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### Shedding light on detritus: Interactions between invertebrates, bacteria and substrates in benthic habitats

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# *Chapter 9*

## Degradation of mangrove-derived organic matter in mangrove associated sponges

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**Abstract:** Sponge communities found in Caribbean mangroves are typical to this habitat, in which sponge communities are partly endemic and very distinct from sponge communities on nearby reefs. A trade-off between resistance to competitors and predators appears to influence success of individual sponge species in mangrove habitats. We speculate that differences in the symbiotic microbial communities may partly be responsible for these differences, as partial degradation of recalcitrant compounds by tannin degrading microorganisms may enhance palatability and facilitate DOM assimilation in the presence of high concentrations of tannins, thereby improving their competitive capabilities. We tested tannase activity and ability to degrade mangrove-derived dissolved organic matter (DOM) in a random set of sponge species collected from mangrove roots in Curaçao and adjacent reefs. Our results suggest that sponges commonly associated with mangrove roots contain bacteria that are capable of degrading mangrove-derived DOM, while bacterial communities associated with sponges that are more typical to reef environments appear less proficient in degrading mangrove-derived DOM. Host specificity of bacterial endobionts capable of degrading mangrove-derived DOM and the presence of high concentrations of recalcitrant organic compounds may lead to ecological separation between mangrove and reef sponge communities.

**Keywords:** *Dissolved organic matter, Sponges, Bacterial symbionts, Reef, Mangrove roots.*

Sponges are the dominant fouling fauna within the epibiotic communities that live on submerged roots of the red mangrove *Rhizophora mangle* (Linnaeus, 1753) throughout the Caribbean. Sponge communities found in most Caribbean mangroves are typical to this habitat, i.e. some species are endemic and the structures of the sponge communities in mangroves and nearby reefs are very distinct. It was recently demonstrated that spongivorous predators can exclude typical mangrove sponges from reef assemblages, while reef sponges are excluded from mangrove sponge assemblages by competition in the absence of predation, suggesting a trade-off in resistance to competitors and predators (Wulff, 2005).

Decomposing mangrove foliage litter as well as leaching of tannins and polyphenolic compounds from mangrove roots are the primary input of organic matter in mangrove ecosystems (Dittmar, 2004; Maie and Jaffe, 2006; Kristensen et al., 2008) and recent evidence clearly demonstrated that mangrove-derived dissolved organic matter (DOM) is the primary carbon source for sponges living in mangrove habitats (Granek et al., 2009). Most sponges form close associations with a wide variety of microorganisms (Taylor et al., 2007), and bacterial symbionts have been shown to play a pivotal role in organic carbon assimilation (de Goeij et al., 2008a,b). However, mangrove-derived DOM consists mainly of tannins and polyphenolic compounds (Maie and Jaffe, 2006). It is well established that tannins are structurally complex and recalcitrant to biodegradation (Field and Lettinga, 1992) and a significant fraction (~50%) of mangrove-derived DOM is relatively resistant to degradation (Koch et al., 2005; Kristensen et al., 2008). Since only a limited number of bacterial and fungal species are able to degrade complex polyphenols and tannins (Bhat et al., 1998), and the structure of endobiotic communities is at least partially host specific in the majority of sponges (Taylor et al., 2007), the structure of the microbial endobiotic community of sponges may be an important component in the macroecology of tropical sponges in the western Atlantic.

It is hypothesized that the presence of tannin-degrading microorganisms within the endobiotic community of mangrove sponges may be partly responsible for the structural differences in reef and mangrove sponge communities as partial degradation of recalcitrant compounds may enhance palatability and thereby facilitate DOM assimilation in the presence of high concentrations of tannins. In contrast, the absence of tannin degraders in the endobiotic community may limit developmental and competitive capabilities of sponges. To begin to test this assumption, we qualitatively explore the presence of tannin-degrading organisms in a random set of species collected from mangrove roots and a nearby reef by assaying tannase activity and evaluate whether endobionts are able to grow on artificial substrate containing mangrove root extracts.

## Methods

### *Sponge and root material*

Material was collected in Curaçao, N.A., southern Caribbean, during fieldtrips in May and September 2009. Twenty mangrove sponges were collected from the inner bays Spaanse Water and Piscaderbaai, and fifteen reef specimens were collected at the shallow reefs in front of the research facility of Carmabi (Caribbean Research and Management of Biodiversity) and prepared for analysis as described below. For detailed maps of the sites see Hunting et al., 2008, in press; De Goeij et al., 2008b. Sponge species were identified based on examination of skeleton structure and spicule morphology, in which we followed the nomenclature of Van Soest et al. (2008). Root segments were haphazardly collected in Spaanse Water and Piscaderabaai, wrapped in aluminum foil to prevent photooxidation and stored at 20°C until sample preparation as described below.

