analysis. Pellets containing lipophilic and hydrophilic sponge extracts were tested on different days. 15 replicate trials were conducted and no fish was tested more than once. We statistically compared the number of solvent control pellets eaten versus those rejected to the number of extract pellets eaten versus those rejected using a Fisher's exact test. This was done separately for each of the two organic extracts.

3. Results

3.1. Sponge associates

Individuals of *Amphimedon viridis* collected from a shallow seagrass community in Saint Joseph Bay, Florida revealed a sponge-associated community comprised primarily (82%) of crustaceans (amphipods, tanaids, decapods, isopods), along with much smaller numbers of polychaetes (Table 1). Because of the small numbers and small sizes of decapods and polychaetes we included these in the "mesofauna" category. When standardized to numbers of sponge-associated mesofauna per unit sponge volume, mean \pm 1 SD abundance was 53 \pm 36 individuals per 100 cm³ of wet sponge tissue (range = 19–121 mesofauna per 100 cm³ wet sponge tissue). The most common sponge-associated mesofaunal group was comprised of the amphipods (78% of total mesofauna, 89% of total crustaceans). 35% of the amphipods were found to occur within the interstices of sponges, while the balance (65%) occurred on sponge surfaces (Table 2). *Laticorophium baconi* comprised the greatest fraction of total amphipods (67%). This species also comprised the largest fraction of sponge-associated mesofauna (49%) and was the numerically dominant sponge-associate in all but two of the 15 sponges examined. Other amphipod species included *Elasmopus levis* (26% of total amphipods), *Erichthonius brasiliensis* (5% of total amphipods), and *Bemlos setosus* (<1% of total amphipods). The tanaid *Hargeria rapax* (3.8% of total mesofauna) and small individuals of the decapod spider crab *Libinia dubia* (3.8% of total mesofauna) also occurred in association with sponges. One small *L. dubia* was found in association with each of 10 of the 15 sponges examined (66%). One isopod species (*Paracerceis caudata*) comprised 1.5% of total mesofauna. Three species of polychaetes (14% of total mesofauna) were observed including the eunicid

Table 3Shannon diversity index, Shannon evenness index and species richness calculated for 15 sponge samples of *Amphimedon viridis*

	Shannon Diversity Index	Shannon's Evenness Index	Species Richness
Range	0.63–1.41	0.40–0.98	3–9
Mean \pm SD	1.07 \pm 0.24	0.71 \pm 0.17	4.9 \pm 1.51

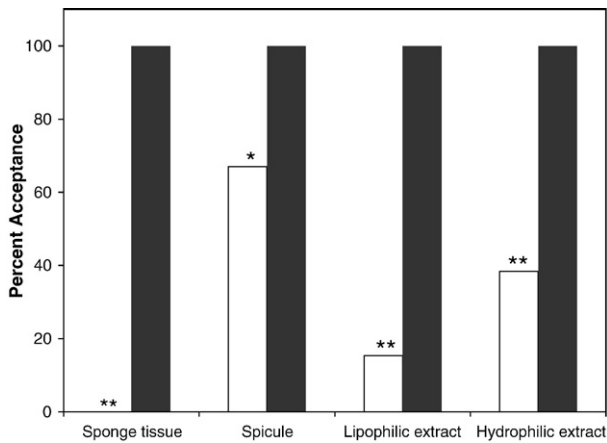


Fig. 1. Pinfish (*Lagodon rhomboides*) feeding bioassays employing whole tissues and alginate food pellets containing natural concentrations of spicules or extracts of the tropical sponge *Amphimedon viridis*. Percent acceptance of pieces of whole sponge tissue by fish when compared to squid mantle tissue controls ($n=13$). Percent acceptance of alginate food pellets containing natural concentrations of spicules by fish when compared to alginate control food pellets ($n=13$). Percent acceptance of alginate food pellets containing natural concentrations of lipophilic and hydrophilic extracts by fish when compared to alginate solvent control food pellets ($n=15$). Single and double asterisks indicate statistical significance at the 0.05 and <0.01 levels, respectively. Experimental treatments are depicted in open bars, control treatments in darkened bars.

