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

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Chapter 8

Mangrove-sponge associations: a possible role for tannins

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Abstract: A positive correlation between sponge coverage and tannin concentrations in prop roots of *Rhizophora mangle* L. has previously been reported. However, the ecological role of tannins within the mangrove sponge association remains speculative. This study investigated whether tannins play a role in sponge recruitment and assessed tannin and polyphenol production in *R. mangle* roots in response to sponge colonization. We demonstrated in a field experiment using artificial substrates with different tannin concentratrions that tannins are positively involved in larval recruitment of the sponge *Tedania ignis,* and that roots significantly enhanced tannin and polyphenolic content in response to natural and experimental sponge fouling. Differential recruitment in response to tannins may have been the result of a behavioral response in sponge larvae. It is also possible that tannins affected the structure of the fouling microbial biofilm on the artificial substrate, or tannins affected the post-settlement dynamics of sponge recruits. Elevations in concentrations of tannins and polyphenolic compounds upon coverage with sponges, combined with differential recruitment of *T. ignis* in response to differences in tannin concentrations, may indicate a positive feedback in recruitment. This may in part explain the typical heterogeneity in sponge coverage and community composition among roots.

***Keywords**: Substrate selection, Sponges, Tannins, Polyphenols, Mangroves, Recruitment*

Sponge communities in mangrove systems consist of species typical to this habitat but are spatially heterogeneous, i.e. neighboring roots can vary greatly in their sponge coverage and composition. This variability can partly be attributed to low recruitment rates, limited larval availability and interactions among sponges (Sutherland 1980; Bingham, 1992; Engel and Pawlik 1995), or may be the result of chemically induced interactions between sponges and mangroves. Tannins and polyphenolic compounds constitute the greater portion of carbon leachates from mangrove leaves (Maie et al. 2006) and concentrations of tannins and polyphenols may vary depending on tissue, growth stage and environmental conditions (Northup et al., 1998; Lin et al., 2006). A positive correlation between sponge coverage and tannin concentrations in the roots of *R. mangle* was previously reported, in which roots with considerable sponge cover (>40%) contained elevated tannin concentrations compared to unfouled roots (Hunting et al., 2008). This suggests that tannins and polyphenolic compounds in general are potentially involved in the mangrove sponge association.

The mechanism responsible for the observed relation remains speculative since the ecological functioning of tannins is ambiguous. Firstly, it has been speculated that tannins act as a settling cue for sponge larvae (Hunting et al., 2008) or provide a carbon source for epibiontic sponges (Ellison et al, 1996) as leached DOC precipitates are also a source of particulate food for metazoans (Baylor and Sutcliffe, 1963; Kuznetsova et al., 1984). Secondly, increases in tannin concentrations may be a physiological response of mangrove roots to sponge fouling. A substantial number of studies have suggested that grazing and fouling are strongly correlated to increases in polyphenol concentrations in which polyphenols may form recalcitrant complexes that are resistant to biodegradation, thereby reducing palatability and inhibiting growth of fouling organisms (e.g. Schmitt et al. 1998). Thirdly, sponge presence may positively or negatively affect nutrient availability for *R. mangle* roots, thereby causing differences in secondary metabolite production. It has been hypothesized that excess carbon may be allocated to carbon rich secondary metabolites in the absence of nutrients (Bryant 1987), while several studies have provided evidence that nutrient enrichment enhances production of total phenolics and tannins in *R. mangle* (Feller 1995; Feller et al. 2003; Feller & McKee 2003).

The objective of this study was to evaluate whether tannins play a role in sponge recruitment and whether roots of *R. mangle* enhance production of tannins and total phenolics in response to sponge colonization. These aspects were addressed by performing *in situ* recruitment and translocation experiments.