### *Tannase activity*

A comparison was made between tannase activity in sponges originating from either mangrove roots or adjacent reefs. Approximately 1 cm<sup>3</sup> sponge tissue was sampled and incubated with seawater containing 0.5 g.L<sup>-1</sup> tannic acid for 3 hours at ambient temperature. Seawater was subsequently discarded, and samples were stored at -20°C until analysis. Tannase activity was assayed using methyl gallate, MG, as described by Osawa and Walsh (1993). In brief, a subsample of the sponge tissue (0.1 cm<sup>3</sup>) was added to 20 mL substrate medium (pH 5) containing NaH<sub>2</sub>PO<sub>4</sub> buffer (33mM) and MG (20mM). Samples were incubated at 37°C for 24 hours under anaerobic and dark room conditions. Methyl gallate is colorless and turns greenish-brownish when hydrolyzed. Coloration of the medium is judged as a positive result for tannase activity. Negative controls containing either sponge tissue and buffer, or MG and buffer, were included to validate that color formation was due to sponge derived tannase activity and to correct for extraction of sponge pigments.

### *Degradation of mangrove-derived DOM.*

In order to determine whether sponge endobionts are able to degrade mangrove-derived DOM, we inoculated bacterial extracts from sponges on agar containing *R. mangle* root extract. Sponge endobionts were extracted from subsamples of sponge tissue (0.1 cm<sup>3</sup>) in a Precellys® 24 lysis/homogenizer (Bertin Technologies, France) using Ø0.5 mm beads and subsequent centrifugation for 30 s at 11000 g. Tannins and polyphenols were extracted from freeze-dried and ground *R. mangle* roots (40g DW) with 70% aqueous acetone for 48 hours. Extracts were centrifuged (4000

rpm 15 minutes) and pellets were air-dried in a flow cabinet. Extracts were subsequently dissolved in deionized water (1L final volume) containing agar (2 g.L) and NaCl<sub>2</sub> (35 psu) and autoclaved for sterilization. This mixture was poured into 50 mL sterile centrifuge flasks to obtain a total substrate volume of 10 mL. After solidification, we added 1 mL of endobiotic extract and artificial seawater 35 psu NaCl<sub>2</sub> to a final volume of 50 mL. Samples were incubated for 72 hours at 37°C under anaerobic conditions. Negative controls did not contain endobiotic bacteria. Overall respiration was subsequently determined measuring DIC accumulation in the overlying water (TIC-TOC analyzer, OI-analytical). Developed biofilms were examined microscopically for presence of bacteria and fungal hyphae. Viability and activity was determined by measuring electron transport system activity (ETSA) following the reduction of 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) to formazan (INTF) sensu Smith and McFeters (1997). Developed biofilms were harvested from the agar surface by scraping and redissolved in 200 µL dH<sub>2</sub>O. Cell integrity was subsequently disrupted by 15 min sonication at room temperature (Branson, 1510). An aqueous INT solution (200 µL; 1 mM with 0.05% dimethylsulfoxide, DMSO) was added and the samples were mixed and incubated for 2 hours at 20 °C under dark room conditions. Enzyme activity was stopped by adding 500 µL DMSO and measured spectrophotometrically at 490 nm (Shimadzu, 1601-UV). Controls were treated with formalin (final concentration 5%) to correct for abiotic reduction of INT.

## Results and Discussion

A total of 15 sponge species were identified from 35 samples (Table 1). Sponge species collected from the mangrove habitat, including *Tedania ignis* (Duchassaing and Michelotti, 1864), *Mycale microsigmatosa* (Arndt, 1927), *Chelonaplysilla erecta* (Row, 1911), *Callyspongia pallida* (Hechtel, 1965), *Haliclona caerulea* (Hechtel, 1965) and *Dysidea etheria* (de Laubenfels, 1936) are commonly found on roots of *R. mangle* throughout the Caribbean (Voss, 1976; Van Soest, 1978, 1980, 1984). Two species, i.e. *Desmapsamma anchorata* (Carter, 1882), *Ircinia strobilina* (Lamarck, 1814) were collected that are known to occur in both habitats (Voss, 1976; Van Soest, 1978, 1980, 1984; Rützler et al, 2000) and were collected from both habitats during this investigation. *Scopalina ruetzleri* (Wiedenmayer, 1977) and *Halisarca caerulea* (Vacelet and Donadey, 1987) were collected from the reef, but are known to occur in both the mangrove and reef environment. The remaining species, *Aplysina archeri* (Higgin, 1875), *Aiolochoxia crassa* (Hyatt, 1875), *Pandaros acanthifolium* (Duchassaing and Michelotti, 1864), *Cribrorhiza vasculum* (Lamarck, 1814) and *Callyspongia*

