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

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Chapter 10

Substrate as a driver of sponge distributions in mangrove ecosystems

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Abstract: Caribbean mangrove-associated sponge communities are very distinct from sponge communities living on nearby reefs, but the mechanisms that underlie this distinction remain uncertain. This study aimed to elucidate the relative importance of substrate and habitat in the ability of sponges to persist in mangrove ecosystems, and to evaluate the role of bacterial symbiont composition and carbon uptake in sponge distribution. Two reef species (Aplysina archeri and Desmapsamma anchorata) were transplanted to mangrove roots and PVC tubes at a mangrove stand and a reef site. The mangrove species Mycale microsigmatosa was transplanted to both substrates in mangroves as control, showing complete survival. In contrast, reduced survival was observed for D. anchorata with lowest survival on roots in mangroves, intermediate survival on both PVC in mangroves and roots on the reef, and complete survival on PVC on the reef. A. archeri had reduced survival in all treatments, but was most affected by the root substrate in mangroves. These results reveal that the inability of typical reef species to survive in mangrove ecosystems is related to habitat and substrate. The symbiotic bacterial communities were host specific and very similar before and after transplantation. The metabolic diversity of bacterial communities in A. archeri, M. microsigmatosa and D. anchorata strongly separated between host species and the surrounding water. It is speculated that compositional differences in dissolved organic matter (DOM) composition and symbiotic bacteria are potentially important in structuring sponge communities, explaining the exclusion of typical reef species and persistence of mangrove species in mangrove ecosystems.

Keywords: Dissolved organic matter, Sponges, Bacterial symbionts, Reef, Mangrove roots.
Submerged roots of mangroves along (sub-) tropical Caribbean coasts serve as a substrate for a diverse and dense sponge community. It is well documented that the species composition of these mangrove-associated sponge communities is very distinct from sponge communities living on nearby reefs (e.g. Van Soest 1978, 1980, 1984, Wulff 2004, Diaz et al. 2012), but the mechanisms that underlie this distinction remain uncertain (for review see: Wulff 2012). Transplantation experiments of typical reef sponges to roots on off-shore mangrove stands embedded in coral reefs revealed that reef species were able to grow well on roots and compete with typical mangrove sponges in the presence of spongivorous fishes (Wulff 2005). This suggests a major role for biological interactions steering sponge species composition. In contrast, typical reef species deteriorated quickly after transplantation to coastal mangroves in the absence of predation (Farnsworth & Ellison 1996, Wulff 2004, Pawlik et al. 2007), suggesting that abiotic factors are also important for sponge survival and perseverance in mangroves ecosystems, although it remained uncertain which abiotic factor was the key controlling variable.

Previous studies indicated that mangrove sponge community assembly mainly relies on small scale (i.e. among root scale) processes (Guerra Castro et al. 2011), which might be directly related to the root substrate (Hunting et al. 2010a,b). Mangrove-derived organic matter leaching from the roots and decomposing litter are the primary carbon sources for sponges living in mangrove habitats (Granek et al. 2009), and bacterial symbionts play an important role in assimilating these organic carbon sources (de Goeij et al. 2008a,b, Ribes et al. 2012). However, mangrove-derived dissolved organic matter (DOM) consists mainly of tannins and polyphenolic compounds (Maie & Jaffe 2006), which are structurally complex and recalcitrant to biodegradation (Field & Lettinga 1992, Koch et al. 2005, Kristensen et al. 2008). Increasing evidence suggests that only a limited number of bacterial and fungal species are able to degrade complex polyphenols and tannins (Bhat et al. 1998), and it has indeed been demonstrated 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 typical to reef environments appear less proficient in degrading mangrove-derived DOM (Hunting et al. 2010b). It is therefore hypothesized that the presence of bacterial endosymbionts that are capable of degrading mangrove-derived DOM may explain the observed differences in species composition between mangrove and reef sponge communities.