Methods

Study site

This study was conducted within Spaanse Water (12°04’21.78’’N, 68°51’38.87’’W), an inner bay of Curaçao, N.A., Southern Caribbean sea (Figure 1), in the period of March until June 2008. Detailed information on physico-chemical characteristics is provided elsewhere (Hunting et al. 2008: and references therein). In brief, the site is moderately eutrophic with low turbidity. Distance to the nearest reef is 2.2 km. Salinity is around 35 psu and pH is around 8. Tidal ranges are approximately 10 cm and resident sponge communities do not emerge during low tide. Sponge coverage is on average little over 10 percent of the total root substrate.

Recruitment experiment

The role of tannins in recruitment of sponge larvae was assessed with artificial substrates made from polymeric gels. This method proved useful in studies focusing on the retention of larval settlement (e.g. Henrikson & Pawlik 1995; Browne & Zimmer 2001). Tannins embedded in a matrix of agar diffuse into the overlying water. A total of 30 mimicry gels (surface area 88 cm^2, volume 440 cm^3; 20 $g.L^{-1}$ agar and 60 µM ascorbic acid) consisted of 3 different treatments containing either 0, 0.3 or 1.8 nM tannic acid (purity > 96%, Sigma-Aldrich). Although the majority of phenolic compounds in woody plants are condensed tannins we used tannic acid as a representative of hydrolyzable tannins, the second major group of phenolics that occur primarily in young, rapidly growing tissues (e.g. prop roots of *R. mangle*) of woody plants (Haukioja et al., 1998). The mimicry gels were vertically installed with plastic rope throughout the mangrove fringes at ± 0.8 m depth in a random fashion in order to minimize the effect of variable physico-chemical conditions (e.g. flow conditions). Gels were placed within cages (mesh-size 1 cm^2) to prevent spongivory. The average leaching rates of tannic acid from agar gels were approximated from a 7 week incubation in aquaria containing artificial seawater with starting concentrations of 0.3 and 1.8 nM. Leaching rates decreased about 40% over the course of the experiment, but were still detectable after 7 weeks. The average leaching was 4 (± 0.26 s.d.) $mgC.d^{-1}$ and 15 (± 0.54 s.d.) $mgC.d^{-1}$, respectively. The average leaching rates are comparable to leaching rates of total phenols (on average 2.4 $mgC.d^{-1}$) reported by Maie et al. (2006), and dissolved organic carbon (on average 10 $mgC.d^{-1}$) reported by Camilleri and Ribi (1986). Conversion of reported leaching rates followed an empirical relation between root dry mass and root length reported by Ellison and Farnsworth (1996). Mimicry gels were collected after 7 weeks and brought to the lab. Gels remained submerged in seawater during transport to prevent exposure to air. Recovered recruits were prepared for microscopy in canada balm. Species identification was based on microscopic examination of skeleton structure and spicule morphology following the nomenclature of Hooper & Van Soest (2002).

Transplantation experiment

A transplantation experiment with specimens of *Tedania ignis* was performed in order to determine whether sponge fouling can induce enhanced tannin and polyphenol production. Fifteen roots were selected for each of the following treatments: **(1)** natural root cover, in which sponge coverage exceeded 40 % (primarily *T. ignis*); **(2)** bare roots that were not covered by any fouling organism during sampling; **(3)** bare roots used for transplantation of specimens of *T. ignis* (collected from neighboring roots and adjacent benthic substrata) attached with plastic cable ties as described by Ellison et al. (1996), in which we aimed to obtain a coverage that was comparable to treatment 2 (>40%); and **(4)** bare roots that were wrapped in plankton net (mesh size 500 µm), to allow natural turbidity to occur while preventing larval recruitment and isopod invasion, thereby ensuring that roots remained unfouled over the course of the experiment. The latter treatment served as an extra control for treatment 2, for which there was no guaranteed absence of fouling organisms during the experiment. Mortality of transplants was < 5%. Any losses within the first 2 weeks of the experiment were replaced. Samples (15 cm root segments) were collected after 8 weeks, wrapped in aluminum foil and immediately stored on ice. All samples were stored at -20°C within 1 hour after collection and remained frozen until analysis.