Table 1. Tannase activity, bacterial activity and rates of mangrove derived organic matter degradation of individual sponge species collected from mangrove roots and adjacent reefs. Indicated is where the collected sponges were sampled and where they naturally occur, i.e. generalist species, occurring in mangrove habitats and adjacent reefs, and reef species, absent from the mangrove environment. **ETSA**, Bacterial activity and **DIC**, rates of mangrove derived organic matter degradation in individual sponge species collected from mangrove roots and adjacent reefs (Provided are mean  $\pm$  S.D.). Previously published data on the presence of microbial symbionts is included.

Species (number of samples)	Sampling habitat <sup>a</sup>	Natural habitat <sup>b</sup>	Tannase activity <sup>c</sup>	ETSA ( $\mu\text{M}\cdot\text{d}^{-1}$ )	DIC ( $\mu\text{g}\cdot\text{d}^{-1}$ )	Presence of symbionts <sup>d</sup>
<i>Tedania ignis</i> (6)	M	G	++	0.144 $\pm$ 0.29	0.76 $\pm$ 0.21	Present <sup>1</sup>
<i>Mycale microstigmata</i> (4)	M	G	+++	0.152 $\pm$ 0.14	1.11 $\pm$ 0.34	Present <sup>2</sup>
<i>Calyspongia pallida</i> (1)	M	G	+	0.091	0.72	Present <sup>3,10</sup>
<i>Chelonophylla erecta</i> (3)	M	G	++	0.082 $\pm$ 0.024	0.41 $\pm$ 0.06	Uncertain <sup>11</sup>
<i>Haliclona caerulea</i> (4)	M	G	+	0.183 $\pm$ 0.171	0.73 $\pm$ 0.26	Present <sup>4</sup>
<i>Dysidea etheria</i> (1)	M	G	+	0.051	0.39	Present <sup>7</sup>
<i>Desmopsamma anchorata</i> (3)	M & R	G	++	0.139 $\pm$ 0.041	0.81 $\pm$ 0.49	Present <sup>6</sup>
<i>Ircinia strobilina</i> (3)	M & R	G	+	0.950 $\pm$ 0.79	0.48 $\pm$ 0.12	Present <sup>3</sup>
<i>Halisarca caerulea</i> (1)	R	G	+	0.190	0.89	Present <sup>2</sup>
<i>Scopalinia reutzleri</i> (1)	R	G	-	0.002	0.08	Absent <sup>5</sup>
<i>Aplysina archeri</i> (4)	R	R	-	0.007 $\pm$ 0.03	0.12 $\pm$ 0.13	Present <sup>7</sup>
<i>Cribrochalina vasculum</i> (1)	R	R	-	0.039	0.25	Present <sup>8</sup>
<i>Allochoxia crassa</i> (1)	R	R	-	0.008	0.22	Present <sup>3</sup>
<i>Panderos acanthifolium</i> (1)	R	R	-	0.029	0.08	Present <sup>9</sup>
<i>Calyspongia vaginalis</i> (1)	R	R	-	0.007	0.03	Present <sup>3</sup>

<sup>a</sup> Abbreviations: M, Mangrove roots; R, Reef

<sup>b</sup> Abbreviations: G, Generalist species, occurring in mangrove habitats and adjacent reefs; R, Reef species

<sup>c</sup> (-) Indicates no color development occurred, and (+, ++, +++ +++) represents an increase in color intensity

<sup>d</sup> Observed directly with electron microscopy or inferred from numerical abundance in sponge extracts or fatty acid profiles. References: 1. Stenle et al. (1988); 2. De Goeij et al. (2008b); 3. Weisz et al. (2008); 4. Maldonado (2007); 5. Rützel et al. (2003); 6. Carballera and Shalabi (1994); 7. Lee et al. (2001); 8. Carballera and Reyes (1990); 9. Schmitz et al. (1981); 10. Uncertain for this particular species, but members of this genus contain bacteria; 11. Very few reports provide anecdotal evidence for the presence of bacteria.

(*Cladochalina vaginalis* (Lamarck, 1814), were collected from the reef and are very common on shallow reef systems throughout the Caribbean, but are absent from mangrove habitats (Voss, 1976; Van Soest, 1978, 1980, 1984; Rützler et al, 2009).