Diopatra cuprea, an unidentified serpulid, and an unidentified errant species.

Shannon diversity indices for the fifteen individual sponges collected from the population at Saint Joseph Bay ranged from 0.63 to 1.41 (mean \pm 1 SD = 1.07 ± 0.24) (Table 3). Shannon evenness indices ranged from 0.40 to 0.98 (mean = 0.71 ± 0.17). Sponge-associated species richness values ranged from 3 to 9 species with a mean of 4.9 ± 1.5 species among the 15 sponges examined.

3.2. Fish feeding deterrent properties of sponges

Small pieces of whole sponge tissues of *Amphimedon viridis* were highly deterrent ($P < 0.001$) to pinfish (Fig. 1). In no instances, were any of the sponge pieces presented to pinfish ingested. Instead, pinfish would approach the introduced piece of sponge, take it within their mouth, and then rapidly spit it out. Individuals did not mouth the sponge piece more than once. In all cases, controls consisting of similar sized piece of squid mantle tissue were readily ingested.

In contrast, pinfish presented alginate food pellets containing natural volumetric concentrations of spicules displayed a weak, but statistically significant ($P = 0.045$), deterrent response (Fig. 1). Thirty % of the spicule pellets were rejected by fish, while all control alginate food pellets were readily ingested. Pellets with spicules were often mouthed by individual pinfish, chewed slightly, and then spit out. Pinfish did not return to mouth pellets with spicules a second time.

Alginate food pellets containing hydrophilic or lipophilic sponge extract were highly deterrent to pinfish (hydrophilic $-P < 0.001$, lipophilic $P < 0.01$) (Fig. 1). Pellets containing sponge extracts were mouthed and then spit out and pinfish did not attempt to consume pellets after an initial tasting. In contrast, pinfish consumed all of the control pellets in both sponge extract feeding bioassays.

4. Discussion

Amphimedon viridis sampled from shallow seagrass habitats of Saint Joseph Bay, Florida possess a moderately diverse assemblage of sponge-associated organisms whose sizes generally fall into a mesofaunal categorization. A total of 10 sponge-associated taxa were detected with a mean \pm 1 SD species richness values of 4.9 ± 1.5 species per sponge. When standardized to abundances of sponge-

associates per unit volume sponge, the densities of sponge-associates ranged from 19 to 121 per 100 cm^3 wet sponge tissue. This is 1–2 orders of magnitude higher than densities of sponge-associates found to occur in the interstices of two species of the tropical sponge *Haliclona* sp. (0.004 to 4.4 individuals per 100 cm^3 wet sponge; Abdo, 2007). Even if we restrict our comparison to the mesofauna we found to occur in the interstices of *A. viridis* (4 to 39 individuals per 100 cm^3 sponge), abundances are still an order of magnitude higher when compared with those of *Haliclona* spp. Shannon diversity values of *A. viridis* sponge-associates ranged two fold within the sponge population sampled, indicative of a generally diverse community of sponge-associated fauna that varied somewhat from sponge to sponge. This pattern was also similarly reflected in moderate Shannon species evenness values.

Amphipods, by far the dominate sponge-associated taxa (78% of total mesofauna) were found on both the surfaces and interstices of sponges, with approximately one third of them occurring within sponges. This ratio is a general estimate as it was not possible to prevent the likely event that some mesofauna moved out of or into sponges when disturbed during collections. Nonetheless, sponge-associated mesofauna are clearly exploiting both the interstices and the outer surfaces of *A. viridis* as substrate. Among representatives of the Amphipoda, one species in particular, *Laticorophium baconi*, dominated the samples comprising about two thirds of the total amphipods. Three other amphipod species occurred in association with sponges, including *Elasmopus levis*, which comprised about a quarter of the total amphipods, with *Erichthonius brasiliensis* and *Bemlos setosus*, both quite rare, making up the balance.