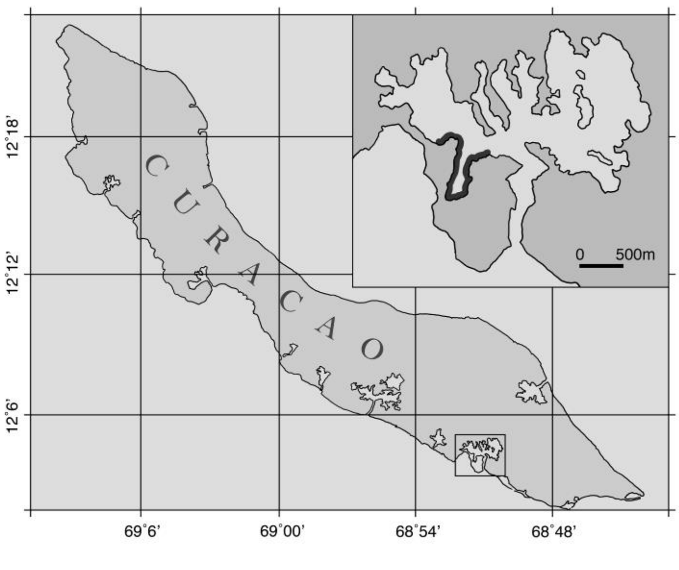

Fig. 1: Map of Curaçao and Spaanse water (inset) showing location of the study site (shaded area).

Analytical techniques

Root samples (15 cm) were freeze-dried and ground with an electric coffee grinder (particle sizes ranged 200 – 300 µm). Tannins and polyphenolic compounds were extracted from 100 mg of ground sample as described previously (Hunting et al. 2008). Analysis of the protein precipitating fraction of tannins is described in detail elsewhere (Hagerman 1987; Hunting et al. 2008). Total phenolics were determined using Folin-Ciocalteu reagents as described by Ragazzi & Veronese (1973). Prior to this assay, 10 µL extracts were put in open aliquots in a flow cabinet to allow vaporization of aceton. Phenolics were subsequently resuspended in 10 µL deionized water and assayed. Absorbance was measured at 740 nm (Nanodrop, ND1000). Tannic acid was used for calibration and results were expressed as Tannic Acid Equivalents ($gTAE.gDW^{-1}$). Treatments were compared by performing a one-way ANOVA with a Tukey-Kramer post hoc test for multiple comparisons of means (Matlab v.7.0).

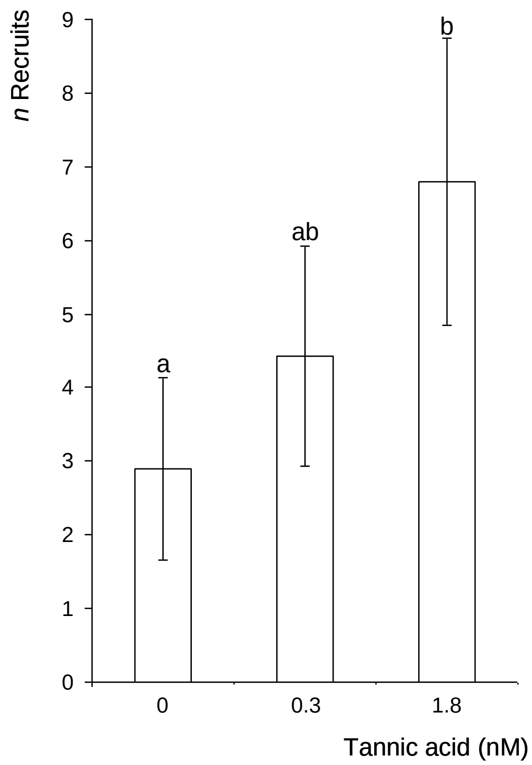

Fig 2. Average number of sponge recruits on mimicry gels with different tannin concentrations. Provided are means (± s.e.). Corresponding letters indicate statistical similarity (α < 0.05; n = 10 per treatment).