Tannase activities of sponge species and their corresponding sampling and natural habitat are presented in table 1. Sponge species are divided in 2 groups based on their natural occurrence: generalist species, occurring in both mangrove and reef environments; and reef species, occurring in reef environments, but absent in mangrove environments. Color development was variable among specimens collected from the reef and mangrove roots. Except for *S. Ruetzleri*, tannase activity was present in all generalist species collected from both mangrove roots and the reef. All the presented generalist species have also been regularly observed in Caribbean mangrove systems. It should be noted, however, that *D. anchorata* appears more common in mangrove systems in Curaçao (Keunen and Debrot, 1995; Hunting et al., 2008) compared to other mangrove sites in the Caribbean (e.g. Rützler et al, 2000). The species collected from both the reef and mangrove roots, i.e. *D. anchorata* and *I. Strobilina* showed comparable color development, irrespective of sampling habitat. In contrast, color development was clearly absent in sponge species that seem restricted to reef environments.

Sponge endobionts were inoculated on substrate containing mangrove extracts. Microscopic examination of the developed biofilm revealed that bacterial cells were more numerous, morphologically diverse, and motile in treatments containing sponge endobionts that naturally occur in mangrove habitats compared to endobiontic communities retrieved from sponge species typical of reef environments (data not presented). Apart from bacterial cells, we were unable to detect fungal hyphae, thereby we suspect that tannase activity and remineralization of mangrove-derived DOM in our incubations had a bacterial origin. We did not examine the taxonomic identities of the bacteria and whether these bacteria were actual symbionts of the investigated sponges (e.g. Transmission Electron Microscopy), and therefore we can not exclude the possibility that bacteria resided outside sponge tissue. In addition, the presented enzymatic activities may be an overestimation of actual activities due to the relatively long periods of incubation (24 h and 72 h). Current knowledge on sponge-microbe associations remains fragmented (Taylor et al., 2007), and previous studies on sponge-microbe symbiosis of sponge species included in this study often fail to provide evidence that bacteria are present within sponge tissue. Although information on bacterial presence in tissues of the collected sponges retrieved from primary literature (provided in table 1) suggests that, except for *S. Reutzleri*, all sponges appear to have

associations with bacteria, future work should resolve whether these bacteria are true symbionts.

The ability of the sponge microbial community to grow on substrates containing mangrove-derived DOM and the inherent degradation rates of mangrove extracts as approximated by DIC accumulation and ETSA are presented in table 1. The results suggest that sponges commonly associated with mangrove roots contain bacteria that are capable of degrading tannins, while bacterial communities associated with sponges that are more typical to reef environments appear less proficient in degrading mangrove tannins. Mangrove leachate is a complex mixture of compounds that are relatively resistant to microbial degradation and degradation of tannins is restricted to several bacterial and fungal species. Sponges attached to mangrove roots are in the direct vicinity of root leachates and exposed to high concentrations of tannins if tannins are allowed to accumulate. The presence of tannin-degrading symbionts may provide typical mangrove sponges a competitive advantage, provided that symbionts play an important role in the assimilation of organic matter (Weisz et al., 2008; de Goeij et al., 2008a). It has been demonstrated that typical mangrove species grow more rapidly on mangrove roots compared to typical reef species (Wulff, 2005). This growth rate advantage resulted in overgrowth of reef species by mangrove species, which eventually eliminated reef species from the mangrove environment (Wulff, 2005). Future research efforts should elucidate whether differences exist between sponge species from different natural habitats with respect to organic matter assimilation in order to validate the ecological relevancy of the presented observations.

A number of biotic and abiotic variables are considered important in mangrove-sponge endemism and community dynamics. The principal variables include current, temperature, salinity, tidal ranges, storm events, water quality, competition, predation and proximity to source populations (Wulff, 2005; Pawlik et al., 2007; Hunting et al., 2008), in which physico-chemical parameters become progressively variable with increasing distance from the equator. Our results suggest that the structure of the endobiotic community of sponges may contribute to the structural differences between mangrove and reef ecosystems. Although knowledge on the degree of host specificity in sponges remains fragmented, evidence exist that the structure of the endobiont communities is at least partly host specific in the majority of sponge species (Taylor et al., 2007: and references therein). Moreover, host specificity was shown to be important in sponge performance under differing physico-chemical conditions (e.g. Roberts et al., 2006). Host specificity of bacterial endobionts capable of degrading mangrove derived DOM and the presence of high concentrations of recalcitrant organic compounds in mangrove habitats

may improve competitive abilities of mangrove sponges and therefore lead to ecological separation between mangrove and reef communities.