Amphipods are common associates of marine sponges (Biernbaum 1981, Duffy and Paul 1992, Spanier et al., 1993; Seger and Moran, 1996; Crowe and Thomas, 2002; Riberio et al., 2003; Richards et al., 2007). Those found to occur in association with *Amphimedon viridis* did not belong to taxa known to be strict sponge endocommensals such as species within *Colomastix* and *Leucothoe* genera (S. LeCroy, pers. comm.). Rather, the amphipods found in association with *A. viridis* have been reported in seagrass and rubble habitats in moderate to high salinity environments, and appear to opportunistically occur in association with sponges (LeCroy, 2002, 2004). The high abundance of *Laticorophium baconi* suggests this species may prefer *A. viridis* as a substrate, although this needs to be experimentally evaluated. *Laticorophium baconi* is distributed widely in the Gulf of Mexico, Caribbean, Atlantic, Pacific, and the South China Sea and has been reported from a broad spectrum of habitats including hard substrates, seagrass beds, *Sargassum* rafts, and sand and mud bottoms (LeCroy, 2004). Thus, *L. baconi* certainly does not show obligate specificity to sponges.

Regardless of whether they are strict endo- or ecto-commensals or simply opportunistically exploiting sponges as habitat, amphipods and the other sponge-associated mesofauna observed in the present study may derive benefits from associating with *Amphimedon viridis*. These benefits may include using sponges as a direct or indirect source of nutrition (Oshel and Steele, 1985; Duffy and Paul, 1992; Crowe and Thomas, 2002; Beccero et al., 2006; Crawley and Hyndes, 2007). While not investigated in the present study, it is possible that some mesofauna associated with *A. viridis* feed directly on its tissues. Indeed there are well documented examples of sponge predators that have the ability to consume toxic compounds in the sponge with which they associate (e.g., Pawlik et al., 1988). Mesofauna occurring within the interstices of *A. viridis* may also exploit organic particles brought to them via water currents generated by choanocytic activity (Riberio et al., 2003). Alternatively, some mesofauna may exploit sponges to predate other mesofauna.

As all amphipods brood their young (Munguia et al., 2007) it is possible that their associations with *A. viridis* may provide some resource relative to reproduction. In the present study, approximately 20–30% of the two most abundant species of amphipods (*Laticorophium*

baconi and *Elasmopus levis*) were observed carrying broods (J.P. Huang pers. obs.). Moreover, small numbers of juvenile amphipods were evident suggesting that sponges may provide a suitable habitat for recruitment. Taken together this suggests that some amphipod reproductive activities may be correlated with an association with *A. viridis*. Other mesocrustaceans are known to utilize sponges as “reproductive centers”. For example, the isopod *Paracerceis sculpta* exploits *Leucetta losangelensis* for its reproductive interactions (Shuster, 1989). Moreover, shrimp in the *Synalpheus* genus exploit sponges as a refuge for complex eusocial behaviors including those tied to reproduction (Spanier et al., 1993; Duffy, 2003).

A more parsimonious explanation for the association of mesofauna with *Amphimedon viridis* is in the provision of refuge from predators. The sparid pinfish *Lagodon rhomboides* is a generalist that feeds aggressively on mesocrustaceans in seagrass habitats (Livingston, 1982; Strong and Livingston, 1984). Juvenile pinfish are known to especially target small crustaceans such as amphipods. As the majority of the mesofauna we found in association with *A. viridis* occurred exposed on sponge surfaces, pinfish are likely predators capable of biting at sponge surfaces to try and consume these surface-associated mesofauna. A related sparid, *Diplodus holbrooki*, that occurs in sympatry with pinfish in seagrass habitats, has been found with sponge material in its gut, perhaps incidental to attempts to capture associated mesofauna or from feeding directly on sponge tissues (Strong and Livingston, 1984).