Results

Sponge recruitment on mimicry gels containing tannic acid is presented in Fig. 2. An average of 7 recruits was recovered from gels with high tannic acid contents (1.8 nM), which was significantly ($p<0.05$) higher compared to an average of 3 recovered recruit in mimicry substrates that did not contain tannic acid. Figure 2 suggests that there is enhanced recruitment in the lower ranges of tannic acid content (0.3 nM), however, there was no statistical difference between the low concentration treatment and that of the zero and high concentration (1.8 nM) treatments. Next to sponge recruits, the mimicry gels also contained some Bryozoa, and each treatment contained a patchy, yet unspecified microbial biofilm. All recruits were 2 - 3 mm in size. Over 90% of the recruits recovered from the mimicry gels were identified as *Tedania (Tedania) ignis* (Duchassaing and Michelotti) and the remaining species included *Desmapsamma anchorata* (Carter), *Dysidea janiae* (Duchassaing & Michelotti) and *Ircinia felix* (Duchassaing & Michelotti).

Total phenolic and tannin concentrations in mangrove roots of the different treatments are presented in Figures 3A and 3B, respectively. Following an 8 week incubation period, roots covered with sponges contained approximately 20% more phenolic compounds and 22% more tannins than roots that were not covered by sponges. Roots covered with transplants contained significantly higher ($p<0.05$) concentrations of total phenols, and tannins compared to roots covered with plankton net. Furthermore roots that were naturally covered with sponges expressed a significant ($p<0.05$) increase in total phenol content compared to roots covered with plankton net.

Discussion

The present study demonstrates that recruitment of the sponge *Tedania ignis* is enhanced when substrates contain higher tannin concentrations. Sponges have short living lecithotrophic larval stages and hydrology and stochasticity are considered the principal factors explaining large scale spatial patterns, while active habitat selection becomes progressively more important at small (< 1m) spatial scales (Pawlik 1992; Mariani et al., 2006). Since roots of *Rhizophora mangle* generally provide the only stable substrate in mangrove ecosystems, substrate localization is a critical process for the successful reproduction of mangrove associated sponges. In order to actively select an appropriate substrate, sponge larva should have the ability to detect and discriminate substrate specific cues. Enhanced recruitment associated with increased tannin concentrations may be the result of a behavioral response of sponge larvae to tannins. However, although larvae of several sponge species are known to actively