Evaluating the role of habitat, substrate and symbiotic bacteria in driving sponge distributions thus requires experiments that discriminate effects of substrate and habitat (i.e. surrounding water) on the survival and perseverance of sponges in mangrove ecosystems, and assess symbiotic
bacterial community composition and metabolic diversity upon transplantation. We therefore 1) monitored survival and condition of typical mangrove and reef sponge species after in situ reciprocal transplantation to DOM-releasing mangrove roots and DOM-free surrogate roots (PVC tubes) in both mangrove and reef environments; 2) determined the structure and stability of the symbiotic bacterial community in the sponge host before and after transplantation; and 3) evaluated carbon utilization patterns of the symbiotic bacterial communities of the individual sponge species.

**Methods**

**Study site**

For this study, a location was chosen where reef and mangrove ecosystems were closely connected. Therefore, experiments were performed at the “Spaanse water” [N12°4′14.5″, W68°51′36.8″], and “Caracasbaai” [N12°4′11.4″, W68°51′43.8″], on the island of Curaçao, Netherlands Antilles, Caribbean Sea (Fig. 1). The inner bay “Spaanse Water” is connected to the open sea by a small channal, and monopolized by the red mangrove *Rhizophora mangle*. Sponges are the dominant epibionts on fringing roots (on average >10% coverage per root (Hunting et al. 2008)). Tidal ranges are approximately around 10 cm, which does not cause emergence of the resident sponge community. Further details on physicochemical characterization are provided elsewhere (Hunting et al. 2008). “Caracasbaai” is an adjacent reef dominated by corals and sponges. Both sites were used for sponge collection and as transplantation sites.

![Map of study area](image)

*Fig. 1 Map of study area; 1) “Spaanse Water” (mangrove site) and 2) “Caracasbaai” (reef site) on Curaçao. Inset: shaded areas indicate study area.*
Reciprocal transplantation experiment

Three sponge species were selected to investigate substrate and habitat effects upon transplantation in April and July 2010. 1) Mycale microsigmatosa inhabits mainly mangrove habitats and is a dominant species in mangroves in the Western Atlantic (Hunting et al. 2008, Wulff 2009). 2) Desmapsamma anchorata is an opportunistic species that abounds on reefs, but sometimes also occurs in mangrove habitats (McLean and Yoshioka 2008). 3) Aplysina archeri, a vasiform species, is commonly found on Caribbean reefs but is absent in mangroves, and therefore chosen to represent a typical reef species. Fragments of M. microsigmatosa (2-3 cm²) were collected from mangrove roots and adjacent substrata in “Spaanse Water” at depths ranging from 0.9-2.3 m. Fragments of A. archeri (6-10 cm³) and D. anchorata (2-6 cm³) were collected from the “Caracasbaai” reef at depths ranging from 9.8-22.3 m and 4.8-8 m, respectively. Sponge fragments were transported in 25 L containers with natural seawater.

To distinguish between possible substrate and habitat effects, we used freshly cut prop roots of R. mangle and PVC tubes (Ø 40mm) as surrogate roots. PVC tubes were placed at both the mangrove site and reef site approximately one week before the start of the experiment to allow development of a biofilm required for sponge attachment (Ellison et al. 1996). Cleaned prop roots were cut two days before the start of the experiment. All substrates were placed at 2 m depth in both habitats. Fragments of the reef sponges A. archeri (n=80 per treatment) and D. anchorata (n=80 per treatment) were subsequently transplanted to both cut mangrove roots and PVC tubes in both the mangrove site and reef site (total of 320 specimens). Fragments of M. microsigmatosa (n=80 per treatment) were transplanted to both cut mangrove roots and PVC tubes within the mangrove habitat as control to exclude potential effects of transplantation and cutting of the root substrate (total of 160 specimens), although this does not rule out potential negative effects of root cutting on reef sponges. Plastic cable ties were used for attachment, which did not affect the sponges during the experiment. Survival was visually inspected after 0, 2, 4, 6, 8, 10, 14, 18, 24, 32 and 42 days. Confidence limits (CL95%) of the obtained proportions were approximated following Newcombe (1998).