In the present study, small bite-size pieces of whole tissues of *Amphimedon viridis* were highly deterrent to pinfish. The basis of this rejection response by pinfish could be related a low nutritional value of the food item, or the presence of some physical or chemical deterrent. The nutritional hypothesis is unlikely as sponges are well known prey for a number of marine invertebrate and grazing fish species (Randall and Hartman, 1968; Meylan, 1990; Dunlap and Pawlik, 1998), and are known to possess sufficient levels of protein, carbohydrate and lipid to make them attractive prey (e.g., McClintock, 1987). Thus, it is more likely that the rejection response is related to the presence of structural or chemical defenses.

Monoaxon spicules isolated from *Amphimedon viridis* and imbedded in alginate food pellets at an ecologically relevant concentration only weakly deterred feeding in pinfish. Other reports are mixed with respect to the effectiveness of spicules as fish deterrents. Chanas and Pawlik (1995) found that sponge spicules from eight Caribbean sponges were ineffective feeding deterrents against the generalist wrasse *Thalassoma bifasciatum*. Other studies have found deterrent effects. For example, Burns and Ilan (2003) reported spicules isolated from two out of six Caribbean sponges and four out of six Red Sea sponges deterred feeding when presented in food pellets at natural concentrations to the generalist wrasse *Thalassoma klunzingeri*. The weak deterrent response noted in our study may be attributable to the small size of the monoaxons (approx 4 µm length). Burns and Ilan (2003) noted that only sponge spicules larger than 250 µm in length deterred predation in the wrasse *T. bifasciatum* or *T. klunzingeri*. However, Chanas and Pawlik (1995) found large spicules (>250 µm) in some sponges were consumed by fish. Thus, large spicule size per se is not necessarily a fish deterrent.

Food pellets containing natural concentrations of lipophilic or hydrophilic extract from *Amphimedon viridis* elicited strong feeding deterrent responses in pinfish. Similarly, Burns et al. (2003) did not test individual extracts but did find that a combined lipophilic and hydrophilic extract of *A. viridis* from the Red Sea was deterrent to wrasse. Berlinck et al. (1996) described a halitoxin from the tissues of *A. viridis* collected along the Brazilian coast, and a mixture of halitoxin and amphitoxin were isolated from *A. viridis* in the Red Sea (Kelman et al., 2001). While no bioassays were conducted to evaluate whether these cyto- and neurotoxic alkyl pyridine alkaloids from *A. viridis* cause feeding deterrence in pinfish, this seems likely. It has been demonstrated that amphitoxin isolated from a congener, *Amphimedon compressa*, is deterrent against sympatric generalist fish (Albrizio

et al., 1995). As alkaloids are lipophilic, the fact that the hydrophilic extract in the present study was also deterrent to pinfish suggests that additional secondary metabolites may be present that contribute to feeding inhibition.

Several studies have detected a synergistic relationship between spicules and chemical deterrents in marine sponges (e.g., Burns et al., 2003; Hill et al., 2005). In the present study, the feeding deterrent impact of spicules on fish was low, while the impact of chemical deterrents on palatability was high. Thus, it is unlikely that combining spicules with organic extracts from *A. viridis* would prove synergistic. For example, Jones et al. (2005) found that in the marine sponge *Agelas clathrodes*, there was 100 percent rejection of pellets containing organic extracts and only a 9 percent rejection of pellets containing spicules. In subsequent experiments, they found no synergistic effect at natural concentrations of both extracts and spicules. Even though there was no clear evidence of host-sponge specialization among the mesofauna associated with *A. viridis*, a trait suggested to increase defense in amphipods associating with chemically defended macroalgae (Hay et al., 1990), it is likely that even opportunistic associations may yield significant benefits to mesofauna.

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