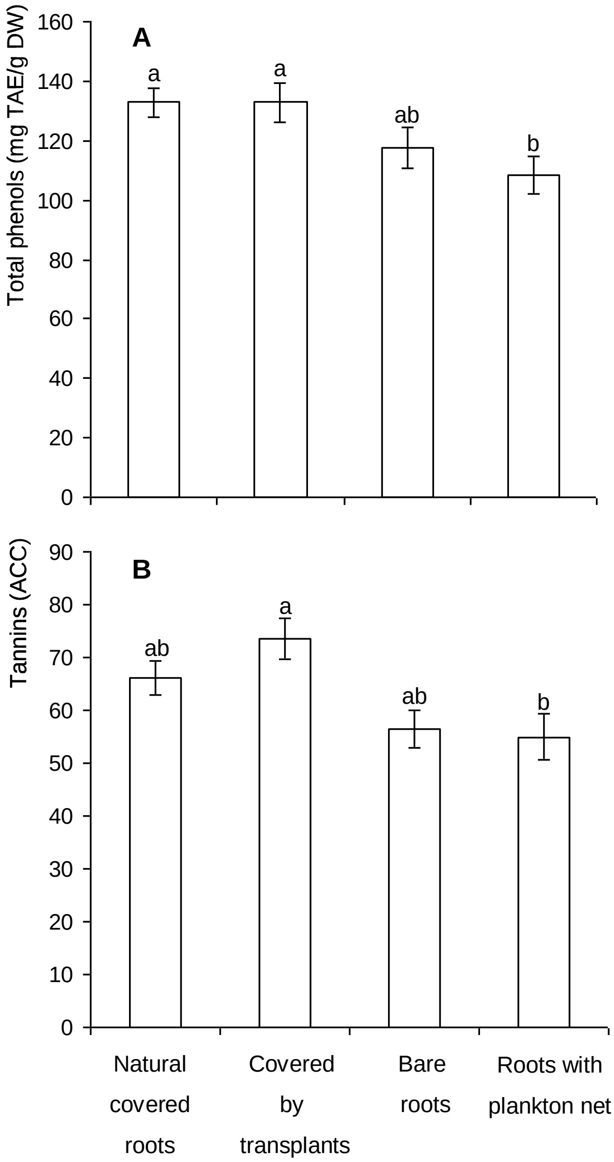

Fig. 3. Concentrations of (A) total phenolic compounds (expressed as Tannic Acid Equivalents,TAE) and (B) protein precipitating tannins (expressed as Albumin Complexing Capacity, ACC) of mangrove roots after 8 weeks of incubation with four different treatments in Spaanse Water, Curaçao. Treatments include natural covered roots, natural unfouled roots, roots covered with transplants and roots wrapped in plankton net. Bars indicate means (± s.e.) and corresponding letters indicate statistical similarity ($\alpha < 0.05$; $n = 15$ per treatment).

examine and select an appropriate substrate and chemical stimuli are often considered a cue in selective settlement (Maldonado 2006, Whalan et al. 2008), no unequivocal evidence exists that clearly demonstrates chemotaxis and chemical regulation of settlement in sponge larvae. Alternatively, tannins may have altered the structure of the fouling microbial consortium and larvae of *T. ignis* may have subsequently responded to differences in chemical, textural or structural aspects of the microbial biofilm. In addition, differences in tannic acid contents between treatments may have caused differences in post-settlement dynamics. It is possible that higher tannic acid concentrations may have reduced mortality of sponge recruits due to increased nutrient availability or reduced predation. Irrespective of the mechanism, a direct or indirect influence of tannins on sponge larvae may suggest that secondary metabolites of plants and algae play a pivotal role in substrate selection or post-settlement dynamics and henceforth polyphenolic compounds may be involved in structuring epibiont communities. Future research efforts should elucidate whether larvae of *T. ignis* respond directly to dissolved tannins or polyphenolic compounds or indirectly to possible differences in the fouling microbial assemblages. It is also necessary to evaluate the early stages of sponge community assembly and post-settlement dynamics in relation to tannins and polyphenols in order to gain insight in the ecological relevancy of tannins and polyphenols in sponge community patterns.

Sponge colonization promoted tannin and phenol production in *R. mangle* roots. We can not exclude the possibility that elevations in tannin concentration are physiological responses to stress (e.g. injury). However, we speculate that the elevated concentrations of tannins and polyphenolic compounds upon coverage, combined with differential recruitment of *T. ignis* in response to differences in tannin concentrations, may result in a positive feedback in recruitment, i.e. settlement induces elevation in secondary metabolites, which, in turn enhances the number of recruits. This type of mechanism may help explain among root heterogeneity and would complement a facultative mutualism between *R. mangle* and common root fouling sponges as proposed by Ellison et al. (1996). These authors presented evidence that *R. mangle* roots produce adventitious rootlets generally involved in nutrient uptake that ramify tissue of several sponge species, including *T. ignis*. They also found that roots of *R. mangle* obtain nitrogen from sponges and that sponges obtain carbon from *R. mangle* roots, while both sponge and tree exhibit enhanced growth rates upon association. In addition, enhanced eutrophic state and experimental fertilization has been shown to result in elevations of *R. mangle* phenolics (Feller 1995; Feller et al. 2003; Feller & McKee 2003; Hunting et al. 2008). Elevations of phenolic compounds in this study may therefore indicate that sponge coverage enhances nutrient availability to *R. mangle* roots.

This study aimed to evaluate the role of tannins within the association of sponges and prop roots of the red mangrove *R. mangle*. The results presented here indicate that tannins are directly or indirectly involved in the recruitment of the sponge *T. ignis* and suggest that the ecological role of tannins within the mangrove-sponge association is more complex than previously anticipated.