D. anchorata is known for its unusual fast growth (Wulff 2005) and therefore an additional reciprocal transplantation experiment was performed to monitor changes in sponge tissue after transplantation of D. anchorata (n=20 per treatment) to mangrove roots and PVC-tubes in both the mangrove and reef habitat. Transplants were photographically monitored (Nikon digital underwater camera) on five occasions during two weeks, and images were evaluated for survival, necrosis (formation of
white patches), and development of new oscula. Confidence limits (CL95%) of the obtained proportions were approximated following Newcombe (1998).

**Bacterial community structure**

Subsamples were taken from the transplanted sponges to detect changes in bacterial community composition upon transplantation. Triplicate samples of all treatments were sampled immediately upon transplantation, and 25 days after initial sampling for *M. microsigmatosa*. Triplicates were sampled for *A. archeri, D. anchorata* at the point that >60% of the reef sponge transplants died (50 and 29 days after initial, respectively). Subsamples were taken from the internal healthy tissue (without noticeable necrosis) and stored in 100% Dimethylsulfoxide (DMSO) as described by Dawson et al. (1998). Bacterial DNA of the sponge-bacterial consortia was isolated as described by Hardoim et al. (2009). In brief, sponges stored in 100% DMSO were washed in 6.5 mL autoclaved water for 15 to 90 minutes and ground under liquid nitrogen to disrupt the sponge tissue and endoskeleton (mesohyl, mineral spicules and spongin fibers). DNA of ground samples was subsequently isolated using the UltraClean® Microbial DNA Isolation Kit (MO BIO, Carlsbad, New Mexico, US). An additional seawater sample (50mL) at both sites was sampled as control. DNA from filtered seawater samples (0.2μm Cellulose nitrate filter, Whatman®; NC 20) was isolated using PowerWater® DNA Isolation Kit (MO BIO, Carlsbad, New Mexico, US). Isolated DNA was amplified using the general bacterial forward primer F357 (GC CGC CCG CCG CGC GGC GGG CGG GGC GGG GGC ACG GGG CCT ACG GGA GGC AGC AG), containing a GC clamp (CGC CCG CCG CCG CCC GCG CCC GGC CCG CCG CCC CCG CCC C) at the 5' end, and reverse primer R518 (ATT ACC GCG GCT GCT GG) (Muyzer et al. 1993), amplifying the variable V3 region of the 16S ribosomal RNA gene using the following conditions: Initial denaturation: 94°C, 5 min. Cycling steps: 94°C, 30 sec., 54°C, 30 sec., 72°C, 1 min.; 35 cycles; Final elongation 72°C, 8 min.; Cooling 10°C for 15 min. on a MJ Research PTC-200 Thermo Cycler™ (St. Bruno, Quebec, Canada). Denaturing Gradient Gel-Electrophoresis (DGGE) was performed on an Bio-Rad DCode™ system using 1 mm thick gels consisting of 8% (w/v) polyacrylamide (37.5 : 1 acrylamide : bisacrylamide) with a linear denaturing gradient from 30% to 55%, where 100% denaturing solution contained 7 M Urea and 40% (v/v) deionized formamide. The gels were run in 1x TAE buffer (Tris, Acidic acid and EDTA) at 60°C for 4 hours at 200 V and stained with Ethidium Bromide. Images of banding patterns were subsequently analyzed with Gelcompar II (Applied Maths, Kortrijk, Belgium), in which banding patterns of individual species were correlated by means of the unweighted pair group method with arithmetic mean
(UPGMA) and Pearson’s correlation coefficients, and differences between sponge species were tested by a Mann-Whitney U test, where the within-group similarities were tested against the between-group similarities for each combination of species.

**Metabolic diversity of sponge bacterial symbionts**

In order to detect differences in C-resource utilization of the bacterial symbionts of the targeted sponge species, triplicate samples of *Mycale microsigmatosa*, *Desmapsamma anchorata* and *Aplysina archeri* were additionally collected in January 2013 and stored at 4°C for 1 week until analysis. Triplicate samples of the water of both the mangrove site (Spaanse Water) and the reef site (Caracasbaai) were included as control. Sponge samples were ethanol (70%) rinsed (30s) to reduce the potential contribution of superficial bacteria in the analysis. Bacterial endosymbionts of the sponge specimens were extracted from subsamples of sponge tissue (0.1 cm³) in a Precellys® 24 lysis/homogenizer (Bertin Technologies, France) using Ø 0.1 and 0.5-mm beads and subsequent centrifugation for

![Fig. 2 Survival percentages of A. archeri (n=320, t=42 d) and D. anchorata (n=320, t=15 d) after transplantation from the reef site to mangrove roots and PVC tubes at the reef and mangrove site, and M. microsigmatosa (n=160, t=57 d) after transplantation within the mangrove site to mangrove roots and PVC tubes. Error bars indicate approximated 95% confidence limits (C.L.). Bars sharing the same letters are not significantly different at the p < 0.05 level.](image-url)
30 s at 11,000 g (Hunting et al. 2010b). We assessed bacterial metabolic diversity by community level physiological profiling (CLPP) using Biolog® GN microplates containing 95 unique single substrates (Biolog, Inc., Hayward, USA) (Garland and Mills 1991, Garland 1997). The 95 substrates in the Biolog GN plate are comprised of simple, common substrates (e.g. Sucrose, Mallose and Citric Acid), selected on their ability to discriminate among bacterial isolates (Bochner 1989). Biolog GN plates do not include recalcitrant substrates or substrates typical of mangrove DOM, and therefore this approach can only be used to illustrate that microbial communities are functionally similar or distinct (Garland 1999). Samples were incubated for 48h and utilization patterns of 95 different single carbon sources were analyzed using a Jaccard-based cluster analysis and one-way ANOSIM and a bonferroni corrected pair wise comparison (Hammer et al. 2001).

Results
Survival and condition of sponge species

In the control treatments, almost all specimens (>90%) of the sponge *M. microsigmatosa* survived transplantation to mangrove roots and PVC tubes in the mangrove system (Fig. 2), with no significant (p>0.05) difference observed in survival between treatments. In contrast, only a small proportion (17%) of transplants of the sponge *A. archeri* survived transplantation to mangrove roots in the mangrove habitat, which was twice as low compared to transplants to mangrove roots and PVC tubes on the reef or onto PVC tubes in mangroves, (all 57% survival) (Fig. 2). The poor survival of *A. archeri* in all treatments (max 57%) indicates that this species was negatively affected by the transplantation. The lowest survival (17%) observed on mangrove roots in the mangrove site suggests that only substrate effected the survival of *A. archeri*. Transplants of the opportunistic sponge *D. anchorata* performed better compared to transplants of *A. archeri*. *D. anchorata* showed a more gradual mortality compared to *A. archeri*, with lowest survival on roots in mangroves, intermediate survival on PVC tubes in mangroves and roots on the reef (Fig. 2), and complete survival was observed for specimens transplanted to PVC tubes at the reef site. These results indicate a combined effect of substrate and habitat on the survival of *D. anchorata*.

In the photographic monitoring experiment, clear differences were also visible in the development of *D. anchorata* after transplantation, indicated by survival, necrosis and oscula formation. Representative photographs of the most dominant changes per treatment are shown in figure 3, and proportions are presented in figure 4. In the mangrove-root, mangrove-PVC and reef-root treatments, necrosis was already clearly
**Fig. 3:** Photographic recordings of the development of the sponge *D. anchorata* after transplantation from the reef site to mangrove roots and PVC tubes at the reef site and the mangrove site. Photographs were taken on day 0, 4 and 15. The photographs are representative of all replicates for each treatment.
visible on day 4, eventually resulting in total mortality in mangrove-root transplants and partial survival in specimens transplanted to mangrove-pvc and reef-root. The stress caused by the mangrove habitat and/or mangrove root was visible as tissue degradation and alteration. In contrast, all specimens transplanted to PVC at the reef site appeared healthy and formed a number of new oscula at the end of the experiment (day 15), which was also the case for the majority of specimens (~60%) that survived transplantation to mangrove roots at the reef site. Sponge condition generally reflected the same ranking as shown in Fig. 2, i.e. the worst condition was observed on roots in mangroves, intermediate conditions on PVC tubes in mangroves and roots on the reef (Fig. 2), while all specimens transplanted to PVC tubes at the reef site performed very well.

**Bacterial community structure and metabolic diversity**

Cluster analysis of the symbiotic bacterial community composition for replicates (n=3) of the three sponge species (M. microsigmatosa; D. anchorata and A. archeri) and their surrounding waters revealed that the bacterial community composition depended largely on host species (for all species: Mann-Whitney U; p < 0.001), irrespective of sampling time and habitat (Fig. 5). Samples of the surrounding waters of the reef and mangrove ecosystem also clustered together and were distinct from sponge endosymbiont communities (Fig. 5). DGGE patterns of individual sponge species are provided as supplementary material (Fig. S1-3)\(^1\).

![Fig. 4: Condition of the sponge D. anchorata after transplantation from the reef site to mangrove roots and PVC tubes in the mangrove site and the reef site. Condition is categorized by mortality (after 15 d), necrosis (during experiment), and oscula development (after 15 d) as percentage of total number of transplants. Error bars indicate approximated 95% confidence limits (C.L.). Bars sharing the same letters are not significantly different at the p < 0.05 level.](image-url)
Bacterial communities derived from the three sponge species shared a number of carbon sources, but also used a number of unique carbon sources (Supplementary table 1), including e.g. Glycerol and 2-Amino-Ethanol (A. archeri), D,L-Camitine and Succinic acid (D. anchorata), and Phenylethylamine and Hydroxy-L-proline (M. microsigmatosa). Consequently, cluster analysis of metabolic diversity of bacterial communities in the sponge species A. archeri, M. microsigmatosa and D. anchorata, and the control water samples revealed a strong separation between sponge host species as well as the water samples (Fig. 6) (ANOSIM, bonferroni corrected pair wise comparison: p<0.05).

Fig. 5: Pearson’s based cluster analysis of sponge-associated bacterial community composition of samples of M. microsigmatosa, D. anchorata and A. archeri before and after transplantation from the reef site to mangrove roots and PVC tubes at the reef site and the mangrove site. Microbial community composition was significantly different between species (for all species: Mann-Whitney U; p < 0.001).
Discussion

Transplantation of the typical reef sponge *A. archeri* and the opportunistic sponge *D. anchorata* on roots in both the reef and mangrove ecosystem revealed high mortality. This result is in line with previous transplantation experiments where typical reef species were similarly transplanted to mangrove roots, in which potential effects of competition were excluded (Ellison & Farnsworth 1992, Pawlik et al. 2007). Photographic recordings of *D. anchorata* revealed substantial necrosis and mortality in all mangrove and root related treatments, while specimens transplanted to PVC-tubes on the reef developed very well. This outcome illustrates that, in addition to biotic interactions like predation and competition for space (Wulff 2005) and extreme fluctuations in abiotic conditions (Pawlik et al. 2007), root substrate is also critically important in limiting survival of typical reef species in mangrove systems. A large part (~50%) of the *A. archeri* transplants did not survive transplantation in all treatments. Since *A. archeri* typically occurs much deeper than the depth used in our study, it is possible that *A. archeri* transplants were adversely affected by e.g. stressful light intensities. Despite this, survival of *A. archeri* was clearly reduced when transplanted to roots in mangroves, thereby suggesting a substrate effect. *D. anchorata* developed well on PVC tubes on the reef, but survival of *D. anchorata* was reduced when transplanted.
to roots on the reefs site and PVC in mangroves, demonstrating that besides the root substrate, the surrounding water or habitat in general also affected D. anchorata in mangrove ecosystems. Both substrate and abiotic constraints thus affected survival and hampered perseverance of typical reef sponges in mangrove ecosystems.

The question remains why typical mangrove sponge species such as Mycale microsigmatosa are capable of maintaining viable populations on mangrove roots in coastal mangrove ecosystems, and why typical reef species are negatively affected by the root substrate. We hypothesize that bacterial symbionts play an important role. Although there is a growing amount of literature on community composition of sponge endosymbionts, little is known about shifts in these communities induced by biotic and abiotic factors and over time (Thacker & Freeman 2012). Although we only sampled two time points, profiling bacterial communities by means of DGGE suggested that the symbiotic communities in the sponges remained mainly host specific, irrespective of time and treatment (substrate and habitat). This is in line with other studies that considered symbiotic bacterial communities over larger temporal scales (e.g. Hentschel et al. 2002, Yang et al. 2011, Erwin et al., 2012).

Our study further revealed that the host specific bacterial symbionts used different carbon sources, suggesting that each sponge symbiotic bacterial community has its own specific resource niche, as frequently observed for free living environmental bacterial communities (e.g. Salles et al. 2009, Gravel et al. 2011). However, the use of Biolog plates comes with limitations, as ecological relevant substrates are not captured in this assay, and therefore we can not directly relate substrate utilization profiles to distribution patterns of sponge-bacterial consortia. Nonetheless, we speculate that differences in resource niches could potentially be important, especially considering that DOM, although not characterized in this study, often differs both in composition and concentration between mangrove and reef habitats (e.g. Dittmar et al. 2006). It has been demonstrated that the variation in bacterial communities within sponges coincide with metabolic differences between sponge species (Weisz et al. 2007), and that rates of Dissolved Organic Carbon (DOC) and nutrient uptake by sponges largely depend on the concentration of symbiotic bacteria (Ribes et al. 2012). Evidence for sponge resource preferences is also evident from studies that considered isotope ratios. For instance, Van Duyl et al. (2012) showed that encrusting sponges in reef cavities feed mainly on DOM derived from crustose coralline algae and coral mucus, while mangrove-derived organic matter is an important C-source for sponges that typically occur in mangrove ecosystems (Granek et al. 2009). Although the utilization profiles of simple substrates obtained in this study do not directly relate to mangrove DOM, they complement a previous
study that reported differences in the ability of bacterial symbionts to degrade mangrove DOM (Hunting et al. 2010b). Bacterial symbionts of mangrove sponges appeared proficient in degrading mangrove DOM, while symbionts of reef sponges were less capable of degrading mangrove DOM. Earlier studies also showed that common mangrove-associated sponges grew faster when attached to mangrove roots compared to PVC tubes, while typical reef species performed better on PVC than on the root substrate (Ellison et al. 1996, Wulff 2005). Although this remain speculative and requires further investigation, this suggests that typical mangrove species potentially have a competitive advantage over reef species when growing on mangrove roots, and that the composition of DOM can be of general importance to the performance of sponge-bacterial consortia. However, since mangrove DOM consists of a complex mixture of structurally diverse compounds, a major challenge remains the identification of specific bacterial gene-clusters (e.g. pyrosequencing) and relevant compounds in DOM (e.g. gas chromatography – mass spectrometry, GC-MS), and ultimately how these components interact and relate to the overall performance of sponge-bacterial associations under natural conditions.

In conclusion, increased mortality and poor development upon reciprocal transplantation revealed that the inability of typical reef species to survive in mangrove ecosystems is due to a combined effect of abiotic constraints and the root substrate. Our results further suggested that bacterial symbionts are largely host specific and have a specific DOM resource niche, hinting that differences in DOM composition and corresponding differences in symbiotic bacterial communities potentially are important in structuring sponge community composition, which would explain the exclusion of typical reef species and the persistence of mangrove species in mangrove ecosystems.

1Supplementary data related to this chapter can be found at:
www.int-res.com/articles/suppl/m486p133 supp.pdf